Lipid Composition of *Herrania* and *Theobroma* Seeds

D.R. Carpenter[a], J.F. Hammerstone, Jr.[a], L.J. Romanczyk, Jr.[a,*] and W. Martin Aitken[b]
[a]M&M/MARS, Hackettstown, New Jersey 07840 and [b]Almirante Center for Cocoa Studies, 45630—Itajuipe, Bahia, Brazil

The seeds of nine *Herrania* and nine *Theobroma* species were surveyed for fatty acid, sterol, tocopherol and tocotrienol compositions. Principal component and cluster analyses suggested that these analytes could be used collectively as chemotaxonomic criteria to differentiate the *Herrania* species from the *Theobroma* species, as well as to provide subgroup distinctions within each genus for comparison to the existing classification schemes.

KEY WORDS: Cluster analysis, fatty acids, *Herrania*, principal component analysis, Sterculiaceae, sterols, *Theobroma*, tocopherols, tocotrienols.

Seventeen *Herrania* (1) and twenty-two *Theobroma* (2) species have been described and assigned to the Sterculiaceae (3). The *Herrania* are morphologically similar to the *Theobroma* (2) and, until Schultes' research (1), the *Herrania* were considered a section of the *Theobroma*. *Theobroma cacao* L. is the only species of major economic importance, because its fat-rich seeds are the unique source of cocoa solids and cocoa butter used by the confectionery industry. Although the seeds from several *Theobroma* species have been investigated for their lipid composition (4–7), little information is available for the *Herrania* (8), and knowledge of the sterol and tocol compositions within each genus is largely unknown. Therefore, we report our findings on the fatty acid, sterol, tocopherol and tocotrienol compositions obtained from a survey of nine *Herrania* and nine *Theobroma* species for multivariate analysis to determine chemotaxonomic relationships between these genera.

MATERIALS AND METHODS

Plant material. Herrania and *Theobroma* pods were obtained from the Centro Agronomico Tropical de Investigacion y Enseñanza (CATIE) germplasm collection at Turrialba, Costa Rica, and from the Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC) cocoa germplasm collection (Belém, Brazil).

Reagents. Pyridine, N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane, cholesterol, campesterol, stigmasterol, β-sitosterol, α-tocopherol and δ-tocopherol were obtained from Sigma Chemical Company (St. Louis, MO). β-Tocopherol was obtained from Matreya (Pleasant Gap, PA) and γ-tocopherol from Fluka (Ronkonkoma, NY). Cycloartenol and 24-methylene cycloartanol were purified from hydrolyzed γ-oryzanol (Farmingdale, NY) as described by Rogers *et al.* (9). Tocotrienols (α-, γ- and δ-isomers) were a generous gift from Hoffman La Roche (Basel, Switzerland).

Sample preparation. Seeds with pulp were removed from *Herrania* and *Theobroma* pods and freeze-dried on a Labconco (Kansas City, MO) Freeze Dry System. The pulp and hulls were manually removed, and the freeze-dried seeds were ground to a fine powder with a Tekmar Mill (Cincinnati, OH). The ground mass was subjected to overnight extraction with redistilled petroleum ether (b.p. 38–39.6°C) in a Soxtec apparatus (Fisher Scientific, Springfield, NJ). The solvent was carefully removed by slow evaporation under a stream of nitrogen, and the resultant extracts were stored at −40°C.

Gas chromatography of fatty acid methyl esters (FAME). FAME were prepared by alkali-catalyzed transmethylation (10). FAME separations were achieved on a 30 m × 0.25 mm i.d. Supelco (Bellefonte, PA) SP2340 fused-silica capillary column programmed at 90°C for 3 min, then 5°C/min to 210°C for 20 min on a Hewlett-Packard (Palo Alto, CA) Model 5880A gas chromatograph. The injector and flame-ionization detector temperatures were set at 220 and 250°C, respectively. Helium was used as the carrier gas at a linear velocity ($\bar{\mu}$) of 30 cm/s. One-μL injections were split 50:1.

Sterols derivatization. Preweighed samples (0.1 g) containing cholesterol (0.2 mg) as the internal standard (ISTD) were saponified at 80°C for 1 h with 0.5 mL of 50% KOH in ethanol. After cooling to room temperature, 1.5 mL distilled water was added, and the free sterols were extracted two times with 5 mL redistilled *n*-hexane. The combined extracts were dried over Na_2SO_4 and taken to dryness under a stream of nitrogen. Dry pyridine (0.1 mL) was added, followed by an equal volume of BSTFA reagent. Trimethylsilyl (TMS) ether derivatives of cholesterol, campesterol, stigmasterol, β-sitosterol, cycloartenol and 24-methylene cycloartanol were similarly prepared.

Gas chromatography of sterol-TMS ether derivatives. Sterol-TMS ether derivatives were separated on a 25 m × 0.25 mm i.d. Quadrex (New Haven, CT) 50% methylphenylsilicone fused-silica capillary column, programmed at 250°C for 37 min, then 10°C/min to 300°C for 5 min on a Hewlett-Packard Model 5890A gas chromatograph. The injector and flame-ionization detector temperatures were set at 250 and 300°C, respectively. Helium was used as the carrier gas at a linear velocity ($\bar{\mu}$) of 25 cm/s. One-μL injections were split 50:1. Quantitation was achieved by the ISTD technique (11). Peak identifications were made by comparison to the retention time (t_R) of authentic sterol-TMS ether derivatives and by mass spectral analysis.

Mass spectrometry (MS). Analyses were performed on a Hewlett-Packard Model 5987A GC-MS System. Electron ionization-MS (EI-MS) of the sterol-TMS ether derivatives was performed at 70 eV with a source temperature of 200°C, a scan range of 50–600 amu at a rate of 1.2 scans/s. Chromatographic conditions were identical to those described above.

High-performance liquid chromatography (HPLC) of tocols. Analyses were performed on a Hewlett-Packard Model 1090 HPLC System, equipped with a Hewlett-Packard Model 1046A programmable fluorescence detector. Tocol separations were achieved on a 25 cm × 4.6 mm, 5μ Supelcosil (Supelco) LC-Si column held at 45°C. The mobile phase consisted of 8% (by vol) redistilled *tert*-butylmethyl ether in redistilled *n*-hexane at a flow rate of 1.8 mL/min. Components were detected by fluorescence where excitation (λ_{ex}) and emission (λ_{em}) wavelengths were set at 290 and 325 nm, respectively. Fifty μL of 2.5% (wt/vol) fat solutions in redistilled *n*-hexane were injected. Tocols

*To whom correspondence should be addressed at M&M/MARS, 800 High St., Hackettstown, NJ 07840.

were quantitated by the external standard technique (12), and peak identifications were made by comparison to t_Rs of authentic tocopherol and tocotrienol standards.

Statistical analysis. Principal component analysis (PCA) and cluster analysis were performed with the Multi-Variate Statistics Package (MVSP Plus, version 2.0; Kovach Computing Services, Anglesey, Wales, United Kingdom) by following the user manual instructions. Data entries for fatty acid, sterol, tocopherol and tocotrienol compositions were made for all the species examined except for *T. gileri* and *T. mammosum*, because their data sets were incomplete. Values of zero were entered for all analytes listed as none detected (see Tables 2–5 and 7,8 later in text). The α and δ tocotrienol isomers were excluded from PCA because none could be detected from any surveyed species. A value of 0.02 was entered for analytes listed as trace (see Tables 7,8). For cluster analysis, distance and similarities were calculated with the Gower general similarity coefficient (13,14), and the resultant tree description was presented as a dendrogram.

RESULTS AND DISCUSSION

The yields of fat (Table 1) obtained from the *Herrania* were generally consistent and ranged from 52.5% for *H. mariae* to 70.0% for *H. nitida*. Those obtained from the *Theobroma* were more variable and ranged from 1.2% for *T. gileri* (7) to 64.0% for *T. obovatum*. Our specimen of *T. mammosum* was an exception because immature seeds were the only available material for study.

The *Herrania* fatty acid profiles (Table 2) were consistent for members assigned to each section of the genus, except for *H. mariae*. A distinctive feature for the *Herrania* was the high percentage of arachidic (20:0) acid. This was considered unusual because this fatty acid occurs only in small amounts in many edible seed oils but attains major proportions in members of the Sapindaceae and in some members of the Leguminaceae (15). Appreciable amounts of arachidic acid were also found in most members of the *Andropetalum*, *Glossopetalum* and *Telmatocarpus* sections of *Theobroma* (Table 3), but not at the levels observed in the *Herrania*. In contrast to the *Theobroma*, much higher levels of linoleic (18:2) acid were also found within the *Herrania*. Variations with these and with other fatty acids suggested that members of either genus were characterized by different desaturase and elongation pathways. Some species might therefore be useful candidates to study lipid storage biosynthesis and the mechanisms that regulate fatty acid composition.

Campesterol, stigmasterol, β-sitosterol, cycloartenol and 24-methylene cycloartanol were the major sterols identified in *Herrania* (Table 4) and *Theobroma* (Table 5). EI mass spectra (Table 6) for two 4,4-dimethyl sterols (cycloartenol and 24-methylene cycloartanol) exhibited low molecular ion abundances relative to their $(M - 90)^+$ ions. They also exhibited the characteristic loss of *m/z* 212, as well as abundant $(M - 90 - CH_3)^+$ ions. The EI mass spectra for three Δ5-unsaturated 4-demethylsterols (campesterol, stigmasterol and β-sitosterol) exhibited abundant M^+ and *m/z* 129 ions, as well as the characteristic $(M - 90)^+$ ions. β-Sitosterol was the dominant sterol for both genera, and no distinguishing distribution patterns were evident except for a tenfold increase in total sterols content obtained from immature *T. mammosum* seeds.

TABLE 1

Fat Content of *Herrania* and *Theobroma* Species

Species	Origin	% Fat (reference)
H. albiflora	Costa Rica	54.9
H. balaënsis	Costa Rica	61.6
	Ghana	64.4 (8)
H. columbia[a]	Costa Rica	65.0
H.cuatrecasana	Costa Rica	65.3
H. mariae[b]	Brazil	52.5
	Ghana	64.1 (8)
H. nitida	Costa Rica	70.0
H. nycterodendron	Costa Rica	67.0
H. purpurea	Costa Rica	69.8
H. umbratica	Costa Rica	60.7
T. angustifolium	Costa Rica	48.1
	Mexico	46.0 (6)
	—[c]	58.6 (7)
T. bicolor	Costa Rica	29.6
	Brazil	36.1 (4)
	Brazil	34.1 (7)
	Costa Rica	27.0 (7)
	Ecuador	38.0 (5)
	Mexico	34.2 (6)
T. cacao	Brazil	54.5
T. gileri	—[c]	1.2 (7)
T. grandiflorum	Brazil	56.7
	Brazil	60.5 (4)
	—[c]	36.7 (7)
T. mammosum[d]	Costa Rica	3.1
	—[c]	49.7 (7)
T. microcarpum	Brazil	5.3
T. obovatum	Brazil	64.0
T. speciosum	Costa Rica	25.8
T. subincanum	Brazil	48.8

[a]*Herrania columbia* is a questionable species because Schultes (1) makes no reference to it. We are uncertain of this specimen's true identity.
[b]*Herrania mariae* also referred to as *T. mariae* (2; cf. also Ref. 20).
[c]Origin of species not specifically mentioned.
[d]Immature seeds.

Tocopherol and tocotrienol distributions within the *Herrania* (Table 7) were distinctly different from those obtained from the *Theobroma* (Table 8). A majority of *Herrania* contain approximately equal quantities of α- and γ-tocopherol as the dominant isomers. The notable exceptions were *H. mariae*, where very low tocol levels were found, and *H. nycterodendron*, where α-, γ- and δ-tocopherols were found in nearly equal amounts. For a majority of the *Theobroma*, γ-tocopherol was the principal isomer, except for *T. microcarpum* and *T. obovatum*, which had high δ-tocopherol levels. Trace quantities of γ-tocotrienol could be detected in most *Herrania* but only in *T. cacao*.

PCA was used to determine systematic variations within each separate analyte matrix (16,17). In PCA, a matrix consists of the measurements obtained from a given set of variables and objects (e.g., individual species). A geometrical interpretation for each object is given as a point in dimensional space, where each variable defines an orthogonal axis. The component loadings are scaled to unity, so that the sum of squares of an eigenvector (component coefficient) equals one, and the component scores are scaled so that the sum of squares equals the eigenvalue (percentage of total variation). Multivariate methods then search and plot the structure of the data to

TABLE 2

***Herrania* Seed Fatty Acid Composition**

Species	Composition (%)									
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C24:0
Section *Herrania*										
H. albiflora	ND[a]	6.2	ND	25.8	9.6	37.3	0.5	19.2	1.1	0.2
H. purpurea	ND	6.4	ND	28.4	9.4	33.9	0.4	20.1	1.1	0.1
H. umbratica	ND	5.8	ND	25.3	10.3	38.7	0.5	18.0	1.1	0.1
Section *Subcymbicalyx*										
H. balaënsis	0.2	8.6	0.2	18.4	14.2	39.2	0.6	16.6	1.7	0.2
H. cuatrecasana	0.2	9.7	0.4	22.4	10.2	38.8	0.5	16.3	1.2	0.2
H. mariae[b]	0.4	5.4	0.1	25.8	33.3	14.5	0.2	17.6	2.0	0.4
H. nitida	0.3	7.9	0.1	26.4	18.7	27.5	0.5	16.9	1.4	0.2
H. nycterodendron	0.2	7.9	0.3	23.4	11.4	37.1	0.5	17.4	1.5	0.3
H. columbia[c]	ND	6.2	ND	23.1	12.8	39.1	0.5	17.1	1.0	0.1

[a]Not detected.
[b]See footnote *c* in Table 1.
[c]See footnote *b* in Table 1.

TABLE 3

***Theobroma* Seed Fatty Acid Composition**

Species (reference)	Composition (%)								
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C24:0
Section *Oreanthes*									
T. speciosum	38.9	ND[a]	17.7	28.2	12.3	0.4	2.0	0.3	0.2
Section *Telmatocarpus*									
T. gileri (7)	11.8	0.8	12.2	34.2	27.5	2.4	11.2	NR[b]	NR
T. microcarpum	38.5	0.4	24.0	28.8	6.7	0.2	0.9	0.1	0.1
Section *Glossopetalum*									
T. angustifolium	4.4	ND	24.8	46.4	6.4	0.9	12.5	4.3	0.2
(6)	5.0	NR	25.0	49.7	5.1	NR	11.3	3.9	NR
(7)	4.8	ND	25.9	48.8	7.5	ND	12.7	NR	NR
T. grandiflorum	6.7	ND	35.2	41.6	3.4	0.4	11.0	1.5	ND
(4)	11.5	NR	31.8	40.3	5.6	1.0	9.8	NR	NR
(7)	10.0	0.2	21.7	47.4	8.6	ND	12.1	NR	NR
T. obovatum	6.0	ND	30.1	47.2	5.7	0.7	8.6	1.4	0.1
T. subincanum	5.7	0.1	32.5	41.5	2.8	0.1	13.4	2.5	0.3
Section *Andropetalum*									
T. mammosum[c]	33.3	ND	17.2	26.6	15.8	2.7	1.7	1.2	1.4
(7)	5.6	0.2	25.9	44.1	10.7	0.5	13.0	NR	NR
Section *Theobroma*									
T. cacao [Criollo]	27.4	0.2	34.2	33.6	3.1	0.1	1.0	0.2	ND
Section *Rhytidocarpus*									
T. bicolor	5.6	0.2	46.7	42.0	2.9	0.1	2.1	0.2	ND
(4)	9.8	NR	41.4	43.2	3.8	ND	1.9	NR	NR
(5)	6.6	ND	42.9	45.1	3.0	NR	2.0	NR	NR
(6)	6.1	NR	50.4	39.4	2.8	NR	1.3	NR	NR
(7)[d]	6.8	ND	43.5	43.4	4.3	ND	1.9	NR	NR
(7)[e]	7.8	ND	34.0	51.8	5.0	ND	1.9	NR	NR

[a]None detected.
[b]Not reported.
[c]Immature seeds.
[d]Costa Rican origin.
[e]Brazilian origin.

determine whether any recognizable patterns exist. In this case, PCA was performed with all 17 species by using each separate analyte data set [fatty acid (nine variables), sterol (five variables) and tocols (five variables)], followed by the combined analyte data set (16 variables). PCA of each matrix provided a two-significant principal component model that accounted for 60, 74, 65 and 48% of the total variance obtained from the fatty acid, sterol, tocol and combined analyte data sets, respectively.

Figures 1–3 represent plots of the scores obtained from the fatty acid, sterol and tocol data sets for each species set on the x–y plane, where the x-axis represents the first principal component and the y-axis the second. The results clearly showed a separation of the *Herrania* from the

TABLE 4

***Herrania* Seed Sterols Composition**

Species	Campesterol (mg/100 g oil)[a]	Stigmasterol (mg/100 g oil)	β-Sitosterol (mg/100 g oil)	Cycloartenol (mg/100 g oil)	24-Methylene cycloartanol (mg/100 g oil)	Unidentified (mg/100 g oil)
Section *Herrania*						
H. albiflora	19 ± (1)	81 ± (3)	276 ± (5)	19 ± (2)	18 ± (1)	13 ± (1)
H. purpurea	11 ± (1)	59 ± (2)	160 ± (4)	9 ± (1)	3 ± (1)	5 ± (1)
H. umbratica	30 ± (2)	74 ± (2)	342 ± (6)	5 ± (1)	3 ± (1)	14 ± (1)
Section *Subcymbicalyx*						
H. balaënsis	15 ± (2)	71 ± (2)	138 ± (3)	ND[b]	ND	2 ± (1)
H. cuatrecasana	17 ± (1)	83 ± (3)	174 ± (4)	ND	8 ± (1)	15 ± (1)
H. mariae[c]	16 ± (2)	92 ± (4)	188 ± (5)	ND	4 ± (1)	9 ± (1)
H. nitida	8 ± (1)	55 ± (3)	94 ± (4)	2 ± (1)	4 ± (1)	16 ± (1)
H. nycterodendron	15 ± (2)	64 ± (3)	139 ± (5)	3 ± (1)	3 ± (1)	14 ± (2)
H. columbia[d]	9 ± (1)	47 ± (3)	146 ± (3)	5 ± (1)	ND	20 ± (2)

[a]Values reported as the means of duplicate analyses, SD in parenthesis.
[b]Not detected.
[c]See footnote *c* in Table 1.
[d]See footnote *b* in Table 1.

TABLE 5

***Theobroma* Seed Sterols Composition**

Species	Campesterol (mg/100 g oil)[a]	Stigmasterol (mg/100 g oil)	β-Sitosterol (mg/100 g oil)	Cycloartenol (mg/100 g oil)	24-Methylene cycloartanol (mg/100 g oil)	Unidentified (mg/100 g oil)
Section *Oreanthes*						
T. speciosum	20 ± (2)	73 ± (4)	200 ± (5)	5 ± (1)	2 ± (1)	32 ± (3)
Section *Telmatocarpus*						
T. gileri	Specimen not available					
T. microcarpum	22 ± (2)	82 ± (4)	250 ± (6)	3 ± (1)	6 ± (1)	26 ± (3)
Section *Glossopetalum*						
T. angustifolium	8 ± (1)	17 ± (1)	142 ± (4)	10 ± (1)	ND[b]	11 ± (1)
T. grandiflorum	9 ± (1)	23 ± (2)	196 ± (5)	3 ± (1)	ND	17 ± (2)
T. obovatum	11 ± (1)	49 ± (3)	150 ± (5)	29 ± (2)	9 ± (1)	58 ± (4)
T. subincanum	8 ± (1)	40 ± (2)	122 ± (4)	9 ± (1)	2 ± (1)	12 ± (1)
Section *Andropetalum*						
T. mammosum[c]	129 ± (4)	390 ± (18)	2,611 ± (34)	17 ± (2)	ND	15 ± (2)
Section *Theobroma*						
T. cacao [Criollo]	18 ± (2)	48 ± (3)	139 ± (4)	8 ± (1)	2 ± (1)	13 ± (1)
Section *Rhyditocarpus*						
T. bicolor	8 ± (1)	22 ± (2)	207 ± (5)	3 ± (1)	2 ± (1)	13 ± (2)

[a]Values reported as the means of duplicate analyses, SD in parenthesis.
[b]Not detected.
[c]Immature seeds.

TABLE 6

EI Mass Spectra for *Herrania* and *Theobroma* Sterol-TMS Ether Derivatives[a]

Sterol-TMS ether derivative	*m/z* (relative intensity)
Campesterol-TMS ether	M^+ 472(13.4), 382(13.4), 343(21.6), 255(5.7), 215(2.1), 145(17.0), 129(62.0), 107(17.4), 105(18.1), 73(100.0)
Stigmasterol-TMS ether	M^+ 484(10.0), 394(11.6), 255(22.5), 213(9.4), 159(22.9), 145(21.9), 129(72.0), 107(19.9), 105(23.3), 83(100.0)
β-Sitosterol-TMS ether	M^+ 486(10.0), 396(21.5), 357(28.7), 255(8.0), 213(6.9), 159(14.4), 129(100.0), 107(28.5), 105(23.2), 75(32.9)
Cycloartenol-TMS ether	M^+ 498(0.0), 408(19.7), 393(23.2), 365(18.9), 339(18.0), 286(6.3), 173(17.3), 159(18.9), 95(51.0), 73(100.0)
24-Methylene cycloartanol-TMS ether	M^+ 512(0.0), 422(14.2), 407(11.1), 379(14.1), 300(4.9), 203(10.7), 175(18.3), 159(17.6), 95(42.1), 73(100.0)

[a]Abbreviations: EI, electron ionization; TMS, trimethylsilyl.

TABLE 7

***Herrania* Seed Tocols Composition (μg/g oil)[a]**

Species	Tocopherols				Tocotrienols		
	α	β	γ	δ	α	γ	δ
Section *Herrania*							
H. albiflora	74 ± (3)	5 ± (1)	57 ± (3)	8 ± (1)	ND[b]	trace[c]	ND
H. purpurea	91 ± (4)	8 ± (1)	125 ± (5)	23 ± (2)	ND	trace	ND
H. umbratica	141 ± (5)	9 ± (1)	107 ± (4)	18 ± (2)	ND	trace	ND
Section *Subcymbicalyx*							
H. balaënsis	85 ± (3)	14 ± (1)	84 ± (3)	40 ± (3)	ND	ND	ND
H. cuatrecasana	83 ± (3)	7 ± (1)	87 ± (3)	20 ± (2)	ND	ND	ND
H. mariae[d]	8 ± (1)	ND	37 ± (2)	6 ± (1)	ND	ND	ND
H. nitida	127 ± (6)	12 ± (1)	82 ± (3)	22 ± (2)	ND	trace	ND
H. nycterodendron	90 ± (4)	35 ± (3)	93 ± (4)	87 ± (3)	ND	trace	ND
H. columbia[e]	122 ± (5)	6 ± (1)	125 ± (3)	16 ± (2)	ND	trace	ND

[a]Values reported as means of duplicate analyses, SD in parenthesis.
[b]Not detected.
[c]Trace (<0.02 μg/g oil).
[d]See footnote [c] in Table 1.
[e]See footnote [b] in Table 1.

TABLE 8

***Theobroma* Seed Tocols Composition (μg/g oil)[a]**

Species	Tocopherols				Tocotrienols		
	α	β	γ	δ	α	γ	δ
Section *Oreanthes*							
T. speciosum	8 ± (1)	2 ± (1)	248 ± (7)	32 ± (3)	ND[b]	ND	ND
Section *Telmatocarpus*							
T. gileri	Specimen not available						
T. microcarpum	ND	4 ± (1)	80 ± (3)	230 ± (8)	ND	ND	ND
Section *Glossopetalum*							
T. angustifolium	4 ± (1)	ND	329 ± (11)	25 ± (2)	ND	ND	ND
T. grandiflorum	trace[c]	ND	122 ± (5)	6 ± (1)	ND	ND	ND
T. obovatum	ND	ND	126 ± (5)	92 ± (4)	ND	ND	ND
T. subincanum	ND	ND	100 ± (4)	24 ± (3)	ND	ND	ND
Section *Andropetalum*							
T. mammosum[d]	Insufficient sample for analysis						
Section *Theobroma*							
T. cacao [Criollo]	14 ± (1)	ND	266 ± (6)	6 ± (1)	ND	8 ± (1)	ND
Section *Rhytidocarpus*							
T. bicolor	ND	ND	78 ± (2)	8 ± (1)	ND	ND	ND

[a]Values reported as means of duplicate analyses, SD in parenthesis.
[b]Not detected.
[c]Trace (<0.02 μg/g oil).
[d]Immature seeds.

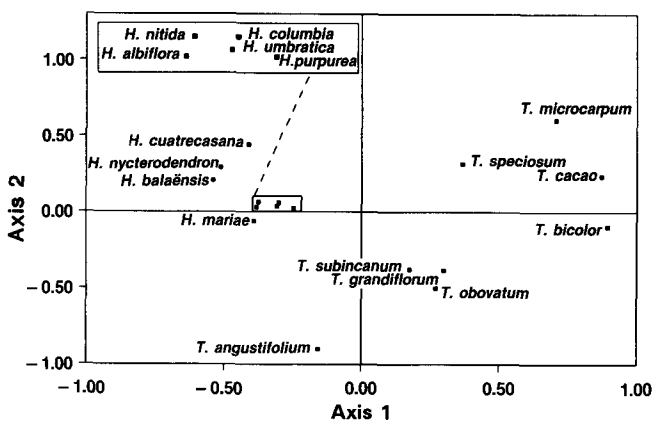

FIG. 1. Plot of the first two principal components of the *Herrania* and *Theobroma* species by using the fatty acid data set.

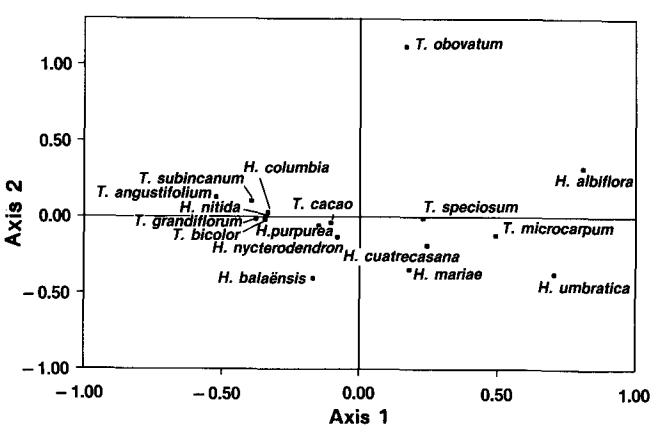

FIG. 2. Plot of the first two principal components of the *Herrania* and *Theobroma* species by using the sterol data set.

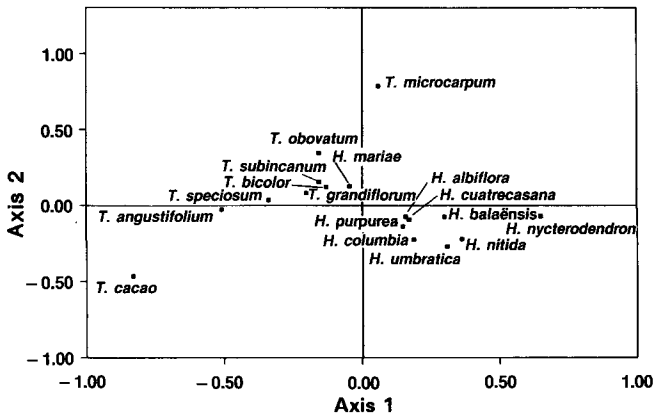

FIG. 3. Plot of the first two principal components of the *Herrania* and *Theobroma* species by using the tocol data set.

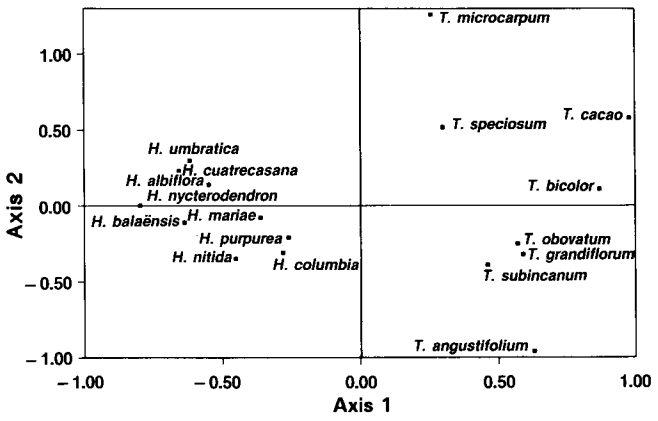

FIG. 4. Plot of the first two principal components of the *Herrania* and *Theobroma* species by using the combined analyte data sets.

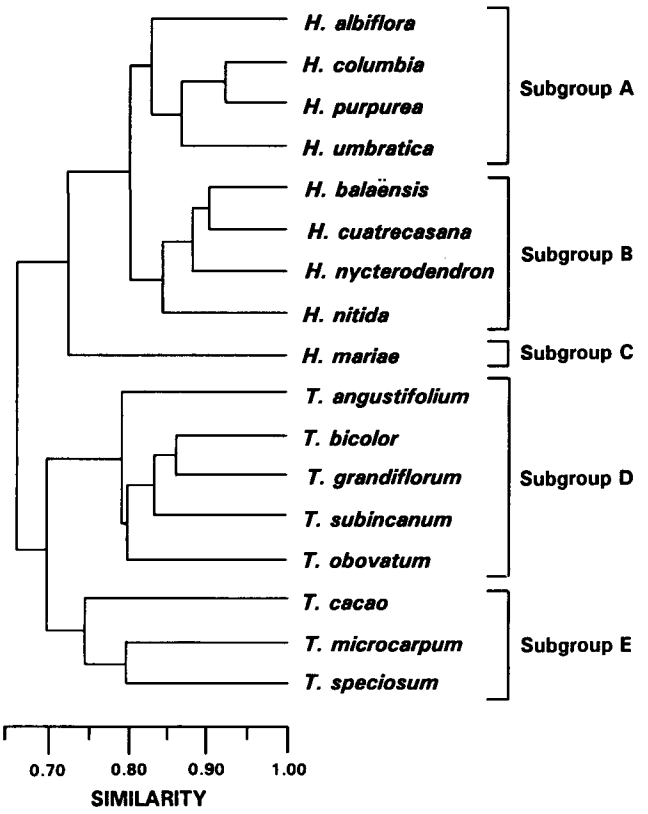

FIG. 5. Dendrogram of *Herrania* and *Theobroma* species.

Theobroma, except in the case of the sterols (Fig. 2). A possible explanation for this result is that the sterol values were derived from saponified fats. This procedure results in the loss of information regarding the natural composition of sterol fatty acid esters, sterol glycosides and acyl sterol glycosides (18), which could be more useful in chemotaxonomy than the sterol values derived from these compounds. As Alberghina *et al.* (19) have shown that the sterol contents are useful for the classification of olive oils, the sterol data set was combined with the other analyte data sets to produce a summary plot (Fig. 4). As with the fatty acid and tocol data sets, a clear separation of the *Herrania* from the *Theobroma* was observed.

Gower general similarity coefficients were calculated for each species and used in a cluster analysis to produce a dendrogram (Fig. 5), which clearly showed a separation of the *Herrania* from the *Theobroma*, as well as five subgroup distinctions. Each subgroup contained species that loosely agreed with the reported classification schemes (1,2). With the exception of *H. columbia*, whose true species' identity is not known, the *Herrania* comprising subgroups A and B were consistent for those assigned to the *Herrania* and *Subcymbicalyx* sections of the genus (1). The average similarity for the *Herrania* clustered in these subgroups was 83 and 84%, respectively. *Herrania mariae* was another exception because it separated into a more distant subgroup (C) whose average similarity to subgroups A and B was 80%. Because *H. mariae* has been reported (2; cf. also Ref. 20) to occur in multiple morphological forms with characteristics of more than one species, our specimen may represent a genetic variant of the "Mariae" complex (20). The five sections that comprise the *Theobroma* were not resolved by the species found within subgroups D and E. Subgroup D contained representatives of the *Glossopetalum* (*T. angustifolium, T. grandiflorum, T. obovatum* and *T. subincanum*) and *Rhytidocarpus* (*T. bicolor*) sections of the genus, where the average similarity for this subgroup was 79%. Members of these sections are morphologically regarded as the most ancient and primitive representatives of the *Theobroma* (2). Subgroup E contained representatives of the *Theobroma* (*T. cacao*), *Telmatocarpus* (*T. microcarpum*) and *Oreanthes* (*T. speciosum*) sections of the genus, where the average similarity for this subgroup was 73%. Members of these sections are regarded as more recent derivatives of the genus (2).

The results presented in this initial survey suggested that the fatty acid, sterol, tocopherol and tocotrienol compositions can be used collectively as chemotaxonomic criteria to differentiate the *Herrania* from the *Theobroma*, but not at the individual section levels. To complement and extend these initial findings, further investigations of additional specimens and species, an examination of known F1 *Theobroma* interspecies crosses (21), a more detailed analysis of the sterols fraction, and an examination of the *Guazuma* (22), as another near relative of

Theobroma and *Herrania*, are collectively required for more rigorous comparisons of the current classification schemes.

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