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Adaptation in Congo Brazzaville of *Eucalyptus citriodora* from Australia. Tree-to-tree yield and composition variability

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The adaptation of five lines of *Eucalyptus citriodora* from Australia (14713, S and W of Mt. Garnet OLD, Australia), introduced in Congo-Brazzaville in 1989 was evaluated on a seeded plantation using starter fertilizer (NPK,13-13-21, at 300 g/plant), set up in 2003 and monitored for about 2 years. Here, we report results for the first 25 months of growth. The analysis of a large number of samples of essential oils obtained showed that their chemical composition was highly stable. Their content varied by harvesting season and tree age, with an average production ranging from 9.83 to 13.28 kg/ha of essential oil. The best results were obtained in the rainy season. The essential oil contents of the different lines were, in descending order: Z444(3.17%), Z442(3.11%), Z439(2.88%), Z443(2.83%), and Z438(2.46%). The level of citronellal, the major constituent of these essential oils, ranged from 54 to 88% in trees 12 years old, and from 81 to 94% in test plants aged 6 to 25 months. Citronellal, citronellol and isomers of pulegol together made up more than 95% of the essential oil.

Key words: *Eucalyptus citriodora*, sylviculture, essential oil composition, citronellal, Congo-Brazzaville.

INTRODUCTION

Eucalyptus citriodora is one of the species of eucalyptus, native to Australia, that has been widely cultivated throughout the world, in particular for the essential oils that can be extracted from its leaves in generally high yields (>3%). Out of about 60 species brought to Congo to set up industrial plantations for paper pulp or poles for electricity supply cables, 30 have adapted to various degrees to the soil and climate of the coastal savannah in

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the region of Pointe-Noire. Hybrids, both naturally-occurring and created by controlled cross-breeding, have made it possible to set up more than 40,000 ha of industrial plantations. Some species that are especially resistant to vegetative propagation have proved more difficult to develop for paper making. This is the case for *E. citriodora*, although this species can be crossed with others to breed high-performance hybrids.

As part of a systematic study of non-timber uses of the eucalyptus forest of Pointe-Noire (Silou et al., 2009; Loumouamou et al., 2007; Loumouamou et al., 2009), we looked at how the sylviculture of *E. citriodora* might be developed for the large-scale production of essential oils (Mapola, 2006).

First, we undertook the characterization of the parent individuals that presented potential utility for this purpose, as regards both essential oil content and chemical composition. A preliminary study of the variation, both individual and seasonal, of the essential oil content and chemical composition conducted on some 20 trees of Australian origin acclimatized at Pointe-Noire showed that *E. citriodora* acclimatized in Congo-Brazzaville presented appreciable essential oil contents throughout the year, with a peak in the rainy season (>5%), and that this essential oil was mostly made up of citronellal (54 to 89%), citronellol (0.3 to 15%) and isomers of pulegol (0.7 to 11%) (Silou et al., 2002). Out of the 4 Australian chemotypes described by Penfold and Willis (1953), only the citonellal/citronellol chemotype was brought to Congo-Brazzaville.

Thus, we found five lineages of particular interest, seeds from which were used to set up a sylvicultural test series.

Here, we report on the evolution, during the first 25 months of culture, of the quantitative and qualitative production of essential oil of these five lines from 14713 (S and W of Mt. Garnet OLD, Australia) introduced in 1989. Our purpose was to evaluate the potential of growing this species from seeds in village plantations for the production of essential oils.

MATERIALS AND METHODS

Plant materials

The experiment was conducted on plantations of the research unit for the productivity of industrial plantations (UR2PI) at Pointe-Noire (Congo-Brazzaville). The region of Pointe-Noire is located on the Atlantic coast at 4°45’ S by 12° E. Its altitude is 90 to 120 m. The climate is of the lower Congo type, with an annual rainfall of 1200 mm, an average humidity of about 85% and air temperatures of around 25°C. It is characterized by a dry cool season (June-September) alternating with a warm rainy season (October-May), with a drier spell in December and January (Samba-Kimbata, 1978). The study area was located in the tertiary sedimentary coastal basin (pliocene), in a series of circuses of clayey sandstone, sand and clay. The soils were poor, strongly desaturated ferrallitic on a sandy to sandy-clayey substratum (Vennetier, 1968). These poor coastal savannahs are being improved by the industrial plantation of eucalyptus.

The experimental field, located 29 km from Pointe-Noire, at the Kissoko station, was a savannah, never previously cultivated, supporting essentially *Loudetia arundinaceae*, *Hyparrhenia diplandra*, *Annona arenaria* and *Hymenocardia acida*.

The ground was prepared as for the UR2PI industrial plantations. This involved: (i) cutting and uprooting bushes, (ii) applying herbicide to the grassy top stratum (15 to 20 L of water for 0.6 to 1.0 L of Roundup® (glyphosate), according to the amount of grass, (iii) burning dried biomass, (iv) mechanical cross-ploughing the whole plot to a depth of about 20 cm with a disk plough and (v) applying starter fertiliser with NPK (13-13-21) at 300 g/plant, that is, 39 g of nitrogen per plant.

The young nursery seedlings were replanted at a density of 1111 plants/ha, that is, 3 × 3 m. The plant material consisted of 5 out of the 10 lines of *E. citriodora* (Z438, Z439, Z442, Z443, and Z444) from 14713 (S and W of Mt. Garnet OLD, Australia) introduced in 1989. These lines were growing on the UR2PI experimental plot at Luvuiti (Pointe-Noire, test origin 1989: L90-80G).

The above-ground biomass production was evaluated at 6 to 15 months on the trees. Samples of leaves were collected regularly to follow the quantitative and qualitative evolution of the essential oils. The first harvest was carried out 6 months after planting and the plant material was sampled every 2 months up to 12 months, every 3 months up to 15 months and every 6 months up to 36 months.

Essential oil extraction

The essential oils were extracted by steam distillation. In a 6-L container, 300 g of dried leaves of *E. citriodora* partly immersed in 3 L of water was heated for 1 h, 30 min. The distillate was collected from a condenser and the essential oil was recovered by decantation.

Gas chromatography (GC) and GC/mass spectrometry (GC/MS) analysis

GC analysis was performed on a Delsi 121 C instrument equipped with a 25 m × 0.25 mm Cowax 52 CB column. Temperature was programmed from 50 to 220°C at 2°C per minute. Injector and detector temperatures were, respectively 240 and 255°C. The carrier gas was nitrogen at a flow rate 1 ml/min.

GC/SM was performed on a Sigma 300 chromatograph equipped with a 60 m × 25 mm Supelcowax 10 column and an HP 5970 300 mass spectrometer (EI mode, 70 eV). Temperature was programmed from 50 to 220°C at 2°C per minute. Injector and detector temperatures were respectively 240°C and 255°C. The carrier gas was nitrogen at a flow rate 1 ml/min.

Retention indices of all constituents were determined according to Kovats’ method using *n*-alkanes as standards. The constituents were identified by comparison of their Kovats’ indices with those of literature (Jemings and Shibamoto, 1980). Identification were confirmed by comparing mass spectra of essential oil constituents with those in data banks (Adams, 1995; McLafferty and Stauffer, 1989) and with the stored laboratory mass spectral library.

Statistical treatments

The statistical treatments were performed using XLSTAT 2006 (Addisoft), a Microsoft Excel add-in. To evaluate the difference at 5% significance level, of biomass or oil production, t-test (Student test) was used for only 6 trees samples; 2 or 3 trees samples are too small:

$$t = (Mean_1 - Mean_2)/(n_1 + n_2)^{1/2}$$

Table 1. Dry matter from total biomass per tree for the five lines studied.

Age (months)	Line	Number of trees studied	Mass (kg)/tree mean (SD)
	Z438	6	0.31(0.03)[a]
	Z439	6	0.34(1.44)[a]
6	Z442	6	0.34(0.27)[a]
	Z443	6	0.32(0.17)[a]
	Z444	6	0.47(0.14)[a]
	Z438	3	0.85(0.22)
	Z439	3	0.20(0.42)
8	Z442	3	0.49(0.25)
	Z443	3	0.55(0.09)
	Z444	3	0.34(0.23)
	Z438	3	2.56(0.70)
	Z439	3	0.64(0.53)
12	Z442	3	2.12(0.60)
	Z443	3	1.08(0.67)
	Z444	3	0.90(0.74
	Z438	6	2.48(1.19)[b]
	Z439	6	0.89(1.03)[b]
15	Z442	6	1.97(0.01)[b]
	Z443	6	1.49(0.47)[b]
	Z444	6	1.76(1.22)[b]

[a,b]: Values with same letter are no significantly different at 5% level.

If t (calculated) > t (observed), means are no significantly different at this level.

RESULTS AND DISCUSSION

Production of biomass

Table 1 groups the values for the total dried above-ground biomass of the trees belonging to the five lines studied. At age 6 months we note that the total above-ground biomass per individual varied among lines, although it remained of the same order of magnitude. From 8 months differences began to appear, line Z439 falling behind. This trend became stronger at 12 and 15 months when Z439 presented the lowest production and Z438 the highest production. The production of the other lines lay between these two extremes.

However, these variations remain weak and the averages are not significantly different at 5% level between the different lines for the same age of trees. For the same line, this difference is significant between 6 and 15 months age (Table 1).

Figure 1 (average of 90 trees in the test aged 15 months) gives the distribution of dry matter per individual in the different plant compartments for the five lines studied.

The leaves, which form the raw material for the extraction of the essential oils, exceeded 2 kg per tree at age 15 months in 4 out of the 5 lines. In addition, as the leaves were generally distilled with branches, the sum of the biomasses of the leaves and branches would be more representative of the quantity of raw material produced.

Quantitative production of essential oils

On the basis of an average extraction yield of 3.57% relative to dry matter and 1111 trees per hectare, the estimated production of the plantation studied is given in Table 2. The production of dried leaves, which ranged from 1699.83 to 3110.80 kg/ha, gave 9.83 to 13.28 kg/ha of essential oils for an average of 11.21 kg/ha over the test.

Of the 5 lines, Z442 and Z444 were among the most productive, with respective averages of 12.31 and 13.28 kg/ha. As for the biomass, the gap between the extremes of essential oil production was narrow for any particular age and the averages are not significantly different at 5% level between the different lines; it is, for the same line at 5 and 15 months.

Essential oils were extracted from leaves sampled

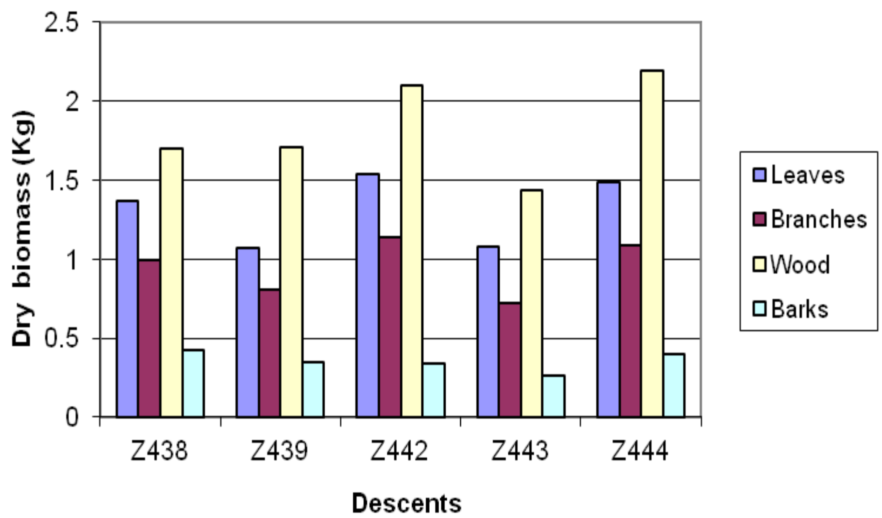

Figure 1. Distribution at 15 months of the dry biomass of the different compartments per individual (in kg) for five lines.

Table 2. Essential oil production (kg/ha) per line.

Age (months)	Line	Oil production (kg/ha)
6	Z438	8.20[a]
	Z439	9.60[a]
	Z442	10.37[a]
	Z443	9.43[a]
	Z444	10.87[a]
8	Z438	11.97
	Z439	10.43
	Z442	15.23
	Z443	9.90
	Z444	13.87
12	Z438	9.43
	Z439	11.10
	Z442	10.30
	Z443	10.43
	Z444	15.16
15	Z438	9.73[b]
	Z439	11.23[b]
	Z442	13.33[b]
	Z443	10.47[b]
	Z444	13.20[b]

[a,b]: Values with same letter are significantly different at 5% level.

indiscriminately in each geographical orientation (N, S, E and W) from one tree in each of the 5 lines, except for Z444 for which 4 trees were studied. The results obtained are presented in Table 3. The variation in the essential oil content according to the orientation of the leaves on the tree was low for Z438 and Z439 (CV < 26%) and very low

Table 3. Intra-tree variation in essential oil (%) for the five lines studied.

Line	Mean	CV (%)**
Z438	2.10*	22.55
Z439	2.42*	25.89
Z442	3.60*	14.06
Z443	4.70*	10.06
Z444 (North)	4.81[a]	14.46
Z444 (South)	5.15[a]	5.48
Z444 (East)	3.16[a]	11.03
Z444 (West)	3.63[a]	6.18

[a,b]: Values with same letter are no significantly different at 5% level. *Mean of four geographical orientations; **CV: coefficient of variation.

Table 4. Individual variation in essential oil (%) for the five lines in six trees aged 6 months.

Line	Number of trees	Mean % essential oil	Coefficient of variation
Z438	6	2.46[a]	17
Z439	6	2.88[a]	6
Z442	6	3.11[a]	25
Z443	6	2.83[a]	22
Z444	6	3.17[a]	20
Mean		2.89	
Coefficient of variation (%)		9	

[a,b]: Values with same letter are no significantly different at 5% level.

for Z442, Z443 and Z444 (CV $<$ 15%). The production of essential oil was homogeneous over the whole tree.

Table 4 gives the results obtained from the study of the individual variation of essential oils of the 5 lines in trees aged 6 months. The essential oil contents of plants grown from seeds aged 6 months (2.46 to 3.17%) were comparable to those of parent trees aged 12 years (2.3 to 3.7%). Intra-lineage variability was generally higher (6 to 25%) than inter-lineage variability (9%). The oils studied displayed a degree of homogeneity. The 5 lines studied presented similar productions of essential oils, but the most promising levels were found, as for parent trees, in 2 of the 5 lines, Z442 and Z444.

Table 5 groups the results obtained for the seasonal variation of essential oil levels in trees grown from seeds for the 5 lines studied. Although the average oil content varied little from one line to another, irrespective of the study period, the inter-tree variation could sometimes be large. Hence, it would be safer to consider as homogeneous only production of essential oil from an indiscriminate sample combining all the leaf biomass of the plantation.

Qualitative production of essential oils

Table 6 gives the composition of essential oils extracted from trees of the 5 lines studied. Two of the 12 compounds identified (>0.01%) made up more than 90% of the essential oil: citronellal (88.29 to 89.60%) and citronellol (4.98 to 6.39%). The cumulated isomers of pulegol took third place (0.67 to 2.33%), and all the other constituents presented individual levels lower than 0.5%. These levels were mean values obtained over the four orientations of the leaves studied on the same tree. The low values of the standard deviations for the major constituents reflect the homogeneity of the composition of the oils from a given tree, irrespective of the geographical orientation. The coefficients of variation were, respectively 1.59 and 22.51% for citronellal and citronellol. A typical chromatogram of the essential oils studied is given in Figure 2.

Table 7 shows the profile of the essential oils extracted from leaves of the trees belonging to the five lines studied. Most of the 20 compounds identified were present in trace amounts (<0.5%). As the study of intra-tree variations, citronellal and citronellol made up more than 90% of the essential oil, and the isomers of pulegol took third place (3 to 5%).

We have taken into account only citronellal, citronellol and the isomers of pulegol, which made up more than 95% of the essential oil. The results obtained from monitoring the variation in the composition of the essential oils for the 5 lines over 25 months are grouped

Table 5. Seasonal variation of essential oil content in test trees grown from seeds at 6 to 25 months.

Cutting season	Harvesting date	Line	Number of trees	Mean*	Coefficient of variation (%)
		Z438	6	2.46[a]	18.70
		Z439	6	2.88[a]	27.08
	04/06/2003	Z442	6	3.11[a]	27.65
		Z443	6	2.83[a]	24.03
		Z444	6	3.17[a]	21.45
Dry season					
		Z438	3	3.60	35.28
		Z439	3	3.09	41.75
	18/08/2003	Z442	3	4.60	3.04
		Z443	3	3.30	2.73
		Z444	3	4.04	5.69
		Z438	3	3.19	17.55
		Z439	3	3.62	26.24
	27/11/2003	Z442	3	3.14	48.73
		Z443	3	2.94	8.16
		Z444	3	4.58	37.12
Rainy season					
		Z438	6	3.06[b]	27.12
		Z439	6	3.12[b]	36.22
	26/02/2004	Z442	6	4.09[b]	17.60
		Z443	6	3.09[b]	38.51
		Z444	6	4.03[b]	41.94
		Z438	3	3.35	6.87
		Z439	3	3.18	23.90
Transition	26/05/2004	Z442	3	4.51	11.31
		Z443	3	3.18	23.90
		Z444	3	4.82	14.73
		Z438	2	2.64	26.52
		Z439	2	2.54	29.13
Rainy season	13/01/2005	Z442	2	3.38	13.91
		Z443	2	3.86	27.20
		Z444	2	4.96	17.74
		Z438	2	2.23	7.62
		Z439	2	1.84	25.00
Dry season	28/06/2005	Z442	2	3.05	32.79
		Z443	2	2.39	63.18
		Z444	2	2.52	26.19

[a,b]: Values with same letter are no significantly different at 5% level. *Mean of lines.

in Table 8. We note a stable oil composition as the tree ages, irrespective of the season of the year.

In this work we analyzed the oils from leaves taken from 90 *E. citriodora* trees acclimatized at Pointe-Noire in Congo-Brazzaville. The quantity and quality of the essential oils in the leaf biomass were estimated. The characterization of these oils reveals a citronellal chemotype. Although the essential oil content varied according to the harvesting season and the age of the trees, the best yields being obtained in the rainy season, its chemical composition proved remarkably stable, irrespective of the location of the leaves on the

Table 6. Intra-tree variation in the chemical composition of essential oils for the five lines studied [mean of four geographical orientations (standard deviation)].

KI	Constituent	Z438	Z439	Z442	Z443	Z444
939	Alpha-pinene	0.03(0.01)	0.04(0.02)	0.06(0.05)	0.05(0.03)	0.03(0.01)
980	Beta-pinene	0.130.06)	0.16(0.02)	0.12(0.05)	0.12(0.09)	0.10(0.04)
991	Myrcene	0.04(0.01)	0.04(0.01)	0.04(0.02)	0.05(0.01)	0.05(0.03)
1033	1,8-cineole	0.21(0.05)	0.26(0.04)	0.28(0.05)	0.21(0.07)	0.18(0.04)
1098	Linalool	0.75(0.43)	0.09(0.04)	0.36(0.12)	0.25(0.20)	0.14(0.05)
1146	Pulegol (1)*	0.96(0.90)	1.58(1.12)	1.32(1.27)	1.47(0.63)	1.71(0.81)
1153	Citronellal	89.77(1.04)	88.29(1.04)	88.52(1.05)	89.15(2.05)	89.62(1.93)
1160	Pulegol (2)**	0.40(0.07))	0.41(0.08)	0.59(0.32)	0.34(0.04)	0.42(0.08)
1228	Citronellol	5.13(0.69)	6.39(1.30)	5.98(1.30)	5.79(1.63)	4.98(1.44)
1356	Eugenol	0.35(0.31)	0.39(0.33)	0.45(0.23)	0.49(0.35)	0.17(0.14)
1401	Methyl eugenol	0.12(0.02)	0.15(0.04)	0.09(0.03)	0.10(0.03)	0.13(0.03)
1581	Caryophyllene	0.37(0.07)	0.36(0.04)	0.31(0.07)	0.30(0.07)	0.31(0.09)

*(1) Isopulegol + isomers (before citronellal); **(2) Isopulegol + isomers (after citronellal).

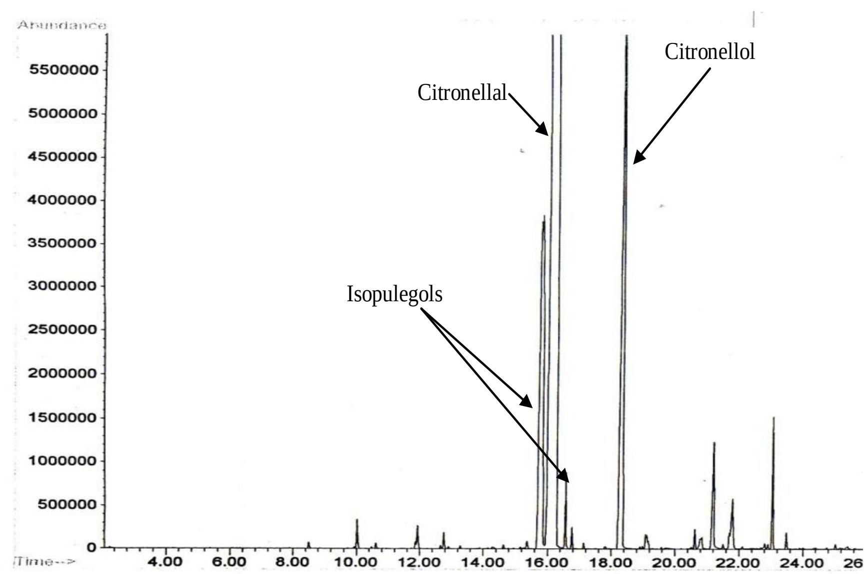

Figure 2. General profile of chromatogram of essential oils from *E. citriodora* acclimatized in Congo-Brazzaville.

Table 7. Individual variation in chemical composition of essential oils from young trees grown from seeds.

IK	Identified constituent	Z438	Z439	Z442	Z443	Z444
939	α-pinene	0.07	0.08	0.07	0.05	0.08
976	Sabinene	0.06	0.02	0.02	0.02	0.12
980	β-pinene	0.21	0.15	0.27	0.20	0.18
991	Myrcene	0.05	0.05	0.05	0.06	0.06
1031	Limonene	0.03	0.02	0.04	0.04	0.04
1031	1,8-cineole	0.05	0.20	0.08	0.11	0.04
1055	Bergamal	0.28	0.51	0.43	0.23	0.48
1098	Linalool	0.37	0.35	0.52	0.55	0.37
	Cis-rose oxide	0.09	0.03	0.14		0.05
1146	Pulegol (1)*	2.18	1.74	2.37	2.82	1.76
1153	Citronellal	88.67	86.72	85.01	88.50	86.21
1160	Pulegol (2)**	1.07	0.72	1.35	0.89	1.63
1228	Citronellol	4.59	5.42	5.72	4.76	4.91
1255	Geraniol	0.35	0.34	0.21	0.18	0.16
1292	Hydroxy citronellal	0.16	1.56	0.85	1.04	1.11
1356	Eugenol	0.26	0.30	0.41	0.45	0.50
1401	Methyl eugenol	0.23	0.32	0.20	0.37	0.37
1404	Caryophyllene	0.13	0.20	0.17	0.22	0.31
1576	Spathulenol			0.14	0.57	
1581	Caryophyllene oxide	0.14	0.27	0.14	0.19	0.35

*(1) Isopulegol + isomers (before citronellal); **(2) Isopulegol + isomers (after citronellal).

Table 8. Seasonal variation in the amounts of major constituents (citronellal, citronellol and isopulegols) at 6 to 25 months in trees grown from seeds.

Period	Major constituent level (%)														
	Z438			Z439			Z442			Z443			Z444		
	1*	2**	3**	1	2	3	1	2	3	1	2	3	1	2	3
04/06/2003	91.09	3.56	1.13	90.71	3.67	1.05	89.78	3.89	1.18	89.01	4.20	1.52	89.62	3.66	1.20
18/08/2003	86.60	5.10	4.36	86.05	5.32	3.2	88.63	4.22	3.55	85.40	5.19	4.87	89.83	3.48	3.37
27/11/2003	87.90	3.22	6.42	88.16	3.55	5.14	83.41	5.85	8.13	88.57	3.60	5.44	90.61	3.16	5.00
26/02/2004	91.10	3.96	2.06	90.14	4.49	2.35	90.96	4.58	2.01	90.97	4.40	1.71	90.53	4.13	3.11
26/05/2004	67.55	8.08	2.63	48.27	13.16	2.21	59.53	10.74	3.44	54.78	10.95	3.29	68.70	11.78	2.18
13/01/2005	89.77	5.13	1.36	88.29	6.39	1.99	88.52	5.98	1.91	89.15	5.79	1.81	89.62	4.98	2.13
28/06/2005	85.55	7.98	1.43	86.42	6.68	1.13	86.74	6.58	1.18	85.58	7.71	1.29	82.33	7.75	1.80
Mean	87.73	4.93	2.54	85.73	5.71	2.48	86.43	5.55	3.06	86.14	5.54	2.85	85.89	5.17	2.68
ET	7.97	1.73	2.74	12.08	2.76	1.30	8.24	2.03	2.25	11.72	2.38	1.58	7.52	2.92	1.17
CV	9.08	35.09	107.87	14.09	48.34	52.42	9.53	36.58	73.53	13.61	42.96	55.44	8.76	52.51	43.66

* 1= Citronellal; **2 = citronellol; ***3 = isopulegols.

tree and the harvesting period, and even from one tree to another. The essential oil content presented comparable values in all the lines; in decreasing order: Z444(3.17%), 2442(3.11%), Z439(2.88%), Z443(2.83%) and Z438(2.46%).

The amounts of citronellal, the major constituent of these oils, ranged from 54 to 88% in parent trees aged 12 years, and from 81 to 94% in the young test plants aged 6 to 15 months, an average improvement in the first descendants of 10 to 15%. Citronellal, citronellol and the isomers of pulegenol together made up more than 95% of the essential oil.

This successful adaptation of *E. citriodora* suggests that this species could be profitably cultivated in Congo-Brazzaville for its essential oil.

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