Introduction

Galls have fascinated and intrigued generations of biologists because, among other reasons, each of the species leading to their formation produces a morphologically specific and often very complex anatomical structure. Gall development has an effect on the anatomical structure of the host organs (Isaias et al. 2014). Galls on plants grow through induction by several different groups of organisms, among them microorganisms, nematodes, and mites as well as more than 13,000 species of insects (Espírito-Santo & Fernandes 2007). Galls produced by insects are the result of the special relationship between them and the host plant, and the ability to form galls is one of the most efficient strategies for the use of plants by herbivores (Roskam 1992). The development of host cells is characterized by a species-specific structure induced by the gall insect, in which the larvae growing inside obtain shelter, food, and protection against both natural enemies and abiotic factors (Rohfritsch & Anthony 1992). The characteristic gall morphology often can determine the genus or species of the insect that created the gall. The role of galls is explained via three hypotheses: nutrition hypothesis (the body that causes the formation of a gall), protection hypothesis (the role of galls is to protect against the influence of the microclimate), and metabolism hypothesis (the cells that cause the gall uses and regulates the nutritional value of the plant tissues for their needs), microenvironment hypothesis (the gall protects against the influence of the microclimate), and protection against enemies hypothesis (assumed to reduce mortality in a population of gall inducers) (Price et al. 1987). Gall ontogeny is a set of complex interactions between the cells of plants and organisms that cause the formation of a gall (Rohfritsch 1992). They induce cytogenetic and morphogenetic changes, which together lead to the formation of a gall (Mani 1964). The host tissue is highly modified and incorporated into the structure of the developing gall to form an inseparable whole.

Gall development can be divided into three main phases: initiation, growth and differentiation, and maturation. In fully differentiated galls caused by Cynipidae, the larval chamber is usually lined with feeder cells, which are surrounded by a layer of sclerenchyma cells, closing the chamber (Stone et al. 2002). Nutritive tissue consists of large thin-walled cells in which the protein content and physiology is similar to the seed (Hartley 1998; Schönrogge et al. 2000). These cells, to a certain point, are the exclusive larval food source (Shorthouse & Rohfritsch 1992). Larval chambers are similar in all Cynipidae, but there are huge differences in the construction of the external tissues of the gall (Shorthouse & Rohfritsch 1992).

In studies of galls very important is the answer to the question of how galls are constructed and what their structural relationship with the plant–host is. Although there has recently been a significant increase in researchers interested in the anatomy of galls (Meyer & Maresquelle 1983; Alvarez et al. 2009; Oliveira & Isaias 2010; Oliveira et al. 2011), we still lack information about the structure of galls produced by certain species. Despite the substantial amount of literature, data on the galls formed by the representatives of the Cynipidae and knowledge about gall anatomy and histochemistry are still very modest, even in the case of species forming galls as large as Cynips quercusfolii. Knowing gall construction and its connection with the leaf may also be important to study the physiology of leaves, particularly in species in which galls are abundant and have the potential to modify the functioning of the leaf. The aim of the study was to characterize the anatomical traits of galls in the development stage formed by C. quercusfolii on sessile oak leaves.

Materials and methods

Leaves of the sessile oak (Quercus petraea (Mattuschka) Liebl.) with galls caused by the agamic generation of the gall wasp (C. quercusfolii L.) were collected in July of 2011 (Figure 1). This period is optimal because galls have already reached almost the maximum size, and the larval chamber is still very small so at cross sections one can see the whole gall, undamaged by the development of the larvae. The
concentrations of ethanol, embedded in Paraplast Plus® and sectioned at room temperature for over 24 h according to Ruzin (1999). After removing the Paraplast Plus® by xylene, the sections in microscope. Cells on the tubercles part of gall were similar as the cells in different parts of the gall outside the bulges.

Slight tubercles occur on the surface of the gall. These bulges are visible also on sections in microscope. Cells on the tubercles part of gall were similar to the cells in different parts of the gall outside the bulges.

The gall surface is covered with small raised tubercles. These structures are visible on sections in microscope. Cells on the tubercle part of the gall were similar as the cells in different parts of the gall outside the tubercles. Gall is initially green, but develops a red or pink blush as it matures, particularly when the gall is in the sun. The larval chamber is located in the center of the gall, 3–4 mm in diameter and thin-walled. Parasitized galls are commonly much smaller, often slightly misshapen and without the characteristic surface markings or texture. Near the gall (area from the gall to the edge of the leaf blade), we found a change in the color of the leaf. It was clear yellow, while the rest of the leaf was morphologically intact and undamaged and was characterized by an intense green color. The structure of the oak leaf was typical. It was possible to identify the upper epidermis, palisade parenchyma, spongy parenchyma, lower epidermis, on which stomata occur (Figure 2), leaf vein, without galls covering the epidermis; underneath we distinguished the collenchyma, parenchyma, and ring of sclerenchyma, and inside of this ring, the vascular tissue (Figures 3 and 4). The leaf surface was covered with the cuticle (Figures 2 and 3). The veins connected with the gall had a modified anatomy (Figures 5 and 6). Also is visible the variation of cells and tissues; the hypertrophy and the hyperplasia of tissues are visible. The collenchyma cells were located under the well-developed upper epidermis. In the central part of the vein, a wide, multicellular sclerenchyma band created a stable connection between the gall and leaf. The outer layers of the vein near the gall, consisting of the sclerenchyma cells, form a continuous outer sclerenchyma of the gall. Vascular bundles, shifted to the adaxial side, were visible in a longitudinal section through the leaf vein, and the area they occupied was reduced. Well-developed vascular bundle strands were adjacent to the core cell sclerenchyma and passed to the gall (Figures 7–9). The leaf developed vascular bundles, which were formed continuous with the bundles of the gall. The sclerenchyma exhibits strong expanding, and the lower side of the vein has become the basal part of the gall (Figures 5–9).

Anatomical preparations indicate the characteristic connection of the gall with the leaf. Leaf vein tissues are highly modified. In the central part of the vein, a broad band of sclerenchyma cells that make up the continuity with the sclerenchyma band is visible at the base of the gall (Figures 5–9). Continuity of the leaf vascular system with the gall is also retained (Figures 7–9).

Galls on longitudinal and cross sections have a circular or slightly oval shape. Galls observed by light microscopy are characterized by differentiated tissue structure; various tissue types take the form of rings with an uneven thickness. An irregular chamber is inside the gall (Figure 9). Galls on the outside are surrounded by the epidermis. The gall epidermal tissue is homogeneous and monolayered; there were neither hairs nor stomatal cells within it. Thick-walled epidermal cells are alive and are the same size, and the cell walls are lignified (Figures 9–13). The epidermis is covered by a thin layer of cuticle. The number of epidermal cells observed by light microscopy revealed large vacuoles, which stain in an intensely dark color, suggesting the presence of tannin compounds.

A continuous ring of thick-walled cells, forming the outer layer of the sclerenchyma, is under the epidermis (Figures 10–12, 14). This layer is almost the entire circumference of the width of two to three cells and enlarges only in the basal part. There is a clear continuity of the sclerenchyma layer of the leaf nerve and gall (Figure 7). The next layer is a wide ring of thin-walled, non-lignified parenchyma cells (Figures 10, 11, 14, and 15). The vascular bundles are located among them (Figure 15). These cells have a structure that is

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**Results**

It is unilocular, usually in groups on the abaxial surface of the leaf; spherical, reaching a diameter of 15–25 mm when mature. Galls were formed on fully developed leaves (Figure 1). The surface texture and density of the gall tissue vary significantly across oak hosts (and hence geographic regions). The typical gall has a parenchyma soft and spongy, and the gall is easily compressed between finger and thumb. The gall surface is covered with small raised tubercles. These structures are visible also on sections in microscope. Cells on the tubercles part of the gall were similar as the cells in different parts of the gall outside the tubercles. Gall is initially

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**Figure 1.** Spherical-shaped, yellow-green gall (*Cynips quercusfolli*) localized at the abaxial surface of the leaf blade on the lateral vein on fully developed leaves. Slight tubercles occur on the surface of the gall. These bulges are visible also on sections in microscope. Cells on the tubercles part of gall were similar as the cells in different parts of the gall outside the tubercles.
typical of the parenchyma; we observed many starch grains. Within the parenchymatic ring, we can distinguish the sub-layer of cells containing numerous amyloplasts (Figure 14). The number of cells containing starch as well as the number of amyloplasts per cell increases toward the larval chamber (Figure 14). There are two areas with particularly large numbers of amyloplasts. The first is located on the outer side of the inner sclerenchyma layer and creates a ring of cells with uneven thickness, which is particularly wide over the larval chamber. A second region with a large number of amyloplasts

Figures 2–9. (2) Cross section through the part of the leaf blade without a gall. The bifacial leaf is composed of palisade mesophyll (the adaxial part of chlorenchyma) and spongy mesophyll (the abaxial chlorenchyma). Lignin autofluorescence in vascular bundles (vb), stomata (arrow) in the lower epidermis (le), and tannin autofluorescence in the upper epidermis (ue) are visible. CLSM, Bar = 50 µm. (3) Cross section through the normal midrib. Blue – autofluorescence (excitation UV) – mainly lignin; Red – autofluorescence (excitation 488 nm) – mainly chlorophyll. CLSM, Bar = 400 µm. (4) Cross section through the normal vein. Blue – autofluorescence (excitation UV) – mainly lignin; Red – autofluorescence (excitation 488 nm) – mainly chlorophyll; ub – upper epidermis, le – lower epidermis, sc – sclerenchyma, xy – xylem. CLSM, Bar = 300 µm. (5) Leaf vein with basal part of gall; anatomy of vein strongly modified, centrally located sclerenchyma cells (red), forming a connecting pin with the gall. Tissue continuity of vein and gall is clearly visible. Staining by safranin (red – lignin) and fast green (green – cellulose cell wall and live protoplast), LM, Bar = 250 µm. (6) Same piece as Figure 5, strong autofluorescence of lignin (excitation UV). CLSM, Bar = 250 µm. (7) Basal part of the gall. Red – lignified cell walls, green – cellulose cell walls and live protoplasts. e – epidermis, pc – parenchyma, sc – sclerenchymatic pin, * – nutrient tissue, cells of parenchyma full of amyloplasts, LM, Bar = 195 µm. (8) Enlarged detail of Figure 6. Red – cells of sclerenchyma around vascular bundles, xylem tracheary elements are visible (arrow). LM, Bar = 90 µm. (9) Same piece as Figure 6. Lignin autofluorescence is visible (excitation UV), xylem tracheary elements with characteristic thickenings (arrow). CLSM, Bar = 90 µm.
in the cells is located on the inner part of the inner sclerenchyma ring in the basal region of the larval chamber (Figures 7, 14, 16, and 17). Amyloplasts fill almost the whole cell (Figure 17). This part of the gall is a nutritive tissue for the larvae. The nutritive tissue is interrupted and irregular and provides the inner layer of parenchyma cells, which pads the larval chamber. Inside the chamber, we can also find single degeneration of such cells or groups of cells. Inner parenchyma cells are adjacent to sclerenchyma cells with thick, lignified cell walls, which form the inner ring of sclerenchyma with an uneven thickness, the interior of which is the larval chamber (Figures 6, 7, 14, and 16).

Discussion
The design of the gall based on concentrically arranged layers seems to be widespread (Stone et al. 2002). A similar plan of gall structure to *C. quercusfolii* was reported for *Neuroterus*...
**quercusbaccarum** (Koncz et al. 2011), *Diplolepis rosaefoli*, (LeBlanc & Lacroix 2001) and *Schizomyia macrocapillata* (Sá et al. 2009). In the structure of the gall formed by *C. quercusfolii* on the leaves of *Q. petraea*, we have distinguished: (1) the protective ‘first contact zone’ created from epidermal cells containing tannin compounds and a sub-epidermal sclerenchyma ring, (2) the wide parenchymatous zone, (3) the internal protective zone created by the sclerenchyma ring, and (4) the nutritional zone consisting of cells filled with amyloplasts containing starch. Such a gall structure seems to be quite versatile and optimally provides developing larvae both a safe haven and a constant source of high-energy food. The analyzed galls were located only on the abaxial (lower) side of the leaf blade, usually on the lateral leaf veins. Giertych et al. (2013) reported that galls were located at a fixed distance from the edge of leaves irrespective of leaf size, but the distance from the gall to the leaf petiole depended significantly on leaf size. They suggested that location is optimal for galls in order to receive the right amount of nutrients.

There were no differences in the anatomical parts of the leaf blade near the gall and unchanged parts of the leaf. In both cases, in the cross section of the leaf blade, the typical leaf cell system was visible. However, both the leaf discoloration observed in the neighborhood of the gall and the chemical analyses performed indicate a physiological change in the region (Giertych & Karolewski 2014).

The structure of the vein, which is located on the gall, is highly modified. It expands the sclerenchyma, which is located on the lower side of the vein, and forms a kind of stable stand, which contains the gall, creating a stable connection of the gall and leaf. The gall, in the form of a sphere, is raised above the surface of the leaf, creating a close anatomical and physiological relationship with the leaf. The continuity of the conductive tissues of the leaf and the gall ensure the proper supply to the developing gall (Kenoyer 1936). Due to the interaction of the plant and insect, the gall is built from typical plant tissue and becomes an integral part of the leaf.

The galls of *C. quercusfolii* are characterized by their green–yellow color when there are young, which may indicate...
the presence of chlorophyll; however, in our preparations, we have not identified any chloroplasts. Significantly lower levels of chlorophyll-related compounds in the leaf tissue were also noted in the gall tissue that is caused by some Cecidomyiidae species (Huang et al. 2014). Lack of chloroplasts means that the tissues of galls live at the cost of the remaining assimilative organs of the plants. During growth, the gall is a great sink for nutrients and carbohydrates (Stone et al. 2002). On the other hand, amyloplasts were observed in many of the parenchyma cells. These regions, in which the cells are filled with amyloplasts, are storage parenchyma. The central cross section of the gall is clearly marked with a gradient of starch, which increases toward the larval chamber. The high concentration of starch in the reserve tissue of galls was noted also for Cecidomyiidae. However, in that case, the concentration decreased toward the larval chamber, and there was a lack of starch in the nutritive tissue (Koncz et al. 2011; Oliveira et al. 2011). In our study, we also found the presence of amyloplasts in the nutritive tissue. It has not been widely reported that there is an accumulation of starch in the nutritional tissues of galls produced by Cynipidae. The gradient of nutrients from the larval chamber toward the border gall (decreasing in the case of proteins and lipids and increasing the gradient to the stalk) was shown by histochemical methods for the gall caused by N. quercusbaccarum (Koncz et al. 2011). Gall tissue concentrates photoassimilates by mobilizing these resources from neighboring regions of the plant. This makes it possible to prepare for developing C. quercusfolii larval stocks as starch stored in the amyloplasts. It creates the same kind of energy bank, in which resources can be released gradually, depending on the needs of the growing insect. These resources are located partially inside the larval chamber (number of cells with amyloplasts), making it especially easy to access. The presence of galls strongly modifies the functioning of the leaf, and the gall may even cause increased photosynthesis productivity of the infected plant (Fay et al. 1993). It seems that the main source for starch synthesis is supplied with leaf assimilates. Galls embedded directly on the vein, which are integrated with the leaf tissue (also conductive), would be a kind of sink, taking the glucose, which is a product of photosynthesis, of the leaf parenchyma (Janikiewicz et al. 1970). On the other hand, the green color of the gall indicates that, in these tissues, photosynthesis can also occur. A centripetal starch gradient was found in the gall. An increasing number of grains of starch were observed near the larva chamber in the parenchyma cells.

Conclusions

As a result of the interaction of insects and plants, we established the structure with a characteristic anatomy, which is an integral part of the leaf. The creation of the gall had no destructive effect on the leaf structure. Anatomical changes were found only on the vein, which was embedded with the gall. The continuity in the tissues in the close anatomical relationship between the leaf vein and the gall has been shown. Extensive vein sclerenchyma creates a kind of picket connecting the gall with the leaf and providing stability. Galls are characteristic anatomical structures. Their structure can be divided into the epidermis, the sclerenchyma sub-epidermal zone, a broad zone of parenchyma, the inner ring of sclerenchyma, and the nutritional zone surrounding the larval chamber. Storage material in the developing gall is starch, which is probably the result of both the exports from the leaf as well as a product of photosynthesis in the cell’s gall parenchyma. A centripetal starch gradient was found in galls, and its contents increased from the gall edge toward the larva chamber, where the highest content was observed in the parenchyma cells in the basal part of the larval chamber.

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