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Original article

Proanthocyanic polymorphism in holm oak (*Quercus ilex* L) in the Mediterranean region of France

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Summary — We studied the proanthocyanic diversity of holm oak in France. The percentage of prodelphinidin remains constant for each individual tree, independent of age or location of the leaves. By contrast, this content can be significantly different between trees within the same population. An in-depth analysis of a Languedocian population showed a good relationship between observed and predicted sample structure, according to the hypothesis of 2 alleles, prodelphinidin 'weak' and 'strong', respectively, governing the biosynthesis of this polyphenol. We have probably observed a biochemical polymorphism comparable to that previously demonstrated for several coniferous species.

holm oak / prodelphinidin / polymorphism

Résumé — Polymorphisme proanthocyanique du chêne vert *Quercus ilex* L dans la région méditerranéenne française. La diversité proanthocyanique d'une population languedocienne de chêne vert *Quercus ilex* a été étudiée. La teneur foliaire relative en prodelphinidine est constante pour un individu, indépendamment de son âge et de la situation des feuilles; elle peut par contre différer significativement entre arbres de la même population. Une analyse détaillée montre une bonne corrélation entre effectifs observés, et ceux calculés conformément à l'hypothèse de l'existence de 2 allèles, respectivement «faible» et «fort», gouvernant la synthèse de la prodelphinidine. Il s'agit donc probablement ici de polymorphisme biochimique, analogue à celui déjà mis en évidence chez plusieurs conifères à l'aide du même marqueur phénolique.

chêne vert / prodelphinidine / polymorphisme

INTRODUCTION

The holm oak *Quercus ilex* L is the most prevalent and characteristic preforest and forest species in the western Mediterranean basin. The species has a large geographical range from Morocco (where it is very widespread) to Turkey (where its presence is more fragmentary). The holm oak in France has penetrated northwards to above the 45th parallel, to the Vendée and the southern extremity of the Dombes (Ain).

Its ecological adaptability is also noteworthy, as the species can be found from the edge of the sea, on the northern side of the Mediterranean, up to an altitude of 2 500–2 600 m in the Atlas Mountains (Barbero *et al*, 1992). It grows in temperate to very cold Mediterranean bioclimates between the semi-arid and the damp, one. The species grows equally well on limestone or siliceous soils, though this adaptability cannot be clearly linked to any ecophysiological or morphological characteristics of the populations concerned.

Q. ilex participates in the structure of numerous potential vegetational series represented by pre-steppe or forest structures. By contrast, the numerous preforest landscapes composed of holm oak are an expression of the anthropogenic constraints of the agro-sylvo-pasture systems in which it is involved. Moreover, this species possesses considerable morphological variability as regards its leaves, to such an extent that there are occasionally more differences within a given individual than between close neighbors or even between distinct populations.

Such variability has an obvious impact on the systematics of holm oak, considered by some authors as a specific large taxon and by others as being organized into several entities, the 2 most important being *Quercus rotundifolia* in the western

part of the area, and *Quercus ilex* ss in the eastern part, between Italy and Turkey.

Because of the above ecological and biological particularities of holm oak, we considered it of interest to investigate to what extent proanthocyanic 'marking' – the systematic use of which has thrown light on several conifers – was able to help clarify such a complex situation, through a more objective expression of the biological diversity of this species. The data presented here are taken from Nader (1990).

MATERIALS AND METHODS

Sampling strategy

Forty-four specimens were taken from 1 population at Valliguières (Gard) in the French Mediterranean zone of holm oak. The sampling strategy was based on spatial and temporal parameters.

Spatial parameters

Bioclimatic parameters were evaluated indirectly by analyzing the significant floristic complex growing alongside the holm oak. The Valliguières population is located at the top of the meso Mediterranean level and can even reach the base of the upper Mediterranean; *Buxus sempervirens* and *Coronilla emerus* are common here. Edaphic parameters revealed compact and dolomitic limestone. Analyses were performed on samples from neighboring individuals (A, B and C); the samples were taken at different heights of the same tree or taken in isolation in relatively clear or densely wooded areas. The effects of the organization and spatial-temporal evolution of populations on the biochemical structure of individuals is described elsewhere (Barbero *et al*, 1991).

Temporal parameters

The hypothesis that the biochemical structure of adult leaves varies with the season was also tested by taking samples from 3 individuals: A,

B and C, during different seasons from the summer of 1985 to the autumn of 1986 inclusive. In addition, young and old leaves were compared in 16 individuals, to evaluate any differences in proanthocyanic composition.

Biochemical aspects

The leaves of holm oak always contain the 2 proanthocyanidins: procyanidin and prodelphinidin, but in variable absolute (mg/g) and relative (%) quantities depending on the specimen (the second molecule differs from the first only by the presence of a supplementary vicinal hydroxyl group on the lateral phenyl ring. The analytical technique, based on treatment with hot hydrochloric acid followed by visible photometry then high-performance liquid chromatography, is described in detail elsewhere (Nader, 1990)). Studied separately at other French sites, this variability was shown to be comparable in all cases, and therefore characterizes the *Q. ilex* species considered globally in the biogeographic zone concerned. In the Valligüères population, mean values were 3.88 mg/g ($n = 41$ individuals; standard deviation 0.48 mg/g) for the proanthocyanic pool, and 39% (standard deviation 14%) for relative prodelphinidin content; extreme values were 2.30 and 4.25 mg/g, 20 and 68%, respectively.

The in-depth investigation of 3 individuals, A, B and C, showed that proanthocyanic composition was quite independent of the height at which leaf samples were taken in the tree (table

I). The difference noted between high and low leaves for a given individual was virtually zero, less than the deviation of the biochemical assay technique (A: $+0.04 \pm 0.23$ mg/g; B: $+0.08 \pm 0.20$ mg/g; C: $+0.01 \pm 0.20$ mg/g).

Total levels of proanthocyanins in adult leaves (> 5 months) from these same individuals were also found to be practically constant ($< \pm 10\%$), as shown by the mean values of 11 samples taken between 5 August, 1985 and 24 December, 1986 (A: 3.30 ± 0.32 mg/g; B: 3.22 ± 0.27 mg/g; C: 3.40 ± 0.21 mg/g). The results were even more demonstrative as regards relative prodelphinidin content (A: $41 \pm 2\%$; B: $52 \pm 2\%$; C: $20 \pm 1\%$).

However, when data were grouped for each season, absolute values were seen to be slightly lower in winter (table II, fig 1). In addition, more pronounced differences were noted between young (< 4 months) and adult leaves from the same individual. The former were shown to contain markedly lower levels of total proanthocyanin and a little more relative prodelphinidin, resulting from active biosynthesis during foliar ontogenesis (table III, fig 2).

In conclusion, the proanthocyanic composition of holm oak leaves is an individual characteristic which is dependent, to a secondary degree, on seasonal ontogenesis. Nevertheless, the relative level of prodelphinidin (LD %) appears to be a particularly reliable and sensitive marker in adult leaves, where it has been shown to be independent of the morphological height and the date the sample was taken. It is therefore quite possible to describe each individual

Table I. The influence of the leaf location in the tree on the proanthocyanic composition of 3 syntopic individuals of holm oak (Valligüères, adult leaves, 1985–1986) (mean values; standard deviation in parentheses).

Adult leaves Valligüères 1985–1986	Tree's level: high		Tree's level: low	
	LA (mg/g)	LD%	LA (mg/g)	LD%
Individual A ($n = 9$)	3.24 (0.33)	40 (3)	3.19 (0.31)	41 (1)
Individual B ($n = 7$)	3.11 (0.21)	52 (2)	3.03 (0.21)	50 (1)
Individual C ($n = 6$)	3.32 (0.17)	20 (1)	3.31 (0.19)	20 (1)

LA: proanthocyanidins; LD%: relative level of prodelphinidin.

Table II. Seasonal variations in the proanthocyanic composition of 3 synthopic individuals of holm oak (Valliguières, adult leaves, 1985–1986) (mean values).

Season	Proanthocyanidins (mg/g)			Prodelphinidin (%)		
	A	B	C	A	B	C
Spring 1985	3.44	3.30	3.57	41	49	21
Autumn 1985	3.22	3.06	3.36	41	53	21
Winter 1985–1986	2.87	2.99	3.18	41	52	20
Spring 1986	3.22	3.05	3.24	41	52	20
Summer 1986	3.54	3.46	3.59	41	53	21
Variation coefficient	± 10%	7%	6%	≤ 1%	4%	3%

by a small number, even a single biochemical analysis, independent of the weight and dryness of the sample.

POPULATION ANALYSIS

Values for the LD% marker in the 3 individuals A, B and C, at Valliguières (see table II) enable us to differentiate between the 3 individuals without any ambiguity, and are probably the expression of biochemical

polymorphism. The study of the 38 other individuals in the same population has confirmed the diagnosis and yielded further details. It would seem permissible to subdivide the population into 3 subsets: I, II and III, with limits of < 31, 31–46, and > 47% prodelphinidin content. The frequency histogram (fig 3) and the proanthocyanic plane LD/LC (mg/g) (fig 4) illustrate this point. The number of individuals within the limits of each subset is 10, 19 and 12 (24, 46 and 29% of the total, respectively).

Table III. The influence of age on the proanthocyanic composition of holm oak (Valliguières, 1986, young and adult leaves) (mean values; standard deviations in parentheses).

Sample	Proanthocyanidins (mg/g)		Prodelphinidin (%)	
	Young leaves	Adult leaves	Young leaves	Adult leaves
1) Individual A	0.72 – 3.40	3.51 (0.34)	46 (1)	43 (2)
Individual C	0.77 – 3.32	3.52 (0.19)	29 (4)	20 (2)
2) 16 individuals				
Young leaves	1.71 (0.36)		40 (10)	
Adult leaves	3.56 (0.46)		41 (11)	
Difference	$t = 12.3$ $p \leq 0.001$		NS	

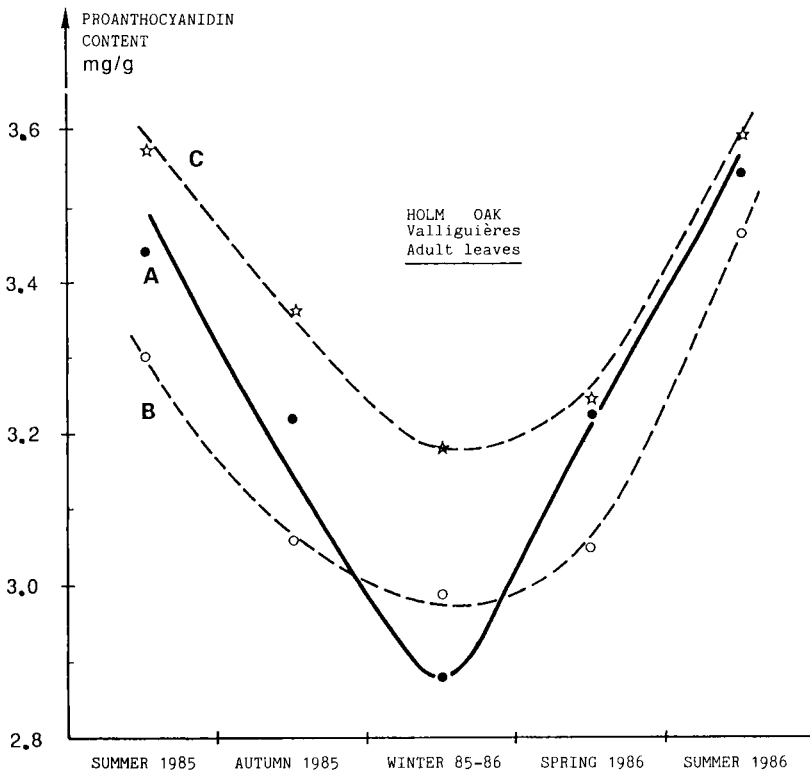


Fig 1. Seasonal evolution of the proanthocyanidin content of adult leaves in 3 holm oak individuals (Valliguières, 1985–1986).

Proanthocyanic polymorphism of the holm oak therefore appears to be structured, and we can put forward the hypothesis of a genetic model in conformity with that demonstrated for the same prodelphinidin marker in *Juniperus thurifera* (Gauquelin *et al*, 1988) and in *J oxycedrus* (Lebreton *et al*, 1991). The results suggest that the gene(s) governing the synthesis of this molecule is present as 2 alleles, prodelphinidin 'weak' d (< 31%) and 'strong' D (> 47%), explaining the appearance – in the panmictic hypothesis – of 3 pheno-

types dd, dD and DD whose numbers should conform with the well known Hardy–Weinberg binomial formula.

The conformity between observed and calculated sample structures (table IV) seems sufficiently good to accept the implicit genetic model. The allelic frequency obtained ($p(d) = 0.48$) suggests a process of hybridization, virtually equilibrated in this population. It should be noted that this biochemical polymorphism cannot be directly linked to the morphological 'polymorphism' of the leaves of the same individuals.

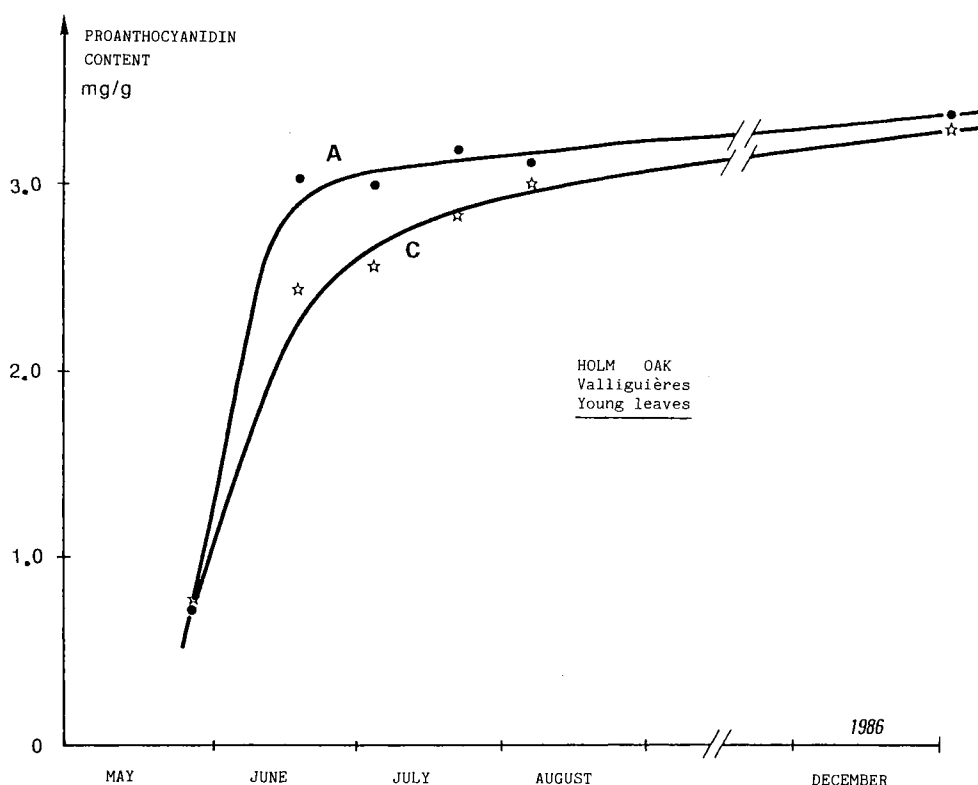


Fig 2. Seasonal evolution of the proanthocyanic content of young leaves of 2 holm oak individuals (Valliguières, 1986).

Table IV. Conformity ($\chi^2 = 0.05$, corrected for continuity) between phenotypic sample size (relative prodelphinidin content), both calculated ($p(d) = 0.476$) and observed (Valliguières, June 1987, 41 individuals, adult leaves).

Number of individuals	Phenotype subsets		
	dd	dD	DD
Theoretical	9.3	20.4	11.3
Experimental	10	19	12
Difference	$p(d) = 0.48$		$\chi^2 = 0.05$

GENERAL DISCUSSION

The ubiquitous nature and variability of the holm oak are such that considerable research has been performed in the hope of establishing a realistic and convenient taxonomy. The populations in France are even more interesting in that, for biogeographers and ecologists, they are derived from the hybridization of the 2 eastern (*Q ilex ss*) and western (*Q rotundifolia*) taxa, which only accentuates the biological and systematic complexity of the question.

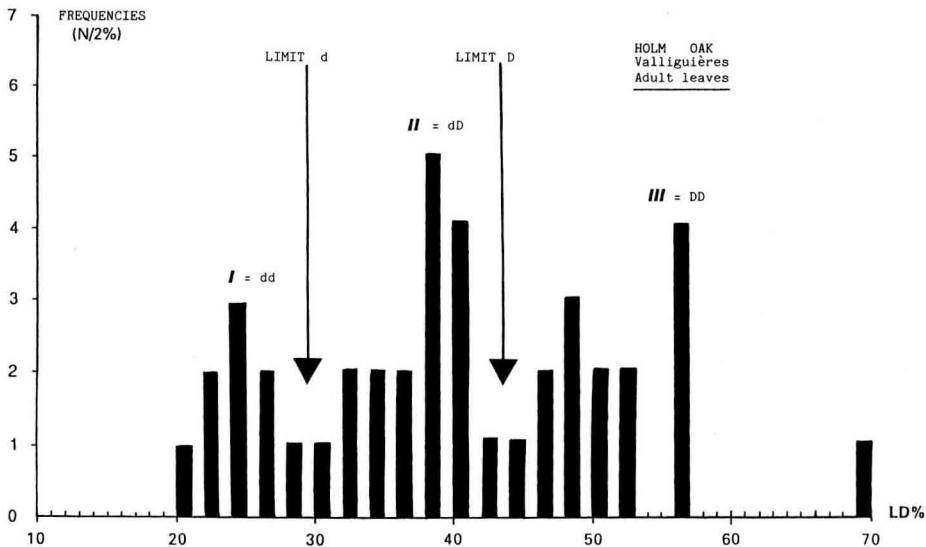


Fig 3. Distribution of 41 syntopic individuals of holm oak into 3 subsets based on the relative prodelphinidin content (Valliguières, 1987, adult leaves).

Madjidieh (1982) used morphological data (including foliar surface indices), collected from the whole western Mediterranean area, to establish that the populations of holm oak present notable differences, though no abrupt discontinuities are apparent. The south of France is the site of extreme types: *Q ilex* and *Q rotundifolia*, but with a continuum of intermediary types. *Q ilex* ss is more frequently found on siliceous soils (north-facing slopes, deep soil), whereas *Q rotundifolia* type seems to be dominant on limestone substrates (zones of summer hydric stress) (Madjidieh, 1987).

From a biochemical point of view, Yacine (1987) used alloenzyme frequencies to show the differentiation (unsuspected by biometrics) between Italian and Spanish populations. Similarly, Afzal-Rafii (1988) used electrophoretic analysis of isoenzymes (13 peroxidase bands demonstrat-

ed) to show a possible distinction between Spanish and Italian populations; the latter appear to be more homogeneous from a foliar biometrics point of view. The lipid composition of the acorns has also been envisaged as an aid in the systematics of holm oak (Pelleau, 1984; Rafii *et al*, 1991).

Already demonstrated by Touati (1985), the variability of holm oak polyphenols completes the picture, especially since it seems to be the expression of genetically determined polymorphism. Moreover, this variability can be brought to light using very simple methods of sampling and biochemical analysis, which could, therefore, be applied in a large ecogeographic sweep. As with other lignous species whose prolonged period of immaturity renders classical genetic experiments very difficult, early biochemical diagnosis can be considered a particularly realistic alternative.

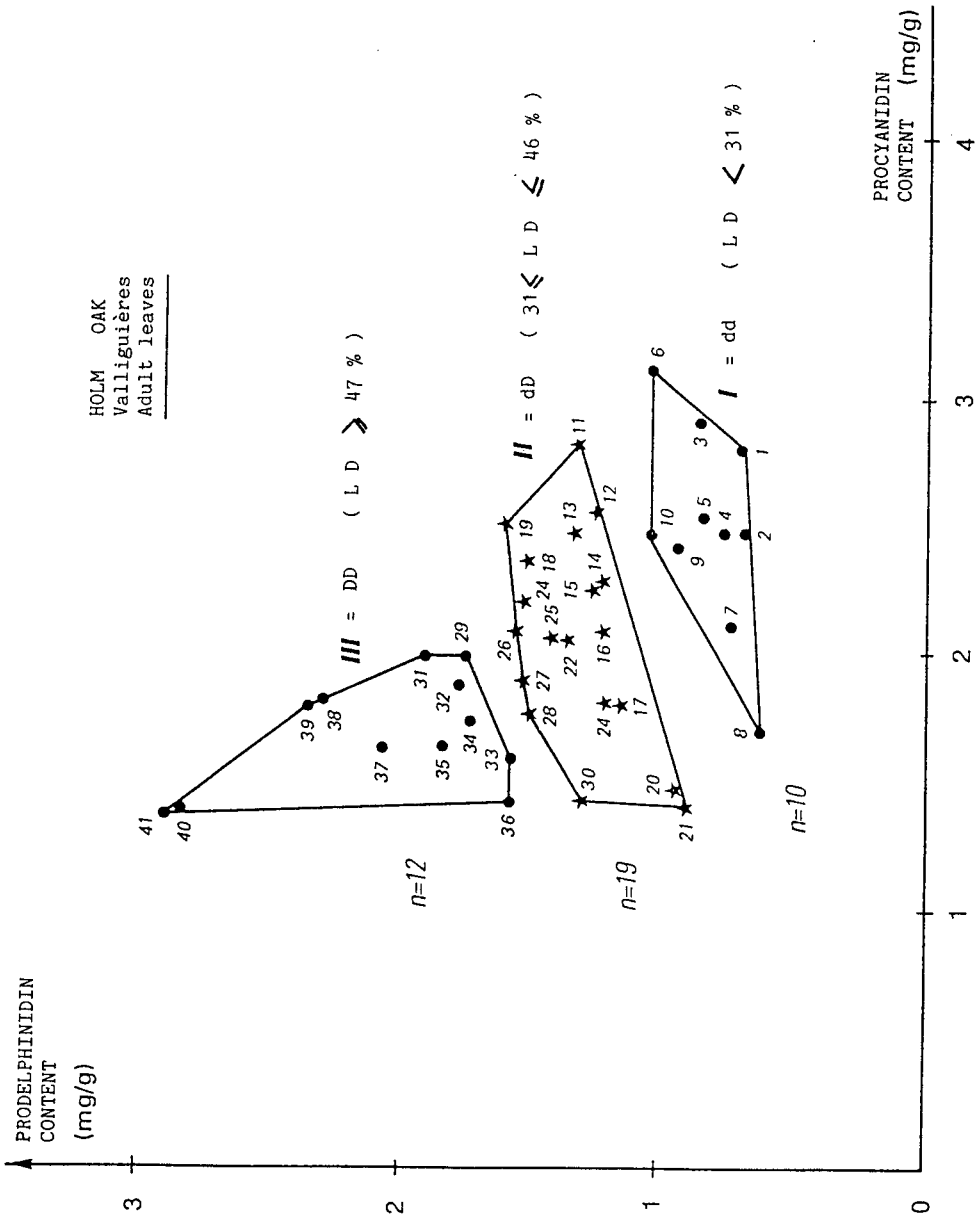


Fig 4. Distribution of 41 syntopic individuals of holm oak into 3 subsets based on absolute prodelphinidin and procyanidin contents (Valliguères, 1987, adult leaves).

Our results, however, are currently limited to French sites, and therefore possess a preliminary character that should prelude further research in an attempt to: 1) test the biochemical and genetic model proposed here on other populations; 2) test the usefulness of the prodelphinidin marker (very effective in conifers) in biogeographical studies throughout the whole area of the species considered; 3) search for correlations between other biochemical and/or morphological characteristics, in order to establish a synthetic expression describing the general polymorphism of this taxon; 4) describe in systematic terms the reality of this polymorphism in order to define reliable taxonomic entities which could be used for biogeographical and phytoecological purposes and, in general, contribute to the establishment of a modern biosystematic of oaks.

Again, very generally, these approaches should contribute to a reduction in the difficulties met in characterizing polymorphic species within the entire *Quercus* genus.

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