Biochemical Systematics and Ecology 37 (2009) 206–213

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Essential oil composition of *Bothriochloa* Kuntze (Poaceae) from South America and their chemotaxonomy

Lidia R. Scrivanti*, Ana M. Anton, Julio A. Zygadlo

Instituto Multidisciplinario de Biología Vegetal (CONICET – Universidad Nacional de Córdoba), Casilla de Correo 495, 5000 Córdoba, Argentina

ARTICLE INFO

Article history:
Received 9 December 2008
Accepted 24 March 2009

Keywords:
Poaceae
Andropogoneae
Bothriochloa
Aromatic grasses
Essential oils
South America
Chemotaxonomy
Multivariated statistical analysis

ABSTRACT

The essential oils compositions of the 13 taxa (twelve species and two varities) of *Bothriochloa* have been studied. Two entities are endemics: *Bothriochloa eurylemma* and *Bothriochloa meridionalis*. The other nine taxa have disjunt distribution between North and South America and are representatives of two complexes: the *Bothriochloa barbinodis* complex and the *Bothriochloa saccharoides* complex. Multivariated statistical analysis (Principal Component Analysis, Hierarchical Cluster Analysis) applied to GC–MS data seems to have systematic significance for the delimitation of the species, independently of their morphological characteristics. However, some affinities with chromosome complement and mode of reproduction (amphimixis or sexuality) can be recognized.

1. Introduction

The genus *Bothriochloa* Kuntze (Poaceae: Andropogoneae) comprises about 40 species widespread in warm-temperate areas throughout the world. Twenty eight species inhabit in the Americas where about 12 native taxa have disjunct distribution in North and South America (Vega, 2000) and three are endemic of the South America (Argentine and Brazil).

According to their morphological and cytological characteristics, the American species belong to two complexes. In the *Bothriochloa barbinodis* complex, the spikelets have a glandular pit in the first glume (although in some collections only some of the glumes are pitted), spikelets more than 5 mm long with a long awn, and a chromosome complement of 180 or 220. The species of the *Bothriochloa saccharoides* complex have the first glume lacking a glandular pit, spikelets usually less than 5 mm long, with short awns and a chromosome complement of 60 or 120. Nevertheless, polymorphic species of difficult circumscription exist in both complexes (De Wet, 1968; Allred and Gould, 1983).

Bothriochloa is a well known aromatic grass, although different compositions of their essential oils from Old World (Pinder and Kerr, 1980; Melkani et al., 1984; Bhandari et al., 1993; Kaul and Vats, 1998) have been previously reported, no previous studies were found on the essential oil composition from America. The essential oils of *Bothriochloa* species from Old World were characterized by dominance of three sesquiterpenes: intermedeol, neointermediol and acorenone-B. According to the dominating sesquiterpene in the essential oil, Zalkow et al. (1980) propose to divide the genus in three groups.

The aims of this study were: (a) to determine the essential oil composition of the thirteen taxa of *Bothriochloa* from South America (Argentine, Brazil and Uruguay) (b) to examine their potential chemotaxonomic significance. The constituents of all the essential oils were subjected to Multivariate Statistical Analysis by means of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

* Corresponding author. Tel.: +54 351 4331056; fax: +54 351 4332104.
E-mail address: rscrivanti@imbiv.unc.edu.ar (L.R. Scrivanti).

0305-1978/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2009.03.009

2. Materials and methods

2.1. Plant materials

Table 1 contains information concerning the species of *Bothriochloa* studied, the voucher numbers of the specimens at CORD, the site and date of collection; the plants analyzed in this study were also maintained in the glass house. All the plant samples were collected at bloom stage, and the whole plant material was used.

2.2. Essential oil isolation

Dried plants were hydrodistilled in Clevenger-like apparatus. The oils obtained were dried over anhydrous sulphate and stored in a refrigerator until analysis.

2.3. Gas chromatography analyses

Analyses were performed in a Shimadzu GC-R1A (FID) gas-chromatograph, fitted with a 30 m × 0.25 mm (0.25 μm film thickness) fused silica capillary column coated with a phase 5% phenyl 95% dimethylpolysiloxane, non-polar DB-5 column, and then we used a polar Supelcowax 10 capillary column, phase polyethyleneglycol. The GC operating conditions were as follows: oven temperature programmed from 40 to 230 °C at 2 °C/min, injector and detector temperatures 240 °C. The carrier gas was nitrogen at a constant flow of 0.9 ml/min. The oil components were identified by comparison of their retention indexes (RI), mass spectra with those of authentic samples, by peak enrichment, with published data (Adams, 1995) stored in the mass spectra library of National Institute of Standards and Technology (NIST 3.0) and our mass spectra library which contains references of mass spectra and of retention indices of volatile compounds. GC–MS analyses were performed with a Perkin–Elmer Q-700 equipped with a SE-30 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm film). The analytical conditions were: oven temperature from 40 to 230 °C at 2 °C/min, the carrier gas was helium at a constant flow of 0.9 ml/min, the source was at 70 eV.

All the analyses were performed in triplicate.

2.4. Data analyses

All data were statistically analyzed using statistical InfoSat software (version 1.1). Hierarchical cluster analysis was carried out based on essential oil components present in different taxa. Standard Euclidian distance matrix was generated, and clusters among taxa were made with average linkage method by using InfoStat software (version 1.1). Principal component analysis was carried out to find oil constituents with maximum loading in the first and second components.

3. Results

3.1. Essential oil composition

The compositions of the oils isolated from the thirteen taxa of *Bothriochloa* are shown in Table 2. The oils were complex mixtures of sesquiterpenes, monoterpenes and non-terpenes: 104 components were identified in the essential oils of the thirteen entities studied.

Table 1
Species of *Bothriochloa* from South America studied, origin and voucher.

Plant species	Voucher[a]
B. alta (Hitchc.) Henrard	Argentina. Córdoba: Dept. Santa María, Anizacate, 26-IV-2004, Scrivanti 173.
B. barbinodis (Lag.) Herter	Argentina. Córdoba: Dept. Punilla, La Cumbre, 26-IV-2004, Scrivanti 179.
B. edwardsiana (Gould) Parodi	Argentina. Córdoba: Dept. Punilla, La Cumbre 10-V-2004, Scrivanti 190.
B. eurylemma M. Marchi & Longhi-Wagner	Argentina. Entre Ríos: Dept. Federación, Federación, 15-III-2005, Scrivanti 243.
B. exaristata (Nash) Henrard	Argentina. Corrientes: San Martín, Yapeyú, 15-III-2006, Scrivanti et al. 278.
B. imperatoides (Hack.) Herter	Brazil. Rio Grande do Sul: Uruguaiana, CORD 36.
B. laguroides (DC.) Herter var. *laguroides*	Uruguay. Maldonado, Punta del Este, 13-III-2005, Scrivanti 234.
B. laguroides var. *torreyana* (Steud.) M. Marchi & Longhi-Wagner	Brazil. Rio Grande do Sul: Uruguaiana, CORD 44.
B. longipaniculata (Gould) Allred & Gould	Argentina. Entre Ríos: Dept. Federación, Federación, 15-III-2005, Scrivanti 242.
B. meridionales M. Marchi & Longhi-Wagner	Brazil. Rio Grande do Sul: Uruguaiana, CORD 39.
B. perforata (Trin. ex Fourn.) Herter	Argentina. Córdoba: Dept. Capital, 06-II-2004, Scrivanti 46.
B. saccharoides (Sw.) Rydb. var. *saccharoides*	Argentina. Córdoba: Dept. Punilla, La Cumbre, 10-V-2004, Scrivanti 191.
B. springfieldii (Gould) Parodi	Argentina. Córdoba: Dept. Punilla, Carlos Paz, 10-V-2004, Scrivanti 187.

[a] Housed at CORD.

Table 2
Yield (%) and composition (%) of the essential oils of *Bothriochloa* species studied.

Composición	RI	1	2	3	4	5	6	7	8	9	10	11	12	13	14[b]	15[b]	16[b]
tricycline	915	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–	–
α-thujene	931	–	–	tr	–	–	–	tr	–	–	–	–	–	–	–	0.7	0.9
α-pinene	940	–	–	tr	–	–	–	tr	–	0.6	–	–	–	tr	1.4	2.7	0.8
α-fenchene	953	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
camphene	954	–	–	tr	–	–	–	tr	–	–	–	–	–	–	7.4	3.2	5.9
sabinene	975	–	–	–	–	–	–	–	–	0.3	–	0,1	–	0.1	–	–	–
β-pinene	981	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7
myrcene	991	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	0.3	–
butyl butanoate	995	0.1	–	–	–	–	–	tr	–	0.4	–	–	0,1	0.1	–	–	–
ethyl hexanoate	998	–	–	–	–	–	–	tr	–	0.7	–	–	0.3	0.2	–	tr	–
decane	1000	–	–	–	–	–	–	4.5	–	2.0	8.1	–	0.7	0.3	–	–	–
α-terpinene	1019	–	–	–	–	–	–	tr	–	–	–	0.8	–	–	–	–	–
p-cymene	1025	–	–	–	–	–	–	tr	–	–	–	–	–	–	–	0.1	0.4
limonene	1030	–	–	–	–	–	–	–	–	0.2	–	–	–	–	14.9	6.7	7.1
beta phellandrene	1030	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	0.1	–
1,8-cineole	1032	–	–	–	–	–	–	–	–	–	–	2.3	–	–	–	tr	–
(Z)-β-ocimene	1039	–	–	–	–	–	–	–	–	0.1	–	–	–	–	0.8	–	–
isobutyl hexanoate	1044	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–
isopentyl butanoate	1058	–	–	–	–	–	15.1	0.1	–	–	–	–	–	–	–	–	–
γ-terpinene	1063	–	–	–	–	–	–	–	4.2	–	–	–	–	–	–	–	–
propyl hexanoate	1079	–	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
camphenilone	1082	–	–	–	–	12.9	–	–	–	–	5.6	–	–	–	–	–	–
terpinolene	1090	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–
linalool	1097	–	–	–	–	–	–	–	–	–	–	9.6	–	–	0.5	–	0.4
n-undecane	1100	–	–	–	–	–	0.2	5.2	–	1.3	–	–	1.4	0.8	–	–	–
methyl octanoate	1127	–	–	–	–	–	–	2.3	–	–	–	–	0.1	0.1	–	tr	–
camphor	1147	–	–	–	–	–	–	tr	–	0.4	–	–	–	–	0.1	0.3	–
isoborneol	1163	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–	–	0.3
borneol	1170	–	–	–	–	–	–	tr	–	0.6	–	0.3	–	–	2.3	–	0.4
4-terpineol	1178	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4	0.2	0.5
C10H15	1179	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5	–	–
butyl-n-hexanoate	1188	0.1	–	–	–	–	–	2.2	–	0.4	–	–	0.1	tr	0.4	0.3	–
α-terpineol	1189	–	–	–	–	–	–	–	–	–	–	–	–	–	1.4	–	0.3
hexyl butanoate	1193	tr	–	0.2	–	–	0.6	3.2	–	–	–	–	–	–	–	–	–
2*E*-hexenyl butanoate	1194	–	–	–	–	–	11.5	1.1	–	–	6.7	–	–	–	–	–	–
ethyl octanoate	1197	–	–	–	–	–	–	0.2	–	0.4	–	–	0.1	tr	–	0.2	0.3
3-decanol	1197	–	–	–	–	–	–	tr	1.2	1.1	–	–	–	–	–	–	–
n-dodecane	1200	–	–	–	–	–	0.3	12.7	2.2	0.9	3.2	–	1.7	1.6	–	–	–
trans carveol	1218	0.2	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–
isobornyl acetate	1226	–	–	–	–	–	–	–	–	–	–	–	–	–	2.0	0.3	–
cis carveol	1230	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	0.3	–
isoamyl hexanoate	1238	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–
isobornyl formate	1240	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–	–
carvone	1246	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.8	1.2
nonanoic acid	1271	2.4	–	–	–	–	–	tr	–	6.3	5.8	–	0.4	0.2	–	–	–
perillaldehyde	1271	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7
no conocido 1	1275	–	–	–	–	–	–	–	–	–	–	–	–	–	1.3	–	–
methyl nerolate	1280	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
trans-anethole	1285	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
thymol	1290	0.2	–	–	–	–	–	–	1.8	–	–	–	–	–	–	–	–
tridecene	1292	–	–	–	–	0.4	1.0	0.9	–	–	–	–	–	–	–	–	–
geranyl formate	1298	–	–	11.6	–	–	–	–	–	–	–	–	–	–	–	–	–
methyl geranate	1326	1.6	–	6.2	–	–	–	–	–	–	–	–	–	–	–	–	–
δ-elemene	1340	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–
trans-2-hexenyl-n-hexanoate	1368	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.7	–
butyl octanoate	1373	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–
β-patchoulene	1381	0.4	–	–	1.4	–	–	–	–	–	–	–	–	–	–	–	–
geranyl acetate	1381	–	–	0.4	–	–	–	–	–	–	–	–	–	–	0.5	2.6	–
(E) β-damascenone	1385	–	–	–	–	–	–	–	–	–	46.8	–	–	–	–	–	–
β-cubebene	1388	0.2	–	0.1	–	–	0.2	tr	–	–	–	–	–	–	–	–	–
β-bourbonene	1388	–	–	–	–	–	–	-	1.6	–	–	–	0.7	0.2	0.3	–	1.6
1-tetradecene	1389	–	–	–	–	0.7	1.6	10.0	15.5	0.8	–	–	0.3	0.3	–	–	–
β-elemene	1391	0.4	–	1.1	–	15.5	–	tr	–	5.3	–	–	0.4	0.3	–	–	–
methyl eugenol	1404	–	–	–	–	–	–	–	–	–	–	1.8	–	–	–	–	–
longifolene	1409	0.3	–	1.0	–	–	–	0	–	–	–	–	0.1	1.1	–	–	–
(E)-β-damascone	1414	–	–	–	–	–	10.7	–	–	–	–	–	–	–	–	–	–
β-caryophyllene	1421	0.2	–	0.7	–	–	–	Tr	–	2.3	–	–	0.5	0.4	3.1	0.3	3.9
no conocido 2	1430	–	–	–	–	–	–	–	–	–	–	–	–	–	1.6	–	–

Table 2 (*continued*)

Composición	RI	1	2	3	4	5	6	7	8	9	10	11	12	13	14[b]	15[b]	16[b]
isoamyl octanoate	1433	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–
β-gurjunene	1434	0.4	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–
β-humulene	1439	2.3	–	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–
9-decene-1-ol	1443	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–
aromadendrene	1445	0.1	–	–	–	–	–	tr	–	0.8	–	–	0.6	0.8	–	–	1.0
2-pentadecanone	1451	1.2	–	–	–	–	–	–	6.8	–	–	–	–	–	–	–	–
α-humulene	1455	0.1	–	–	–	–	–	tr	–	–	–	–	0.2	0.2	0.7	–	–
geranyl acetone	1455	0.1	–	–	–	–	–	–	–	0.2	–	–	–	–	–	tr	–
E-β-farnesene	1457	0.1	40.6	0.1	–	–	–	1.6	–	–	–	–	17.7	4.7	–	–	–
allo-aromadendrene	1460	–	–	–	–	–	–	–	41.7	0.2	–	–	0.2	0.1	–	–	1.03
γ-gurjunene	1477	0.7	–	14.3	0.3	40.2	–	–	–	2.3	–	–	32.6	8.6	–	–	–
geranyl n-propanoate	1478	–	–	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–
γ-muurolene	1480	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
germacrene D	1485	0.2	–	13.6	–	–	–	–	–	1.1	–	–	0.3	0.2	–	–	–
β-ionone	1489	–	–	–	–	–	0.5	–	0.9	–	–	–	–	–	–	–	–
β-selinene	1490	0.7	–	2.6	–	1.0	–	–	–	1.8	–	–	0.3	0.1	–	–	–
δ-selinene	1493	4.2	–	–	–	–	–	–	1.1	1.2	–	–	0.7	0.4	–	–	–
valencene	1496	1.9	–	–	–	1.0	–	–	–	4.3	–	–	1.4	1.6	0.5	–	–
viridiflorene	1497	–	–	2.9	–	–	–	–	–	–	–	–	6.9	–	–	–	–
α-selinene	1498	0.1	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	4.9
bicyclogermacrene	1500	0.5	–	–	–	–	–	–	–	5.5	–	–	0.5	0.2	–	–	–
α-muurolene	1500	2.3	–	–	–	–	–	–	–	–	–	0.3	–	0.3	–	–	–
β-bisabolene	1506	–	–	0.5	–	0.6	–	–	0.2	–	–	–	–	–	–	–	–
germacrene A	1510	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–
γ-cadinene	1514	0.1	–	–	–	–	–	–	–	–	–	0.8	0.3	0.3	–	–	0.6
cubebol	1517	tr	–	–	–	–	–	tr	–	–	–	–	–	–	–	–	–
7-*epi*-α-selinene	1522	1.2	–	–	–	–	–	–	–	–	–	–	0.1	0.5	–	–	–
kessane	1522	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6	–	3.8
δ-cadinene	1523	0.2	–	1.2	–	–	–	tr	–	2.4	–	0.6	–	4.2	–	–	1.1
trans-calamenene	1529	tr	–	–	–	–	–		–	–	–	–	–	–	–	–	–
cis-nerolidol	1533	–	18.1	10.6	1.7	–	–	tr	–	1.4	–	1.9	0.7	5.5	–	–	–
no conocido 3	1539	–	–	–	–	–	–	–	–	–	–	–	–	–	1.7	–	–
α-cadinene	1540	19.2	–	0.3	–	–	–	tr	–	–	–	–	–	–	–	–	–
α-calacorene	1546	–	–	–	–	–	–	tr	–	–	–	–	–	–	–	–	–
selina-3,7 (11) diene	1547	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
α-agarofuran	1550	0.4	–	–	–	–	2.7	tr	tr	–	–	–	–	–	–	0.5	–
elemol	1550	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.6	0.5
(cis)-muurolol 5-2n-4-α-ol	1561	–	–	0.6	–	–	–	–	–	–	–	–	–	–	–	–	–
dodecanoic acid	1567	2.8	–	–	4.5	–	2.4	0.8	–	14.1	3.8	–	4.8	2.7	–	–	–
caryophillene epoxide	1572	–	–	–	–	–	–	–	–	–	–	–	–	–	0.9	4.5	–
germacrene-D-4-ol	1576	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–
spathulenol	1580	–	–	–	–	–	–	–	1.3	–	–	–	–	–	–	–	–
hexadecene	1590	–	–	–	–	1.9	1.1	17.0	–	–	–	–	–	–	–	–	–
viridiflorol	1593	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–
ethyl dodecanoate	1597	–	–	–	–	–	–	3.9	–	–	–	–	1.0	0.8	–	–	–
tetradecanal	1613	0.1	–	–	–	–	–	–	–	0.4	–	–	–	–	–	–	–
10-*epi*-γ-eudesmol	1625	2.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
γ-eudesmol	1633	0.8	–	–	–	–	–	tr	–	2.0	–	–	1.5	1.7	–	–	–
epi-α-cadinol	1641	0.3	–	19.4	–	–	–	–	–	–	–	39.7	0.8	19.9	–	–	–
α-muurolol	1646	5.2	–	–	4.8	–	0.5	–	–	–	–	7.9	0.1	tr	–	–	–
cubenol	1648	–	–	0.3	1.7	–	–	–	–	–	–	–	–	1.8	–	–	–
β-eudesmol	1651	–	–	1.0	–	–	–	–	–	–	–	–	0.3	0.7	1.0	8.4	–
α-cadinol	1654	–	–	9.4	0.8	–	0.4	tr	6.6	1.6	–	–	0.06	2.1	–	–	–
selin-11-en-4-α-ol	1660	–	–	–	–	7.1	–	–	–	–	–	–	–	–	–	–	–
neointermedeol	1660	0.6	–	–	–	0.3	–	tr	tr	–	–	–	–	–	0.1	3.5	4.3
intermedeol	1667	32.3	–	–	–	4.3	–	tr	tr	0.1	–	–	0.1	tr	24.7	–	12.1
acorenone	1693	tr	–	–	–	–	–	–	–	–	–	–	–	–	9.8	18.2	–
farnesyl acetate (Z, E)	1701	–	–	–	7.4	–	12.8	1.1	12.2	16.7	–	–	0.5	4.9	–	–	–
farnesol (E,E)	1725	11.9	41.3	0.2	74.0	8.4	31.9	33.0	0.6	18.9	17.1	29.8	20.3	27.2	–	8.5	0.8
methyl linoleate	2096	–	–	–	–	–	–	–	1.3	–	–	–	–	–	–	–	–
6-methyl-5-hepten-2-one	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
trans-p-menth-2,8-dien-1-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–
P-cymen-8-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
propyl octanoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–
isobutyl octanoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5	–
cyperene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–
trans-B-farnesene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0
ar-curcumene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6
geranyl butyrate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7.2	–
humulene epoxide	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–

(*continued on next page*)

Table 2 (*continued*)

Composición	RI	1	2	3	4	5	6	7	8	9	10	11	12	13	14[b]	15[b]	16[b]
selineol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.2	–
farnesal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.5	–
C15H24O	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4.7
C15H24O	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.9
2-methyl-2-butenyl hexanoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5	–
2-methyl-2-butenyl octanoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.5	–
Oxygenated sesquiterpene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26.8
sesquiterpene ketone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.2
δ-terpinene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–
Yield (%, v/p)[a]		1.3	0.4	3.1	0.2	0.9	0.5	1.0	1.3	1.3	2.0	0.5	0.4	0.5			

tr = less than 0.05%.
1: *B. alta*; **2:** *B. barbinodis*; **3**: *B. edwardsiana*; **4**: *B. eurylemma*; **5**: *B. exaristata*; **6**: *B. imperatoides*; **7:** *B. laguroides* var. *laguroides*; **8:** *B. laguroides* var. *torreyana*; **9:** *B. longipaniculata*; **10:** *B. meridionalis*; **11:** *B. perforata*; **12:** *B. saccharoides* var. *saccharoides*; **13:** *B. springfieldii*; **14:** *B. pertusa*; **15:** *B. bladhii* (1); **16:** *B. bladhii* (2).
[a] The yield was calculated in ml of oil in 100 g of dry weight of distilled material.
[b] *B. pertusa*, *B. bladhii* (1) from Nanital and *B. bladhii* (2) from Nepal, data of Kaul and Vats (1998).

The sesquiterpenes compounds made up the higher contribution (67%) with oxygenated dominating (52%). The content of monoterpenes amounted to 17%. Other common constituents were nonterpene derivatives such as hydrocarbons, esters, fatty acids and phenolic compounds.

A great variability was found in the oil composition being (*E*,*E*)-farnesol, γ-gurjunene and *epi*-α-cadinol the major components and in the yield, which ranged from 0.2% in *Bothriochloa eurylemma* to 3.1% *Bothriochloa edwardsiana* (Table 2).

The major essential oil component (*E*,*E*)-farnesol was detected in *Bothriochloa alta*, *B. barbinodis*, *B. eurylemma*, *Bothriochloa imperatoides*, *Bothriochloa laguroides* var. *laguroides*, *Bothriochloa meridionalis*, *Bothriochloa perforata*, *B. saccharoides* var. *saccharoides* and *Bothriochloa springfieldii*. Other dominant compound in *B. alta*, *B. edwardsiana*, *Bothriochloa exaristata*, *Bothriochloa longipaniculata*, *B. saccharoides* var. *saccharoides* and *B. springfieldii* was γ-gurjunene, while *epi*-α-cadinol was recorded in *B. edwardsiana*, *B. perforata* and *B. springfieldii*.

The sesquiterpenes made up the highest contribution (83.6%) in the essential oil of *B. alta* (cfr. Table 2). The main compounds in the essential oil were intermedeol (32.3%), α-cadinene (19.2%) and *E*,*E*-farnesol (19%). The esters and monoterpenes amounted to 8 and 4%, respectively. The content of hydrocarbons and fatty acids was low amounting to 2.1%.

Only three main compounds have been found in the essential oil of *B. barbinodis*, two sesquiterpenes oxygenated (*E*,*E*-farnesol and nerolidol cis) amounted to 67% of the total; the content of hydrocarbons sesquiterpenes (*E*-β-farnesene) was 33% (cfr. Table 2).

B. edwardsiana was characterized by higher quantity of essential oil (3.1%) in which sesquiterpenes compounds dominated (85%), while the monoterpenes amounted to 15% (cfr. Table 2). The main sesquiterpenes oxygenated were *epi*-α-cadinol (19.4%), geranyl formate (11.6%), nerolidol cis (10.6%), α-cadinol (9.4%) and methyl geranate (6.2%). The hydrocarbons sesquiterpenes were γ-gurjunene (14.3%) and germacrene D (13.6%).

The major components in the essential oil of *B. eurylemma* were oxygenated sesquiterpenes amounting to 78% with *E*,*E*-farnesol (74%) as the main compound; the sesquiterpenes hydrocarbons amounted to 22% (cfr. Table 2).

In the essential oil of *B. exaristata* the content of sesquiterpenes, monoterpenes and hydrocarbons in the oil sample amounted to 80%, 6.7% and 13.3%, respectively (cfr. Table 2). The dominant sesquiterpenes were γ-gurjunene (40.2%), β-elemene (15.5%) and camphenilone (12.9%).

In the essential oil of *B. imperatoides* the contents of sesquiterpenes, hydrocarbons and esteres amounted to 70.6, 11.8 and 17.6%, respectively (cfr. Table 2). The sesquiterpenoid fraction contained the highest proportion of oxygenated compounds (90%). *E*,*E*-farnesol (31.9%), isopentyl butanoate (15.1%), farnesyl acetate (*Z*,*E*) (12.8%), 2-*E*-hexenyl butanoate (11.5%) and damascone (*E*)-β (10.7%) were dominant among them.

The content of sesquiterpenes compounds amounted to 63.6% in the essential oil of *B. laguroides* var. *laguroides*. The content of monoterpenes amounted to 16% and the content of esteres was 13.6%, while the hydrocarbons compounds amounted to 6.8% (cfr. Table 2). The major sesquiterpenes oxygenated were the *E*,*E*-farnesol (33%) and hexadecene (17%). Among the hydrocarbons n-dodecane (12.7%) and 1-tetradecene (10%) had the highest presence.

The sesquiterpenoid fraction contained the highest proportion of oxygenated compounds (83.3%) in the essential oil of *B. laguroides* subsp. *torreyana* (cfr. Table 2), while sesquiterpenes hydrocarbons were low amounting to 17%. The contents of monoterpenes, esteres and hydrocarbons amounted to 5.2, 10.5 and 15.8%, respectively. The main compounds were alloaromadendrene (41.7%), 1-tetradecene (15.5%) and farnesyl acetate (12.2%).

In the essential oil of *B. longipaniculata* the contents of sesquiterpenes, monoterpenes, hydrocarbons and esteres amounted to 57.5, 17.5, 12.5 and 7.5%, respectively (cfr. Table 2). In addition, phenolic compunds and fatty acids were counting to 2.5%. The content of the hydrocarbons (48%) and oxygenated compounds (52%) was similar in the sesquiterpenes fraction. The major compounds were *E*,*E*-farnesol (18.9%), farnesyl acetate (16.7%) and dodecanoic acid (14.1%).

In the essential oil of *B. meridionalis* the contents of sesquiterpenes, hydrocarbons, monoterpenes, esteres and fatty acids amounted to 37.5, 25.0, 12.5, 12.5 and 12.5%, respectively (cfr. Table 2). The content of oxygenated sesquiterpenes amounted to

100% in the sesquiterpenes fraction, while sesquiterpene hydrocarbons were not detected. The main sesquiterpenes were damascenone-(*E*)-β (46.8%) and *E*,*E*-farnesol (17.1%).

The major components in the essential oil of *B. perforata* were sesquiterpenes amounting to 66.7% and the monoterpenes with 33.3% (cfr. Table 2). The content of the hydrocarbons (45%) and oxygenated compounds (55%) was similar in the sesquiterpenes fraction. The main monoterpene was linalool (9.6%), while *epi*-α-cadinol (39.7%), *E*,*E*-farnesol (29.8%) and α-muurolol (7.9%) were the major sesquiterpenes found.

The contents of sesquiterpenes, esteres, hydrocarbons and fatty acids amounted to 76.3, 10.5, 10.5 and 2.6%, respectively in the essential oil of *B. saccharoides* var. *saccharoides* (cfr. Table 2). The content of the hydrocarbons (45%) and oxygenated compounds (55%) was similar in the sesquiterpenes fraction. The principal compounds were γ-gurjunene (32.6%), *E*,*E*-farnesol (20.3%) and *E*-β-farnesene (17.7%).

In the essential oil of *B. springfieldii* the major components were sesquiterpenes amounting to 71.7% and the monoterpenes amounted to 8.7%. The content of the hydrocarbons, esteres and fatty acids amounted to 8.7, 8.7 and 2.2%, respectively. The main sesquiterpenes were *E*,*E*-farnesol (27.2%), *epi*-α-cadinol (19.9%) and γ-gurjunene (8.6%) among others.

3.2. Chemotaxonomic significance of the oils obtained from Bothriochloa taxa

The hierarchical cluster analysis based on essential oil composition placed *Bothriochloa* species from the Old World (*Bothriochloa bladhii* and *Bothriochloa pertusa*) distantly from all the South American species (Fig. 1). *B. bladhii* and *B. pertusa* contain high intermedeol (ranging from 12.1 to 24.7%) and low (*E*,*E*)-farnesol (less than 8.52%), while γ-gurjunene and *epi*-α-cadinol were not detected (Table 2).

The rest of the taxa forming the main cluster are distributed into two clusters. The first cluster (C I) includes *B. imperatoides*, *B. exaristata*, *B. meridionalis*, *B. perforata*, *B. springfieldii* and *B. eurylemma*, and is distinguished by its high (*E*,*E*)-farnesol content in its essential oils (31.9, 8.4, 17.1, 29.8, 27.2, and 74%, respectively) (Table 2). The cluster C I separates into two subclusters: SC1 and SC2. The subcluster SC1 includes *B. imperatoides* and *B. exaristata*. *B. exaristata* presents low content in (*E*,*E*)-farnesol (8.4%) and high content in γ-gurjunene (40.2%); nevertheless, according to its camphenilone, tridecene and hexadecene content was placed to *B. imperatoides*. The other subclaster SC2 includes *B. meridionalis*, *B. perforata*, *B. springfieldii* and *B. eurylemma*. All the taxa had important amounts of *epi*-α-cadinol (0.8–39.7%).

The second cluster (C II) includes *B. laguroides* var. *laguroides*, *B. saccharoides* var. *saccharoides*, *B. barbinodis*, *B. longipaniculata*, *B. edwardsiana*, *B. laguroides* var. *torreyana* and *B. alta*. It is distinguished by its high γ-gurjunene and *epi*-α-cad0inol content and low (*E*,*E*)-farnesol (cfr. Table 2). *B. laguroides* var. *laguroides* and *B. barbinodis* belong to this cluster in spite of their high concentration in (*E*,*E*)-farnesol, on account of the presence of hydrocarbons 1-tetradecene, n-dodecane and

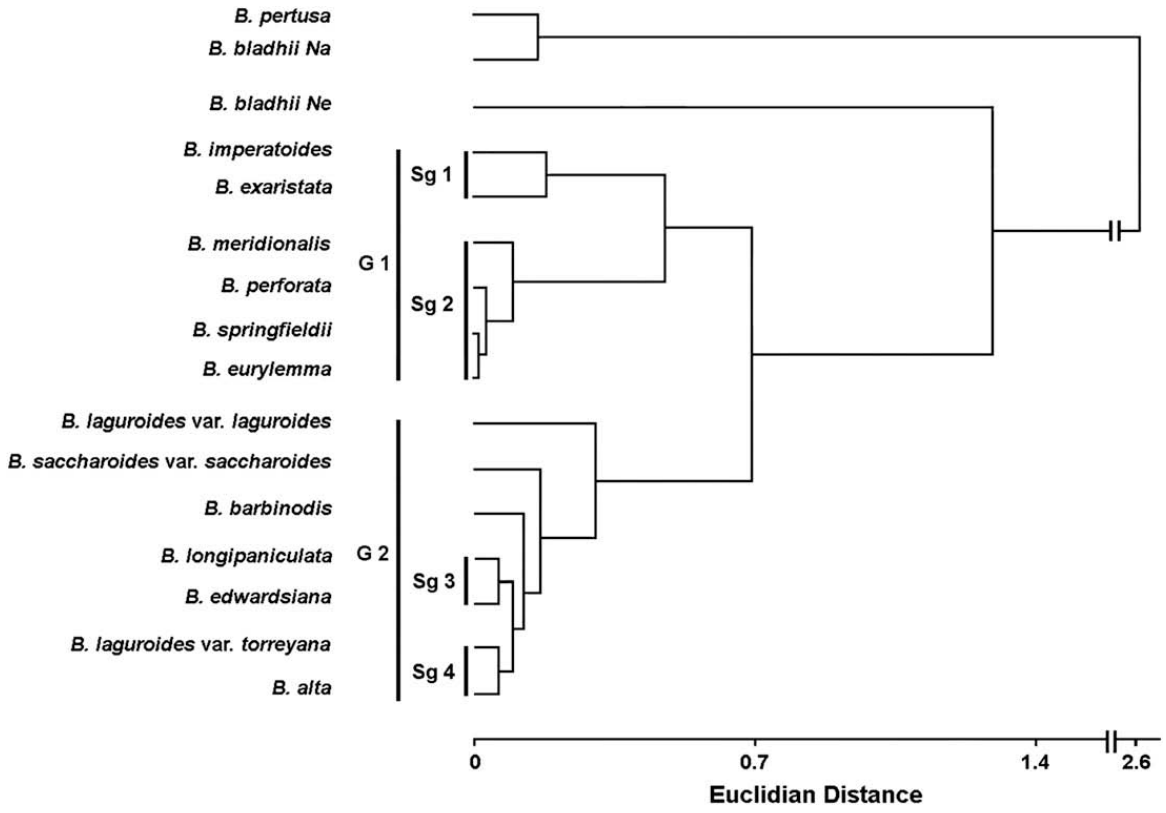

Fig. 1. Hierarchical clusters of *Bothriochloa* taxa based on oil constituents.

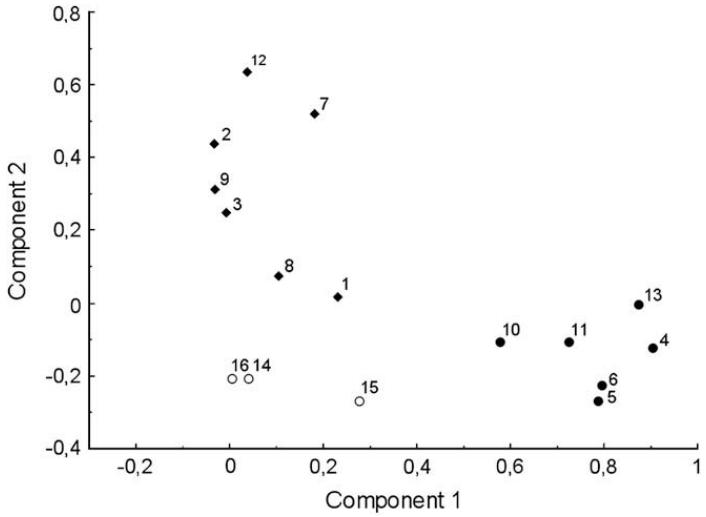

Fig. 2. Principal component analysis of the volatile metabolites of *Bothriochloa* from South America and Old World. References. 1: *B. alta*; 2: *B. barbinodis*; 3: *B. edwardsiana*; 4: *B. eurylemma*; 5: *B. exaristata*; 6: *B. imperatoides*; 7: *B. laguroides* var. *laguroides*; 8: *B. laguroides* subsp. *torreyana*; 9: *B. longipaniculata*; 10: *B. meridionales*; 11: *B. perforata*, 12: *B. saccharoides* var. *saccharoides*; 13: *B. springfieldii*; 14: *B. pertusa*; 15: *B. bladhii* de Nanital; 16: *B. bladhii* de Nepal. White symbol, Old World species; black symbol, South American species.○ high intermedeol content; ● high (*E*,*E*)-farnesol content; ◆ high γ-gurjuneno content and low (*E*,*E*)-farnesol content.

n-undecane and other sesquiterpenes. *B. laguroides* var. *laguroides*, *B. saccharoides* var. *saccharoides* and *B. barbinodis* are the first in separating. *B. longipaniculata* was closer to the *B. edwardsiana* according to their quantity of cis-nerolidol (SC3). On the other hand, *B. laguroides* var. *torreyana* was similar to *B. alta* because of the thymol predominance (SC4).

Principal component analysis (Fig. 2) revealed that significant differences exist between the American and the Old World species when the composition of the essential oils is considered (data from Kaul and Vats, 1998). The highest positive loading is shown by (*E*,*E*)-farnesol in the principal component 1 while in the principal component 2 highest positive loading corresponds to γ-gurjunene and *epi*-α-cadinol. The lowest loading was shown in component 1 by intermedeol.

The scattered plot based on the matrix developed from both components (Figs. 1 and 2) showed the taxa grouping in a similar way to the hierarchical cluster analysis. The taxa with high (*E*,*E*)-farnesol content clustered together and the taxa with less (*E*,*E*)-farnesol and high in γ-gurjunene and *epi*-α-cadinol contents grouped separately. The Old World taxa formed an independent cluster on account of the high intermedeol.

4. Discussion

Although in works published so far only six species from the Old World have been explored in the essential oil composition, it can be said that the sesquiterpenes were always dominant while the quantity of monoterpenes, hydrocarbons and esteres was very small (De Wet and Scott, 1965; Pinder and Kerr, 1980; Zalkow et al., 1980; Melkani et al., 1984; Bhandari et al., 1993; Kaul and Vats, 1998), which agrees with our results. However, in those works the oxygenated sesquiterpenes intermedeol, neointermedeol and acorenone-B were determined as dominant components in the *Bothriochloa* species from the Old World, but they did not exceed as exception at 4.3% (intermedeol) in the American entities. Although, *B. alta* was the only specie with intermedeol (32.3%) in its essential oil.

According to the dominant sesquiterpene (intermedeol, neointermedeol or acorenone-B), Zalkow et al. (1980) recognized for Old World *Bothriochloa* three major groups. Our results showed that the American bluestem's cluster conforms to others markers: (*E*,*E*)-farnesol, γ-gurjunene and *epi*-α-cadinol. Therefore, the *Bothriochloa* species from America cannot be chemically associated with the groups that Zalkow et al. (1980) recognized in species of the Old World. Cytological and embryological data confirmed this point of view (De Wet et al., 1963; De Wet, 1968).

In the hierarchical cluster (Fig. 1), the concentration of the oil constituents [(*E*,*E*)-farnesol, γ-gurjunene and *epi*-α-cadinol], was taken as the major variable to construct the dendrogram. Two clusters, one with high (*E*,*E*)-farnesol content, the other with low (*E*,*E*)-farnesol content and high γ-gurjunene and *epi*-α-cadinol quantities were established. Cytogenetical and embryological studies indicated that diploid ($2n = 20$) representatives of the genus reproduced sexually, while polyploids are usually apomicts (De Wet et al., 1963 and refs. therein). The Old World polyploids are mostly gametophytic apomicts, while the New World and several Australian polyploids, reproduced sexually. It is interesting to note that the diploid species are restricted endemic to India, while polyploids are widely distributed. Thus, the sexual species from the Americas and some Australian ones are only distantly related to Old World apomicts (De Wet, 1968).

The great chemical variation existing between the *Bothriochloa* species from the Old World and America might be sustained by genetic factors. In addition, the groupings resulted independent from the morphological, cytological and ecological

similarities. Thus, it seems that the complexes formation (*B. barbinodis* and *B. saccharoides* complexes) has no correlation with the grouping based on the volatiles of these plants.

In agreement to De Wet and Scott (1965) the essential oil can be used as a taxonomic character with systematic significance in reflecting the taxonomic relationships among the different *Bothriochloa* taxa.

Genetic and molecular studies would be necessary to establish if the presence of sesquiterpenes arises from a common ancestor or if it must be considered a derived character.

Additionally, as the majority of the species inhabit disturbed ecosystems where the competition is great, the presence of essential oils would be important for the establishment of the populations, inhibiting the growth of other species in the plant community. In fact, the grasses congregate an important number of entities where allelopathy has been reported (Chou and Young, 1975; Rietveld, 1975, 1977; Rice, 1976; Bokhari, 1978; Navqi, 1972; Hussain et al., 1982; Hussain and Abidi, 1991; Li et al., 2005) and *B. pertusa* is among them (Hussain et al., 1982). We can infer that the chemical compounds here revealed in other *Bothriochloa* species, operate in a similar manner. Nevertheless, activity against herbivory or in extenuation of high solar radiation cannot be discarded.

Acknowledgements

CONICET and SECyT-UNC are acknowledged for financial support.

References

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography and Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, IL.
Allred, K.W., Gould, F.W., 1983. Systematics of the *Bothriochloa saccharoides* complex (Poaceae: Andropogoneae). Syst. Bot. 8, 168–184.
Bhandari, R., Shah, G.C., Mathela, C.S., 1993. New constituents of *Bothriochloa bladhii*. J. Essent. Oil. Res. 5, 325–327.
Bokhari, U.G., 1978. Allelopathy among prairie grasses and its possible ecological significance. Ann. Bot. 42, 127–136.
Chou, C., Young, C., 1975. Phytotoxic substances in twelve subtropical grasses. J. Chem. Ecol. 1, 183–193.
De Wet, J.M.J., 1968. Biosystematic of the *Bothriochloa barbinodis* complex (Gramineae). Am. J. Bot. 55, 1246–1250.
De Wet, J.M.J., Borgaonkar, D.S., Richardson, W.L., 1963. Chromosome number and mode of reproduction in the Bothriochloininae. Caryologia 16, 47–55.
De Wet, J.M.J., Scott, B.D., 1965. Essential oils as taxonomic criteria in *Bothriochloa*. Bot. Gaz. 126, 209–214.
Hussain, F., Abidi, N., 1991. Allelopathy exhibited by *Imperata cylindrica*. Pakistan J. Bot. 23, 15–25.
Hussain, F., Navqi, H.H., Ilahi, I., 1982. Interference exhibited by *Cenchrus ciliaris* L. and *Bothriochloa pertusa* (L.) A. Camus. Bull. Torrey Bot. Club 109, 513–525.
Kaul, V.K., Vats, S.K., 1998. Essential oil composition of *Bothriochloa pertusa* and phyletic relationship in aromatic grasses. Biochem. Syst. Ecol. 26, 347–356.
Li, H., Huang, J., Zhang, X., Chen, Y., Yang, J., Hei, L., 2005. Allelopathic effects of *Cymbopogon citratus* volatile and its chemical components. Ying Yong Sheng Tai Xue Bao 16, 763–767.
Melkani, A.B., Mathela, C.S., Dev, B., 1984. Constituents of the essential oil of *Bothriochloa bladhii* (Retz.) S.T. Blake. J. Sci. Food Agric. 35, 878–880.
Navqi, H.H., 1972. Preliminary studies of interference exhibited by Italian rye grass. Biologia 18, 201–210.
Pinder, A.R., Kerr, S.K., 1980. The volatile essential oil of five *Bothriochloa* species. Phytochemistry 19, 1871–1873.
Rietveld, W.J., 1975. Phytotoxic Grass Residues Reduce Germination and Initial Growth of Ponderosa Pine. USDA Forest Ser. Paper No. RM-153. USDA Rocky Mt. For. and Range Stn., Fort Collins, Colorado.
Rietveld, W.J., 1977. Phytotoxic effects of bunchgrass residues on germination and initial growth of yellow Sweetclover. J. Range Mang. 30, 39–43.
Rice, E.L., 1976. Allelopathy and grassland improvement. In: Ester, J.R., Tyrl, R.J. (Eds.), The Grasses and Grasslands of Oklahoma. Ann. Okla. Acad. Sci., vol. 6. Noble Foundation, Ardmore, Oklahoma, pp. 90–111.
Vega, A.S., 2000. Revisión taxonómica de las especies americanas del género *Bothriochloa* (Poaceae: Panicoideae: Andropogoneae). Darwiniana 38, 127–186.
Zalkow, L.H., Baxter, J.T., MacClure, R.J., Gordan, M.M., 1980. A phytochemical investigation of *Bothriochloa intermedia*. J. Nat. Prod. 43, 598–608.