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Variability in Foliar Phenolic Composition of Several *Quercus* Species in Northern Mexico

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Abstract. Quantitative and qualitative composition of the foliar phenolic compounds were investigated in 81 individual specimens of several white oak species (*Quercus* spp.). The trees were growing in twelve locations in Durango, Mexico. The phenol profiles were determined by HPLC-DAD and a Folin-Ciocateu procedure. The results revealed that: (i) the foliar phenol profiles of all species analysed were complex and formed by 6 to 30 compounds, (ii) the flavonols mostly quercetin glycoside, isorhamnetin glycoside, kaempferol glycoside and phenolic acids were the main identified compounds, (iii) there was a high intra and inter-specific variability in the foliar phenol profiles both at the quantitative and qualitative levels, and (iv) the foliar phenol profiles indicated a slight species-specific tendency for phenols to be accumulated, although this was not clearly distinguished. Significant differences ($P < 0.05$) in the content and composition of the foliar flavonoids between species were observed due to the large environmental and soil conditions variability between localities.

Keywords: *Quercus chihuahuensis*, *Quercus arizonica*, *Quercus grisea*, *Quercus undata*, *Quercus convallata*, foliar phenol profiles

Introduction

Quercus is the most important and largest genus of the Fagaceae family (Rodríguez and Romero, 2007). Two centres of diversity of *Quercus* are recognised i.e. around 125 species found in Southeast Asia and about 161 species in Mexico (Valencia, 2004). *Quercus* species after *Pinus*, constitute the second most important timber yielding group in Mexico. Approximately 86 of the Mexico's species are endemic (González-Rodríguez *et al*., 2004), and distributed mainly in the temperate mountainous regions (Rzedowski, 2006).

Phenolic compounds are ubiquitous secondary metabolites in plants. They cover a large group of biologically active ingredients (more than 8000 compounds) ranging from simple phenol molecules to polymeric structures with molecular mass of more than 3000 daltons (Marinova *et al*., 2005). The foliar phenolic composition of plants is important for several reasons, e.g., for inhibiting phytophagous insects and for attracting pollinators (Hadacek, 2002). The broad biological activity of phenolic compounds, e.g., as antioxidants (Falleh *et al*., 2011; Almaraz-Abarca *et al*., 2007; 2006) and antibacterial compounds (Bangou *et al*., 2011), has also led to studies of phenolic composition in many species of plants. However, the phenolic composition of a few *Quercus* species have been studied, mainly with the aim of determining their biological activity including *Q. robur* (Salminen *et al*., 2004), *Q. alba* (Miller *et al*., 1992), *Q. rubra* (Warren *et al*., 2002), *Q. miyagii* (Ishimaru *et al*., 1987), *Q. dentata* (Wang *et al*., 2010), *Q. cerris* L. (Romussi *et al*., 1988), *Q. mongolica* (Ishimaru *et al*., 1988), *Q. suber* and *Q. pubescens* (Romussi *et al*., 1991), *Q. incana* (Iftikhar *et al*., 2009), *Q. ilex* (Karioti *et al*., 2011), *Q. acutissima* (Yeon *et al*., 2011), *Q. infectoria* (Gurpreet *et al*., 2008), *Q. petraea* (Meyer *et al*., 2009), *Q. salicina* (Jung-Il *et al*., 2008), *Q. coccifera* (Karageorgou and Manetas, 2006), *Q. sideroxyla*, *Q. eduardii*, and *Q. resinosa*

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(Rivas-Arreola *et al*., 2010). These species represents around 4% of the 531 species that are included in this genus (Borazan and Babaç, 2003).

Flavonoids represent the most common and widely distributed group of plant-food phenolics, and their contents and compositions are associated with the antioxidant properties of different fruits and vegetables (Harborne and Williams, 2000). Flavonoid profiles have been used as significant taxonomic markers for establishing a system of classifying several plant species (Almaraz-Abarca *et al*., 2006; Emerenciano *et al*., 2001; Fiasson *et al*., 1997; Abdala and Seeligmann, 1995). These chemical markers were found to be rather species-specific (Almaraz-Abarca *et al*., 2006; Míka, 2005). Changes in phenolic composition caused by environmental factors influence directly the quality of the plant material for potential uses (Santos *et al*., 2006).

The aim of the present study was to investigate the variability in foliar phenolic composition of the following eight *Quercus* species that are among the most abundant species in the temperate forests of Durango, Mexico: *Q. arizonica s.l*., *Q. chihuahuensis*, *Q. grisea*, *Q. undata*, *Q. convallata*, *Q*. aff. *convallata*, *Q. arizonica* intro. *coccolobifolia*, and *Q. arizonica* aff. *transmontana*. Quantitatively and qualitatively intra- and inter-specific variability in phenolic compounds were studied and their taxonomical significance was verified.

Materials and Methods

Plant material. The foliar phenol composition of individual specimens of *Q. arizonica s.l*., *Q. chihuahuensis*, *Q. grisea*, *Q. undata*, *Q. convallata*, *Q*. aff. *convallata*, *Q. arizonica* intro. *coccolobifolia*, and *Q. arizonica* aff. *transmontana* was analysed. The phenol composition was interpreted considering prior to morphological identification of the samples. Ecogeographic and soil information were also recorded when sampling (Table 1). Foliar samples were collected from individuals growing under variable ecological conditions. Leaves from 6 to 7 adult individuals of healthy appearance were collected in all cases. A total of 81 specimens were sampled at 12 locations. Authentication of each specimen sampled was done at the CIIDIR Herbarium in Durango City, Mexico.

Phenol extraction. Each sample was analysed individually and 2 g of dried and grounded leaves of each sample were macerated with 40 mL 60% methanol (v/v) in darkness and at room temperature for 24 h. The extracts were centrifuged (8000 rpm) for 10 min at room temperature. The supernatants were separated and pellets re-extracted in 7 mL 60% methanol (v/v) in darkness and at room temperature for 2 h. The extracts were centrifuged under the same conditions. Similar supernatants were combined to form the total extracts, and these were concentrated to dryness by rotary evaporation and then re-dissolved in 3 mL methanol. Aliquots were removed for determination of the phenol content and HPLC-DAD analysis.

Total phenol contents. The amount of total phenols in the methanol foliar extracts of each sample of *Quercus* was determined by the Folin-Ciocalteu method (Lozoya-Saldaña *et al.,* 2007). The following values were obtained from the standard curve for gallic acid (32-260 µg/mL vs. absorbance): $A_{760\,nm} = -0.011 + 0.0004$ (gallic acid) and correlation coefficient, r = 0.9989. Absorbance values were recorded at 760 nm after 120 min of incubation at darkness. Total phenol contents were expressed as mg of gallic acid equivalents GAE/g dry weight (Dw).

Analysis by HPLC/DAD. The individual HPLC-DAD phenol profiles were obtained following the method described by Campos and Markham (2007). Extracts (20 µL) were analysed in a Perkin Elmer Series 200 HPLC system, with a Perkin Elmer Brownlee analytical C18 column (4.6×250 mm, 5 µ) and a gradient of acidified water and acetonitrile. The flow rate was 0.8 mL/min. Standard chromatograms were plotted at 280 and 340 nm. Spectral data for all peaks were accumulated in the range of 200-400 nm using a diode-array detector (DAD) (Perkin Elmer Series 200). The structural information on each resolved compound was obtained by direct comparison of retention times (RT) and UV spectra with those of standards (quercetin, quercitrin, naringenin, hesperidin, epicatechin, phthalic acid, vanillic acid, gallic acid and trans-cinnamic acid) and according to the data compiled by Mabry *et al*. (1970) and Campos and Markham (2007). Quantitative determination of the major flavonoids was made from a stock solution of quercetin, which was prepared (120, 250, and 500 µg/mL)

Table 1. Collection sites for *Quercus* species and hybrids

Individuals	Reference number: Species	Eco-geographic information (location, latitude N, longitude W, altitude (masl), average temperature (°C), annual precipitation (mm), soil type, date
1-6	36286: *Q. chihuahuensis*, 36319: *Q .chihuahuensis* × *grisea*, 36315: *Q. chihuahuensis*, 36316: *Q. chihuahuensis*, 36321: *Q. chihuahuensis* and 36327: *Q. chihuahuensis*	Site I: 35 Km Durango-Mazatlán Highway, 23°56'08.5", 104°51'58.3", 2270, 13, 733, eutric regosol, May 2008
7-13	36387: *Q. arizonica s.l*., 36515: *Q. arizonica* intro. *grisea*, 36385: *Q. arizonica* intro. *grisea*, 36419: *Q. grisea*, 36426: *Q. arizonica s.l*., 36396: *Q. arizonica s.l.* and 36466: *Q. grisea s.l.*	Site II: 29 Km Durango-Mazatlán Highway, 23°57'12.4", 104°51'01.5", 2207, 14, 711, 2207, entisoil, May 2008
14-20	36410: *Q. chihuahuensis*, 36403: *Q. undata*, 36400: *Q. chihuahuensis*, 36401: *Q. chihuahuensis*, 36404: *Q. undata*, 36402: *Q. chihuahuensis* and 36394: *Q. undata*	Site III: 13 Km Durango-Mazatlán Highway, 23°59'11.4", 104°44'53.7", 2082, 16, 644, leptosol, May 2008
21-27	36760: *Q. arizonica s.l*., 36739: *Q. arizonica s.l*., 36737: *Q. arizonia s.l*., 36736: *Q. arizonica s.l*., 36743: *Q. arizonica s.l*., 36725: *Q. arizonica s.l.* and 36726: *Q. arizonica s.l*	Site IV: 187 Km Tepehuanes-Guanaceví Road, 25°28'25.4", 105°47'51.5, 2167, 14, 632, eutric regosol, Jun 2008
28-33	36762: *Q. grisea*, 36730: *Q. arizonica s.l*., 36784: *Q. aff.arizonica*, 36783: *Q. aff. arizonica*, 36741: *Q. arizonica s.l.* and 36742: *Q. arizonica s.l.*	Site V: 191 Km Tepehuanes-Guanaceví Road, 25°29'31.4", 105°47'11.2", 2079, 15, 606, eutric regosol, Jun 2008
34-40	36727: *Q. arizonica s.l*., 36728: *Q. arizonica s.l*., 36750: *Q. arizonica s.l*., 36780: *Q. arizonica s.l*., 36781: *Q. arizonica s.l*., 36761: *Q. grisea* and 36782: *Q. arizonica s.l.*	Site VI: 193 Km Tepehuanes-Guanaceví Road, 25°30'11.6", 105°47'14.6", 2098, 15, 611, eutric regosol, Jun 2008
41-47	38820: *Q.* aff. *convallata,* 38821: *Q.* aff. *convallata,* 38822: *Q*. aff. *convallata,* 38823: *Q*. aff. *convallata* intro. *arizonica,* 38824: *Q*. aff. *convallata,* 38825: *Q*. aff. *convallata* and 38826: *Q*. aff. *convallata*	Site VII: 6 Km El Tecuán-Regocijo Road, 23°52'37.2", 105°00'58.7", 2168, 13, 765, regosol, Jun 2008
48-54	38827: *Q.* aff. *convallata,* 38828: *Q.* aff. *convallata,* 38829: *Q. convallata* introgresión *arizonica,* 38830: *Q. convallata,* 38831: *Q. convallata,* 38832: *Q. convallata* and 38833: *Q. convallata*	Site VIII: 8 Km El Tecuán-Regocijo Road, 23°51'41.9", 105°00'12.9", 2188, 13, 773, district regosol, Jun 2008
55-61	38834: *Q.* aff. *convallata,* 38835: *Q.* aff. *convallata,* 38836: *Q. arizonica* x *convallata,* 38837: *Q*. aff.*convallata,* 38838: *Q. arizonica s.l,* 38839: *Q. arizonica s.l.,*and 38840: *Q*. aff. *arizonica* x *convallata*	Site IX: 10 Km El Tecuán-Regocijo Road, 23°50'54.4", 105°00'01.6", 2172, 13, 777, district regosol, Jun 2008
62-68	36734: *Q. arizonica* intro. *coccolobifolia,* 36733: *Q. arizonica s.l.* intro. *coccolobifolia,* 36825: *Q. arizonica* aff. *transmontana,* 36836: *Q. arizonica* aff. *transmontana,* 36837: *Q. arizonica s.l.,* 36834: *Q. arizonica* aff. *Transmontana* and 36838: *Q. arizonica s.l.*	Site X: 39 Km Durango-Mezquital, 23°46'35.6", 104°25'23.7", 2098, 16, 602, leptosol, Jun 2008
69-75	36860: *Q. arizonica s.l.,* 36845: *Q. arizonica s.l.,* 36816: *Q. arizonica* intro. *coccolobifolia,* 36788: *Q. arizonica s.l.,* 36858: *Q. arizonica* aff. *transmontana,* 36859: *Q. arizonica* intro. *coccolobifolia* and 36843: *Q.* aff. *arizonica* x *coccolobifolia*	Site XI: 41 Km Durango-Mezquital, 23°45'45.3", 104°25'05.9", 2043, 16, 588, leptosol, Jun 2008
76-81	36830: *Q. arizonica* aff. *transmontana,* 36835: *Q. arizonica* x *coccolobifolia,* 36831: *Q. arizonica* intro. *coccolobifolia,* 36735: *Q. arizonica* intro. *coccolobifolia,* 36829: *Q. arizonica* intro. *coccolobifolia and* 36832: *Q. arizonica* intro. *coccolobifolia*	Site XII: 36 Km Durango-Mezquital Highway, 23°47'01.4", 104°25'33.2", 2061, 16, 593, calcium afisol, Jun 2008

for construction of the calibration curve ($A_{280\ nm}$ = 413389 + 25881[quercetin], r = 0.9999) by plotting the standard concentrations against the peak area in the HPLC chromatograms. The concentration of each individual compound was expressed in µg of quercetin equivalents (EQ quercetin µg/g Dw) (here 395.9 µg/g Dw).

Data analysis. The individual phenol profiles comprised of all compounds detected in the respective HPLC-DAD chromatograms. Each compound was treated as a single chemical character and assessed in a binary matrix coded by 1 (presence) or 0 (absence) in each population. A dendrogram obtained from a cluster analysis (Ward´s method) based on chemical marker data was calculated

using PAST 1.43 (Hammer *et al*., 2001). Because phenolic contents were not normally distributed, the non-parametric Kruskal-Wallis test was used to evaluate intra and inter-specific variability of these compounds either at the quantitative or the qualitative levels (Kruskal and Wallis, 1952). The test was performed with the NPAR1WAY procedure of SAS/ETS® (SAS Institute Inc., 2004).

Results and Discussion

***Quercus* foliar phenolic compounds.** A total of 73 compounds were determined by HPLC-DAD analysis of the leaves of the species under study (Table 2). The analysis revealed 20 phenolic acids and 53 flavonoids. The flavonoids present in the foliar tissues included 34 flavonols, 5 flavones, 4 dihydroflavonoids and 10 un-identified flavonoids. The flavonols included seven quercetin derivatives, four myricetin derivatives, seven kaempferol derivatives and two isorhamnetin derivatives. The flavones included two tricitin derivatives and one luteolin derivative. Compounds f 13 (quercetin glycoside), f 31 (isorhamnetin glycoside) and f 37 (kaempferol glycoside) were the most abundant phenolic compounds. The results of the present study are consistent with those

Table 2. Retention time and λ_{max} of the phenolic compounds present in the foliar tissues of the analysed *Quercus* species

Compound	Identification	Retention time (min)*	λ_{max} (nm)
f01	Fl	36.40±0.00	255, 360
f02	Fl	37.87±0.00	255, 360
f03	U	41.83±0.00	280, 360
f04	Qg	44.18±0.00	255, 299 sh, 350
f05	Fl	63.12±0.17	255, 370
f06	Fl	67.72±0.27	250, 301sh, 370
f07	Fl	68.50±0.31	255, 367
f08	Fl	68.46±0.22	265, 285sh, 355
f09	Fl	69.59±0.31	255, 360
f10	Qg	69.97±0.00	255, 294 sh, 355
f11	Lg	70.07±0.00	265, 295 sh, 360
f12	U	70.27±0.44	265, 290 sh, 355
f13	Qg	70.65±0.27	252, 290 sh, 350
f14	Mg	70.81±0.00	253, 265 sh, 299 sh, 353
f15	U	71.50±0.36	253, 270 sh, 360
f16	Qg	72.60±0.26	255, 300 sh, 360
f17	Df	72.56±0.30	280
f18	Td	72.91±0.01	266, 293 sh, 350
f19	U	72.97±0.00	265, 350
f20	U	73.43±0.26	266, 297 sh, 353
f21	Fl	74.43±0.25	254, 305 sh, 364
f22	Kg	74.76±0.13	260, 350
f23	U	74.82±0.16	267, 285sh, 360
f24	Qg	75.27±0.21	256, 300 sh, 357
f25	Mg	75.66±0.33	255, 297 sh, 352
f26	Fl	75.17±0.00	267, 360
f27	Tg	75.66±0.22	250, 262 sh, 353
f28	Fl	75.66±0.00	250, 360
f29	Qg	76.44±0.39	256, 266 sh, 292sh, 356
f30	U	76.43±0.18	270, 357
f31	Ig	76.56±0.36	255, 267 sh, 355
f32	Ig	77.18±0.00	255, 267sh, 355
f33	U	77.50±0.36	267, 285 sh, 356
f34	Df	77.74±0.09	281
f35	Fl	78.93±0.05	266, 296 sh, 351
f36	U	78.72±0.21	255, 269 sh, 355
f37	Kg	78.74±0.32	265, 350
f38	Fl	80.13±0.15	267, 350
f39	Kg	80.62±0.36	266, 351
f40	U	80.43±0.00	270, 287 sh, 361
f41	Kg	81.45±0.42	265, 295 sh, 349
f42	Df	81.02±0.00	278
f43	Mg	81.07±0.00	255, 267 sh, 297sh, 354
f44	Mg	82.06±0.03	256, 300 sh, 351
f45	Qg	82.48±0.37	256, 267 sh, 300 sh, 351
f46	Kg	82.15±0.25	265, 351
f47	Kg	83.89±0.04	266, 350
f48	Df	88.25±0.31	281
f49	Ca	89.50±0.00	287, 311
f50	F	89.47±0.00	266, 294sh, 314
f51	Pa	93.46±0.07	267, 316
f52	Pa	96.95±0.00	267, 311
f53	Fl	101.37±0.00	266, 364
f54	Fl	103.08±0.01	256, 370
f55	Kg	103.10±0.12	264, 364
f56	Pa	104.66±0.42	266, 314
f57	F	105.63±0.00	266, 297 sh, 313
f58	Pa	108.57±0.21	267, 314
f59	Pa	109.31±0.24	267, 312
f60	Pa	110.35±0.34	267, 311
f61	Pa	111.37±0.26	267, 312
f62	Pa	112.40±0.29	267, 314
f63	Pa	113.30±0.25	266, 311
f64	Pa	114.11±0.05	266, 311
f65	Pa	115.47±0.06	266, 311
f66	Pa	116.60±0.30	268, 311
f67	Pa	117.50±0.32	267, 312
f68	Pa	118.52±0.34	267, 312
f69	Pa	119.52±0.33	266, 313
f70	Pa	120.42±0.26	267, 315
f71	Pa	121.53±0.04	267, 313
f72	Pa	122.35±0.21	266, 314
f73	Pa	123.52±0.39	266, 311

*Retention times are mean values and standard deviations for 1-81 independent samples; Ca = cinnamic acid; Df = dihydraflavonoid; F = flavone; Fl = flavonel; Ig = isorhamnetin glycoside; Kg = kaempferol glycoside; Lg = lutheolin glycoside; Mg = myricetin glycoside; Pa = phenolic acid; Qg = quercetin glycoside; Td = tricitin derivative; Tg = tricin glycoside; U = unidentified.

continued next column →

reported by Karioti *et al.* (2011) who also identified kaempferol glycosides, isorhamnetin glycosides and quercetin glycosides as the most abundant phenolic compounds in extracts of *Quercus ilex* trichomes. Rivas-Arreola *et al.* (2010) and Wang *et al.* (2010) also identified kaempferol glycosides and quercetin glycosides as the most abundant phenolic compounds in other species of *Quercus*. The most complex foliar phenol profile was that of *Q. chihuahuensis*, which included 30 phenols. The least complex profile was that of *Q. arizonica* intro. *coccolobifolia*, with 6 phenols.

Total phenolic contents. Table 3 shows the average total foliar phenolic contents of the species of *Quercus* analysed. According to the non-parametric Kruskal-Wallis test, the total phenol contents varied significantly among *Quercus* species (H = 28.48, P = 0.0002) (Table 4). A comparison among groups of individuals belonging to the same *Quercus* species and that were growing in different sites showed that locality also affected phenol contents of the (H = 62.69, P<0.0001), suggesting variation in phenolic compounds within tree species and localised environmental control of levels of these compounds. As an example phenol contents of *Q. arizonica s.l.* which was recorded in sites II, II, IV, V, VI, IX, X and XI varied significantly in 6 of the 7 pair comparisons tested using the individuals of site II as the reference group. These results could be explained by the effect of site variability in soil composition, temperature, and rainfall (Borges *et al*., 2013; Gobbo-Neto and Lopes, 2007; Monteiro *et al*., 2006). The lowest average level (196.85 mg/g Dw) was estimated for individuals of *Q. convallata* (site IX) and the highest average level (399.61 mg/g Dw) for *Q. arizonica s.l.* (site IV).

Table 3. Average total foliar phenolic contents (gallic acid equivalents) of *Quercus grisea, Q. arizonica s.l., Q. chihuahuensis, Q. undata, Q. arizonica* intro. *coccolobifolia, Q. arizonica* aff. *transmontana, Q.* aff. *convallata*, and *Q. convallata*

Site	Tree species	No. of individuals	Average total concentration
I	*Q. chihuahuensis*	5	369.74
II	*Q. arizonica s.l.*	3	340.00
II	*Q. grisea*	1	367.50
III	*Q. chihuahuensis*	4	280.30
III	*Q. undata*	3	299.97
IV	*Q. arizonica s.l.*	7	399.61
V	*Q. arizonica s.l.*	3	313.73
V	*Q. grisea*	1	311.20
VI	*Q. arizonica s.l.*	6	295.62
VI	*Q. grisea*	1	281.20
VII	*Q.* aff. *convallata*	6	259.97
VIII	*Q.* aff. *convallata*	2	198.75
VIII	*Q. convallata*	4	198.73
IX	*Q.* aff. *convallata*	3	208.73
IX	*Q. arizonica s.l.*	2	196.85
X	*Q. arizonica* aff. *transmontana*	3	239.97
X	*Q. arizonica intro coccolobifolia*	1	255.00
X	*Q. arizonica s.l.*	2	232.45
XI	*Q. arizonica* aff. *transmontana*	1	213.70
XI	*Q. arizonica intro coccolobifolia*	2	210.00
XI	*Q. arizonica s.l.*	3	221.23
XII	*Q. arizonica* aff. *transmontana*	1	247.50
XII	*Q. arizonica intro coccolobifolia*	4	273.75

Results of the non parametric Kruskal-Wallis test among studied *Quercus* species are shown in Table 4. Significant differences (P<0.05) between foliar phenol levels were observed in 16 of 28 pair comparisons. The foliar phenol contents were highest in *Quercus arizonica s.l.* and *Q. chihuahuensis* and lowest in *Q. arizonica* aff. *transmontana, Q. convallata* and *Q.* aff. *convallata*. The foliar phenol contents estimated for the species of *Quercus* analysed in the present study are similar to the levels reported by Karageorgou and Mantas (2006), who estimated levels ranging from 50 to 550 mg/g Dw in the leaves of *Q. coccifera* L., and by Rivas-Arreola *et al*. (2010), who estimated levels ranging from 227 to 537 mg/g Dw in the leaves of another species of *Quercus.*

Individual phenolic concentration. The relative concentrations of the individual phenolic compounds detected in each sample are shown in Table 5. The Kruskal-Wallis test revealed statistically significant differences between the relative concentrations of phenolic compounds in several samples (Table 6). The maximum concentration observed was that of compound f 08 (385.86 µg/g Dw) in *Q. chihuahuensis* (sample 1), and the lowest concentration was that of compound f 72 (37.21 µg/g Dw) in *Q. arizonica* intro. *coccolobifolia* (sample 71).

As shown in Table 6, highly significant differences were observed in the mean relative concentrations of

Table 4. Results of the Kruskal-Wallis test for paired comparisons of foliar phenol contents in the analysed *Quercus* species

Pairs of species compared (mean score in brackets)	H	*Pr*> H
Q. chihuahuensis (19.38) – Q. arizonica s.l. (17.51)	0.22	0.636
Q. chihuahuensis (6.61) – Q. grisea (6.16)	0.03	0.853
Q. chihuahuensis (6.88) – Q. undata (5.33)	0.42	0.516
Q. chihuahuensis (15.27) – Q. aff. *convallata (6.59)*	10.68	0.001**
Q. chihuahuensis (9.00) – Q. convallata (2.50)	7.73	0.005**
Q. chihuahuensis (11.16) – Q. arizonica intro. *coccolobifolia (5.07)*	6.46	0.011*
Q. chihuahuensis (10.0) – Q. arizonica aff. *transmontana (3.0)*	9.00	0.002**
Q. arizonica s.l. (14.94) – Q. grisea (15.50)	0.01	0.914
Q. arizonica s.l. (15.15) – Q. undata (13.66)	0.08	0.774
Q. arizonica s.l. (22.46) – Q. aff. *convallata (10.81)*	8.95	0.002**
Q. arizonica s.l. (17.26) – Q. convallata (4.00)	7.88	0.0050**
Q. arizonica s.l. (18.75) – Q. arizonica intro. *coccolobifolia (10.50)*	4.02	0.044*
Q. arizonica s.l. (17.46) – Q. arizonica aff. *transmontana (8.40)*	4.17	0.041*
Q. grisea (4.0) – Q. undata (3.0)	0.483	0.486
Q. grisea (12.83) – Q. aff. *convallata (6.04)*	6.21	0.012*
Q. grisea (6.00) – Q. convallata (2.50)	5.58	0.0323*
Q. grisea (8.33) – Q. arizonica intro. *coccolobifolia (4.28)*	3.75	0.0527
Q. grisea (7.0) – Q. arizonica aff. *transmontana (3.0)*	5.00	0.025*
Q. undata (12.50) – Q. aff. *convallata (6.13)*	5.47	0.0193*
Q. undata (6.00) – Q. convallata (2.50)	4.66	0.030*
Q. undata (8.33) – Q. arizonica intro. *coccolobifolia (4.28)*	3.77	0.052
Q. undata (7.0) – Q. arizonica aff. *transmontana (3.0)*	5.06	0.0245*
Q. aff. *convallata (9.27) – Q. convallata (4.50)*	3.34	0.067
Q. aff. *convallata (8.45) – Q. arizonica* intro. *coccolobifolia (11.14)*	1.08	0.297
Q. aff. *convallata (8.31) – Q. arizonica* aff. *transmontana (8.90)*	0.051	0.820
Q. convallata (3.25) – Q. arizonica intro. *coccolobifolia (7.57)*	4.34	0.037*
Q. convallata (3.00) – Q. arizonica aff. *transmontana (6.60)*	3.93	0.047*
Q. arizonica intro. *coccolobifolia (7.35) – Q. arizonica* aff. *transmontana (5.30)*	0.95	0.329

*significant differences; **highly significant differences.

phenols between the following pairs of *Quercus* species: *Q.* aff. *convallata* and *Q. arizonica s.l.; Q.* aff. *convallata* and *Q. chihuahuensis*; *Q. arizonica* intro. *coccolobifolia* and *Q. convallata*; *Q. chihuahuensis* and *Q. convallata.* The tree species *Quercus grisea, Q. chihuahuensis, Q. arizonica s.l.* and *Q. undata* accumulated the highest relative concentrations of phenols, whereas *Q.* aff. *convallata* and *Q. arizonica* aff. *transmontana* accumulated the lowest levels.

In summary, phenolic concentrations of studied white oak tree species varied greatly among, species, individuals, and sites. This is in agreement with previous studies on phenolic contents. Significant variation in phenolic concentrations has been reported among tissue types in an individual, among sites, and between species (Van Alstyne *et al*., 1999, Rivas-Arreola *et al*., 2010). The results indicate that *Quercus* species contain larger amounts of phenol compounds than most of the other plants. Rivas-Arreola *et al.* (2010) reported phenolic contents of 537 mg/g, 331 mg/g and 227 mg/g in dried leaves of *Quercus sideroxyla*, *Quercus eduardii* and *Quercus resinosa*, respectively. Amarowicz *et al.* (2004) reported phenolic contents of 55.4, 58, and 67.6 mg/g in dried seeds of red bean (*Phaseolus vulgaris*), red lentil (*Lens culinaris*) and green lentil (*Lens culinaris*), respectively. Marinova *et al.* (2005) reported total phenolic contents of 3.03, 4.29, 3.55 and 6.70 mg/g in fruits of plum (*Prunus domestica*), sour cherry (*Prunus cerasus*), blackberry (*Rubus coesins*) and blueberry (*Vaccinium myrtilus*), respectively.

Cluster analysis. The results of the cluster analysis of the foliar phenol profiles of the individuals analysed

Table 5. Relative concentrations of the individual phenolic compounds identified in the leaves of the *Quercus* species under study (quercetin equivalents)

Compound	Relative concentration (µg/g Dw)							
	Q. grisea	*Q. arizonica s.l.*	*Q. chihuahuensis*	*Q. undata*	*Q. arizonica* intro. *coccolobifolia*	*Q. arizonica* aff. *transmontana*	*Q.* aff. *convallata*	*Q. convallata*
f05	71.2	76	-	-	-	-	-	69.6
f06	323.84	302.12	285	306.42	98.18	96.59	-	80.76
f07	-	-	-	-	152.55	-	-	-
f08	300.88	-	385.86	-	212.20	-	203.49	-
f09	-	344.43	-	-	167.86	-	-	69.28
f12	362.64	336.15	-	-	66.51	-	-	
f13	-	214.73	330.37	-	-	-	66.51	67.30
f15	-	289.64	145.29	-	120.35	120.35	-	-
f17	227.24	306.95	-	-	216.16	-	-	-
f20	-	-	-	-	100.29	-	-	-
f21	-	309.59	-	318.30	184.09	193.72	184.09	179.73
f22	-	160.73	146.22	142.52	-	-	-	-
f23	-	193.19	-	-	-	187.65	180.13	-
f24	-	-	-	102.14	-	100.55	71.26	60.96
f25	-	-	-	-	-	-	146.74	-
f27	-	145.69	-	-	-	-	95.80	62.55
f29	213.78	239.91	-	-	148.85	-	-	-
f31	-	306.95	174.98	-	-	-	128.46	58.59
f34	-	313.02	-	-	-	-	-	-
f36	-	139.35	124.31	-	-	63.34	-	-
f37	-	153.41	146.87	-	72.84	101.54	55.42	-
f39	-	64.13	-	-	59.38	58.59	46.71	-
f45	-	-	-	-	-	-	43.81	-
f46	-	-	-	70.47	-	63.34	58.85	50.41
f55	-	67.46	-	-	-	-	-	-
f58	-	69.10	66.51	-	-	-	-	-
f59	71.26	84.72	99.76	-	-	-	-	-
f60	-	67.3	72.05	-	-	-	-	-
f61	57.8	61.36	82.34	-	-	-	-	-
f62	-	-	59.38	-	-	52.25	45.92	-
f63	-	62.81	-	-	46.71	-	-	-
f65	-	62.29	-	-	-	53.05	-	-
f66	62.55	84.19	-	67.30	-	55.42	49.48	-
f67	57	-	67.30	-	-	-	-	-
f68	-	62.55	-	49.09	-	-	-	-
f69	-	58.59	-	-	44.73	-	-	-
f70	-	57.14	-	-	-	-	-	42.75
f72	-	45.76	-	-	37.21	-	-	-
f73	-	43.15	-	-	-	-	-	-

are shown in Fig. 1. Two main groups can be distinguished: group I, formed by subgroups A and B, and group II, by subgroups C, D, E, F. Clade I excluded samples from sites 2, 4, 5, 6, and 12, which mainly included specimens of *Quercus arizonica s.l.* and *Q. arizonica* intro. *coccolobifolia*. Clade II excluded samples from site 9, which comprised samples of *Q.* aff. *convallata* and *Q. arizonica s. l.* All subgroups were heterogeneous, except subgroup C, which included three samples of the same taxa, *Q. arizonica* intro. *coccolobifolia* from site 12. A third of the samples of *Q. chihuahuensis* were grouped in subgroup A, from

Table 6. Results of the Kruskal-Wallis test for paired comparisons of samples to assess differences in the relative concentrations of phenolic compound in the leaves of the *Quercus* species under study

Pairs of species compared	H	Pr > H
Q. aff.*convallata (33.07) –Q. arizonica* intro. *coccolobifolia (41.87)*	3.02	0.0821
Q. aff. *convallata (59.51) –Q. arizonica s.l. (80.84)*	7.65	0.0057**
Q. aff. *convallata (30.25) –Q. arizonica* aff. *transmontana (34.55)*	0.72	0.3936
Q. aff. *convallata (32.61) –Q. chihuahuensis (48.41)*	9.32	0.0023**
Q. aff. *convallata (31.54) –Q. convallata (27.62)*	0.59	0.4418
Q. aff. *convallata (25.32) –Q. grisea (38.68)*	6.11	0.0134*
Q. aff. *convallata (26.62) –Q. undata (33.50)*	1.62	0.2029
Q. arizonica intro. *coccolobifolia (65.89) –Q. arizonica s.l. (66.66)*	0.00	0.9246
Q. arizonica intro. *coccolobifolia (25.12) –Q. arizonica* aff. *transmontana (20.97)*	1.04	0.3057
Q. arizonica intro. *coccolobifolia (29.37) –Q. chihuahuensis (33.25)*	0.70	0.3999
Q. arizonica intro. *coccolobifolia (26.48) –Q. convallata (15.53)*	7.4	0.0065**
Q. arizonica intro. *coccolobifolia (18.71) –Q. grisea (23.27)*	1.26	0.2611
Q. arizonica intro. *coccolobifolia (20.35) –Q. undata (19.09)*	0.09	0.7549
Q. arizonica s.l. (62.66) –Q. arizonica aff. *transmontana (54.75)*	0.76	0.3803
Q. arizonica s.l. (66.19) –Q. chihuahuensis (79.61)	2.89	0.0891
Q. arizonica s.l. (63.65) –Q. convallata (39.96)	6.43	0.0112*
Q. arizonica s.l. (57.14) –Q. grisea (66.09)	0.71	0.3973
Q. arizonica s.l. (57.75) –Q. undata (60.36)	0.06	0.8047
Q. arizonica aff. *transmontana (20.36) –Q. chihuahuensis (29.75)*	4.51	0.0335*
Q. arizonica aff. *transmontana (20.36) –Q. convallata (29.75)*	4.57	0.0324*
Q. arizonica aff. *transmontana (13.27) –Q. grisea (17.81)*	1.94	0.1634
Q. arizonica aff. *transmontana (14.55) –Q. undata (15.72)*	0.12	0.7191
Q. chihuahuensis (30.57) –Q. convallata (14.71)	12.87	0.0003**
Q. chihuahuensis (23.26) –Q. grisea (22.18)	0.05	0.8121
Q. chihuahuensis (24.45) –Q. undata (18.50)	1.71	0.1910
Q. convallata (10.87) –Q. grisea (18.54)	6.09	0.0136*
Q. convallata (11.40) –Q. undata (17.77)	4.20	0.0404*
Q. grisea (12.22) –Q. undata (10.77)	0.27	0.5988

*significant difference; **highly significant difference.

sites 1 and 3. Subgroup B mainly comprised samples of *Q.* aff. *convalata* from sites 7, 8 and 9, and *Q. arizonica s.l.* from sites 9 and 10. Subgroup D comprised samples of *Q. arizonica s.l.* from sites 2, 4, 5 and 6. Subgroup E included the samples of *Q. arizonica* intro. *coccolobifolia* from sites 10 and 11, and subgroup F included two samples of the same taxa *Q. arizonica* intro. g*risea* from site 2 and *Q. arizonica s.l.* from site 4. Despite the species-specific tendency reported for phenol profiles (Almaraz-Abarca *et al*., 2006; Veit *et al*., 1995), the species of *Quercus* analysed in the present study were not clearly distinguished by their phenol profiles.

Conclusion

The phenols synthesised by foliar tissues of the species of *Quercus* analysed here are diverse and some of the profiles are complex (e.g., that of *Q. chihuahuensis*). The compounds are produced in different ways and can reach very high concentrations. The variability in the phenol composition and the amounts of those compounds in the leaves hampered identification of some trends in the patterns of accumulation. This is the first report on the quantitative and qualitative composition of foliar phenolic compounds in selected *Quercus* tree species of Mexico. Further chemo-taxonomic studies including the analysis of terpenoid profiles in other types of tissues may yield more conclusive results.

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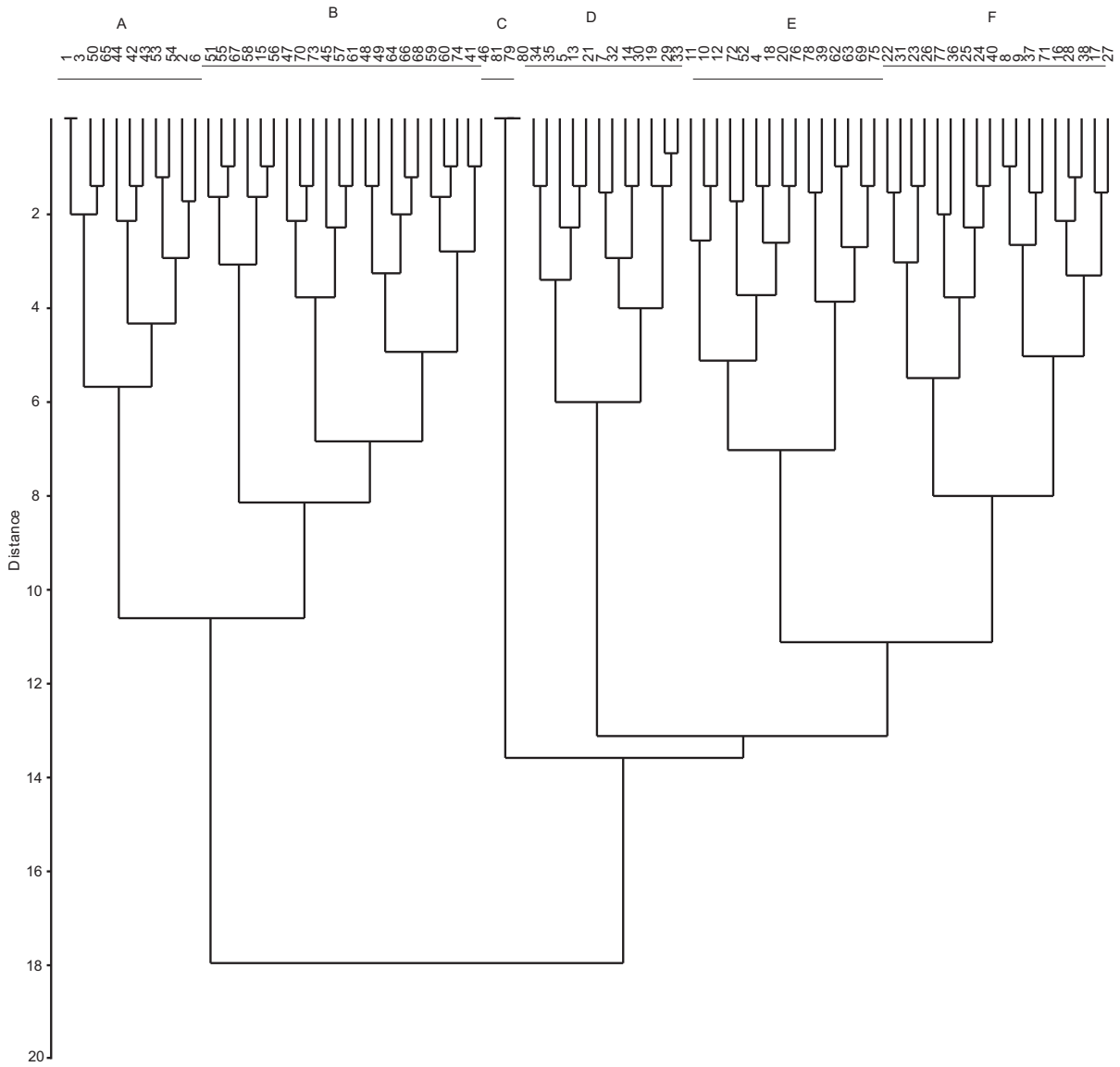

Fig. 1. Results of cluster analysis comparing foliar phenol profiles of 81 specimens of *Quercus arizonica s.l., Q. chihuahuensis, Q. grisea, Q. undata, Q. arizonica intro. coccolobifolia, Q. arizonica* aff. *transmontana, Q. convallata* and *Q.* aff. *convallata*. The numbers of the samples correspond to those shown in Table 1.

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