Acta Bot. Neerl. 22(6), December 1973, p. 655–665.

A SCANNING ELECTRON MICROSCOPICAL STUDY OF THE POLLEN MORPHOLOGY IN THE GENUS QUERCUS

A. SMIT

Hugo de Vries-Laboratorium, afd. Palynologie, Amsterdam

SUMMARY

The pollen morphology of some Eurasiatic species of the genus *Quercus* was studied by means of scanning electron microscopy. Three major pollen types can be distinguished. The relation between these pollen types and the evergreen and deciduous habits is discussed, as well as their taxonomic significance.

1. INTRODUCTION

The genus *Quercus* is represented by more than 300 species widely distributed in the temperate regions of the northern hemisphere and also found at higher altitudes in the tropics. The more than 30 species native in Europe and the countries around the Mediterranean are frequently important constituents of the recent forest vegetation. Palynological and palaeobotanical studies have revealed a considerable representation of the genus *Quercus* in former vegetation. The pollen morphology of the genus has been an object of intensive study. VAN CAMPO & ELHAI (1956), MONOSZON (1961) and PLANCHAIS (1962), who all used conventional transmitted light microscopy, believed to have found criteria to distinguish the pollen grains of several species of *Quercus* from one another. Their method is based on measurements, and on differences in structure and in sculpture. The reports in question dealt with a restricted group of species to be expected as fossils in a certain region. VAN CAMPO & ELHAI (1956) and MONOSZON (1961) attempted to use the results of such studies of the pollen morphology for the identification of fossil *Quercus* pollen to the specific level. However, in the present author's opinion the prerequisite of large numbers of pollen of different *Quercus* species in a fossil sample to be studied, so as to permit an adequate statistical treatment of the variable measurements, is not usually fulfilled. Moreover, the resolving power of the light microscope is marginal at the required magnification. BEUG (1961) gave a more acceptable classification of fossil *Quercus* pollen into two major recognizable types and added a third category "*Quercus indet.*". VAN DER SPOEL-WALVIUS (1963) gave a very detailed description of recent pollen of five *Quercus* species by means of phase contrast microscopy of the surface and by studying thin sections. This author distinguished three pollen types and also indicated the taxonomic significance of her results in relation to the taxonomic treatment of SCHWARZ (1936–1939). YAMAZAKI & TAKEOKA (1959) used transmission electron micro-

scopy (T.E.M.) for the study of pollen from a number of oriental *Quercus* species. Their work revealed for the first time the presence of two different (viz., rounded and elongated) sculptural elements which are more or less superimposed on the coarser scabrate pattern. A study by DUPONT & DUPONT (1972) carried out by means of scanning electron microscopy (S.E.M.) confirmed these findings for a group of seven species occurring in France.

The present study was aimed at an extension of the pollenmorphological research by the examination of a larger group of *Quercus* species in the expectation to arrive at taxonomically useful conclusions. The study of pollen of a larger group of species is also essential for the application of the results to fossil material. It was hoped that the recognition of fossil *Quercus* species by their pollen would permit a more reliable reconstruction of vegetation types and, hence, of the palaeophytogeography of certain regions.

2. METHODS

After acetolysis according to ERDTMAN (1960) fresh pollen is suspended in alcohol or acetone. A drop of the suspension is placed on an S.E.M. stubholder, covered with a thin layer of a mixture of carbon powder and rubber cement. After drying of the pollen, a thin layer of gold is applied by evaporation in vacuo, after which the specimen is ready for observation. The vacuum coating unit used is a model E 12 E Edwards apparatus, with rotatilt. The S.E.M. used was a Cambridge Mark II stereoscan. The best results were obtained with an accelerating voltage of 10 KV.

In some cases the single-stage replica method for transmission electron microscopy of pollen grains, as described in an earlier paper (SMIT & WIJMSTRA 1970), was applied. This method by means of which magnifications of over 25.000 × can be achieved, was chiefly used as a basis of comparison for the scanning microphotographs.

3. RESULTS

The following major pollen types could be distinguished by using characteristics showing in electron microscopical observation alone:

A. *Quercus robur/petraea* type (*plate I*)

The sculpture of this type shows micro-verrucae and flatter parts of the tectum being covered with rounded spinuloid processes of 0.1–0.3 μm high and as much in diameter.

The processes on the micro-verrucae are of a more uniform size and are perhaps to be considered to represent structural units and not only sculptural details. The variability in shape and size of the processes situated directly on the tectum is conspicuously greater in the area close to the colpi. Perforations in the tectum are present, but they are obscured by the presence of verrucae. No intact colpus membranes or pores could be observed.

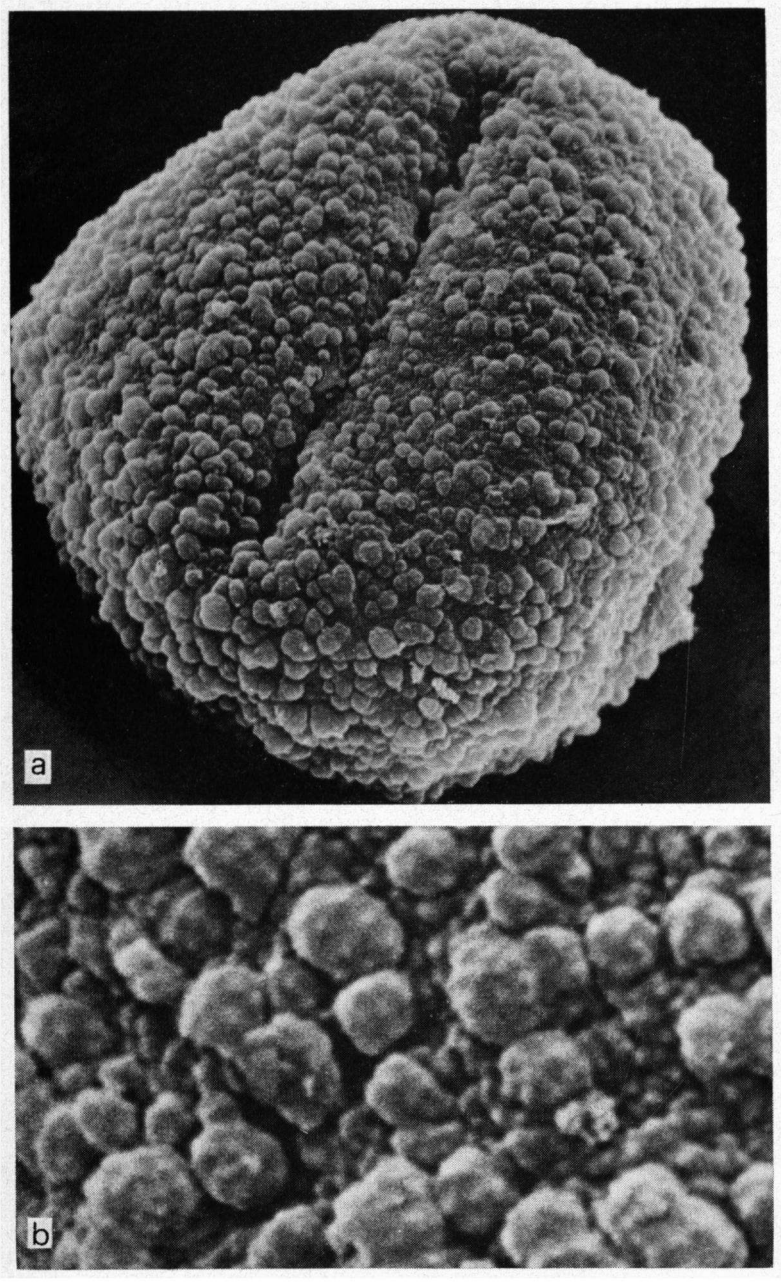

Plate I:
a. Pollen grain of *Quercus robur* L., × 4,000
b. do., surface detail, × 20,000

This pollen type was found to occur exclusively in the following deciduous species:
Quercus robur L.
Quercus petraea (Mattuschka) Liebl.
Quercus pubescens Willd.
Quercus pyrenaica Willd.
Quercus dentata Thunb.
Quercus pontica K. Koch
Although some apparently constant differences in morphology were noticed (such as the smaller polar area and more evenly distributed spinuloid protuberances of *Quercus pontica*), as yet no attempts were made to subdivide this group further.

B. *Quercus ilex/coccifera* type (*plate II*)

The sculpture of this type consists of scattered elongated elements (YAMAZAKI & TAKEOKA 1959: "dispersed rice hulls"; DUPONT & DUPONT 1972: "microrugules"). These elements, 0.4–1.0 μm long and c. 0.1 μm in diam., are straight, hooked or curved, and irregularly grouped in protruding clusters. These clusters contribute to the more or less scabrate sculptural pattern apparent under microscopical observation in transmitted light. ("Il est rare qu'on voie des taches distinctes de scabrae" – VAN DER SPOEL-WALVIUS 1963)

Some perforations in the tectum were observed as well as a colpus membrane with a distinct circular pore. This pollen type must, therefore, be considered to be colporate. The colpus membrane is provided with small (0.1–0.3 μm) granules of a rather indistinct shape.

This pollen type was found to occur in *Quercus ilex* L., *Quercus coccifera* L., and *Quercus calliprinos* Webb, and in the oriental *Quercus phylliraeoides* A. Gray.

All four species can be considered to be persistently evergreen.

C. *Quercus suber* type (*plate III*)

As far as the sculpture of this pollen type can be envisaged, it combines rounded, elliptic, and elongated elements of varying shape and size, and seems to represent an intermediate form between the *Quercus robur/petraea* type and the *Quercus ilex/coccifera* type.

The elongated elements are mostly shorter than 0.5 μm and are sometimes aggregated in clusters, these being rather far (more than 1 μm) apart and thus revealing the tectum covered with, chiefly, rounded and elliptic elements.

Perforations with a diameter of 0.1–0.3 μm are abundant and quite conspicuous. A colpus membrane provided with irregular granules and a well-marked pore were frequently observed (*plate III, b*).

This pollen type must also be considered to be colporate and was found to be present in:
Quercus cerris L.
Quercus suber L.
Quercus crenata Lam. (*Quercus pseudosuber* G. Santi)

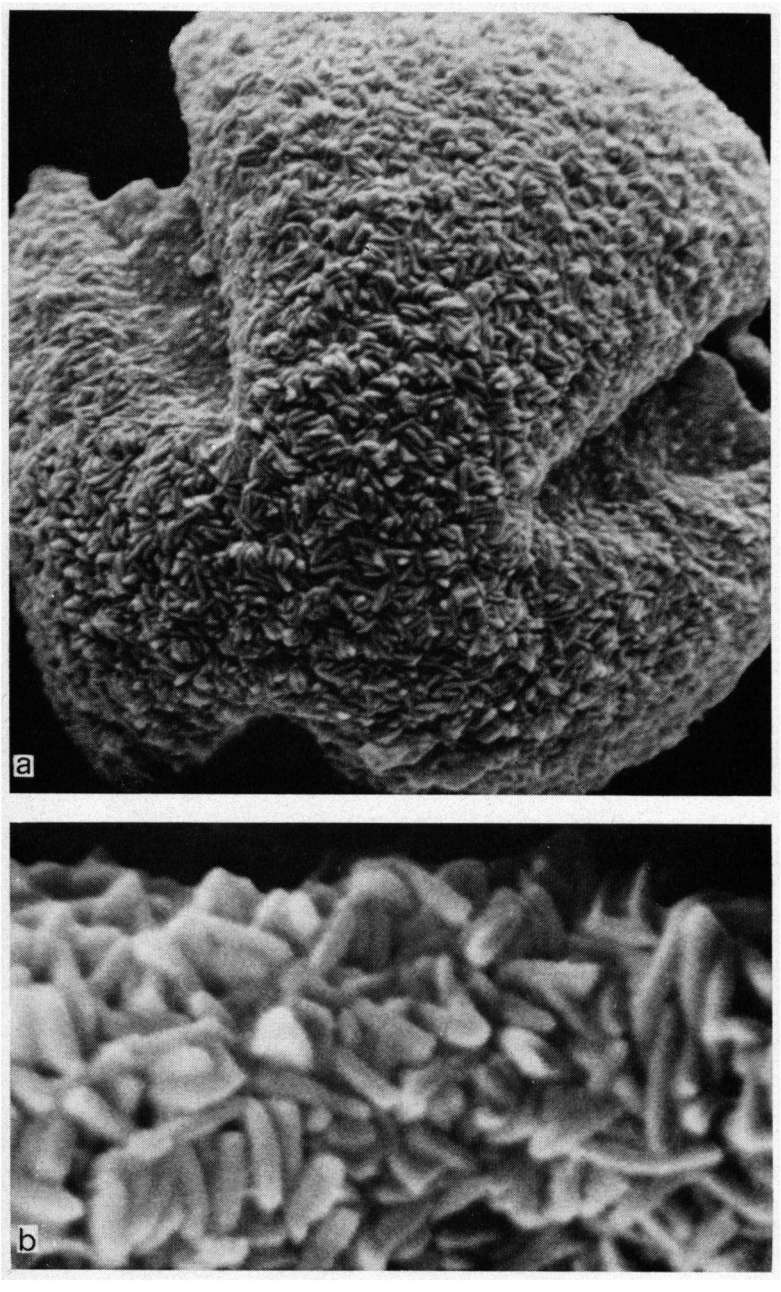

Plate II:
a. Pollen grain of *Quercus calliprinos* Webb, × 6,000
b. do., surface detail, × 25,000

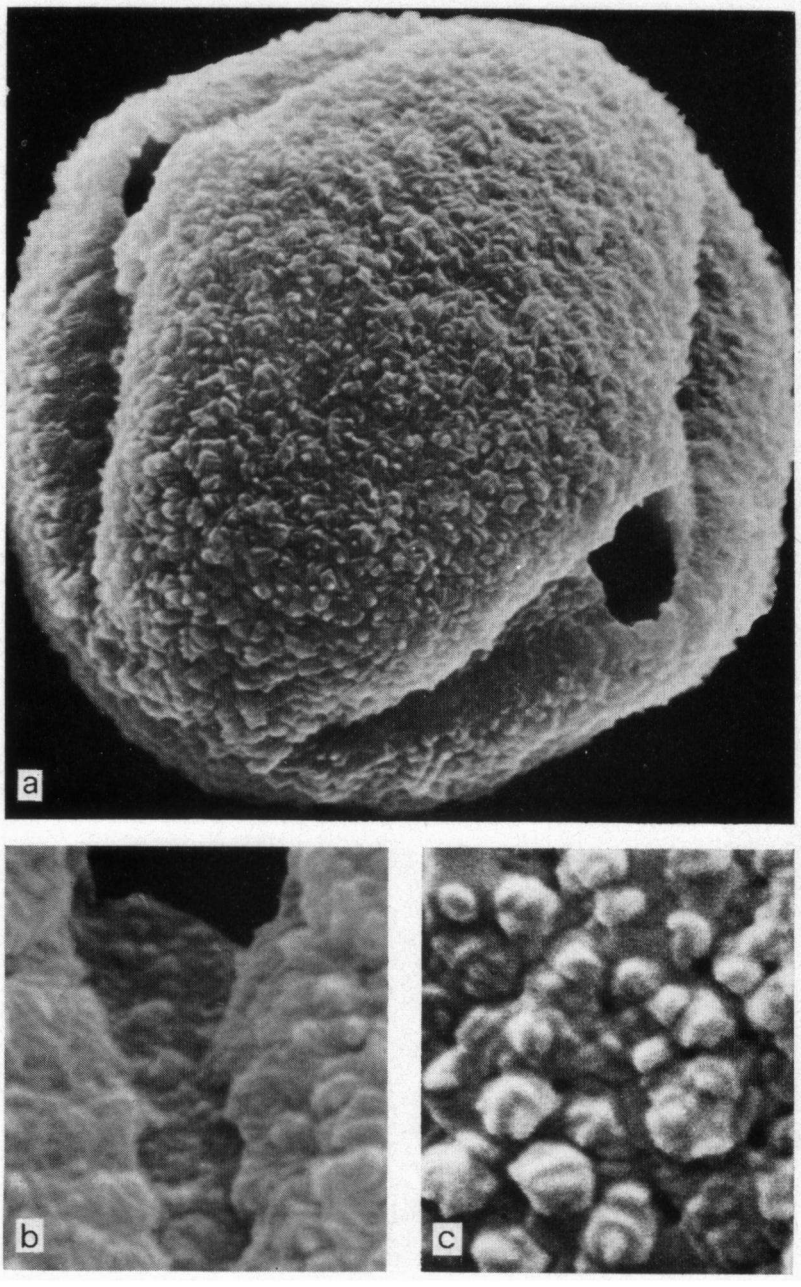

Plate III:
a. Pollen grain of *Quercus crenata* Lam., × 4,500
b. do., colpus membrane, × 15,000
c. Pollen grain of *Quercus suber* L., surface detail, × 20,000

Quercus trojana Webb (*Quercus macedonica* DC.)
Quercus thracica B. Stefanov & S. Nedjalkov
Quercus macrolepis Kotschy
Quercus suber L. × *Quercus cerris* L.

Of these species, *Quercus suber* is evergreen, whereas *Quercus crenata*, *Quercus macrolepis*, *Quercus troiana* and *Quercus thracica*, as well as the hybrid, behave like semi-evergreens. *Quercus cerris* is a deciduous tree, however.

4. DISCUSSION

The taxonomy of the genus *Quercus* has been dealt with by many authors. Only the studies of CAMUS (1936–1939) and of SCHWARZ (1936–1939) will be considered here. The first worker recognized two subgenera of which subgenus *Euquercus* comprises six sections (see *table 1*) chiefly distinguished by the mode of insertion of the ovules and by the length of the styles.

The second author divided the genus into three subgenera on account of a variety of characteristic features of the cupule, the flowers and the leaves, the evergreen behaviour and the rate of hybridization also being considered. (see *table 1*)

The *Quercus* species studied by the present author could be divided into three types, on the basis of their pollen morphology, as indicated in *table 2*, upper part. This subdivision appears to coincide with the taxonomic concept of SCHWARZ (1936–1939), since all species of the *Quercus robur/petraea* type belong to subgenus *Lepidobalanus* (Endl.) Örsted, which is a "deciduous" subgenus as far as the "Old World" species are concerned. The species of the *Quercus ilex/coccifera* type belong to subgenus *Sclerophyllodrys* O. Schwarz. The species with the *Quercus suber* pollen type belong to subgenus *Cerris* (Spach) Örsted. *Quercus thracica* was described later by STEFANOV & NEDJALKOV (1955). According to these authors this species is closely related to the "Cerroid" oaks and must, therefore, also be placed in subgenus *Cerris*.

According to the micrographs and description of DUPONT & DUPONT (1972) and of TAKEOKA & YAMAZAKI (1959), the *Quercus* species studied by these authors could be assigned a place accordingly (*table 2* lower and middle part), which confirms the correlation between the fine pollen morphology and the taxonomic subdivision made by SCHWARZ (1936–1939).

The division of the genus by CAMUS (1936–1939), who referred *Quercus coccifera*, *Quercus suber* and *Quercus cerris* to section *Cerris* of subgenus *Euquercus* and *Quercus ilex*, *Quercus robur* and *Quercus petraea* to section *Lepidobalanus* of subgenus *Euquercus*, is not supported by pollenmorphological criteria.

5. CONCLUSIONS

1. Three pollen types were observed within the genus *Quercus*. *Quercus suber* L. does not occupy an isolated position, permitting a specific determination

Table 1

Fagaceae (after SCHWARZ 1936–1939)		Fagaceae (after CAMUS 1936–1939)	
Subfam.: Quercoidae			
genus: *Quercus*	some examples:	genus: *Quercus*	some examples:
subgenus: Lepidobalanus (Endl.) Örsted		subgenus: Euquercus Hickel & Camus	
section: Roburoides Schwz.	*Quercus pontica* K. Koch *Quercus petraea* Liebl. (= *Q. sessiliflora* Salisb.)	section: Cerris Spach	*Quercus calliprinos* Webb *Quercus phillyraeoides* A. Gray *Quercus coccifera* L. *Quercus suber* L. *Quercus cerris* L.
section: Robur Rchb.	*Quercus robur* L. (= *Q. pedunculata* Ehrh.)		
section: Dascia Ky.	*Quercus pubescens* Willd. *Quercus dentata* Thunb.	section: Mesobalanus Camus	*Quercus pontica* K. Koch
		section: Macrobalanus Örsted	
		section: Lepidobalanus Endl.	*Quercus ilex* L. *Quercus robur* L. *Quercus petraea* Liebl.
subgenus: Cerris (Spach) Örsted			
section: Suber Rchb. & Schwz.	*Quercus suber* L.	section: Protobalanus Trelease	
section: Aegilops Rchb. & Schwz.	*Quercus macrolepis* Kotschy	section: Erytrobalanus Spach	
section: Erythrobalanopsis Örsted			
section: Eucerris Örsted	*Quercus cerris* L.		
subgenus: Sclerophyllodrys Schwz.		subgenus: Cyclobalanopsis (Endl.) Örsted	
section: Ilex (Endl.) Örsted	*Quercus ilex* L. *Quercus phillyraeoides* A. Gray *Quercus coccifera* L. incl. *calliprinos* Webb		
Subfam.: Cyclobalanopsidae			
genus: Cyclobalanopsis			
genus: Erythrobalanus			

Table 2.

Pollen type:	*Quercus robur/petraea* type	*Quercus ilex/coccifera* type	*Quercus suber* type
Subgenus according to SCHWARZ (1936–1939)	Lepidobalanus (Endl.) Örsted	Sclerophyllodrys O. Schwarz	Cerris (Spach) Örsted
Species studied by the present author:	*Quercus pontica* K. Koch *Quercus petraea* Liebl. *Quercus robur* L. *Quercus pubescens* Willd. *Quercus dentata* Thunb. *Quercus pyrenaica* Willd.	*Quercus coccifera* L. *Quercus calliprinos* Webb *Quercus phillyraeoides* A. Gray *Quercus ilex* L.	*Quercus suber* L. *Quercus cerris* L. *Quercus macrolepis* Kotschy *Quercus crenata* Lam. *Quercus trojana* Webb *Quercus thracica* Stefanov & Nedjalkov
Species studied by TAKEOKA & YAMAZAKI (1959). The Cyclobalanopsis group was omitted.	*Quercus dentata* Thunb. *Quercus variabilis* Bl. *Quercus acutissima* Carr. *Quercus mongolica* var. *grosseserrata* Rehd. & Willd. *Quercus aliena* Bl. *Quercus serrata* Thunb.	*Quercus phillyraeoides* A. Gray *Quercus phillyraeoides* var. *crispa* Matsum. This species s.l. was placed by TAKEOKA & YAMAZAKI (1959) in subgenus Lepidobalanus. Since no author of this subgenus was mentioned the taxonomic consequences are not clear.	
Species studied by DUPONT & DUPONT (1972), all occurring in France.	*Quercus pedunculata* Ehrh. (= *Q. robur* L.) *Quercus toza* Bosc. *Quercus pubescens* Willd. *Quercus sessiliflora* Salisb. (= *Q. petraea* Liebl.)	*Quercus ilex* L. *Quercus coccifera* L.	*Quercus suber* L.

as suggested by DUPONT & DUPONT (1972), but belongs as far as the pollen morphology is concerned to a group including also the deciduous species *Quercus cerris* L. (see *table 2*).

2. When one is dealing with fossil samples, the presence of pollen of the *Quercus ilex/coccifera* complex can be established even if very few grains are found, which provides important information for the reconstruction of former vegetation and indicates a Mediterranean type of palaeoclimate. The presence of the *Quercus suber* pollen type indicates the occurrence of South European *Quercus* species, but not necessarily the presence of evergreen trees.
3. The results of the present study, augmented by the findings of VAN DER SPOEL-WALVIUS (1963), support the systematic treatment of the genus *Quercus* by SCHWARZ (1936–1939).

ACKNOWLEDGEMENTS

The author wishes to thank the Netherlands' Organisation for the Advancement of Pure Research (Z.W.O.) for the grant which made the required technical assistance for the S.E.M. possible.

He is also indebted to the management of the Belmonte Arboretum, Wageningen, for supplying him with a considerable part of the pollen material, and to Mr. J. Muller of the Rijksherbarium, Leiden, for his kindly given and useful advice. He also thanks Dr. T. A. Wijmstra and Dr. T. van der Hammen for the stimulating and helpful discussions during the investigation, and Professor A. D. J. Meeuse for his critical revision of the text and valuable advice. The assistance of Mrs. E. J. Thomassen née Beglinger and Mr. G. W. Noldus, who prepared the pollen material, of Mr. H. J. Koerts Meijer, who took care of the photographic work, of Mrs. H. M. den Hertog née Zijm, who typed the manuscript, and especially of Mr. C. Bakker, technician of the Research Group for Scanning Electron Microscopy of the University of Amsterdam, is gratefully acknowledged.

REFERENCES

BEUG, H. J. (1961): Beiträge zur postglazialen Floren- und Vegetationgeschichte in Süddalmatien. *Flora*, **150**: 632–656, Taf. XIII-XV.

VAN CAMPO, M. & H. ELHAI (1956): Etude comparative des pollen de quelques Chênes. Application à une tourbière normande. *Bull. Soc. Bot. France* **103**: 254–260, pl. 1–2, 2 fig.

CAMUS, A. (1936–1939): Les Chênes. Monographie du genre Quercus, Tome I, II. *Encyclopédie économique de Sylviculture*, **6, 7**. (Editions Lechevallier, Paris).

DUPONT, P. & S. DUPONT (1972): Etude de pollens de Chênes (genre Quercus L.) en microscopie électronique à balayage. *C.r. séances Acad. Sci.* 274, Série D, No. 17, 2503–2506.

ERDTMAN, G. (1960): The acetolysis method. *Svensk Bot. Tidskr.* **54**: 561.

MONOSZON, M. KH. (1962): Variations of the morphological characters of pollen from certain species of oak. *Dokl. Akad. Nauk. S.S.S.R.* (Bot. Sci. Sect. Transl.), **140**: 165–168. (Translated from M. Kh. Monoszon, 1961).

PLANCHAIS, N. (1962): Le pollen de quelques Chênes de domaine méditerranéen occidental. *Pollen et Spores* **4**: 87–93, Pl. 1.

SCHWARZ, O. (1936–1939): Monographie der Eichen Europas und des Mittelmeergebietes. *Rep. Spec. Nov. Regni. Veg.*, Sonderbeiheft D, I (1–5): II (1–4). (Herausg. F. Fedde, Berlin).

SMIT, A. & T. A. WIJMSTRA (1970): Application of transmission electron microscope analysis to the reconstruction of former vegetation. *Acta Bot. Neerl.* **19**: 867–876.

SPOEL-WALVIUS, M. R. VAN DER (1963): Les caractéristiques de l'exine chez quelques espèces de Quercus. *Acta Bot. Neerl.* **12**: 525–532.

STEFANOV, B. & S. NEDJALKOV (1955): Quercus thracica nov. spec. – Arbor memorabilis. *Bull. Int. Bot. Sofia*, V. **39**: 39–53.

YAMAZAKI, T. & M. TAKEOKA (1959): Electronmicroscope investigations on the surface structure of the pollenmembrane, based on the replica method. V. Especially on the pollen genus Quercus. *J. Japanese Forest. Soc.* **41**: 125–130. Pl. 1–4, Tab. 1–2.