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Biological and taxonomical investigations of some oak species

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Hermaphroditic flowers blooming unseasonably on an oak tree (»the green oak«) of uncertain hybrid origin prompted a detailed investigation into the biology of the flowering, and the morphology of various traits of the tree, which is growing in an urban area near Zadar, northern Dalmatia, Croatia. Hermaphroditic flowers were also detected on one holm oak tree from Weihenstephan, Germany. Since the first oak tree was described as a hybrid between *Q. cerris* f. *austriaca* × *Q. ilex*, scanning electron microscope investigations of pollen and hairs on the leaves of three species were made, on: *Q. ilex* L., *Q. cerris* L. and the »green oak«. Oak pollen is of elliptical shape and tricolpate. The ornamentation of the pollen grains of *Q. cerris* and the »green oak« were similar concerning the ornamentation of the exine – warty and rough and with small rounded protrusions – as well as the size of the pollen grain. *Q. ilex* pollen was much smaller and the surface lacked the small irregularly shaped protrusions typical of the other oaks. Within samples of holm oak pollen, significant difference in the surface structures was observed. Concerning the different types and number of hairs on the leaf surfaces, *Q. ilex* revealed a much greater number of stellate hairs on the upper leaf side as compared to *Q. cerris* and the »green oak«. Simple uniseriate hairs and simple unicellular hairs were missing on the leaves of *Q. ilex*, but were present on both the other oaks. To sum up the results, the »green oak« and *Q. cerris* reveal some similarities whereas *Q. ilex* does not.

Key words: Green oak, *Quercus ilex*, *Quercus cerris*, hermaphroditic flowers, pollen, trichomes, morphology, scanning electron microscopy

Introduction

A closer look at a tree will very often reveal some unknown and unexpected features important for widening our knowledge of biology and the taxonomy of the species. Urban trees are more suitable for close inspection than trees in the forest. Care for the single tree in the urban environment causes a need for different kinds of expertise and visits from scien-

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tists for their opinions on the improvement of environmental condition, measures against different diseases, pests or surgical treatment. From botanical, taxonomical, urban forestry, arboricultural and other aspects, one particular oak tree of impressive dimensions (Fig. 1) has several times been described in the literature, by JEDLOVSKI 1955, TRINAJSTIĆ 1974, SIEGERT 1995, VRANKOVIĆ and PERNAR 1995, BORZAN *et al*. 1997, BORZAN and PFEIFHOFER 1998, BORZAN 2000, GUTTENBERGER 2000. This tree is known under the vernacular name »the green oak«.

The discovery of hermaphroditic and unseasonable blooming on that tree (BORZAN 2000) prompted a detailed investigation of the morphology of its different traits. In addition, recently, in March 2000, an inflorescence with hermaphroditic flowers was discovered on a holm oak tree (*Quercus ilex* L.) growing in a spacious patio (Fig. 2) inside the Department of Landscape Ecology, Technical University of Munich-Weihenstephan. Since the »green oak« was described by TRINAJSTIĆ (1974) as a hybrid between *Quercus cerris* f. *austriaca* × *Q. ilex* L., and in some other papers (JEDLOVSKI 1955, BORZAN and PFEIFHOFER 1998, BORZAN 2000, GUTTENBERGER 2000) this hybrid formula has been critically discussed, scanning electron microscope investigations of pollen and hairs on leaves were made on specimens of holm oak, as well as of Turkey oak (*Q. cerris* L.) and on the »green oak«, and comparatively analyzed.

Fig. 1. The »Green oak«; a dominating tree (height 20 m) in the village of Islam Latinski, Northern Dalmatia, Croatia.

Fig. 2. Holm oak (height 7 m) in the atrium of the Institute of Landscape Ecology, München-Weihenstephan, Germany.

Materials and methods

Samples of flowers and leaves were collected from the inspected trees on several occasions. The »green oak« tree grows in the village of Islam Latinski, ca 15 km NE of Zadar, Northern Dalmatia, Croatia. Flowers, pollen and leaves from that tree were sampled in November 1998, April 1999 and in May 2001. Pollen and leaves from holm oak trees were collected in four different sites and from six different trees: in urban areas of Zadar in April 1999 from one tree, and in May 2001 from two trees; in Arboretum Trsteno near Dubrovnik from one tree in May 2001; in Posedarje, 20 km NE from Zadar in April 2000 from 1 tree. The holm oak tree growing in a spacious patio inside the Department of Landscape Ecology; in Weihenstephan was inspected and sampled at the beginning of March 2000. The aim of this collection was to investigate the possible differences and variations within one species, among sites and among trees.

Hermaphroditic flowers discovered on the »green oak« tree and on the holm oak tree growing in Weihenstephan were photographed. Pollen grains were stored in petri dishes and air dried. Leaves were fixed in ethanol-acetic acid, transferred to formalin-ethanol-acetic acid, dehydrated and critical point dried. Leaf samples and pollen were mounted on carbon-coated double-sided tape, sputtercoated with gold and investigated with a Philips XL

30 (20kV9) scanning microscope under different magnifications. The lengths of 30 pollen grains from each sample were measured, and measurements analyzed.

Results

On November 3rd, 1998, inside the crown of the »green oak« tree (200 years old, 20.2 m high, 1.6 m at breast height) two branches were observed, flowering unseasonably. Flowers were on 3–5 clustered inflorescence, shorter and thicker than a regular male inflorescence, but much longer than a normal female inflorescence. Each inflorescence had abundant hermaphroditic flowers (Figs. 3a, b). Normal female and male inflorescence of the same tree were sampled and photographed in April 1999 (Figs. 3c, d).

Fig. 3. Flowers of the »green oak« from Zadar and of *Q. ilex* from Weihenstephan; a: hermaphroditic flowers of the »green oak«, November 1998; b: detail of a; c: normal male flowers of the »green oak«, spring 1999; d: normal female flowers of the »green oak«, spring 1999; e: from the left: female, hermaphroditic and male flowers of *Q. ilex* from Weihenstephan, March 2000.

The holm oak tree growing in a spacious patio in Weihenstephan flowered abundantly at the beginning of March 2000. Only one hermaphroditic inflorescence with three bisexual flowers (shown in the middle of Figure 3e) was observed after close-up inspection of each flowering branch within the crown of that small tree, among many other normal male catkins and female inflorescence. This hermaphroditic inflorescence had the size of a normal female inflorescence, and only a close-up view on it could reveal the presence of stamens surrounding a pistillate flower.

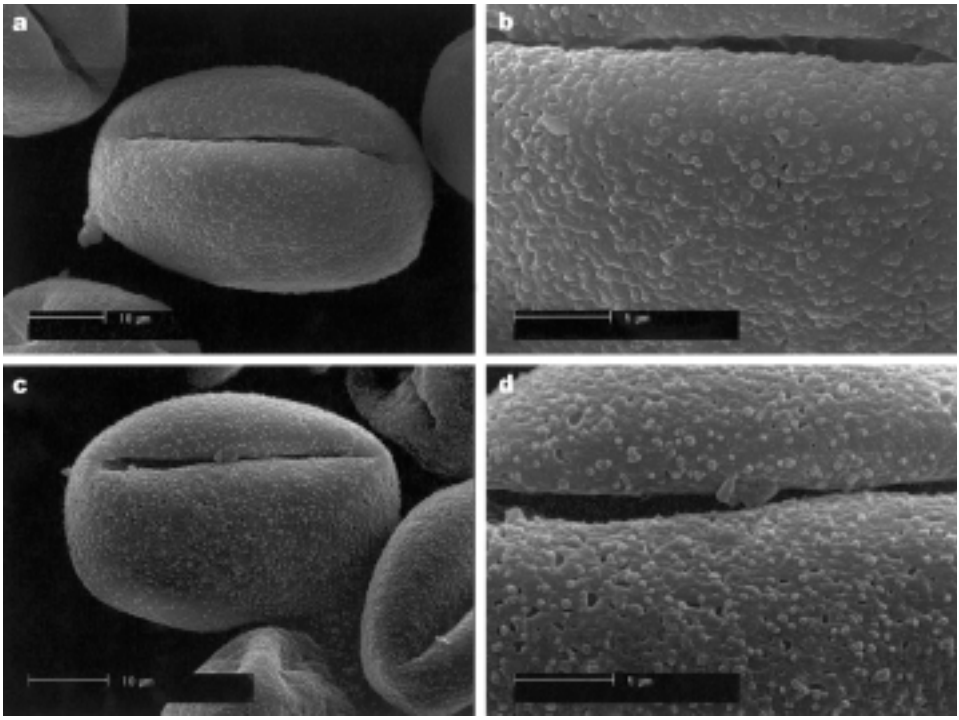

Fig. 4. Scanning electron micrographs of pollen of the investigated oak species; a, b: »green oak«; c, d: *Q. cerris*.

Oak pollen grains are elliptical and tricolpate (Figs. 4, 5). In general, all samples of holm oak trees had significantly smaller pollen grains than Turkey oak and the »green oak« (Fig. 6). Mean pollen length of holm oak for all six samples was 34.25 µm, for Turkey oak 44.39 µm, and for three »green oak« samples 43.63 µm. The length of pollen grains varied in different years, among sites and trees of the same species (Fig. 6). In four cases only, there was no significant difference in the pollen grain length of the sampled trees: between *Q. cerris* (sample No. 4) and the »green oak« (sample No. 3), and among samples of holm oak trees, between samples 5 and 9, 6 and 10 and 7 and 9.

The structure of the pollen grains of Turkey oak and of the »green oak« is similar concerning the ornamentation of the exine – warty and rough, studded with small rounded protrusions (Figs. 4a, b, c, d). Suchlike small, irregularly shaped protrusions were not present

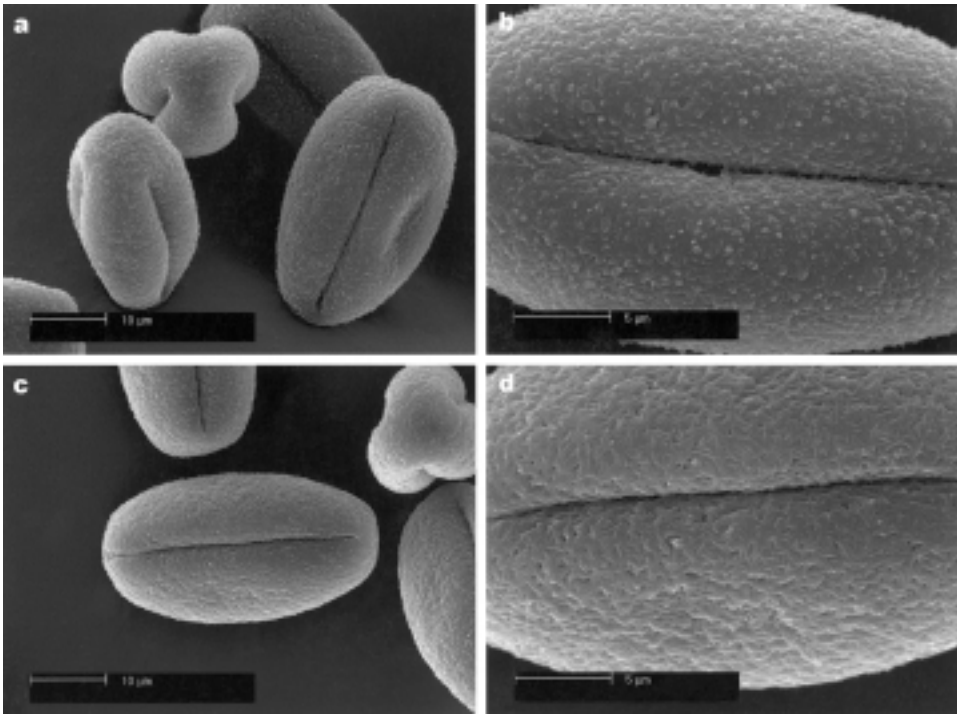

Fig. 5. Scanning electron micrographs of pollen of the investigated *Q. ilex* samples from a; b: Posedarje, near Zadar (middle Adriatic coast); c, d: Arboretum Trsteno (southern Adriatic coast).

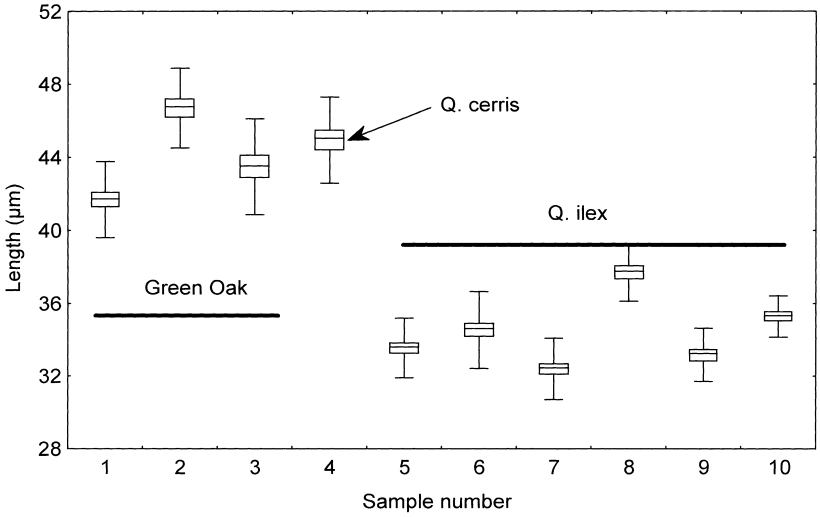

Fig. 6. Length of pollen grains of all investigated trees (10 trees); mean, mean error of mean (Box) and standard deviation; 1–3: »green oak«, 1 – 2001, 2 – 1999, 3 – 2000; 4: *Q. cerris*; 5–10: *Q. ilex*, 5 – Zadar 2001, 6 – Zadar 2001, 7 – Trsteno 2001, 8 – Zadar 1999, 9 – Posedarje 2001, 10 – Weihenstephan 2000.

on the surface of holm oak pollen (Figs. 5b, d). Within samples of holm oak pollen the difference in the pollen structure of the pollen taken from Arboretum Trsteno (Figs. 5a, b) and other samples (Figs. 5c, d) was obvious.

The type and shape of hairs of the *Q. ilex* and *Q. cerris* samples were similar to those described by WESTERKAMP and DEMMELMEYER (1997). Most of the indumentum in all three species covers both leaf surfaces with bundle (stellate) hairs (Figs. 7, 8). Branches of hairs are tip pointed. *Q. ilex* bundle hairs on the adaxial surface were more dense and longer than hairs on the adaxial surface of the »green oak« and *Q. cerris* (Fig. 7). Stellate hairs of *Q. cerris* are lesser branched than those in the two other species (Fig. 7c). Capitate hairs have been found on adaxial surfaces of all three species (Figs. 7a, b, c, and d), but on abaxial surface only with the *Q. ilex* samples (Fig. 8b). Abaxial leaf surfaces were characterized by a dense stellate indumentum, more or less parallel to the smooth leaf surface (Figs. 8a, c, e). On abaxial Turkey oak surfaces, simple hairs with pointed tips (Fig. 8f) were present, and on abaxial surfaces of the »green oak« uniseriate glandular hairs with rounded tips (Fig. 8d). In general, the »green oak« has less dense indumentum on adaxial leaf surfaces than the other two species investigated. All samples had smooth leaf surface under the indumentum.

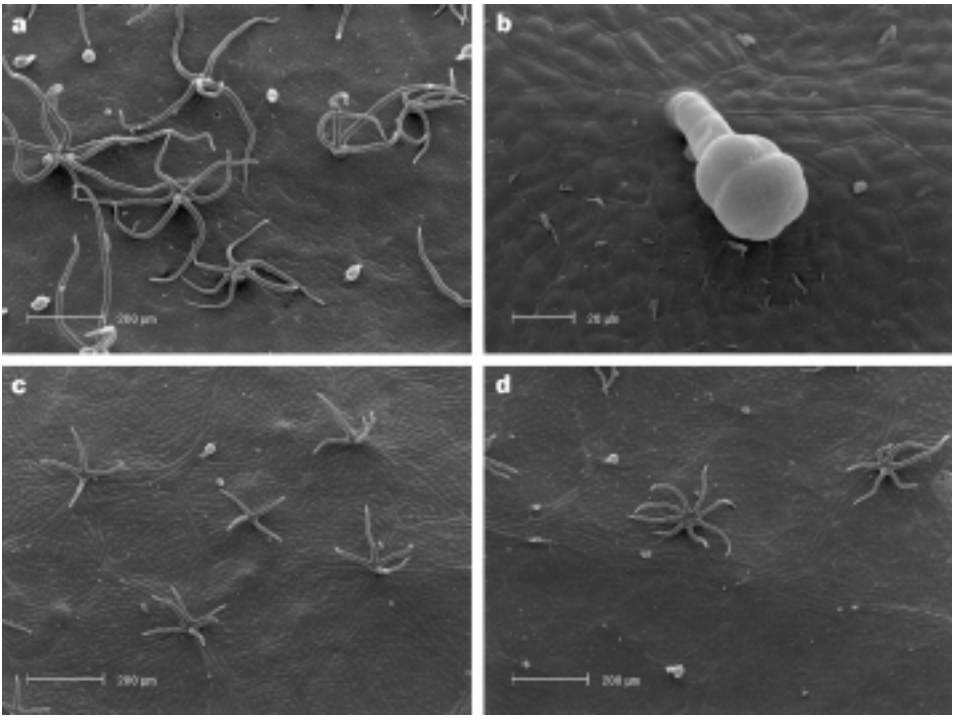

Fig. 7. Scanning electron micrographs of leaves; upper leaf surface (adaxial) – stellate and capitate hairs; a, b: *Q. ilex*; c: *Q. cerris*; d: »green oak«.

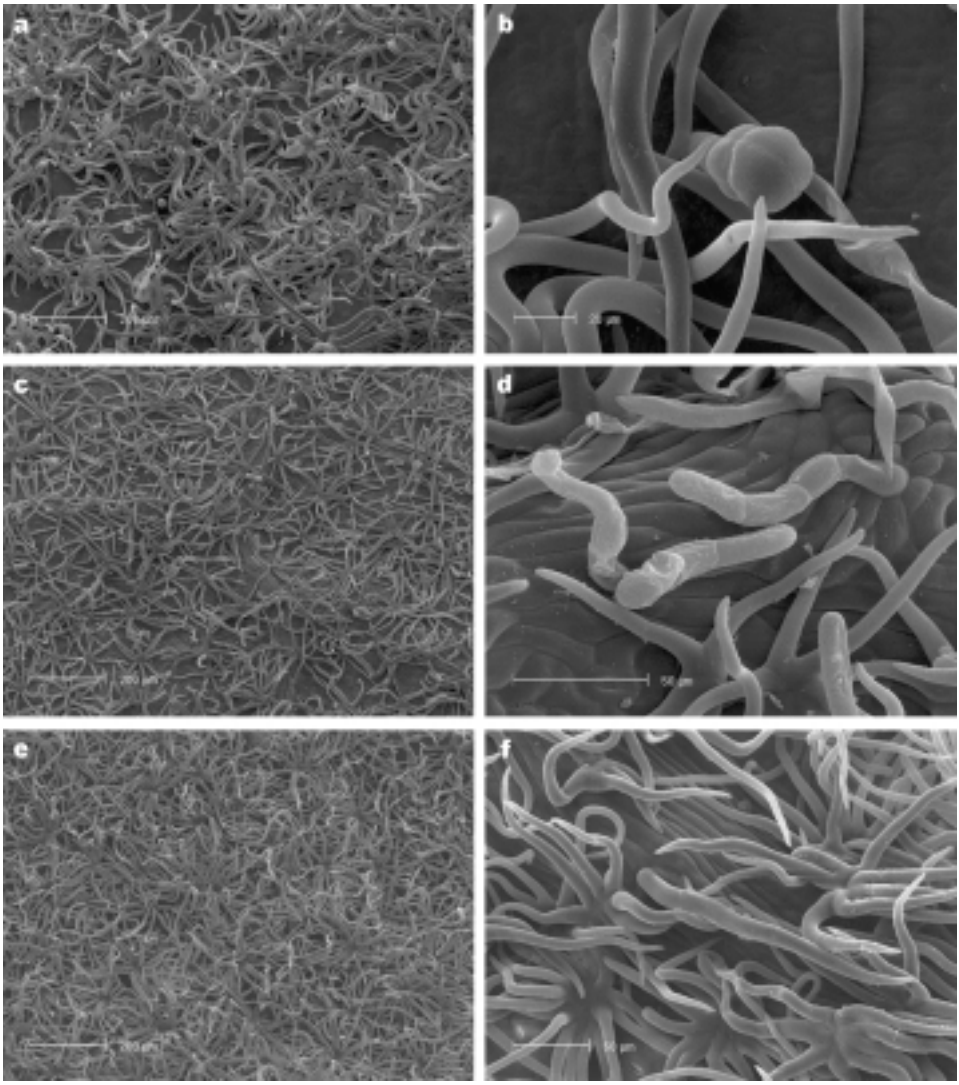

Fig. 8. Scanning electron micrographs of leaves; lower leaf surface (abaxial) – stellate hairs, capitate hairs (b), uniseriate secretory hair (d), single-cell hair, long and sharp-tipped (f); a, b: *Q. ilex*; c, d: *Q. cerris*; e, f: »green oak«.

Discussion

Individual trees in an urban environment are often valuable cultural and natural local monuments. If some new, scientific discoveries are found on those trees, they become even more valuable and important for further propagation and use in urban forestry. Such examples are: the oak tree known under the vernacular name the »green oak« growing near the Croatian town of Zadar (Fig. 1), and the holm oak tree growing in a spacious patio in Weihenstephan (Fig. 2).

In 1955, JEDLOWSKI stated that according to the morphological traits, the »green oak« is most likely a hybrid of *Q. cerris* and *Q. suber*. TRINAJSTIĆ (1974) described the »green oak« as a hybrid *Q.* × *viridis* Trinajstić, hybr. nov. He presumed that this particular tree is a hybrid of »... =? *Quercus cerris* f. *austriaca* × *Q. ilex*«. Since it is unlikely to find a hybrid of two species in which the acorns mature in the second year (*Q. cerris* L.) and in the first year (*Q. ilex* L.), it seemed desirable to examine this tree in detail, define diagnostic features, and investigate it with appropriate scientific methods. A detailed description of the tree was published by BORZAN et al. (1997). The composition of the volatiles obtained by steam distillation of whole leaves from three trees (the »green oak«, *Q. ilex*, and *Q. cerris*) was investigated by BORZAN and PFEIFHOFER (1998). Furthermore, on November 3, 1998, the »green oak« tree was observed to have hermaphroditic flowers (BORZAN 2000). GUTTENBERGER (2000) published investigations results on the wood anatomy, leaf anatomy and on the nuclear DNA-content of the »green oak« and related oak species. None of those investigations has supported TRINAJSTIĆ'S determination of the »green oak« as a hybrid between Turkey oak and holm oak.

Our investigation of the pollen morphology and hairs on leaf surfaces did not support TRINAJSTIĆ'S conclusion that the »green oak« is a hybrid between Turkey oak and holm oak. In our opinion, we can just support the first opinion given by JEDLOVSKI (1955), with an added correction that this tree is not the F_1 hybrid but it could be a progeny of the hybrid between *Q. cerris* and *Q. suber*, repeatedly back crossed to *Q. cerris*. Those results have shown also the suitability of the methods we have used in the discrimination and possible identification of different taxa. The data can be used further, and they can be complemented by further and more complex morphometric measurements for discriminant analysis of questionable oak species or hybrids.

Some of our data on the density of indumentum on investigated species are different than those shown by GUTTENBERGER (2000). That is probably due to the time or season when the samples were collected, or the part of the crown from which the samples were taken, or from the particular part of the leaf – the tip, the middle part, or the leaf-base. It is well known that with aging, leaves lose their hairs, and because of this some data from different research on indumentum density are not comparable.

As a result of this research, a new question has arisen: are there pollen surface differences among trees of different origins, as was shown by samples of holm oak trees from Posedarje (Figs. 5a, b), and from Trsteno (Figs. 5c, d)? If so, does this mean that there are biological differences between the different provenances? A visit to the Herbarium Patavinum, Padua, and to the Erbario Centrale Italiano »Filippo Parlatore«, Secione di Botanica, Museo di Scienze Naturali, Florenze, has revealed that herbarium samples of the »green oak« are very similar to herbarium samples kept in these Italian botanical institutions under the name *Q. crenata* Lamarck and *Q. pseudo-suber* Santi. Comments on those herbarium samples stated that they are hybrids between the Turkey oak and the cork oak (*Q. suber* L.). So, 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.

The discovery of hermaphroditic flowers on the green oak makes the tree even more important and deserving of care from urban foresters. The reason for this is that this tree shows a biological difference from other Mediterranean oak tree species, and is suitable for

further generative and vegetative propagation for use as a long lasting tree with a magnificent crown and appropriate growth as a park or arboricultural tree. The discovery of only one hermaphroditic inflorescence on the holm oak tree in Weihenstephan (Fig. 3e) should point the attention of scientists and urban foresters towards investigating and planting and growing species out of their natural environment. Scientists can expect some irregular behavior in the life of such trees, and urban foresters can find a good approach to enrich the urban area with new specimens to allow for the creating of stories about and the drawing of attention to such trees, to make them more attractive to citizens and visitors.

The unexpected presence of hermaphroditic flowers on both oak specimens can not be explained at the moment. It can be speculated only that this feature can »represent a primitive ancestral condition in the *Fagaceae*, still present in the genome«, as TUCKER et al. (1980) suggested.

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