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## Further Contributions to the Discussion of the Origin of a Particular Oak from the Northern Dalmatia called the “Green Oak”- A Review

By

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With 1 figure

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### S u m m a r y

MÜLLER M., STABENTHEINER E., BORZAN Ž. & GUTTENBERGER H. 2003. Further contributions to the discussion of the origin of a particular oak from the northern Dalmatia called the “green oak” – a review. – *Phyton* (Horn, Austria) 43 (1): 231 – 237, with 1 figure. – English with German summary.

The “green oak” growing near Zadar, northern Dalmatia, is a particular tree of uncertain hybrid origin. The presented mini-review summarizes several papers investigating the origin of this tree where characteristic features of the “green oak” were compared to *Quercus ilex* L. and *Quercus cerris* L. that are discussed to be the ancestors of this hybrid. Results of DNA-measurements, investigations of wood anatomy, leaf anatomy, leaf hairs, pollen grains and the composition of volatile compounds reveal much more similarities between the “green oak” and *Quercus cerris* but not with *Quercus ilex*.

### Z u s a m m e n f a s s u n g

MÜLLER M., STABENTHEINER E., BORZAN Ž. & GUTTENBERGER H. 2003. Weitere Beiträge zur Diskussion um die Herkunft einer speziellen Eiche, die „die grüne Eiche“ genannt wird

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– eine Übersicht. – Phytol (Horn, Austria) 43 (1): 231 – 237, 1 Abbildung. - Englisch mit deutscher Zusammenfassung.

Die nahe Zadar im nördlichen Dalmatien vorkommende "Grüne Eiche" ist ein Hybrid unbekannter Herkunft. Der präsentierte Mini-Review fasst einige Arbeiten zusammen, die sich mit der Herkunft dieses Baumes beschäftigen und in denen Charakteristika der "Grünen Eiche" mit jenen von *Quercus ilex* L. und *Quercus cerris* verglichen werden, die als mögliche Vorfahren dieses Hybrids diskutiert werden. Ergebnisse von DNA-Messungen, Untersuchungen der Holz Anatomie, der Blatt Anatomie, der Haare, der Pollen und flüchtigen Verbindungen zeigen bei weitem mehr Ähnlichkeiten zwischen der "Grünen Eiche" und *Quercus cerris* als zwischen der "Grünen Eiche" und *Quercus ilex*.

## Introduction

On a plateau in Ravni Kotari in the neighbourhood of Zadar, in northern Dalmatia, Croatia one particular oak tree of impressive dimensions is known under the vernacular name the "green oak". JEDLOWSKI 1955 discussed that this tree could be a hybrid between *Q. cerris* and *Q. suber* L. TRINAJSTIC 1974 described the "green oak" as a hybrid *Q. x viridis* Trinajstic, hybr. nov. He presumed it to be a hybrid of *Quercus cerris* f. *austriaca* x *Q. ilex* L., However, hybrids between two oak species, where acorns mature in the second year (*Q. cerris*) and in the first year (*Q. ilex*) are doubtful (BORZAN & al. 1997, BORZAN & PFEIFHOFFER 1998, BORZAN 2000). By contrast to this opinion, however, verified hybrids between oaks with annual and biennial maturation have been reported (COTTAM & al. 1982).

The intermediacy was always evident in verified oak hybrids (COTTAM & al. 1982), so several investigations were performed on the "green oak" and on the possible ancestors *Q. cerris* and *Q. ilex*. These previous investigations are reviewed here to get closer information about the origin of the "green oak".

## Material and Methods

### Plant material

The "green oak" grows in the village of Islam Latinski, 15 km NE of Zadar (44° 12' north, 15° 26' east), Northern Dalmatia, Croatia. Samples of *Q. cerris* and *Q. ilex* were sampled in the vicinity of the "green oak" or in Posedarje, 20 km NE from Zadar.

For sampling, preparation and methodology see publications cited.

### Scanning electron microscopy

The leaves were fixed in ethanol-acetic acid, transferred to formalin-ethanol-acetic acid, dehydrated and critical point dried. Leaf samples were mounted on carbon-coated double-sided tape, sputtercoated with gold and investigated with a Philips XL 30 (20kV9) scanning electron microscope under different magnifications.

## Results and Discussion

### DNA-measurements

DNA measurements did not show big differences between the oak species *Q. cerris*, *Q. ilex*, "green oak" (GUTTENBERGER 2000). The DNA contents of  $1.20 \pm 0.26$  pg for telophases of the "green oak" are in good accordance to results of 2C cells from many other oak species (BENNETT & LEITCH 1995, FAVRE & BROWN 1996, GREILHUBER 1988, OHRI & AHUJA 1990, OLSZEWSKA & OSIECKA 1984). The DNA-contents of the nuclei of the head cells of the trichomes of the "green oak" ( $1.50 \pm 0.68$  pg) and *Q. ilex* ( $1.40 \pm 0.42$  pg) are also within these range. The results of *Q. cerris* ( $2.90 \pm 1.50$ ) were up to two times higher. Head cells of trichomes have often higher DNA contents than telophases of root tips (GUTTENBERGER 1990).

### Wood anatomy

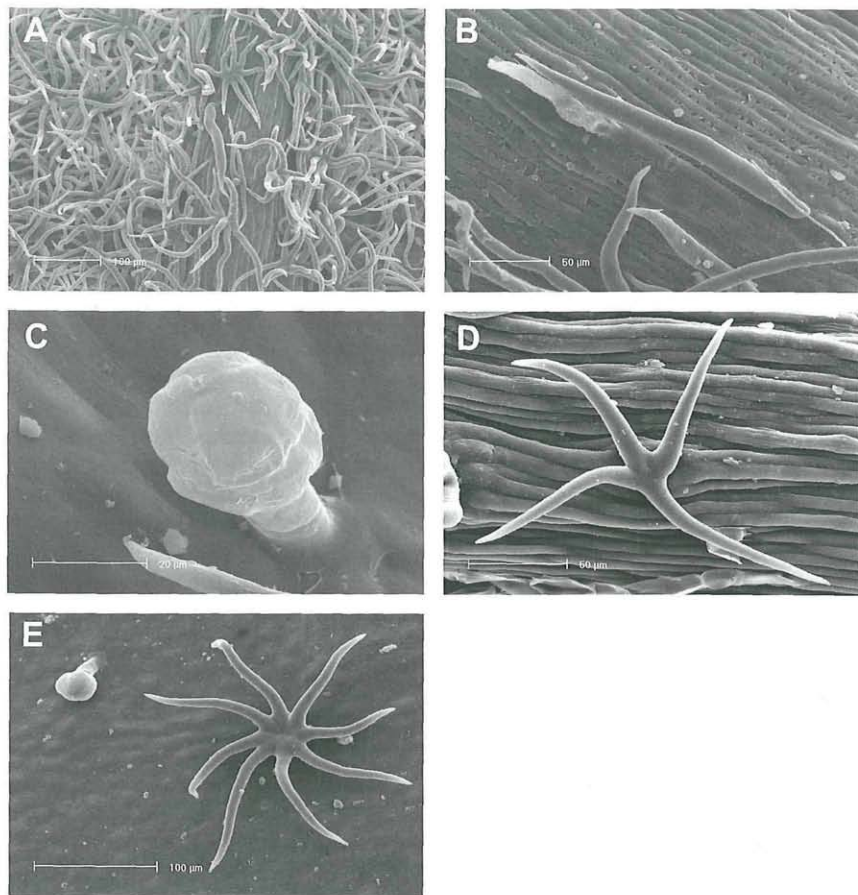
The wood of the "green oak" and *Q. cerris* is very similar (GUTTENBERGER 2000) and characterized for both oak species by ring-porous wood. And the rays are one cell wide and 3 to 20 cells in height. Both also contain in the tangential face triangular ray parenchyma cells that were described for *Q. cerris* (GREGUSS 1959). In contrast *Q. ilex* has diffuse-porous wood and vessels with relatively small diameters. For none of them tyloses could be found, which are described to be typical for *Quercus* species (WAGENFÜHR & SCHEIBER 1989), but heartwood was not investigated in this comparative study. The area of libriform fibres of the "green oak" are smaller than those of the other oak species, the fibres are shorter, but the diameters are nearly the same. These results give an evidence for the similarities between the "green oak" and *Q. cerris*, although there are also some differences.

### Leaf anatomy and epidermal hairs

Leaf anatomy was investigated by GUTTENBERGER 2000 and investigation of epidermal hairs are presented by GUTTENBERGER 2000 and BORZAN & STABENTHEINER 2002.

The leaves of all investigated trees are dorsiventral with several adaxial layers of palisade parenchyma cells and loosened spongy parenchyma – typical for adult sun leaves (ESAU 1969, JURZITZA 1987). The epidermal cells of *Q. ilex* were bigger and showed positive wood reactions in the cell walls, which is characteristic for xeromorphic leaves (LARCHER 1994).

The leaves of the "green oak" bore five different types of hairs – scattered over the abaxial and adaxial surface (Fig. 1A, Tab. 1). The classification of these five different types was done modified according to HARDIN 1976: simple uniserate hairs (Fig. 1B), glandular uniserate hair types, glandular capitate hairs (Fig. 1C), stellate hairs (Fig. 1D), and fused stellate hair types (Fig. 1E). The type and shape of hairs of *Q. ilex* and *Q. cerris* samples were similar to those described by WESTERKAMP & DEMMELMEYER 1997 and could also be found on the leaf surface of the "green oak". However, differences could be found both in the distribution



Figs. 1A-1E: Different hair types of the adaxial and abaxial epidermis of the „green oak“.  
 (A) Overview of the abaxial epidermis with simple uniserate, stellate and fused stellate hairs, bar = 100 μm. (B) Simple uniserate hairs of the adaxial epidermis, bar = 50 μm. (C) Glandular capitate hair of the adaxial epidermis, bar = 20 μm. (D) Stellate hair from the adaxial epidermis, bar = 50 μm. (E) Fused stellate hair and small glandular hair from the adaxial epidermis, bar = 100 μm.

and the size of the hairs. The individual branches of the stellate hairs on *Q. ilex* are longer than those on the surface of the “green oak” and *Q. cerris*. Simple uniserate hairs are very common in genus *Quercus* (HARDIN 1976). These hairs could be investigated on the leaves of the “green oak” and fragments of this hair type could also be observed on *Q. cerris* leaves, for both species abaxial near the veins (Fig. 1A). On *Q. ilex* leaves this hair type was missing. In studies of SAFOU & SAINT-MARTIN 1989, LLAMAS & al. 1995, PENAS & al. 1997, WESTERKAMP &

DEMMELEMEYER 1997, and AAS 1998 it was discussed that *Q. cerris* and *Q. ilex* showed only four different types of hairs. Also LLAMAS & al. 1995, PENAS & al. 1997 did not find this uniserate hair type in *Q. ilex*. WESTERKAMP & DEMMELEMEYER 1997 found hairs with heads, uniserate hairs and peltate hairs in *Q. cerris* leaves, but they observed the uniserate hair type only adaxial.

The amount of hairs, especially stellate and fused stellate hairs on the adaxial and abaxial surface differs in different investigations on the same tree (GUTTENBERGER 2000, BORZAN & STABENTHEINER 2002) – so this feature seems not to be suited for species differentiation. It is well known, that the number of hairs is depending on several environmental parameters. FILELLA & PENUELAS 1999 observed a higher hair density on *Q. ilex* adaxial leaves from a lower sea level (280 – 300 m) than from a higher elevation (1200 m). Drought stress can also lead to an increased number of hairs (BOSABALIDIS & KOFIDIS 2002).

Table 1. Hair types found on the leaves of different oak species. a = adaxial (=upper epidermis), b = abaxial (=lower epidermis); - : not existing; (+): seldom or fragments of this hair type; +: existing.

Species	Hair types									
	simple uniserate		glandular uniserate		glandular capitate		stellate		fused stellate	
	a	b	a	b	a	b	a	b	a	b
"green oak"	-	+	+	-	+	-	+	+	+	(+)
<i>Q. cerris</i>	-	(+)	+	(+)	+	-	-	-	+	+
<i>Q. ilex</i>	-	-	(+)	(+)	+	(+)	-	-	+	+

#### Volatile compounds of the leaves

The composition of the "green oak", *Q. ilex* and *Q. cerris* volatile substances obtained by steam distillation of entire leaves was investigated (BORZAN & PFEIFHOFER 1999). The results showed that the qualitative composition of all three oaks was principally equal. Specific variations were found in the terpenoid patterns, as for example for sesquiterpenes. The "green oak" contained 7, *Q. cerris* 9 and *Q. ilex* 26 sesquiterpenes. Further, some sesquiterpenes of the "green oak" were not detectable in *Q. ilex* or *Q. cerris*. Cluster analysis of the patterns of volatiles of the "green oak", *Q. ilex* and *Q. cerris* did not support the assumption that the "green oak" is a hybrid between *Q. ilex* and *Q. cerris*.

#### Pollen

Oak pollen grains are elliptical and tricolpate (BORZAN 2000, BORZAN & STABENTHEINER 2002). In general, pollen grains of *Q. ilex* are significantly smaller than pollen grains of the "green oak" and *Q. cerris* (mean pollen length of *Q. ilex*:  $34.25 \pm 2.32 \mu\text{m}$ , of *Q. cerris*:  $44.39 \pm 2.36 \mu\text{m}$  and of the "green oak":  $43.63 \pm 3.06 \mu\text{m}$ ). The structure of the pollen grains of *Q. cerris* and the "green oak" is similar concerning the ornamentation of the exine – warty and rough, studded with small rounded protrusions. In contrast, these protrusions were not present on the surface of pollen of *Q. ilex*.

## Conclusion

These presented investigations with the comparison of characteristic features of the "green oak", *Q. cerris* and *Q. ilex* did not support TRINAJSTIĆ'S conclusion 1974 that the green oak is a hybrid between *Q. cerris* and *Q. ilex*. The presented results of all of the studies support the opinion given by JEDLOVSKI 1955, with an added correction that this tree is not the F1 hybrid, but it could be a progeny of the hybrid between *Q. cerris* and *Q. suber*, repeatedly back crossed to *Q. cerris*. Taxonomic relationship between the "green oak" and other oak species has to be investigated further. Further research based on DNA analysis will probably give the final answer to the question of whether the genes of the cork oak are present in the genotype of the "green oak" or not.

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