Triterpenes in elms in Spain

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Abstract: Diethyl ether-petroleum ether extracts were prepared from 49 samples of bark from four elm species (*Ulmus glabra* Hudson, *Ulmus laevis* Pall, *Ulmus minor* Miller, and *Ulmus pumila* L.) and hybrids from crosses between three of these species. Chemical analyses were performed by gas chromatography – mass spectrometry. Ten triterpenes and three sterols were identified. These compounds are discussed in terms of chemotaxonomy of the genus and identification of hybrids, and in relation to the differential attractivity for elm bark beetle feeding. Separation of the four pure species was successfully achieved by the use of the multivariate discriminant analysis. *Ulmus minor* × *U. pumila* hybrids were clearly segregated from their parental species, while *U. minor* × *U. glabra* trees were misclassified as *U. minor* by a multivariate discriminant analysis. Three compounds are described for the first time in the family Ulmaceae and two more in the genus *Ulmus*. Some of the triterpenes and sterols isolated only in *U. glabra* and *U. laevis* may be responsible for the deterrence of bark beetles to feed on these least preferred species.

Résumé : Des extraits dans une solution d'oxyde de diéthyle et d'éther de pétrole ont été préparés à partir de 49 échantillons d'écorce provenant de quatre espèces d'orme (*Ulmus glabra* Hudson, *Ulmus laevis* Pall, *Ulmus minor* Miller et *Ulmus pumila* L.) et d'hybrides issus de croisements entre trois de ces espèces. Les analyses chimiques ont été réalisées par chromatographie en phase gazeuse et spectrométrie de masse. Dix triterpènes et trois stérols ont été identifiés. Les auteurs discutent de ces composés en termes de chimiotaxonomie du genre, de l'identification des hybrides et de la relation avec leur différent pouvoir d'attraction du scolyte de l'orme. Les quatre espèces pures ont pu être séparées en utilisant l'analyse discriminante multivariée. Les hybrides d'*U. minor* × *U. pumila* se différentiaient nettement de leurs parents tandis que les hybrides d'*U. minor* × *U. glabra* étaient à tort classés par l'analyse discriminante multivariée comme étant *U. minor.* Trois composés sont décrits pour la première fois dans la famille des Ulmaceae et deux autres dans le genre *Ulmus*. Certains des triterpènes et des stérols isolés seulement chez *U. glabra* et *U. laevis* ont peut-être un effet dissuasif qui décourage les scolytes à se nourrir sur ces deux espèces qui sont les moins recherchées.

[Traduit par la Rédaction]

Introduction

The genus *Ulmus* comprises more than 40 species worldwide, which are naturally distributed throughout the northern hemisphere in Eurasia, North America, Central America, and northern Africa. The taxonomy of the genus is very complex mainly owing to three factors: broad variations of morphological characters within the genus, intense human use of *Ulmus* since the Roman times, and weak crossability barriers between different species that hybridize naturally (Mittempergher and La Porta 1991). Only species in the section *Blepharocarpus,* such as *Ulmus laevis* Pall, and *Ulmus americana* L., which are genetically isolated from the rest of the genus, do not produce crosses with species in other sections.

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There are three elm species native to western Europe: *Ulmus minor* Miller sensu lato (field elm), *Ulmus glabra* Hudson (witch elm), and *Ulmus laevis* Pall (white elm). The taxonomy and botanical definition of *U. minor* have often been controversial, and five different subspecies or varieties have been defined (Richens 1983). In Europe, the Asiatic elm species *Ulmus pumila* L. (Siberian elm) was introduced in Spain in the 16th century (Gil and García-Nieto 1990). Since the 1950s, this species, which is resistant to Dutch elm disease caused by *Ophiostoma novo-ulmi* Brasier, has been used broadly to replace *U. minor* that were killed by the pathogen (Gil et al. 2003). When hybridization with *U. minor* occurs, seedlings generally show intermediate morphological 1990; Cogolludo-Agustín et al. 2000). Owing to the asymmetric hybridization between *U. minor* and *U. pumila*, with preferential backcrossing to the latter, hybrids are genetically closer to *U. pumila* (Cogolludo-Agustín et al. 2000). Hybrids between *U. glabra* and *U. pumila* have been reported only in laboratory experiments with controlled pollinations (Townsend 1971; Mitterpergher and La Porta 1991).

In the case of *U. minor* × *U. glabra* (*U.* ×*hollandica* Miller), differences are not always clearly observed between parental species and hybrids, which form the × *hollandica* complex. A continuum gradient of similarities in morphology can be observed from *U. minor* towards *U. glabra*, including hybrids, as a result of the true genetic continuity (Ipinza 1990; Machon et al. 1997).

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Table 1. *Ulmus* trees sampled.

U. glabra		*U. laevis*		*U. minor*		*U. pumila*		Hybrids	
Sample	Code	Sample	Code	Sample	Code	Sample	Code	Sample	Code
AV-CA5[a]	3870	GU-CO1[b]	3866	AL-PV1[c]	UPM016	CA-AL1	201	F-BR1[e]	CEM185
AV-CA12[a]	3863	GU-CO2[b]	3867	GR-AR1[c]	UPM061	CA-AL2	202	F-SA1[e]	CEM168
AV-CA13[a]	3864	GU-CO3[b]	3868	MA-PD2[c]	UPM130	CA-AL3	203	F-VN6[e]	CEM103
AV-CA17[a]	3865	M-SL1[b]	3871	P-BL1[c]	UPM138	CA-AL4	204	M-ES3[e]	UPM098
AV-IR2[a]	3872	F-DS4	CEM131	SE-CT2[c]	UPM152	CA-AL5	205	P-VV2[e]	UPM141
AV-IR3[a]	3874	F-HS2	CEM212	CS-CL2[d]	UPM050	CA-AL6	206	M-MT1[f]	UPM103
AV-IR14[a]	3875	LE-BL1	UPM075	M-PH2[d]	UPM107	CA-AL7	207	M-MT2[f]	UPM104
AV-IR15[a]	3873	LE-BL1.4	UPM075	TO-PB1[d]	UPM171	CA-AL9	209	VA-VV1[f]	UPM258
M-RO10[a]	3869	M-QM2	UPM112	TO-PB2[d]	UPM172	CA-AL10	210	VA-VV8[f]	UPM264
C-EU1	na	—	—	ZA-TR3[d]	UPM211	—	—	VA-VV22[f]	UPM277
F-SM4	CEM262	—	—	—	—	—	—	—	—

Note: The codes refer to a European code, except where indicated otherwise. na, European code not available.
[a]Wild collected material.
[b]Cultivated material from other sources, where codes shown are the accession numbers for the voucher specimens retained at the Herbarium of ETSIM, Madrid, Spain (code EMMA).
[c]*Ulmus minor* var. *minor*.
[d]*Ulmus minor* var. *vulgaris*.
[e]*Ulmus minor* × *U. glabra*.
[f]*Ulmus minor* × *U. pumila*.

Different kinds of secondary metabolites are used in the chemotaxonomy of plants, mainly flavonoids, terpenoids, alkanes, and fatty acids. Heimler et al. (1993) successfully used leaf flavonoid glycosides to differentiate between hybrids of different European and Asiatic species. Using several sesquiterpenes in the heartwood of elms, Rowe et al. (1972) only succeeded in differentiating between the section *Madocarpus* Dum. (current section *Ulmus* (Wiegrefe et al. 1994) including *U. minor* and *U. pumila*) and the other four sections within the genus. Triterpenes and related compounds have not been used frequently for chemotaxonomic purposes; however, they have been applied in the chemotaxonomy of several genera, some of them, such as *Rhoiptelea,* related to Ulmaceae (Jiang et al. 1998).

Dutch elm disease has been pointed out as one of the possible causes of the reduction of the existing diversity of *U. minor* (Cogolludo-Agustín et al. 2000). This species is very susceptible to *Ophiostoma novo-ulmi* and very attractive to the elm bark beetles of the genus *Scolytus* that spread the fungus by feeding on elm twigs. On the contrary, *U. glabra* and *U. laevis* are far less attractive than *U. minor* for beetle feeding (Webber and Kirby 1983; Sachetti et al. 1990; Webber 2000).

Factors responsible for such differences in preference are still unknown, but probably involve differences in elm chemicals acting in the processes of host finding and host acceptance. Several compounds from the bark of *U. americana* have been shown to have a phagostimulatory effect on the elm bark beetle *Scolytus multistriatus* Marsh. The first compounds known to have that effect were two pentacyclic triterpenes (Baker and Norris 1967). The formulas of two pentacyclic triterpenes with the same effect were later elucidated by Meyer (1975). Moreover, five other compounds have been shown to produce the same stimulatory effect: *p*-hydroxybenzaldehyde (Baker et al. 1968) followed by (+) catequin-7-β-D-xylopiranoside and (–) lupeil cerate (Doskotch and Chaterji 1970; Doskotch et al. 1973), and two dihydroxibencenes (resorcine and hydroquinone) (Meyer and Norris 1974). Two other benzaldehydes (vanillin and syringaldehyde) are among the compounds that attract *S. multistriatus* to elm bark (Meyer and Norris 1967).

Feeding deterrence was shown in compounds such as juglone (5-hydroxy-1,4 naphtoquinone) (Gilbert et al. 1967) or fraxetin and aesculetin (Norris 1977) extracted from the bark of nonhost trees. Thus, it appears that bark, the feeding substrate for the beetles, should play a major role in host preference, which makes it interesting to study the composition of twig bark in these species in relation to their suitability to the beetle vectors of Dutch elm disease.

In this paper, we report data on triterpene composition from the three Spanish elm species (*U. glabra*, *U. laevis*, and *U. minor*) and from the introduced *U. pumila*, as well as from some of their hybrids. These data are discussed in relation to both elm taxonomy and the attractivity of the elm species to the elm bark beetles.

Materials and methods

Plant material

Trees were sampled from different locations in Spain and from the Dirección General para la Conservación de la Naturaleza – Escuela Técnica Superior de Ingenieros de Montes (DGCN – ETSIM) clone collection at the Forest Tree Breeding Center in Madrid, Spain, between the end of May and the beginning of June 2003. In the clone collection, elm clones and seedlings, 5 to 12 years old, from all over the Iberian Peninsula as well as several exotic elm species are represented. Species sampled were *U. glabra, U. minor*, *U. laevis, U. pumila*, and hybrids of *U. minor* × *U. glabra* as well as *U. minor* × *U. pumila* (Table 1). *Ulmus pumila* samples were grown from cuttings of Chinese origin. All *U. minor* and *U. minor* × *U. pumila* were previously identified by using isozyme markers (Cogolludo-Agustín et al. 2000).

Wild specimens of *U. glabra* were sampled at three different locations in the Central Mountain Range of Spain: Casillas

(samples AV-CA; UTM coordinate 30TUK663651 and 30TUK665248), Rozas de Puerto Real (M-RO; 30TUK737643), and Valle de Iruelas (AV-IR; 30TUK666699, 30TUK671725, and 30TUK671722). Samples of three *U. laevis* trees were also collected from nonpermanent cultivated material in Cogolludo (GU-CO; 30VL926331). Voucher specimens for the elm trees sampled outside the clone collection were deposited in the herbarium at Escuela Técnica Superior de Ingenieros de Montes (ETSIM), Universidad Politécnica de Madrid. From each of the selected trees, 2- to 4-year-old twigs were sampled. Twigs were obtained from three different orientations in the tree crown to homogenize the samples and reduce positional effects. A total of 49 trees were sampled (39 belonging to pure species and 10 to hybrids). Sample sizes were similar to or greater than those used by Heimler et al. (1993) in the discriminant analysis of elm flavonoids.

Preparation of extracts

Samples were brought into the laboratory on the same day of collection; the bark was stripped off the twigs and ground into 3–5 mm pieces. A known mass (15 g) was extracted for 48 h in the dark at room temperature in 75 mL of petroleum ether – diethyl ether (1:1). The extract was then decanted, treated with anhydrous sodium sulfate, and filtered. The solvent was removed from the extract in a nitrogen stream. For the analyses, 10 mg of the dried extract was redissolved in 1 mL of 0.05% triacontanoic acid methyl ester (internal standard) in diethyl ether.

Chromatographic analysis

Identification and evaluation of extracted compounds were achieved by gas chromatography – mass spectrometry (GC–MS) using an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, California, USA) connected to an Agilent 5973N mass detector (electron ionization, 70 eV) (Agilent Technologies, Palo Alto, California, USA) and equipped with a 30 m × 0.25 mm i.d. DB-5MS capillary column (0.25 μm film thickness) (J&W Scientific, Folsom, California, USA). The working conditions were as follows: split (1:20); injector temperature, 250 °C; temperature of the transfer line to the mass spectrometer, 300 °C; column temperature, 60 °C during the split period (2 min), heated to 200 °C at 5 °C/min, heated from 200 to 300 °C at 10 °C/min, and then at 300 °C for 15 min. Electron ionization, mass spectra, and retention times were used to assess the identity of compounds, by comparing them with those in the database (Wiley 275 Mass Spectra Database 2001). Four of the considered compounds were identified by comparison with standards: friedelin, stigmasterol, β-sitosterol, and lupeol (Sigma, Germany).

Quantitative measurements were carried out using triacontanoic acid methyl ester (Sigma, Germany) as the internal standard, which was shown not to interfere with any other compound in the chromatogram. All the data presented refer to the mass of ovendried bark (24 h at 110 °C).

Statistical analysis

Discriminant analysis was the main procedure used. With this method, samples, which each formed an object considered as a data vector of 13 variables represented by the chemical data, were assigned a priori to one of the predefined categories: species and hybrids. A series of discriminant functions were obtained and used to discriminate among samples in each of the categories. The software used was STATGRAPHICS Plus for Windows® (Statistical Graphics Corporation, Rockville, Maryland, USA).

Results

Three sterols and 10 triterpenes were identified in the petroleum ether - diethyl ether bark extracts from different elm species and hybrids The three sterols were stigmasterol (3β-hydroxy-24-ethyl-5,22-cholestadiene), β-sitosterol (24β-ethylcholesterol), and stigmastenone (stigmast-4-en-3-one). The 10 triterpenes were alnulin (D-friedoolean-14-en-3β-ol), β-amyrin (olean-12-en-3β-ol), lupenone (lup-20(29)-en-3-one), lupeol (lup-20(29)-en-3β-ol), moretenol (hop-22(29)-en-3-β-ol), ilexol (D:C-friedours-7-en-3β-ol), epifriedelinol (D:A-friedooleanan-3β-ol), friedelin (D:A-friedooleanan-3-one), methyl betulinate (lup-20(29)-en-3β-hydroxy-28-oic acid, methyl ester), and betulin (lup-20(29)-ene-3β-28-diol). Alnulin, lupeol, and ilexol are new for the family Ulmaceae, and moretenol and betulin are new for the genus *Ulmus*. Table 2 shows the quantitative data of the chromatographic analysis for each taxon (species and hybrids), in micrograms of each triterpene per gram of ovendried bark.

Pure elm species

None of the bark triterpenes identified were exclusive for any elm species. In fact, the majority of the bark triterpenes were common to the four species. Alnulin was the most specific compound, since it was present only in the extracts of *U. glabra* and *U. laevis*. The average concentration of alnulin differed significantly from 86.66 μg/g in *U. glabra* to 199.63 μg/g in *U. laevis*. All the other compounds were identified in at least three of the four species, even though moretenol was detected in *U. laevis* in only one sample. *Ulmus glabra* showed the highest total content of triterpenes (2973.94 μg/g), more than half of it being friedelin (1643.29 μg/g). *Ulmus laevis* showed the second highest total content of triterpenes (1447.86 μg/g), but in this case the most abundant compound was lupeol (879.92 μg/g). *Ulmus pumila* showed the third highest total content of triterpenes and the highest average amount of epifriedelinol (543.32 μg/g). *Ulmus minor* showed the lowest average content of triterpenes (437.66 μg/g), with lupeol as the most abundant triterpene (232.38 μg/g).

The major quantitative differences found in the triterpene composition of the species may allow for the specific discrimination of the samples.

All 13 compounds were used as variables for the discriminant analysis, since the use of this number of variables offered the best results. Three discriminant functions with *P*-values less than 0.01 were obtained in the model. The coefficients for the two first functions are shown in Table 3. The extreme values of the coefficients for friedelin, lupeol, and ilexol indicate that contents of these compounds were the main features of the first discriminant function; similarly, stigmastenone and β-amyrin were dominant in the second discriminant function.

An uneven separation was obtained for the different species (Fig. 1). While *U. pumila* samples were clearly separated from the European elms, *U. minor* samples were located

Table 2. Means and standard deviations (in parentheses) of triterpene and sterol contents (μg/g of ovendried bark) from pure species and hybrids.

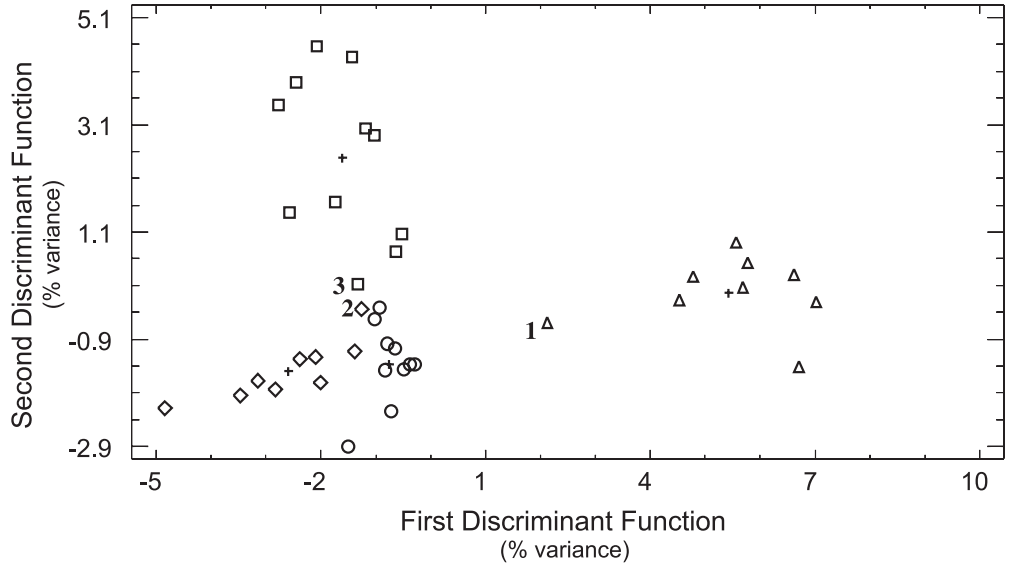
Triterpene or sterol	*U. glabra* (*N* = 11)	*U. laevis* (*N* = 9)	*U. minor* (*N* = 10)	*U. pumila* (*N* = 9)	*U. minor* × *U. glabra* (*N* = 5)	*U. minor* × *U. pumila* (*N* = 5)
Stigmasterol	8.18	3.40	2.60	6.79	2.53	7.59
	(8.69)	(3.46)	(1.47)	(3.04)	(1.28)	(3.80)
β-Sitosterol	101.45	122.39	86.98	73.58	59.23	99.71
	(25.47)	(29.18)	(28.72)	(22.61)	(18.26)	(29.09)
Alnulin	86.66	199.63	0	0	0	0
	(101.24)	(129.32)	—	—	—	—
β-Amyrin	92.68	40.07	9.25	1.20	12.88	23.15
	(54.31)	(19.46)	(7.94)	(2.40)	(17.24)	(32.71)
Lupenone	33.89	16.54	38.96	0	20.59	3.80
	(35.37)	(13.58)	(52.21)	—	(33.49)	(4.61)
Lupeol	592.91	879.92	232.38	20.89	86.51	224.35
	(796.99)	(321.68)	(122.59)	(19.52)	(116.13)	(189.60)
Stigmastenone	0	4.10	2.24	1.85	3.24	0
	—	(2.15)	(2.08)	(2.95)	(4.54)	—
Moretenol	30.53	0.51	41.04	0	70.08	97.63
	(80.65)	(1.53)	(62.40)	—	(128.34)	(117.95)
Ilexol	17.53	1.48	0	21.90	0	17.32
	(14.52)	(4.44)	—	(9.13)	—	(17.54)
Epifriedelinol	294.70	36.65	0	543.32	0.72	8.61
	(193.57)	(20.06)	—	(479.98)	(1.61)	(11.79)
Friedelin	1643.29	96.96	1.11	137.14	0.56	8.28
	(1222.18)	(72.25)	(2.56)	(149.13)	(1.26)	(11.40)
Methyl betulinate	44.14	0	10.82	12.95	8.82	61.96
	(39.62)	—	(10.04)	(25.73)	(12.78)	(89.17)
Betulin	27.97	46.21	12.26	4.88	7.47	13.37
	(31.65)	(13.32)	(3.71)	(7.51)	(10.81)	(15.14)
Total	2973.94	1447.86	437.66	824.52	272.64	565.76
	(1681.01)	(375.33)	(217.65)	(621.66)	(184.36)	(226.47)

Table 3. Coefficients of the first two discriminant functions used to separate the samples in the three analyses considered.

	Coefficients for discriminant functions 1 and 2					
	Elm species[a]		*U. minor*, *U. glabra*, and hybrids		*U. minor*, *U. pumila*, and hybrids	
Triterpene or sterol	1	2	1	2	1	2
Stigmasterol	–0.322	0.197	–1.514	–0.514	1.301	1.133
β-Sitosterol	0.115	–0.018	–0.262	1.515	–0.231	0.169
Alnulin	–0.433	–0.270	–1.198	–1.130	0.000	0.000
β-Amyrin	–0.438	0.480	–0.761	0.309	–0.898	–0.737
Lupenone	0.167	–0.202	0.407	–0.376	0.598	0.032
Lupeol	–1.126	–0.005	0.392	0.249	–0.793	0.125
Stigmastenone	–0.457	–0.600	0.625	–0.645	–0.833	–1.127
Moretenol	0.014	0.319	–0.418	–0.282	0.101	1.031
Ilexol	1.928	0.182	6.496	4.168	1.371	0.146
Epifriedelinol	0.990	0.389	–1.538	–1.722	0.249	–11.146
Friedelin	–2.007	0.224	–4.338	–2.283	1.098	12.184
Methyl betulinate	0.617	0.241	0.643	–0.096	1.648	0.274
Betulin	0.980	0.380	–0.131	0.009	–0.490	0.199

[a]Comparison among all of the pure elm species studied in this paper.

Fig. 1. Discriminant analysis plot of the samples belonging to *U. minor*, ○; *U. glabra*, □; *U. pumila*, Δ; *U. laevis*, ◇; and group centroids, +. Numbered individuals are samples incorrectly classified by the discriminant model as *U. minor*: CA-AL1 (*U. pumila*), 1; F-DS4 (*U. laevis*), 2; and C-EU1 (*U. glabra*), 3.



very close to *U. laevis* and *U. glabra* samples. However, none of the areas of these three species overlapped in the graph. Only three samples, one of *U. glabra*, one of *U. laevis*, and one of *U. pumila* (numbered individuals in Fig. 1), were incorrectly classified as *U. minor* by the model.

Elm hybrids

None of the bark triterpenes identified were exclusive to any of the hybrids (Table 2). The average total triterpene content was lower in *U. minor* × *U. glabra* samples when compared with their parental species (272.64 versus 437.66 μg/g in parental *U. minor* and 2973.94 μg/g in parental *U. glabra*). Some of the characteristic triterpenes of the *U. glabra* samples were either absent in the hybrids, such as alnulin or ilexol, or present in minute amounts, such as epifriedelinol and friedelin,. These hybrids showed a chemical profile close to that of *U. minor* but with a lower concentration in each of the triterpenes, except for moretenol and stigmastenone. In the *U. minor* × *U. pumila* hybrids, the average total triterpene content was intermediate between the profiles of the parental species.

Two independent discriminant analyses were carried out with each type of hybrid and the corresponding parental species. The results for *U. minor* × *U. glabra* samples followed the above-mentioned pattern. Two of the five hybrids occurred within the cluster of *U. minor* samples; the other three hybrids were separated from *U. minor* but were much closer to this species than to *U. glabra* (Fig. 2*a*). Relative weights of the 13 variables in the model showed that contents of ilexol and friedelin are the dominant features for both discriminant functions (Table 3).

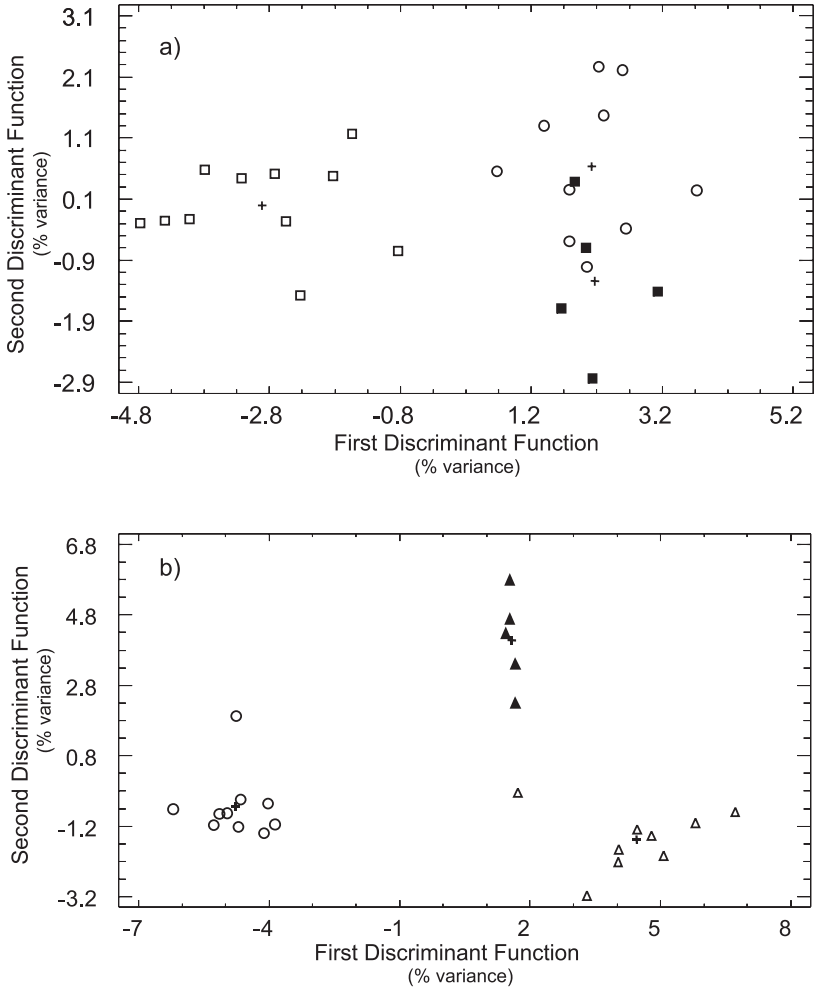
Individuals of *U. minor* × *U. pumila* were clearly segregated from the parental species, with a 100% rate of recognition for the hybrids (Fig. 2*b*). Nonetheless, hybrids appeared to be more closely related to *U. pumila* than to *U. minor*. This is a consequence of the relative weights of each variable in the discriminant model. The contents of methyl betulinate and ilexol were the principal features in the first function, while friedelin and epifriedelinol dominated in the second function (Table 3). Thus, the closer relationship of the hybrids to *U. pumila* than to *U. minor* may be explained by the fact that the bark of the hybrids contained friedelin and epifriedelinol, which were found in the former but not in the latter, except for a small amount of friedelin in one sample.

Discussion

The analysis of the triterpene content in extracts from elm bark showed significant differences between the groups considered, which allowed for the separation of the four elm species analyzed (*U. glabra*, *U. laevis*, *U. minor*, and *U. pumila*). Our results showed that intraspecific variations were highest in *U. pumila* and lowest in *U. minor*, while intraspecific variations in the other two species had a similar magnitude. *Ulmus pumila* is naturally distributed over a large area and this could explain the high variation found. According to the discriminant model, the *U. pumila* triterpene profile differed most from the other three species. European elms, *U. glabra*, *U. laevis*, and *U. minor*, had higher concentrations of lupeol than *U. pumila*, which was characterized by high concentrations of ilexol and epifriedelinol. Although more research is needed, triterpenes and sterols could be used as biochemical markers to distinguish among the four elm species analyzed in this study.

The majority of *U. laevis* trees were sampled in a clone collection, subjected to the same climatic and soil influences, and had similar ages as those of *U. minor* and *U. pumila*. Thus, differences found in the total amount of triterpenes could most likely be explained on a genetic basis. This might not be the case for *U. glabra*, since the majority of samples were taken from older trees in sites that are colder and have more annual rainfall than those in the clone collection.

Fig. 2. Discriminant analysis plot of the samples belonging to (*a*) *U. minor*, ○; *U. glabra*, □; and *U. minor* × *U. glabra,* ■; and (*b*) *U. minor*, ○; *U. pumila*, Δ; *U. minor* × *U. pumila*, ▲; and group centroids, +.



Hybrids of *U. minor* × *U. glabra* were found to have chemical profiles closer to that of *U. minor* than of *U. glabra*, which agrees with the genetic distances found between them using isozyme analysis (Machon et al. 1995). A differentiation between these hybrids and *U. minor* was not possible with the discriminant model applied, but the samples from *U. minor* and from the *U.* ×*hollandica* complex were clearly segregated from the samples of *U. glabra*.

When samples from *U. minor* × *U. pumila* and their parental species were analyzed, the discrimination obtained was much better and the grouping was correct in 100% of the cases. Hybrid trees showed concentrations of the majority of compounds that were intermediate between those of the parental species and occurred in a distinct intermediate position between the parental species, though closer to the *U. pumila* (Fig. 2*b*). This result is in agreement with the fact that these hybrids are also genetically closer to *U. pumila* (Cogolludo-Agustín et al. 2000). Since the most successful hybridizing direction between *U. minor* and *U. pumila* occurs when *U. pumila* is the female parent (asymmetric hybridization) (Mittempergher and La Porta 1991), our results suggest an important maternal effect in the transmission of the hereditary factor linked to the triterpene production.

The discrimination obtained did not separate species according to their degree of attractivity to elm bark beetles: *U. minor* and *U. pumila* on one side, and *U. glabra* and *U. laevis* on the other. However, our data may indicate an inverse relationship between total triterpene content in the bark of elms and suitability to elm bark beetles. Trees of the least attractive species, *U. laevis* and *U. glabra* (Webber and Kirby 1983; Sachetti et al. 1990; Webber 2000), showed total triterpene content between two and six times higher than in trees of *U. minor* and *U. pumila*, which are readily accepted for twig feeding by *Scolytus* beetles. Compounds identified in this study are hydrophobic and occur generally in the epicuticular layer of plants (Baker 1982; Nordby and McDonald 1994; Christie 2003), so they may be perceived by the elm bark beetles through short-range olfaction and gustation.

Some of the compounds found in this study have the same molecular formulas of $C_{30}H_{52}O$ and $C_{30}H_{50}O$ characteristic of the triterpenes isolated by Baker and Norris (1967) and Meyer (1975) from *U. americana*, found to be feeding stimulants for European bark beetles. This coincidence could explain a possible relation of the triterpenes presented here and the feeding behavior of elm bark beetles.

Alnulin, which was exclusively present in the least preferred species *U. laevis* and *U. glabra* (Table 2), may be involved in deterring *Scolytus* from feeding on the twigs of these species (antifeedant activity). Also, β-amyrin, which shows high concentrations in these species, would be a good candidate for study. The absence or low concentrations of these compounds in *U. minor* and *U. pumila* might account for the preference as an acceptable feeding substrate that the beetles have for these tree species.

It can be concluded that the chemical variability of triterpenes in the diethyl ether-petroleum ether extracts from elm bark is of interest in the taxonomy of the genus *Ulmus*. It also may be related to the differential suitability for beetle feeding observed among several elm species. The compounds analyzed here belong to the nonpolar fraction of the bark extracts. Information on the characteristics of the compounds present in the polar fraction of elm bark could complete the results presented.

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