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ORIGINAL RESEARCH PAPER

Oak leaf galls: *Neuroterus numismalis* and *Cynips quercusfolii*, their structure and ultrastructure

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Abstract
Anatomy and surface ultrastructure of the galls induced on oak leaves by the insects – *Neuroterus numismalis* (Ol.) and *Cynips* (*Diplolepis*) *quercusfolii* L. – were investigated using a scanning electron microscope (SEM) and a light microscope (LM). The observations in SEM and in LM enabled a detailed description of these galls and comparison of their structure with that of the typical oak leaf. In *N. numismalis* gall, the external distal tissues were classified as similar to phellem (cork), phellogen, and phelloderm, and a lateral marginal tissue as parenchyma with the likely role of a storage tissue. In the young *C. quercusfolii* gall, the cells of internal, nutritive tissue, on which the larva is grazing, formed globules rising above the surface of larval chamber. Many of them seemed to be destroyed by the larval action. In the gall which attained half of its final size, the tissues near the larval chamber were already partly lignified. The microorganisms (mainly fungi) which live in the oak phyllosphere, occurred also on the galls. We believe that the deep changes in the morphogenetic program of a leaf, which are caused by the gall-forming insects, are impossible without the transfer and the integration of the insect genetic material with that of the host plant. We also postulate that a larva secrets as yet hypothetical substances, which redirect the nutrients transport from the leaf blade towards the gall and support its vital functions.

Keywords
Quercus robur; leaf surface ultrastructure; plant gall anatomy; plant gall induction

Introduction

The morphology and anatomy of plant galls caused by insects (zoocecidia) were described by several authors [1–7]. Nevertheless, the progress in microscopic methods and generally in scientific research makes it possible to supplement the older descriptions.

In this paper, we show the details of the surface ultrastructure and anatomy of oak leaf galls caused by *Neuroterus numismalis* (Ol.) and *Cynips* (*Diplolepis*) *quercusfolii* L., and compare them to the typical leaf structure of the oak (*Quercus robur* L.). The observations were made in a light microscope (LM) and a scanning electron microscope (SEM). Based on more precise descriptions, more detailed suggestions may be put forward of how cynipid galls are induced.

The structure of the *N. numismalis* gall in LM has been described by Meyer and Maresquelle [3] and Meyer [4]. A very detailed description of the gall of related species *N. quercusbaccarum* was presented by Hough [8] and investigated from a histological point of view by Kovácsne Koncz et al. [9]. The latter authors proved the presence of an increasing gradient of proteins and lipids towards the larval chamber.

The first stages of gall development, according to the paper of Harper et al [5] (see also Hough [8]) proceed as follows. After the oviposition, necrosis of some cells located below or beside the egg occurs and the contiguous cells intensively proliferate surrounding the freshly hatched larva. LeBlanc and Lacroix [10] added more details to these observations examining the gall of *Diplolepis rosaefolii*. The cells near the newly deposited egg change showing multiple vacuoles. Shortly after, they undergo the lysis creating a small chamber for the freshly hatched larva. At the same time, some cells of the leaf dedifferentiate. This symptom is characteristic of a plant's response to stress [2]; only later the reprogramming of cell fate leads to cell proliferation, hypertrophy, and the rearrangement of tissues [11,12]. As a result, a completely new, organ-like entity is formed.

The first symptoms of gall formation are relatively early visible: the principal mass of dedifferentiated cells forms the cortical parenchyma of the gall, whereas the cells surrounding the larval chamber differentiate into two strata: the innermost nutritive tissue lining the larval chamber, and the more external nutritive parenchyma [2,3,8]. The cells of the nutritive tissue have fragmented vacuoles, large nuclei and nucleoli; they are rich in lipids and proteins [9]. The cells of the next layer, the nutritive parenchyma, have smaller nuclei and nucleoli, and one large vacuole. They contain a lot of starch, less proteins and lipids [3,10]. Both layers are free of tannins and other phenolics. As the larva consumes the cells of the innermost nutritive tissue, the nutritive parenchyma assumes gradually their function to meet the needs of the larva.

There are several hypotheses of how the cynipid gall is formed. Mani [1] observed that the insect induces the morphogenetic field (cecidozoic field) and suggested that the typical morphogenetic field of a leaf or a shoot gets adjusted to that induced by the cecidozoa. For instance, the gigantism of the cells in some galls is considerable near the cecidozoa but decreases with the distance. He hypothesized on the presence of "cecidotoxin", as yet undefined factor, produced by the host insect during oviposition or by its larva. He also stressed that the tissues of the gall are only partly changed as compared with the typical ones [1]. Maresquelle and Meyer [2] similarly concluded that: "[…] cecidian action is comprised in these three terms: growth stimulation, inhibition of differentiation, possibly also redifferentiation leading to the specific organization: showing complexity, whose etiology remains completely mysterious.". In addition, they carefully examined the possible role of different chemical plant compounds in cecidogenesis.

Raman, in his reviews [6,7], discussed the role of auxins and cytokinins in gall formation as well as the impact of enzymes present in the insect saliva. He also stressed the importance of wounding effects resulting from the larval feeding action. He confirmed, following Hartley [13] and Miles [14] that the question "what specific factor triggers cell enlargement, which works synchronously with cell multiplication in gall development?" – remains unanswered. The most critical, in his opinion, was the nature of the trigger mechanism stimulating the single host-plant cell or the group of 2–3 cells that receive the initial signal directly from the insect.

Investigations on galls may contribute substantially to our general understanding of plant morphogenesis [11] since the form of a gall and its inner structure depend on two hereditary systems, one of the insect and the other of the host plant, the latter remaining under the influence of the signals from the insect.

The purpose of this paper was to present in detail the structure of the galls of *N. numismalis* and *C. quercusfolii* as seen in LM and SEM. Starting from this description and taking into consideration recent progress in biology, we propose new interpretations of the phenomenon of cynipid galls induction and development.

Material and methods

The galls of *N. numismalis* and *C. quercusfolii* were collected near Skierniewice (central Poland) in a mixed oak-pine forest about 25-year-old. Both species of galling insects are common in Poland and in other European countries [15]. The samples of galls were collected every 10–11 days. Those presented here are from the summer and early fall. Typical oak leaves were collected at the same time as the control.

For the studies with SEM, the typical leaves and the galls on a leaf surface were fixed with a CrAF mixture/fixative (chromic acid, acetic acid, and formalin), then dehydrated in ethanol, desiccated with critical point drying CO_2, and sputter-coated with gold [16]. The micromorphology of the gall surface, the oak leaf surface, and the internal structure of galls were analyzed using the scanning electron microscope JEOL JSM 6390LV, in Mossakowski Medical Research Centre, Polish Academy of Sciences in Warsaw.

For a histological study with LM, the galls with oak leaves were also fixed in a CrAF mixture, dehydrated in ethanol, and embedded in paraffin. They were then cut with the rotary microtome Rotary 3002 into cross sections 15-μm thick and stained with safranin and fast green [17] or with Sudan III. The material was analyzed with a light microscope Nikon Eclipse 80i with polarization and the NIS-Elements Br 2.30 program. At least 3–4 samples from different trees were taken for each investigation. The same procedures were repeated in the second year.

Results

To be certain what changes in oak leaf morphogenesis are induced by the gall-forming insect, we first investigated the surface ultrastructure and anatomy of a typical, mature oak leaf.

Surface ultrastructure of a typical oak leaf in SEM

The upper (adaxial) surface of the oak leaf (Fig. 1b) was smooth, covered with a relatively thick layer of cuticle (compare also Fig. 4c). Wax cover was not visible, probably because of its smoothness. There were no stomata or hairs on this side of the leaf. The contours of the cells were clearly visible; the cells were oval and convex. The course of the veins could be easily traced because the surface was elevated above them.

On the lower (abaxial) surface of the leaf (Fig. 1a), numerous stomata were present. The veins were covered with a smooth cuticle and wax, with no stomata above them. The leaf blade surface was covered with wax in a form of rods (Fig. 1a,c,e), which especially on the guard cells of the stomata were very dense and sometimes formed small plates. There were rather few hairs on this side of the leaf (Fig. 1d), each composed of approximately three cells. The single-hair basal cell was covered with wax rods. Some hairs were bent towards the surface of the leaf. Some uniform crystals, about 12–15 μm long and 2–3 μm wide, were dispersed on the surface of the leaf (Fig. 1d). Occasionally they formed groups (compare similar crystals on a gall in Fig. 2c,d). Numerous, ramified hyphae of phyllosphere fungi stuck tightly to the abaxial leaf surface (Fig. 1e; compare [18,19]).

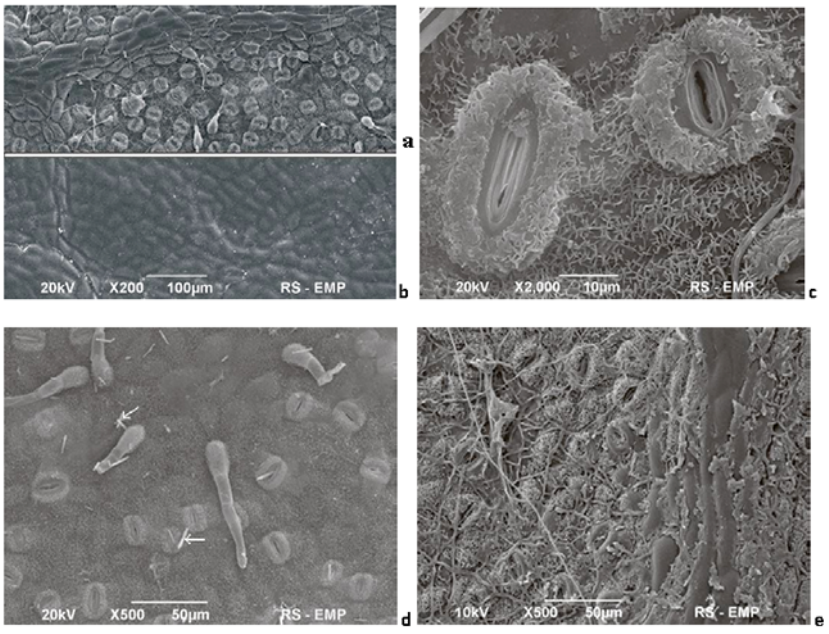

Fig. 1 Typical, mature oak leaf (SEM). **a** Abaxial side – multiple stomata. Note that there are no stomata above a vein. **b** Adaxial side – smooth epidermis without stomata. **c** Wax cover in the form of rods, especially abundant on the guard cells of stomata. **d** The hairs, stomata, and stick-like crystals (arrows). **e** Dense net of phyllosphere fungi on the leaf abaxial side.

Surface ultrastructure of the gall of *N. numismalis* in SEM

The galls of this species were small and their diameter was approximately 1.5–2.5 mm at the end of September. Usually a whole group of these galls,

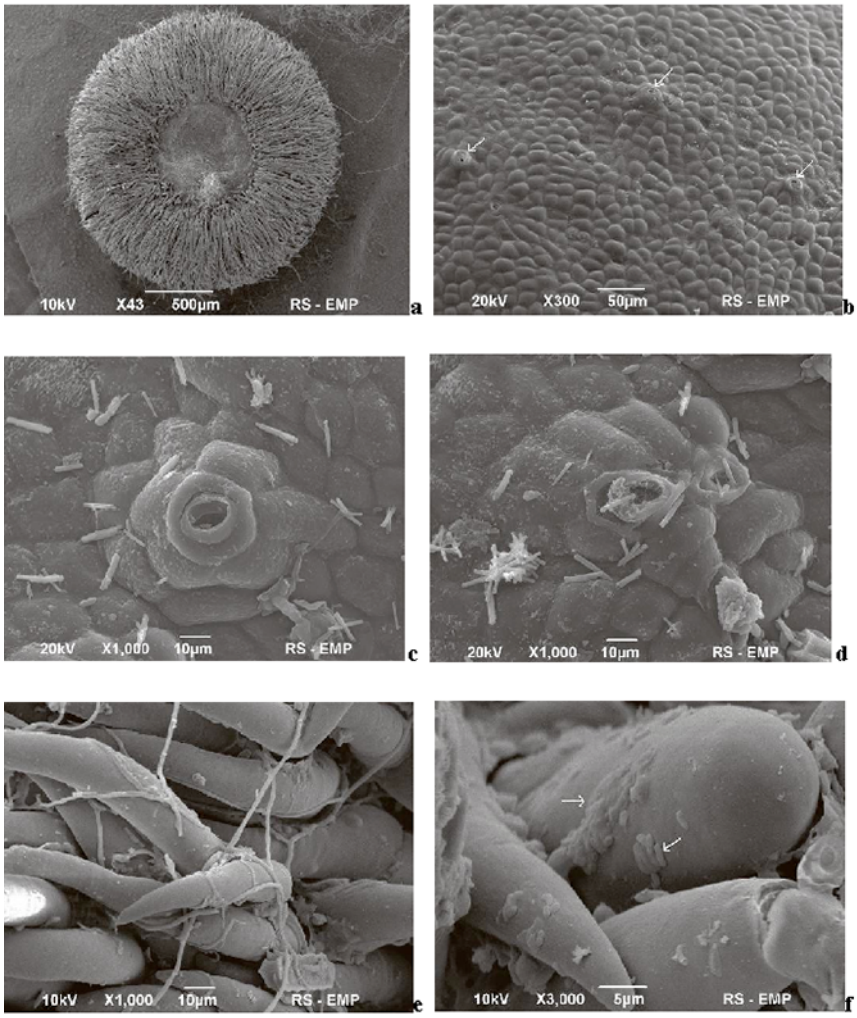

Fig. 2 The gall of *Neuroterus numismalis* (SEM) at the beginning of September. **a** The gall central plane surrounded by a garland of appendices. **b** Magnified surface of the central plane with rarely dispersed, atypical stomata (arrows). **c** Wide open stomata on the hills of cells; visible single crystals or occurring in groups. **d** Two atypical stomata on the central plane of the gall. **e** Hyphae of phyllosphere fungi twinning around the appendices. **f** Phyllosphere bacteria adhering to the appendices (arrows).

which could count up from a few to several dozen individuals, was encountered on a single leaf. They always occurred on the abaxial side of the leaf. In the climate of Poland, they were first visible during the last days of June and shed before oak leaf abscission in late September or in October. The plant material for the microscopic photographs of. *N. numismalis* presented here was collected in late September.

The mature gall showed the central plane (0.75–0.80 mm diameter) surrounded by the garland of appendices (Fig. 2a). They were about 0.5–0.7 mm long and about 0.02 mm thick. Where the appendices casually broke, their internal structure was visible in a form of concentric layers (Fig. 3a). The surface of the central plane resembled that of the abaxial side of the leaf above the veins: the cells were distinct, covered with a smooth layer of cuticle and wax, oval in the outline, and convex (Fig. 2b). Only very few atypical stomata were present in the central plane; they were wide open, usually situated on the "hills" of concentric rings of cells (Fig. 2c), and scarcely covered with wax. Some other atypical stomata were not as elevated and surrounded by the cells arranged less regularly (Fig. 2d). Wax secretion was sometimes visible inside the stomata. Stick-like crystals, similar or identical with those in Fig. 1c, were scattered over the central plane of the gall and sometimes formed groups (Fig. 2c,d). Occasionally, the (unknown) spores were found on the surface of the gall. In addition, the mycelium of unknown species of phyllosphere fungi was frequently encountered on the central plane and penetrated the stomata. The hyphae of fungi were twinning around the appendices (Fig. 2e) and some bacteria adhering to the appendices were visible in higher magnification (Fig. 2f).

The lower surface of the gall (Fig. 3b) was smooth: there were concentric rows of cells around the broken peduncle. In the peduncle, a visible bundle of vascular tissues connecting the gall with the leaf (for its structure see Fig. 6c). No appendices emerged on the underside of the gall. Neither hairs nor stomata were present there. The cross sections of the *N. numismalis* gall (Fig. 3c) revealed that the appendices were also present on the lateral sides of the gall and curved down partly covering the margins of the lower side. The majority of the cells of the gall visible in the cross section were full of content.

Anatomical structure of the typical oak leaf (LM)

The structure of the oak leaf was quite a typical of many dicotyledonous species of the trees growing in temperate climate. It had 1–2 layers of palisade parenchyma and

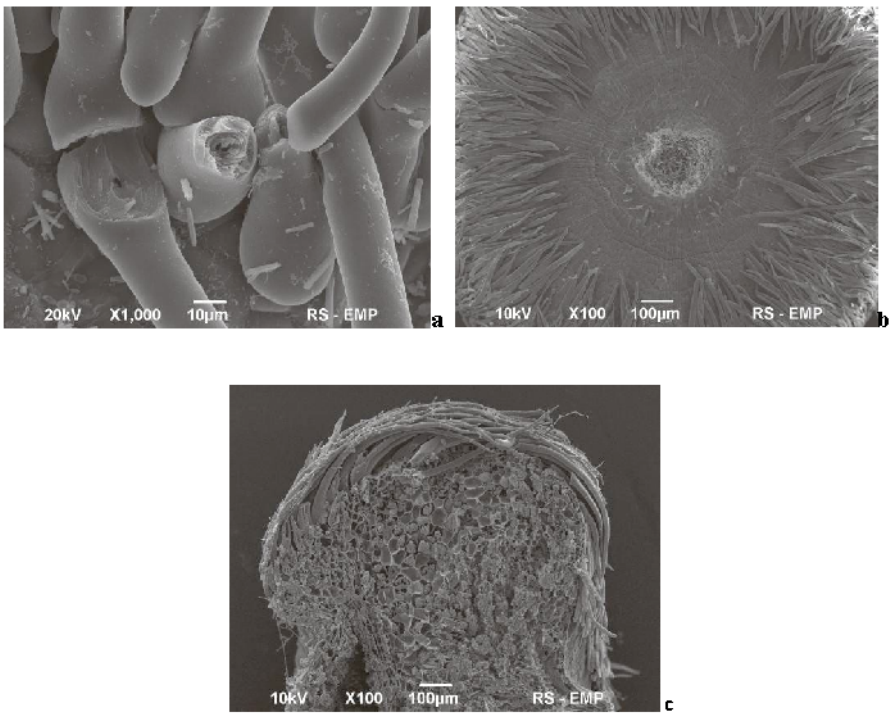

Fig. 3 The gall of *Neuroterus numismalis* (SEM). **a** Broken appendices with visible internal structure of cell wall layers. **b** Underside of a gall surrounded by a garland of appendices. In the center of the gall, a broken attachment to the leaf (the peduncle) is visible. **c** Central cross section through the gall showing that the appendices also emerge on the sides of the gall and adhere tightly to its surface.

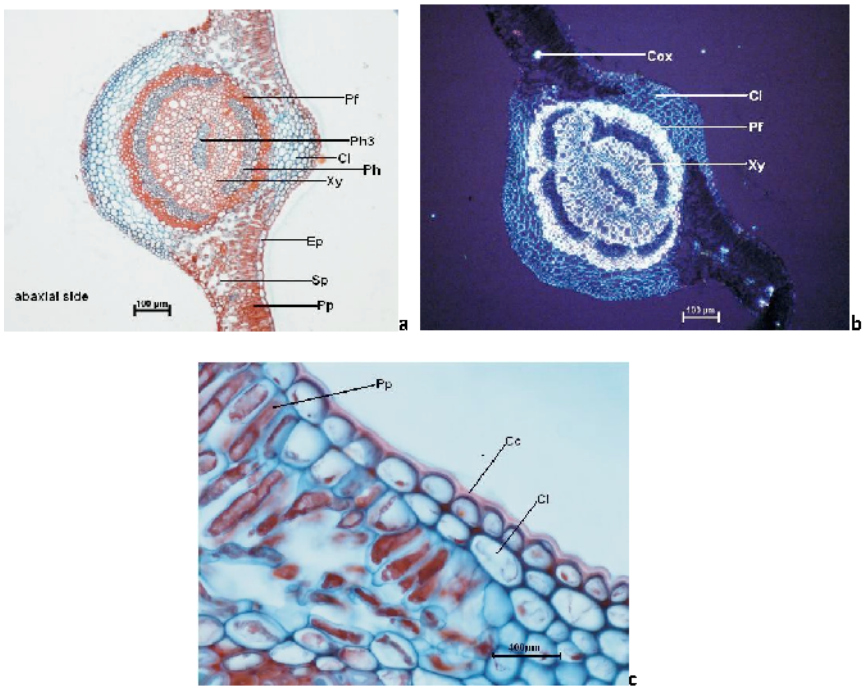

Fig. 4 Typical, mature oak leaf (LM). **a** Cross section of the leaf with the main vein in the center: Ep – epidermis; Pp – palisade parenchyma; Sp – spongy parenchyma; Cl – collenchyma; Pf – phloem fibers; Ph – phloem; Ph3 – additional phloem strand; Xy – xylem. **b** Similar cross section viewed in polarized light. Shining tissues: collenchyma (Cl), phloem fibers (Pf), xylem (Xy); a few dispersed calcium oxalate crystals (Cox) are visible. **c** Magnified fragment of a panel showing details of the leaf structure: thick cuticle (Cc), thick walled collenchyma, and palisade parenchyma (Pp).

well-developed spongy parenchyma (Fig. 4a–c). The epidermis on the adaxial side was composed of flattened cells externally protected by a thick layer of cuticle and wax (Fig. 4c). The epidermal cells on the abaxial side of the leaf (Fig. 4a) were less regularly arranged and had thinner cuticle. The epidermal cells above the veins, on both sides, were smaller, oval or even conical in cross section. The thick layer of collenchyma protected the vein from the upper and lower sides (Fig. 4a–c). The walls of collenchyma cells were bright in polarized light. The vascular bundle was surrounded by an almost uninterrupted ring of phloem fibers which also brightly shone in polarized light (Fig. 4a–c). There was an additional strand of phloem in the center of the central vein (Fig. 4a,b). The xylem with multiple vessels occupied most of the central part of the vein (compare [20,21]).

Anatomical structure of the *N. numismalis* gall (LM)

The transverse sections through the gall in LM (Fig. 5a) showed that the larval chamber was present in the center of the gall. The central plane of the gall was covered by an epidermis with very heavily stained cells (with safranin). Beneath them, there was a stratum of mostly flattened, tightly packed cells arranged in 2–7 radial rows (Fig. 5a,b), with the appearance of cork. Their walls were brightly shining in polarized light (Fig. 5b). The cells of this stratum markedly differed from the cells of the layer below, which showed a meristematic character. They were classified as phellogen or phellogen-like tissue. They had dense cytoplasm and stained dark. The next stratum, below the phellogen-like layer, was composed of rather tightly packed, usually larger cells, thin-walled, and staining blue with the safranin – fast green mixture. They were not bright in polarized light – we recognized this stratum as phelloderm or phelloderm-like tissue. There was a lot of starch grains in the cells of this layer, seen as characteristic crosses in polarized light (Fig. 5e). Near the margins of the gall there was a thick stratum of parenchyma tissue, which would be called the marginal parenchyma; its cells were also rich in starch (Fig. 5e). The extreme, outermost cells were dark, which might indicate their special properties, different from the other cells of the stratum (Fig. 5a). The appendices intensively shone in polarized light, which suggested the crystalline nature of their walls.

The larval chamber was surrounded by two strata of cells (Fig. 5c,d). The stratum which lined the larval chamber – the nutritive tissue, contained the cells full of lipids, staining red with Sudan III (data not shown). In the second, more external stratum, i.e., in the nutritive parenchyma, the cells were larger and, when not stained with Sudan III, they were quite transparent. The larval chamber was protected from above and below by a layer of sclerenchyma, which in transverse section acquired lenticular form (Fig. 5b); in 3D, this sclerenchymatic capsule was cup-shaped with a lid (Fig. 5a,b). The sclerenchyma and some neighboring cells contained large crystals, probably of calcium oxalate. Near the base of the gall, two additional wings of lower sclerenchyma formed the sclerenchymatic calyx protecting the larval chamber from below (Fig. 5b, Fig. 6a,b).

In the more peripheral part of the gall, i.e., behind the larval chamber (Fig. 6a,b), the appendices brightly shining in polarized light were present. Passing inwards, the gall epidermis showing cells with black content was recognized, next, the parenchyma-like tissue gradually changing into the cork, with cell walls shining in polarized light. Then, there was a thin layer of dark tissue in polarized light, presumably a phellogen, and more inwardly, the thick stratum of phelloderm-like parenchyma, full of starch (Fig. 5e). Still more centrally, there was the sclerenchymatic capsule containing many crystals. The nutritive parenchyma inside the capsule stained red with

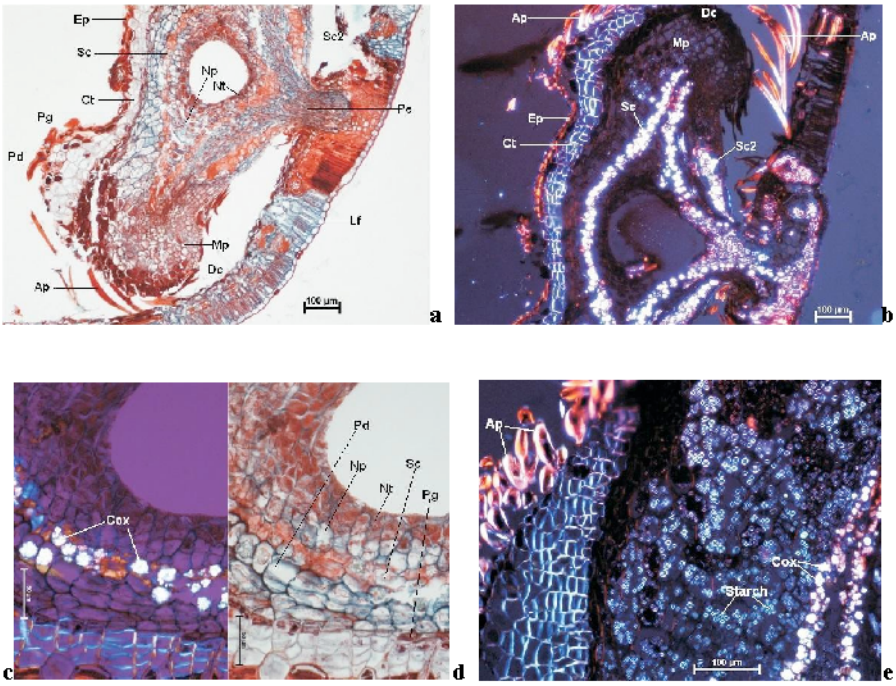

Fig. 5 The gall of *Neuroterus numismalis* (LM). **a** Central cross section through the gall, with the larval chamber in the center: Ep – epidermis of the gall; Ct – cork-like tissue; Pg – phellogen; Pd – phelloderm; Sc – sclerenchymatic capsule; Sc2 –sclerenchymatic lower calyx; Pe – peduncle; Mp – marginal parenchyma; Dc – marginal dark stained cells; Nt – nutritive tissue; Np – nutritive parenchyma; Ap – appendices; Lf – the tissues of the leaf. **b** Similar cross section as in a panel viewed in polarized light. Shining are following structures: Ap – appendices; Ep – epidermis; Sc – sclerenchyma with many crystals; Ct – the cell walls of cork. Marginal parenchyma (Mp) contains the cells with dark substances. **c,d** The tissues near the larval chamber in polarized (**c**) and normal light (**d**); Cox – calcium oxalate crystals. Other abbreviations as above. **e** The cells of phelloderm (Pd) are full of starch (see the crosses, characteristic of starch in polarized light).

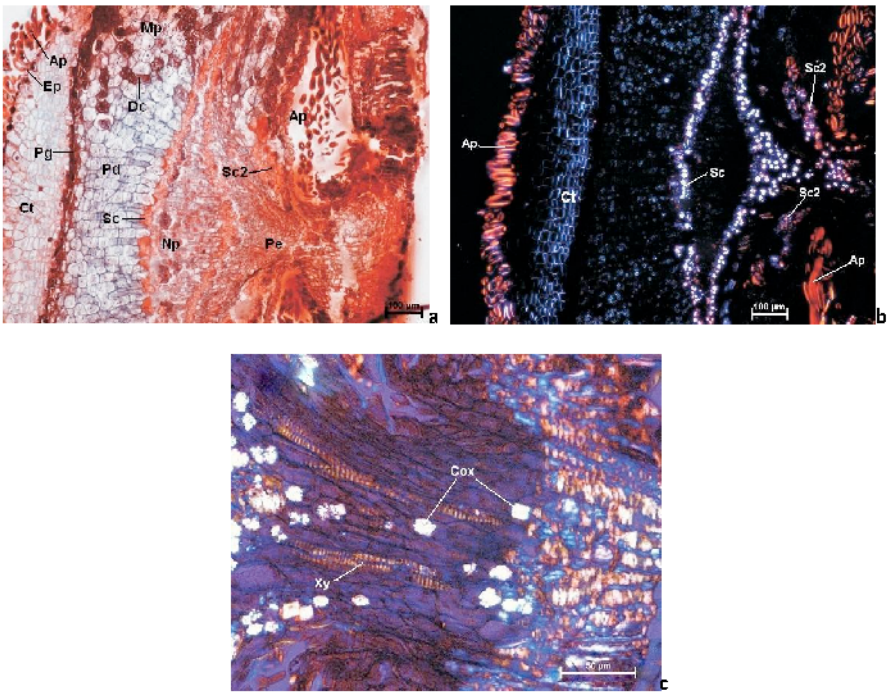

Fig. 6 The gall of *Neuroterus numismalis* (LM). **a** Cross section of the gall made off-center showing the tissues behind the larval chamber; abbreviations are the same as in Fig. 5. **b** The same cross section as in a panel viewed in polarized light: shining are the appendices (Ap), the cork-like tissue (Ct) except for its layers close to the epidermis, the sclerenchymatic capsule (Sc) containing crystals, and the sclerenchymatic lower calyx (Sc2). **c** The peduncle in polarized light with shining druses of calcium oxalate (Cox), the vessels and tracheids (Xy); the thickenings of the vessel walls are mainly of the helical type.

Sudan III (not shown). The peduncle connecting the gall with the leaf (Fig. 6c) contained vessels and tracheids that were clearly visible in polarized light, xylem parenchyma cells, and also phloem strands. The vessels and tracheids were distinguished by clearly visible helical thickenings of their secondary walls. Many crystals were present in the peduncle.

Anatomical structure of the *Cynips quercusfolii* gall (in SEM and LM)

In the very young gall of only 1.75 mm in diameter, collected at the beginning of July (Fig. 7a), the cuticle was smooth and covered with a smooth layer of wax. The contours of the cells were visible but obliterated (Fig. 7b). There were neither hairs nor stomata on its surface. The openings of the unknown function (Fig. 7b) sometimes occurred. In such a young gall, the cells of parenchyma-like tissue were large and radially elongated. The cells close to the gall surface or those located in the vicinity of the larval chamber were smaller and rounder (Fig. 7c). Interesting was the interior of the larval chamber, with its strange globule-like cells. These cells raised up above the surface of the larval chamber wall (Fig. 7d–f). Many of them looked empty, probably destroyed by a grazing larva (Fig. 7d, left side). The torpedo-like shape in the larval chamber was probably a very young larva (Fig. 7d,f) with the mouth of the suction type.

In the older gall, measuring about 8 mm in diameter (less than half of the final size) (Fig. 8a,b), the cells of epidermis were small and of a similar size as the cells of typical leaf epidermis. However, the cells of gall parenchyma were many times larger than those of spongy parenchyma of the typical leaf (Fig. 8a). The cells of gall parenchyma were tightly packed. Their shape changed: from almost round near the surface to more elongated and narrower towards the center. The cells very near to the larval chamber were again different – relatively small, frequently isodiametric, bright in polarized light (Fig. 8b), and stained red with safranin and fast green (Fig. 8c). The cells being a remnants of nutritive parenchyma were scarce and dark both in normal and polarized

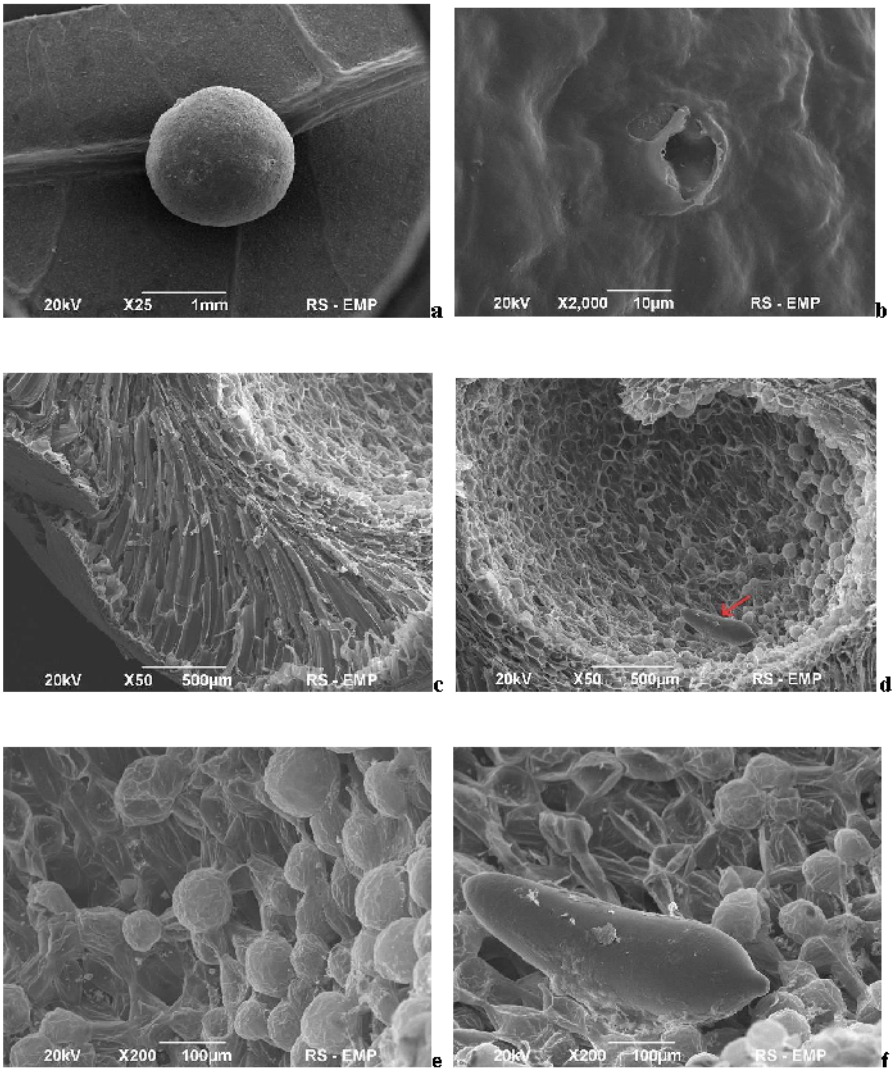

Fig. 7 The gall of *Cynips quercusfolii* (SEM). **a** Very young gall attached to a vein. **b** The opening of an unknown function (probably facilitating ventilation), rare on the gall surface. **c** The parenchyma of the gall composed of elongated cells; smaller and more rounded cells are visible near the larval chamber. **d** Interior of the larval chamber: some cells are destroyed. The globular structures and the larva close to them (pointed with an arrow) are also visible. **e** Magnification of **d** panel presenting the globular cells. **f** The larva; its mouth seems to be of suction type.

light (Fig. 8a,b, pointed with an arrow). In the cells close to the larval chamber, the process of deposition and thickening of secondary walls was already visible at higher magnifications (Fig. 8c). Vascular strands were regularly collocated in gall parenchyma around the larval chamber at some distance to it (Fig. 8a – black arrow, Fig. 8d).

In yet older, almost mature *C. quercusfolii* gall (Fig. 8e), the epidermal cells were of typical size compared to those of the leaf but the cells of the gall parenchyma were gigantic and very loosely arranged. They were supplied by the vessels having annular or helical wall thickenings (Fig. 8f).

In general, the two investigated galls markedly differed as far as their anatomical structure was concerned. Their macro- and microstructure also dramatically differed from that of the typical oak leaf. For instance, in the *N. numismalis* galls, the tissues similar to cork, phellogen, and phelloderm, which normally occur as protective tissues in the shoots, were identified. In addition, the parenchyma cells of the *C. quercusfolii* gall showed the gigantism not found in the ordinary leaf tissues.

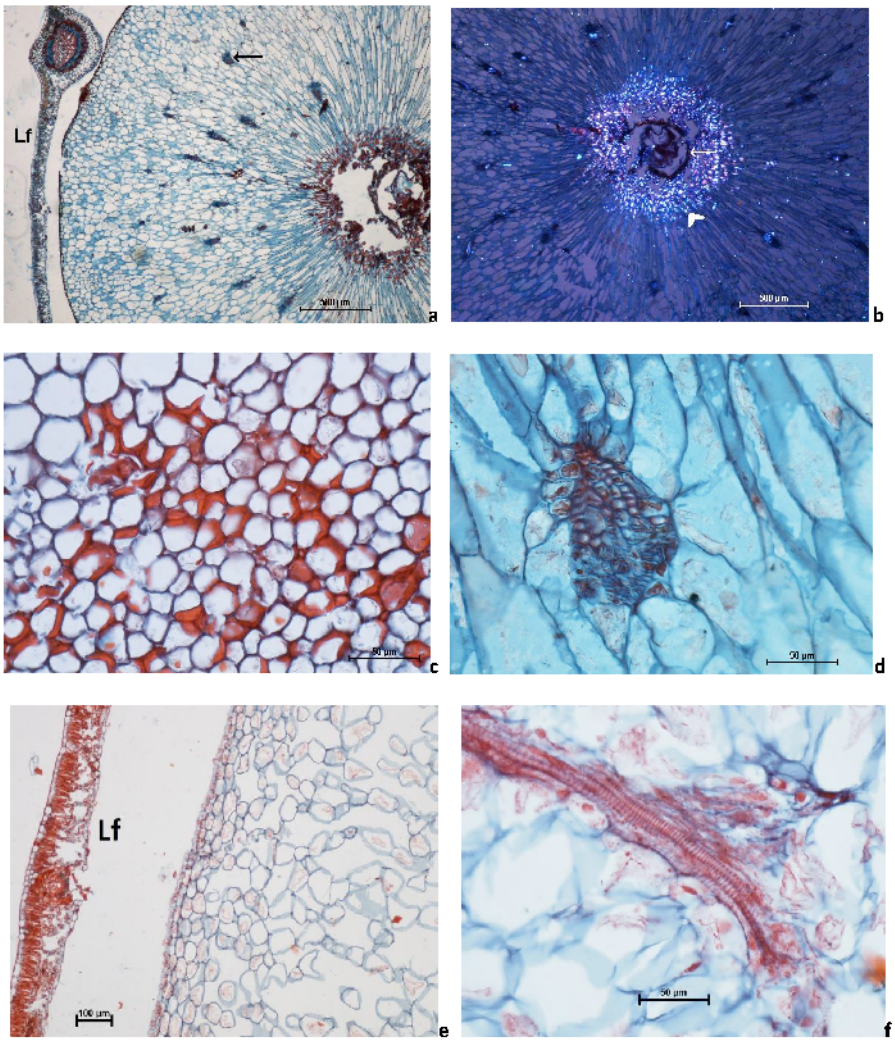

Fig. 8 The gall of *Cynips quercusfolii* (LM). **a–d** The gall about half of the final size (in July). **a** Central cross section through the gall: the cells are very large in comparison to the leaf cells (Lf) and tightly arranged. They are round near the cuticle and gradually more and more elongated towards the center. The cells surrounding the larval chamber are small and isodiametric. The larva and the remnants of the nutritive tissue are in the center. Vascular bundles (black arrow) are regularly spaced in the gall parenchyma. **b** Tissues of a similar gall in polarized light. The remnants of the nutritive tissue and nutritive parenchyma are pointed with a white arrow. The cells of just-forming sclerenchymatic ring surrounding larval chamber are bright in polarized light (arrowhead) due to the presence of already thick, secondary cell walls. **c** The cells close to the larval chamber with thick secondary cell walls stained red by safranin. **d** The cross section of the vascular bundle in gall parenchyma. **e,f** The mature gall. **e** Fragments of the gall and of an oak leaf (Lf) allow comparison of the gigantic and very loosely arranged cells of the gall parenchyma with those of the leaf. **f** The vessels in the gall parenchyma have mostly helical thickenings of their secondary cell walls.

Discussion

The application of SEM together with LM allowed us to describe the galls of *N. numismalis* and *C. quercusfolii* in a more detailed way than it had been done beforehand [2,3]. This made it easier to compare the structure of these two galls between themselves and with the structure of the typical oak leaf.

The gall of *N. numismalis* has the life cycle strictly coordinated with the development of the insect living in it. Our data present the development of agamous generation in early fall only. This gall has a specific and complex external form and also an internal structure. Epidermis covering its central plane is similar to that covering the veins of the typical leaf. On the central plane, there are few transformed stomata, or to be more precise "orifices", which can hardly be classified as stomata. Some of them are on the

hills of cells, the feature which is rarely encountered in nature [22]. As far as we know, the surface ultrastructure of *N. numismalis* gall has not been described earlier.

Like the other cynipid galls, the *N. numismalis* gall contains two strata of tissues which surround the larval chamber [2,3]: the first one, the nutritive tissue lining directly the larval chamber contains the cells full of cytoplasm but show few lipids as it was revealed by staining with Sudan III (not shown). The larva feeds on these cells. When they are consumed, the cells of the next stratum, the nutritive parenchyma, transform into new nutritive cells and start accumulating the nutrients, which will be used by the larva [3]. In fall, the cells of nutritive parenchyma are rich in lipids and tissues located further from the gall center are rich in starch, as it was shown by Kovácsne Koncz et al. [9].

The tissue specific of the *N. numismalis* gall is the cork located directly beneath the epidermis of the central plane [3] and shining in polarized light. The walls of cork tissue show birefringence due to a high content of waxes [21]. It is supposed that this tissue has a protective role for the gall [3]. The formation of such tissue may indeed protect the larval chamber against abrupt changes of temperature during hibernation. Of interest is the very thin layer of living cells full of cytoplasm, which separates the cork-like tissue from the parenchyma stratum located more inwardly. It was not mentioned by Meyer and Maresquelle [3]. The three tissues: phellem (cork), phellogen, and phelloderm constitute the typical periderm, which normally occurs as a protective layer, for instance in woody stems, but their occurrence in the *N. numismalis* gall is rather unusual. The phelloderm and the marginal parenchyma are full of starch so they may serve as storage tissues. There is no substantial difference between them except for the fact that the marginal parenchyma contains dark stained cells which are not encountered in phelloderm. The gall and the larva of *N. numismalis* remain active during the hibernal life below the cover of fallen leaves and markedly grow so the reserves of food accumulated in gall tissues in fall are very important for both [3].

The occurrence of the sclerenchymatic capsule protecting the larval chamber from damage is characteristic of almost all cynipid galls [3]. According to the classification of Meyer and Maresquelle [3], the capsule in *N. numismalis* is of the open type because during the hibernal growth of the larva and the gall, the upper and lower parts of the capsule gradually grow apart.

The peduncle connecting the gall with the leaf contains strands of tracheids or vessels with the helical thickenings of their walls. Albert et al. [23] observed newly differentiated tracheary elements in various galls and found them similar to those formed during cicatrization of wounds.

When the gall of *C. quercusfolii* is very young, its parenchyma resembles, to some extent, the palisade parenchyma of the typical leaf due to a very elongated shape of the cells. The scanning microscope allowed us to look inside the larval chamber. The globular cells in the chamber seem the most interesting. They have not been described by other authors. It is possible that these globules are just the cells full of nutrients, but what is the mechanism making them stick out of the internal wall of larval chamber? It can be speculated that high humidity and relatively stable conditions prevailing inside the larval chamber make the formation of such strange anatomical formations possible. As shown by our study, most of the cells lining the larval chamber are quickly destroyed. It seems strange that so young larva, which measures only about 0.5 mm, could accomplish so serious demolition of cells. Nevertheless, the position of larva is suggestive: it moves towards (and feeds on) still intact globules.

Interestingly, the anatomical structure of both described galls shows characteristic arrangement of tissues around the larval chamber, similar to that in other cynipid galls [3]. The larval chamber is lined with nutritive tissue and contiguous to it is the nutritive parenchyma. At first, the larva intensively feeds on the nutritive tissues and it seems probable that during the rest of its life it grazes on a thick stratum of cells which already have secondary walls.

The stratum of sclerenchyma protects the larval chamber from above and from below in the galls of both species. Apart from these similarities, both galls markedly differ. The gall of *N. numismalis* is small (approximately 2.5 mm in diameter in September or October), forms a garland of appendices, and shows characteristic arrangement of tissues: the cork, phellogen, and phelloderm. The *C. quercusfolii* gall is larger (when mature about 18–20 mm) and shows spongy, very loose parenchyma with giant cells. Therefore, the structure of these two galls essentially differs also from the structure of

the typical leaf. This means that the genetic information which controls their development must clearly differ.

What preliminary conclusions may be drawn from the anatomical investigations presented here?

As it was mentioned, the phenomena related to the gall initiation are of a very local character. They are limited to only one or a few cells near the place where the egg was deposited. The other cells of the leaf remain unchanged and develop normally. As it was shown for *Biorhiza pallida* [24], the oviposed eggs possess the properties of proteolysis, pectinolysis, and lysis of cellulose. Similarly, the first stages of the *N. numismalis* and *N. quercusbaccarum* gall development are connected with the lysis of the cells near the position of the egg [3]. Similar observations were made for *Diplolepis rosaefolii* [10]. Afterwards, the surrounding cells begin to multiply and the sequence of specific developmental events leads to the formation of an adult gall. The cynipid gall is the result of pathological morphogenesis which is no less complex than the morphogenesis of any typical organ of a plant [3,4].

Our commentary on these facts is as follows: it is well known that the fate of each cell in a leaf is strictly determined by the correlative influences coming from other neighboring cells [20]. Without such strict coordination, the leaf would never attain its precise flat shape and internal structure, so well adapted to its photosynthetic function. The gall-forming insect during the oviposition and first stages of larval growth abruptly breaks this coordination in one or a few cells; consequently, these cells start violent proliferation and develop according to a new developmental program different from that of a typical leaf. This program is executed consequently, leading to the formation of an organ-like entity.

It is almost impossible to imagine that such a precisely regulated developmental program could be performed without the injection of a large package of genetic material derived from the insect to the host-plant body. The injected genetic material is probably integrated with the genetic material of a plant and transmitted later to all derived cells during divisions.

Raman [7] explains the induction and formation of the gall in a different way. He states that "the insect saliva flushed on the wounded plant site causes chemical shock. This shock induces osmotic changes which establishes the first recognisable stage in gall induction. To repair the wound and neutralise osmotic change induced stress the plant responds by establishing from one to a few metaplasied cell(s). Localised metabolic changes spread from these cells […]". Further he states: "osmotic-change related stress […] triggers a sequence of plant mediated changes including synthesis of growth promoters.". Raman's interpretation of the mechanism of gall formation includes a possible role of DNA methylation and the role of the elements of broken walls as elicitors of the changes [6,7].

Until recently, the genus *Agrobacterium* [25,26] has been considered as a unique case of the gene transfer between phylogenetically distant organisms. It has been established that the transfer from the bacterium to the host plant is complex and consists of several stages. A well concerted action of both bacterial and host-plant factors, including several special proteins is observed [25,26]. It cannot be excluded that similar phenomena take place in the case of plant galls.

Another analogy, which may be useful for understanding the gall development, is the symbiosis between legume plants and the bacteria of the genus *Rhizobium*, leading to root nodule formation and assimilation of N_2. Both symbionts produce multiple signals that facilitate contact and finally the symbiotic union of both organisms. Consequently, serious morphological and physiological changes occur in both organisms. Several genes from both partners take part in this process [27]. Schönrogge et al. [28] even tried to find an analogy between a gene encoding the nodC factor/protein of *Rhizobium* and some sequences of the gall wasp genomic DNA. Of course, in the case of cynipid gall formation there is no symbiosis but parasitism.

One important problem still remains to be resolved: the gall development stops when the larva is injured or dies [2,6]. This means that besides the postulated injection of the insect-derived genetic material to the plant, a second factor responsible for gall formation

may exist. The larva quite constantly produces a substance(s) or another stimulus which supports typical vital functions of the gall and causes redirection of the nutrients flow towards it. This hypothetical additional factor is probably of a hormonal nature. The experiments with radioactively marked nutrients [6,29] showed that the larva and the tissues lining the larval chamber are the active sinks for nutrients. We do not know the nature of this phenomenon. Usually, the plant organ which produces auxin attracts the nutrients, and other hormones like cytokinins strengthen this effect [30–32].

The investigations on plant galls may essentially contribute to better understanding of leaf morphogenesis [33].

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