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Essential Oil Composition of Diploid and Tetraploid Clones of Ginger (*Zingiber officinale* Roscoe) Grown in Australia

HANS WOHLMUTH,*,† MIKE K. SMITH,‡ LYNDON O. BROOKS,§ STEPHEN P. MYERS,|| AND DAVID N. LEACH⊥

School of Natural and Complementary Medicine, Southern Cross University, P.O. Box 157, Lismore NSW 2480, Australia, Queensland Department of Primary Industries & Fisheries, Maroochy Research Station, P.O. Box 5083, SCMC, Nambour QLD 4560, Australia, Research Methodology Unit, Southern Cross University, Lismore NSW 2480, Australia, Australian Centre for Complementary Medicine Education and Research (ACCMER), Southern Cross University and University of Queensland, Brisbane, Australia, and Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore NSW 2480, Australia

Ginger oil, obtained by steam distillation of the rhizome of *Zingiber officinale* Roscoe, is used in the beverage and fragrance industries. Ginger oil displays considerable compositional diversity, but is typically characterized by a high content of sesquiterpene hydrocarbons, including zingiberene, *ar*-curcumene, β-bisabolene, and β-sesquiphellandrene. Australian ginger oil has a reputation for possessing a particular "lemony" aroma, due to its high content of the isomers neral and geranial, often collectively referred to as citral. Fresh rhizomes of 17 clones of Australian ginger, including commercial cultivars and experimental tetraploid clones, were steam distilled 7 weeks post-harvest, and the resulting oils were analyzed by GC−MS. The essential oils of 16 of the 17 clones, including the tetraploid clones and their parent cultivar, were found to be of substantially similar composition. These oils were characterized by very high citral levels (51−71%) and relatively low levels of the sesquiterpene hydrocarbons typical of ginger oil. The citral levels of most of these oils exceeded those previously reported for ginger oils. The neral-to-geranial ratio was shown to be remarkably constant (0.61 ± 0.01) across all 17 clones. One clone, the cultivar "Jamaican", yielded oil with a substantially different composition, lower citral content and higher levels of sesquiterpene hydrocarbons. Because this cultivar also contains significantly higher concentrations of pungent gingerols, it possesses unique aroma and flavor characteristics, which should be of commercial interest.

KEYWORDS: Ginger; *Zingiber officinale*; ginger oil; essential oil; citral; neral; geranial; tetraploidy

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe; family Zingiberaceae) is a widely used spice, flavoring agent, and herbal medicine and is also employed in the perfume industry. Ginger is a sterile cultigen believed to have originated in India or Southeast Asia and introduced to Europe by Arab traders (*1*, *2*). It was well known in England as early as the 11th century and had become a major item of the spice trade in the 13th and 14th centuries (*3*, *4*). Today ginger is cultivated in many tropical and subtropical areas, the main producers being India, China, Indonesia, and Nigeria (*4*). An estimated 40% of the world's confectionary ginger is grown in a relatively small area of eastern Queensland, Australia (*5*).

Ginger owes its unique flavor properties to the combination of pungency and aroma. The pungency is provided by nonvolatile phenolic compounds, whereas the essential oil gives ginger its characteristic aroma. Ginger rhizome yields two primary extracts: oleoresin and essential (or volatile) oil. The oleoresin is a solvent extract (usually in acetone or ethanol) containing both essential oil and the phenolic compounds responsible for the pungency of ginger, chiefly [6]-gingerol and to a lesser extent [8]- and [10]-gingerol. The corresponding shogaols, which are dehydration products of gingerols formed in heat-treated ginger, are also found in the oleoresin. Ginger oleoresin is used extensively as a flavoring agent in the food and beverage industries.

Commercial ginger oil is normally extracted by steam distillation from dried rhizomes. Typical ginger oil is characterized by a high content of sesquiterpene hydrocarbons, in particular, zingiberene, *ar*-curcumene, β-bisabolene, and β-ses-

* Author to whom correspondence should be addressed [telephone +61-2-66203159; fax +61-2-66203307; e-mail hans.wohlmuth@scu.edu.au].
† School of Natural and Complementary Medicine, Southern Cross University.
‡ Maroochy Research Station.
§ Research Methodology Unit, Southern Cross University.
|| Southern Cross University and University of Queensland.
⊥ Centre for Phytochemistry and Pharmacology, Southern Cross University.

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Table 1. Ginger Clones Studied, Their Genotype, and Their Origin[a]

ID	genotype	cultivar name	origin
Z22	tetraploid∧	(unnamed)	derived from "Queensland" (selection 1) by colchicine treatment
Z23	tetraploid∧	(unnamed)	
Z24	tetraploid*	(unnamed)	
Z25	tetraploid∧	(unnamed)	
Z26	tetraploid*	"Buderim Gold"	
Z27	tetraploid*	(unnamed)	
Z28	tetraploid∧	(unnamed)	
Z29	tetraploid∧	(unnamed)	
Z30	tetraploid$	(unnamed)	
Z31	tetraploid*	(unnamed)	
Z32	tetraploid∧	(unnamed)	
Z33	tetraploid*	(unnamed)	
Z44	diploid	"Queensland" (selection 1)	selected by J. Roscoe, BGL
Z45	diploid	"Queensland" (selection 2)	selected by L. Palmer, BGL
Z46	diploid	"Jamaican"	imported from Jamaica
Z47	diploid#	"Brazilian"	imported from Brazil
Z58	diploid	"Canton"	imported from China

[a] Ploidy: *, confirmed as solid tetraploids by flow cytometry; $, chimera with both diploid and tetraploid tissue sectors; ∧, presumed to be tetraploid from stomatal measurements; #, unknown but presumed to be diploid. BGL = Buderim Ginger Ltd.

quiphellandrene, while important monoterpenoids normally include geranial, neral, and camphene (*6*−*9*). Although these compounds are characteristic of "typical" ginger oils, the literature clearly shows that ginger oil composition is highly variable (*6*−*16*). Factors such as geographical origin, whether the oil is distilled from fresh or dried rhizome material, drying process and temperature, and analytical methodology may all contribute to the disparity of published ginger oil analyses.

Ginger "oil" obtained by supercritical fluid extraction using carbon dioxide is also commercially available, but this product differs radically from steam-distilled oil due to the presence of the pungent gingerols and shogaols (*17*).

Ginger oil is used in the beverage and fragrance industries, and the world production of ginger oil was estimated at 100−200 t in 2000 (*18*). In the classic work, *Perfume and Flavor Materials of Natural Origin*, Arctander described the odor of ginger oil as "warm, but fresh-woody, spicy and with a peculiar resemblance to orange, lemon, lemon-grass, coriander weed oil, etc. in the initial, fresh topnotes [...the] sweet and heavy undertone is tenacious, sweet and rich, almost balsamic-floral" (*19*).

We have previously reported on the content of pungent gingerols in 17 commercial and experimental ginger clones grown in northeastern New South Wales, Australia (*20*). The present Article reports the essential oil composition (analyzed by GC−MS) of these diploid and tetraploid genotypes. This is the first comprehensive survey of steam-distilled Australian ginger oils to be published since the early work by Connell and Jordan (*16*).

MATERIALS AND METHODS

Plant Materials and Distillation. Seventeen commercial and experimental clones of ginger (**Table 1**) were obtained from the Queensland Department of Primary Industries Maroochy Research Station at Nambour, Queensland. Rhizome stock was grown in raised beds under uniform conditions at Southern Cross University, Lismore, New South Wales (latitude 28° 49′ S, longitude 153° 18′ E) for approximately 8 months (*20*). The experimental clones were created from diploid ($2n = 22$) "Queensland" parent material by way of in vitro colchicine treatment of shoots (*5*). Rhizomes were harvested in late July and stored at ambient temperature for approximately 7 weeks before being distilled. Prior to distillation, unpeeled rhizomes were washed and had any diseased tissue removed before being chopped into pieces approximately 1 × 3 × 7 mm. Equal parts of rhizome from three different plants were pooled, and approximately 200 g of this material was hydrodistilled in a Clevenger distillation apparatus for 3 h.

Chemical Analysis. Oils were analyzed on an Agilent Technologies (Palo Alto, CA) 6890/5973 GC−MSD system using helium as the carrier gas at a constant linear flow velocity of 29 cm/s. Oil samples (150 μL) were diluted with pentane (1500 μL), and 1 μL of this solution was injected. The column was a 50 m × 0.22 mm capillary column, 1.00 μm film thickness (BPX-5, SGE Ltd., Melbourne). The split ratio was 25:1. The column oven was programmed from 70 to 280 °C at a ramp rate of 4 °C/min (final hold time 4 min), and the injector temperature was 250 °C. Composition values were recorded as percentage area based on the total ion current chromatogram. Retention indices were determined using a C-8 to C-22 *n*-alkane mixture. Compound identification was based on comparisons with mass spectra and retention indices of authentic reference compounds where possible and by reference to WILEY275, NBS75K, and Adams terpene library (*21*) and published data. Myrcene, 1,8-cineole, linalool, α-terpineol, β-citronellol, citral, geraniol, (−)-bornyl acetate, citronellyl acetate, geranyl acetate, and (*E*)/(*Z*)-nerolidol were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI); (−)-borneol and (Z)-nerolidol were obtained from Fluka Chemie (Buchs, Switzerland); 6-methyl-5-hepten-2-one was obtained from ants (Formicidae) (*22*); and (*E*,*E*)-α-farnesene was obtained from the peel of "Granny Smith" apples (*23*). Germacrene-D and elemol were identified by comparison with these compounds in authentic clary sage (*Salvia sclarea* L.) oil (*24*) and elemi (*Canarium luzonicum* (Miq.) Asa Gray; Berjé Inc., Bloomfield, NJ) oil (*25*), respectively.

Statistical Analysis. Statistical analyses were performed using SPSS (Chicago, IL) for Windows Release 11.5. Mean values, standard deviations, and ranges were calculated for major oil constituents. The oil composition of the 17 clones was the subject of principal components analysis and cluster analysis based on the 10 most abundant constituents. The relationship between neral and geranial was examined by way of a scatter plot and Pearson's correlation coefficient.

RESULTS

Oil Composition. The essential oil composition for the 17 clones is shown in **Table 2**. The mean, standard deviation, and range for the 14 most abundant constituents in the 16 homogeneous or "typical" clones are shown in **Table 3**, which also gives the percentage content for the atypical oil from the cultivar "Jamaican" (Z46). The "typical" oils had a mean citral (neral + geranial) content of 58%, while the five major sesquiterpene hydrocarbons typically found in ginger oil (*ar*-curcumene, (*E*,*E*)-α-farnesene, zingiberene, β-bisabolene, and β-sesquiphellandrene (*10*)) made up only 17%. In contrast, the oil from "Jamaican" had a comparatively low citral content (28%) and contained 35% of the main sesquiterpene hydrocarbons.

Principal Components Analysis. The percentage composition of the 17 oil samples was subjected to principal components analysis based on the 10 most abundant oil constituents (borneol, neral, geraniol, geranial, geranyl acetate, *ar*-curcumene, (*E*,*E*)-α-farnesene, zingiberene, β-bisabolene, and β-sesquiphellandrene). This analysis revealed the presence of three components with eigenvalues exceeding one. These components explained 61.6%, 18.8%, and 10.7% of the variability, respectively. Two components (together explaining 80% of the variability) were retained for further investigation, and Varimax rotation was performed to assist in the interpretation (**Table 4**). The rotated solution revealed a complex structure in which both components had several strong loadings (coefficients relating the variables to the components), but some compounds loaded substantially

Table 2. Composition of Essential Oils of 17 Clones of Ginger Analyzed by GC–MS on a BPX-5 Column[a]

compound	RI	identification method	Z22	Z23	Z24	Z25	Z26	Z27	Z28	Z29	Z30	Z31	Z32	Z33	Z44	Z45	Z46	Z47	Z58
6-methyl-5-hepten-2-one + myrcene	996	*a*	0.34	0.10	0.31	0.43	0.47	0.40	0.38	0.39	0.26	0.26	0.44	0.31	0.31	0.61	0.14	0.41	0.47
β-phellandrene	1058	*b*	0.79	0.12	0.58	0.52	1.59	0.78	0.34	0.23	0.26	0.54	0.44	0.36	0.66	1.39	1.49	1.67	0.48
1,8-cineole	1060	*a*	1.94	0.39	1.28	1.31	2.07	1.70	1.29	1.54	1.12	1.28	1.69	1.27	1.02	1.94	0.79	2.63	1.85
linalool	1112	*a*	0.99	0.98	1.00	1.47	1.21	1.04	1.28	1.55	1.19	1.16	1.34	1.52	1.01	1.22	1.02	0.97	1.42
borneol	1213	*a*	1.84	1.99	2.34	2.60	2.66	2.56	2.89	2.35	2.73	2.94	2.94	2.65	2.06	1.93	3.91	1.94	2.14
α-terpineol	1229	*a*	1.49	1.62	1.59	1.80	1.70	1.68	2.00	2.09	1.96	1.73	2.18	1.98	1.58	1.62	1.13	1.86	2.00
citronellol	1241	*a*	1.71	1.99	1.72	1.86	1.91	2.07	2.12	1.77	2.47	1.71	1.79	1.70	2.14	2.49	1.09	1.48	1.93
neral	1265	*a*	26.49	21.29	21.01	20.23	21.30	19.39	22.05	22.15	21.26	20.77	20.91	21.22	20.33	22.10	10.60	19.71	22.86
geraniol	1268	*a*	4.09	2.73	4.80	5.86	4.54	4.78	5.33	5.79	5.79	4.55	7.30	6.61	3.04	4.13	1.54	3.29	5.86
geranial	1292	*a*	44.31	36.12	34.27	31.62	35.83	31.29	35.09	35.55	34.60	34.85	33.11	34.74	32.80	35.59	17.51	33.02	36.76
2-undecanone	1305	*b*	0.48	0.19	0.33	0.49	0.55	0.39	0.37	0.16	0.39	0.55	0.31	0.32	0.46	0.41	0.93	0.39	0.32
bornyl acetate	1315	*a*	0.00	0.00	0.13	0.24	0.20	0.27	0.24	0.21	0.19	0.20	0.18	0.20	0.15	0.12	0.29	0.00	0.21
citronellyl acetate	1358	*a*	0.00	0.29	0.25	0.41	0.39	0.65	0.31	0.40	0.48	0.40	0.35	0.37	0.37	0.34	0.14	0.12	0.56
geranyl acetate	1388	*a*	1.14	0.99	1.57	2.59	2.24	3.41	1.20	1.62	2.59	2.65	2.60	3.12	1.27	1.30	0.26	0.52	3.45
ar-curcumene	1509	*b*	2.81	5.31	3.17	3.13	2.67	3.36	2.98	3.20	4.48	3.10	3.04	2.87	3.72	2.59	5.72	2.43	2.97
(*E*,*E*)-α-farnesene	1518	*a*	2.16	2.97	3.91	2.78	2.47	2.74	2.25	2.76	2.10	3.14	2.47	2.98	3.81	3.07	4.35	4.30	2.28
zingiberene	1521	*b*	4.29	2.19	7.89	4.11	5.94	5.44	3.50	2.63	1.86	6.93	4.67	5.76	5.19	4.90	11.24	9.00	2.63
germacrene-D	1532	*a*	0.00	0.19	0.36	0.28	0.31	0.34	0.24	0.17	0.00	0.27	0.23	0.22	0.43	0.34	0.73	0.50	0.18
β-bisabolene	1536	*b*	0.97	2.16	1.83	1.39	1.32	1.57	1.26	1.32	1.75	1.61	1.33	1.46	1.79	1.34	4.05	1.90	1.18
β-sesquiphellandrene	1557	*b*	2.93	5.36	5.29	3.68	3.72	4.34	3.40	3.62	4.34	4.62	3.72	4.05	5.04	3.85	9.40	5.62	3.34
(*E*)-nerolidol	1581	*a*	0.00	0.75	0.54	0.54	0.32	0.50	0.50	0.76	0.61	0.38	0.56	0.54	0.61	0.42	1.14	0.54	0.41
elemol	1592	*a*	0.00	0.39	0.24	0.38	0.28	0.4 2	0.35	0.30	0.32	0.24	0.29	0.24	0.56	0.33	0.73	0.36	0.26

[a] Values are percentage content. RI, retention index; a, mass spectral data and retention index as compared to those of reference compound; b, based on mass spectral data and retention index (identification tentative).

Table 3. Content of 14 Constituents in Essential Oils of 16 "Typical" Clones of Ginger and One "Atypical" Clone, "Jamaican" (Z46)[a]

	16 "typical" clones mean ± SD (range)	"Jamaican" (Z46)
1,8-cineole	1.52 ± 0.52 (0.39–2.63)	0.79
linalool	1.21 ± 0.20 (0.97–1.55)	1.02
borneol	2.41 ± 0.39 (1.84–2.94)	3.91
α-terpineol	1.81 ± 0.21 (1.49–2.18)	1.13
citronellol	1.93 ± 0.28 (1.48–2.49)	1.09
neral	21.44 ± 1.63 (19.39–26.49)	10.60
geraniol	4.91 ± 1.28 (2.73–7.30)	1.54
geranial	36.50 ± 3.26 (31.29–44.31)	17.51
geranyl acetate	2.02 ± 0.92 (0.52–3.45)	0.26
ar-curcumene	3.24 ± 0.73 (2.43–5.31)	5.72
(*E*,*E*)-α-farnesene	3.02 ± 0.68 (2.10–4.30)	4.35
zingiberene	4.82 ± 2.03 (1.86–9.00)	11.24
β-bisabolene	1.51 ± 0.31 (0.97–2.16)	4.05
β-sesquiphellandrene	4.36 ± 0.85 (2.93–5.62)	9.40

[a] Mean, standard deviation, and range are shown for the 16 clones. All values are percentage content.

Table 4. Varimax Rotated Component Matrix for a Two-Component Solution for the 10 Most Abundant Ginger Essential Oil Constituents

	component	
	1	2
neral	–0.96	–0.25
geranial	–0.96	–0.15
β-bisabolene	0.89	0.39
borneol	0.87	–0.21
β-sesquiphellandrene	0.78	0.61
ar-curcumene	0.61	0.30
zingiberene	0.55	0.54
geraniol	–0.23	–0.89
geranyl acetate	0.12	–0.87
(*E*,*E*)-α-farnesene	0.45	0.74
percentage of variance explained	49.3%	31.2%

on both components. Neral, geranial, β-bisabolene, borneol, and β-sesquiphellandrene were strongly associated with component 1, indicating a high degree of interrelationship (positive or negative) between the concentrations of these compounds. Similarly, geraniol, geranyl acetate, and (*E*,*E*)-α-farnesene were

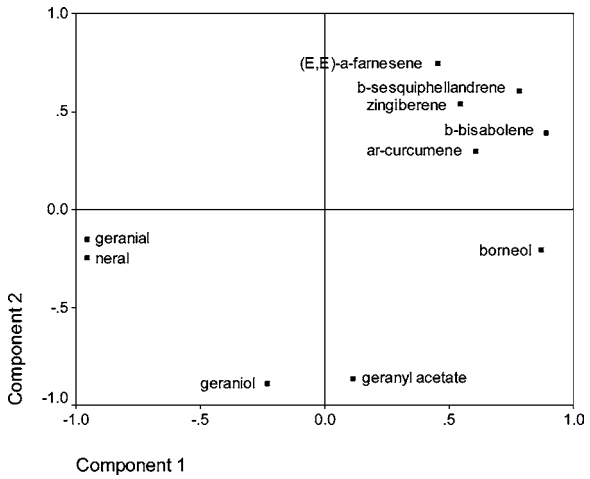

Figure 1. Component plot in rotated space showing Varimax rotated data on two components based on the 10 most abundant constituents of ginger essential oil.

strongly associated with component 2. More broadly, an inverse relationship between levels of citral (geranial + neral) and the sesquiterpene hydrocarbons (zingiberene, *ar*-curcumene, β-sesquiphellandrene, β-bisabolene, and (*E*,*E*)-α-farnesene) was evident by inspection of the component plot in **Figure 1**.

Cluster Analysis. To examine the degree of similarity displayed by the 17 clones in terms of oil composition, a hierarchical, between-groups linkage, cluster analysis based on the 10 most abundant constituents was performed. This is a multivariate procedure that allows for the classification of cases (or variables) into groups based on Euclidian distances between cases. For each constituent, percentage values were rescaled to have a mean value of one, so that all constituents were equally weighted for the purpose of the analysis, and Euclidian distances were calculated between pairs of clones.

Figure 2 shows a dendrogram of the 17 essential oils. This diagram confirms the unique nature of the essential oil from the cultivar "Jamaican" (Z46) when compared to oils from the other 16 clones. The dendrogram also shows that the oil from the clone Z22 stands out from the others. This oil had an

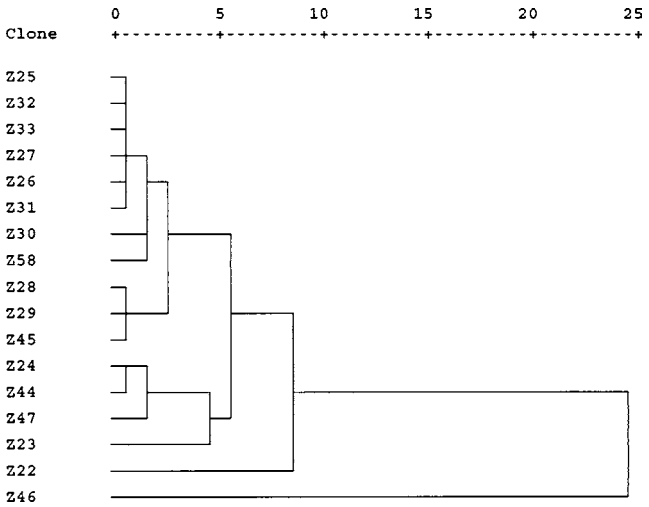

Figure 2. Dendrogram of hierarchical cluster analysis of 17 essential oils of ginger. The diagram shows average linkage (between groups), and values shown along the horizontal axis are Euclidian distances rescaled to an arbitrary scale showing the levels of relative similarity where clusters join.

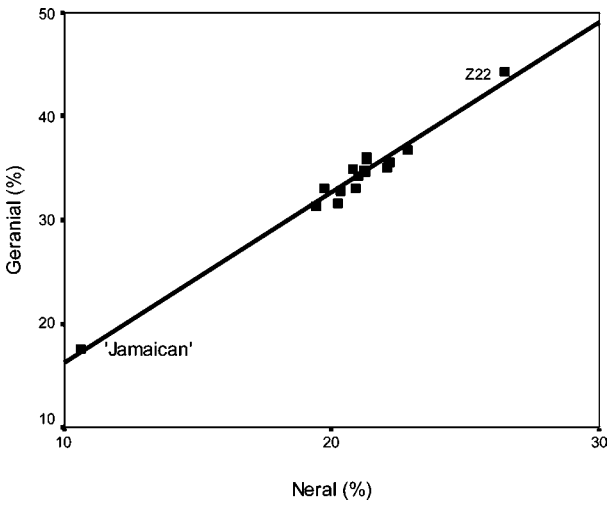

Figure 3. Scatter plot showing the relationship between the percentage content of the stereoisomers neral and geranial in essential oils from 17 clones of ginger.

extremely high citral content (71%). The remaining 15 clones fall into two clusters. The similarity between these two clusters was examined using a multivariate general linear model, comparing the set of clones in cluster 1 (Z25, Z32, Z33, Z27, Z26, Z31, Z30, Z58, Z28, Z29, Z45) and cluster 2 (Z24, Z44, Z47, Z23). Cluster 1 and cluster 2 showed a near-significant difference (Wilks' Lambda = 0.064; $F = 5.838$; df = 10 and 4; $p = 0.052$). In terms of individual constituents, six (borneol, geraniol, geranyl acetate, (*E*,*E*)-α-farnesene, *β*-bisabolene, *β*-sesquiphellandrene) were significantly different between clusters at $p \leq 0.05$ and four (neral, geranial, *ar*-curcumene, zingiberene) were not.

Citral Content. The ginger oils analyzed in the present study had citral contents ranging from 28% in the "Jamaican" cultivar (Z46) to 71% in the tetraploid clone Z22. The mean citral content of the 16 "typical" ginger oils ("Jamaican" excluded) was 57.9% ± 4.9% (range: 50.7−70.8%), which was more than double the corresponding value for "Jamaican" (28%). A similar trend was evident for geraniol, which is a precursor to citral, with a mean concentration of 4.9% ± 1.3% in the "typical" oils as compared to 1.5% in "Jamaican".

Neral-to-Geranial Ratio. The 17 ginger oils contained neral and geranial in a ratio that was remarkably constant. The neral-to-geranial ratio ranged from 0.55 to 0.64, with a mean value of 0.61 ± 0.01. This fixed, linear relationship between the two isomers, which persisted regardless of the percentage content of citral, is illustrated in **Figure 3**. The very strong correlation between the two compounds is quantified by the Pearson's correlation coefficient of 0.987 ($p < 0.001$).

It is particularly interesting to note that the neral-to-geranial ratio also was 0.6 in the cultivar "Jamaican" (Z46), which otherwise yielded an oil that was distinctly different from those of the other 16 clones.

DISCUSSION

Essential oils of 17 clones of Australian ginger were prepared by hydrodistillation of rhizomes and were analyzed by GC−MS. As compared to values in the literature (*6*−*16*), all of the samples were characterized by a very high citral content (28% or greater) and a relatively low content of sesquiterpene hydrocarbons.

Oil Composition. There was no distinct difference in the composition of the oils of the tetraploid clones as compared to the diploid parent cultivar "Queensland" or the cultivars "Canton" and "Brazilian". These oils were characterized by very high levels of citral (geranial + neral) (51−71%) and relatively low levels of the sesquiterpene hydrocarbons characteristic of ginger oil (*10*), zingiberene (4.8% ± 2.0%), *ar*-curcumene (3.2% ± 0.7%), *β*-sesquiphellandrene (4.4% ± 0.9%), *β*-bisabolene (1.5% ± 0.3%), and (*E*,*E*)-α-farnesene (3.0% ± 0.7%). These values contrast with those reported in 1971 by Connell and Jordan, whose analysis of 35 Australian ginger oils found citral levels ranging from 4% to 30% and much higher concentrations of sesquiterpene hydrocarbons (zingiberene 20−28%, *ar*-curcumene 6−10%, *β*-sesquiphellandrene 7−11%, and *β*-bisabolene 5−9%, but no (*E*,E)-α-farnesene) (*16*). The results obtained in the present study are better aligned with more recent analyses of ginger oils from Mauritius (*12*), Sao Tomé e Príncipe (*8*), and Nigeria (*15*) by being high in citral, relatively low in the major sesquiterpene hydrocarbons, and by containing (*E*,*E*)-α-farnesene. In general, the published values for these compounds vary greatly; whether this is due to true natural variability or differences in raw material (fresh or dried, time of harvest), distillation conditions, or analytical methodologies is uncertain.

An inverse relationship between levels of citral, on one hand, and *β*-bisabolene, *β*-sesquiphellandrene, and borneol, on the other, was demonstrated by principal components analysis. Likewise, an inverse relationship was shown to exist between geraniol/geranyl acetate and (*E*,*E*)-α-farnesene. Terpenoids are derived from C_5 isoprene units in the form of the diphosphate (pyrophosphate) esters dimethylallyl diphosphate and isopentenyl diphosphate and are products of the mevalonate pathway (*26*). The common biosynthetic origin of monoterpenoids (such as geranial, neral, geraniol, and geranyl acetate) and sesquiterpenoids (including *β*-bisabolene, *β*-sesquiphellandrene, and (*E*,*E*)-α-farnesene) provides a possible explanation for the inverse relationship seen between mono- and sesquiterpenoids, as the pathways to mono- and sesquiterpenes would compete for common precursors. Similarly, the fact that citral and borneol share the monoterpene precursor geranyl pyrophosphate could explain the inverse relationship observed between these compounds.

The oils analyzed in this study were prepared from fresh rhizomes 7 weeks post-harvest by a short distillation process and analyzed within a short period of time. They may differ

significantly from oils prepared from dried rhizome material by longer distillation processes followed by prolonged storage under conditions that favor oxidation. It has been reported that both zingiberene and β-sesquiphellandrene can undergo oxidation to *ar*-curcumene (*16*, *27*), and high levels of this compound may therefore be indicative of a degraded oil.

The essential oil from the cultivar "Jamaican" (Z46) differed distinctly in composition from all of the others, primarily by having a lower citral content and a higher sesquiterpene hydrocarbon content. This oil contained 28% citral while the total amount of the five main sesquiterpene hydrocarbons was 35%, which is more than double the mean concentration found in the 16 other clones. The distinctiveness of the oil of "Jamaican" was confirmed by hierarchical cluster analysis. As reported previously, this cultivar was also the most pungent of the 17 clones tested due to its high content of gingerols (*20*). The dissimilar essential oil composition coupled with its high pungency gives "Jamaican" flavor and aroma qualities that are distinct from the other clones examined.

Cluster analysis identified one other distinct clone (Z22), characterized by an extremely high (71%) citral content, while the remaining clones fell into two clusters. These two clusters did not appear to reflect breeding history, as the different tetraploid clones produced from the same parent cultivar ("Queensland") were split between the two clusters, as were the other two cultivars, "Brazilian" and "Canton".

Citral Content. The ginger oils analyzed in this study contained remarkably high levels of citral (above 50% in all samples except "Jamaican" and up to 71% in one sample). The highest citral content described previously in ginger oil was 56% in a sample from Fiji (*28*). Although Australian ginger has a reputation for its "lemony" aroma, a 1971 study of 35 Australian ginger oils by Connell and Jordan found considerably lower citral levels, ranging from 4% to 30% (*16*). The same study found only very low citral levels in oils from other parts of the world, and many other studies have reported very low citral levels in non-Australian ginger oils (*7*, *10*, *11*, *14*, *29*). Conversely, several more recent investigations have found substantial citral levels in ginger oils from other parts of the world, for example, from the Central African Republic (35%) (*13*), Mauritius (27%) (*12*), Sao Tomé e Príncipe (22−25%) (*8*), Nigeria (24%) (*15*), and Tahiti (14−25%) (*6*, *30*).

There are several possible, not mutually exclusive, explanations for these somewhat incongruent findings regarding citral content. One relates to the changes in gas chromatography technology (especially detection systems) since the early 1970s, which might explain some of the differences between the results of the current study and those of Connell and Jordan (*16*). It is also likely that ginger oils distilled from fresh rhizomes generally have a higher citral content than oils obtained from dried rhizomes. The notion that sun drying and storage of ginger could cause a loss of citral was put forward by Govindarajan more than two decades ago (*27*), and studies from Sri Lanka (*11*) and Nigeria (*15*) have documented citral losses in ginger oils ranging from 40% to 74%, when rhizomes were dried before distillation. A significant difference in citral content has also been observed in supercritical fluid extracts of fresh and dried Australian ginger, which contained 19.9% and 6.2% citral, respectively (*17*). These data would seem to suggest that many ginger oils contain considerable amounts of citral, provided they are distilled from fresh rhizomes or rhizomes dried at low temperature. On the other hand, the existence of genuine low-citral genotypes of ginger is supported by a recent study from India, where the essential oil distilled from fresh rhizomes contained less than 4% citral (*31*).

A number of other factors could also have contributed to the unexpectedly high citral values obtained in this study. They include the particular growth period (8 months), the harvest time (mid-winter), the 7-week delay between harvest and distillation, and the conditions of cultivation (including climate) at Southern Cross University, which is located some 250 km south of the commercial ginger-growing area in Australia.

Neral-to-Geranial Ratio. Neral (*cis*-citral) and geranial (*trans*-citral) are stereoisomers occurring in many plants in mixtures often referred to simply as citral. The neral-to-geranial ratios found in ginger oils in this study were remarkably constant and close to 2:3 (mean 0.61 $\pm$ 0.01, range 0.55−0.64). The ratios reported in the literature for ginger oil vary greatly, from 0.3 in a Taiwanese oil (*10*) to 4.7 in a Chinese oil (*7*). However, a ratio of 0.6 was also reported for a Mauritian oil made from fresh rhizomes (*12*), a Nigerian oil also prepared from fresh rhizomes (*15*), and an oil made from dried rhizomes from Sao Tomé e Príncipe (*8*), while oils from Madagascar (*32*) and the Central African Republic (*13*) were reported to contain neral-to-geranial ratios of 0.4 and 0.9, respectively. The data obtained in the current study support the hypothesis that neral and geranial occur in ginger in a relatively fixed ratio of approximately 2:3 and call into question the validity of analyses suggestive of very different ratios.

Neral and geranial also occur in other plants in a similar ratio. Species of lemongrass produce essential oil with a citral content typically exceeding 70%. Nine samples of West Indian lemongrass (*Cymbopogon citratus*, family Poaceae) oil from Africa, China, New Zealand, and Cuba had neral-to-geranial ratios ranging from 0.61 (New Zealand) to 0.96 (China) with a mean of 0.76, while four Indian cultivars of East Indian lemongrass (*C. flexuosus*) produced oils with a neral-to-geranial ratio ranging from 0.61 to 0.67 (*33*).

This study has shown that essential oil from the three ginger cultivars "Queensland", "Canton", and "Brazilian" and 12 tetraploid clones derived from "Queensland" had a high degree of compositional similarity and were characterized by a very high citral content. Tetraploid clones were shown to have retained the characteristics of the parent cultivar in terms of essential oil composition. In contrast, the cultivar "Jamaican" produced an oil that was distinctly different, characterized by a lower citral content and higher levels of sesquiterpene hydrocarbons. The distinctive aroma profile of this cultivar, which also contains significantly higher levels of pungent gingerols, should make it of commercial interest to the flavor and fragrance industries.

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