ABSTRACT

A Descriptive Study of Protective Tissue Formation in
Stems of *Quercus buckleyi* Nixon & Dorr (Fagaceae)

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The epidermis and periderm are two components of the protective tissues that cover plant stems and roots. The epidermis is the first protective layer of the plant, and is later replaced by the periderm, which is typically composed of three different tissues: phellogen, phellem, and phelloderm. The objective of my study was to observe and describe the developmental process of protective tissues in stems of *Quercus buckleyi*, commonly known as Texas Red Oak. Scanning Electron Microscopy (SEM) is used in this study to examine *Q. buckleyi* specimens. The epidermis is composed of a single layer of cells that divide anticlinally to compensate for increased diameter. The meristematic phellogen is initiated in the subepidermal layer. The phellogen undergoes both periclinal and anticlinal divisions to create new cells for the periderm. Phellem matures to the outside of the phellogen and is thick walled. It eventually causes the epidermis to slough off during later stages as the periderm replaces the epidermis. No phelloderm to the inside of the phellogen is apparent in the stems of *Q. buckleyi*.

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A DESCRIPTIVE STUDY OF PROTECTIVE TISSUE FORMATION IN

STEMS OF *QUERCUS BUCKLEYI* NIXON & DORR (FAGACEAE)

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By

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CHAPTER ONE

Introduction

Literature Review

Periderm is a layer of secondary tissue in plants that functions as a protective layer for the plant. It serves as a physical barrier for protection against harmful substances such as pathogens and sunlight, as well as prevents desiccation of the plant through its ability to control gas and water exchange. Periderm is composed of three tissues and develops according to several different models, which will be discussed later in greater detail. As the periderm matures it causes the sloughing off of the epidermis, a protective layer formed in early embryonic development (Esau, 1977; Mauseth, 1988).

The epidermis is the outermost layer of the plant and serves as the first layer of protection for the plant during primary growth. Its cells differentiate from the protoderm, one of the three primary meristems that arise from the apical meristem of a plant. It is the interface between the young plant and the environment, and is composed of several cell types including regular epidermal cells, guard cells, and epidermal hairs (trichomes). Epidermal cells are tightly packed together as a way to provide structural support and protection to the plant. Guard cells are found in green plant parts such as leaves and green stems, and surround stomatal airway pores that regulate gas and water exchange. In addition, the epidermis is covered with an outer cuticle that helps prevent water loss. Trichomes are present on younger stems and leaves as another form of protection. The overall function of the epidermis is to protect against harmful agents and participate in the control of water and gas exchange. However, since the epidermis is typically only a single layer of cells it must be replaced by the periderm as the plant increases in diameter.

The periderm, on the other hand, continues to thicken over the course of the plant's lifetime (Esau, 1977; Mauseth, 1988).

As the periderm thickens and replaces the epidermis, it reveals the distinct characteristics of its various layers. The periderm is composed of three tissues: the phellogen (cork cambium), the phellem (cork cells), and the phelloderm. The phellogen has one type of cell, parenchyma cells that are polygonal and flattened radially. It is a lateral meristem that produces both the phellem (to the outside) and the phelloderm (to the inside) by periclinal cell division. It forms initially in localized patches, and with time creates a continuous layer around the plant. While phellogen can arise from parenchyma cells in various areas of the plant, the most common locations for phellogen formation are in the roots and shoots of plants, and the first formed phellogen generally arises just beneath the epidermis (Esau, 1977; Mauseth, 1988).

Phellem is produced on the outer side of the phellogen and contains cells that are more variable in shape and size. While their shape is primarily prismatic, they can easily be elongated vertically, radially, or tangentially within the tangential plane. These cells are packed tightly together and do not have much intercellular space between them. Phellem cells contain a polymer called suberin, which creates a thick suberin layer inside their primary wall. Suberin is an impervious barrier that does not allow water to pass due to soluble lipids in the suberin lamellae as opposed to in the suberin itself. In addition to affecting the cell's permeability to water, suberization of cell walls allows for resistance to chemicals and enzymes that may attack the plant. When phellem cells reach maturation, the protoplasts die, making the layer characteristically composed of dead cells. As the outermost layer, this most likely happens because it is difficult to transport

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nutrients to these cells and is more energy efficient for these cells to not require nutrients and water (Esau, 1977; Mauseth, 1988).

The phelloderm is the layer of cells on the inner side of the phellogen that are positioned in radial files with the phellogen and phellem cells. Phelloderm is a living layer of cells produced by some phellogens in only small amounts, or is not produced at all in some plants. It is typically made of just one thin layer, but can be three to four layers at times. The phelloderm has various roles depending on the type of plant it is in, and looks different depending on whether it is unistratose or multistratose. Unistratose phelloderm is hard to distinguish from the parenchyma that gives rise to the phellogen because it is very thin, whereas multistratose is easily recognizable because the cells form radially with the phellogen cells. The cells can produce for several years in these radial files, indicating that the new cells arise from a division of the cells of the phelloderm from the previous year. In various plants, the phelloderm can produce schizogenous secretory ducts. (Esau, 1977; Mauseth, 1988).

Due to the impermeable nature of the cells in the phellem, plants must have a way to allow for gas exchange between the environment and the inner cells. This is made possible through lenticels, where the cork cells that are produced become rounded so as to pull away from their surrounding cells and create spaces for air. This spongy area is referred to as filling tissue, and the rounded cells are referred to as complementary cells. The lenticels are first created at the same time, or very close to the same time, as the first periderm. They first arise from parenchyma beneath stomata, and with time push outward to rupture the cortex that separates them from the environment. As time goes

on, the lenticels grow deeper into the cortex as well in order to provide gas exchange for the inner cells (Esau, 1977; Mauseth, 1988).

The formation of periderm begins with the development of the phellogen, which gives rise to both the phellem and the phelloderm. The phellogen is typically formed during the first year of growth for the plant, but the specific timing is dependent on the species and environmental effects such as temperature, water availability, and light intensity (Esau, 1977; Mauseth, 1988). Another factor that impacts the timing of phellogen formation is photosynthetic activity in the plant. High levels of photosynthesis can cause a delay in phellogen initiation. Additionally, in a study of periderm development in *Robinia pseudoacacia*, it was found that environmental conditions not only affect the timing of phellogen initiation but can also affect the specific site of initiation (Arzee, Liphschitz & Waisel, 1967).

Once phellogen development has been initiated, living parenchyma cells become meristematic giving rise to the phellogen tissue. This can happen in almost any parenchyma cell, including those in the epidermis, hypodermis, cortex, phloem and xylem (Esau, 1977; Mauseth, 1988). The first phellogen is generally produced during the first year of growth from the subepidermal layer. For example, in *Robinia pseudoacacia* the first phellogen differentiates in the seedling stage from collenchyma cells in the second or third layer beneath the epidermis (Arzee, Liphschitz & Waisel, 1967). Subsequent layers of secondary phellogen can be produced several years later and arise out of deeper layers, including the parenchyma of secondary phloem. Subsequent layers of phellogen tissue are important for replacing the first phellogen, which is pushed outward as the vascular tissue of the plant grows, resulting in increased diameter.

Meristematic parenchyma cells give rise to phellogen uniformly in localized patches at first, and then eventually form a continuous layer as the meristematic cells spread their activity laterally. Lower subsequent layers of phellogen are not always continuous, but can be overlapping sheets of discontinuous layers, often situated beneath the cracks of upper discontinuous layers. Once the phellogen is produced, it becomes meristematic and undergoes a series of divisions in order to produce the phellem and the phelloderm. The primary form of division is periclinal division to the inside and outside of the phelloderm. Upon initial division, the inner cell becomes part of the phelloderm, which is a thin layer made up of few cells, while the outer cell remains as a phellogen cell. Sometimes the phellogen can remain just a single layer of cells that then continue with periclinal divisions to produce the phellem toward the outside of the plant. The amount of phellem and phelloderm produced is variable in different plants. Once the phellem and phelloderm are produced various changes occur to each as they become more characteristic of their function (Esau, 1977; Mauseth, 1988). In *Acacia raddiana* brown-colored tannins enter phellem cells and the cells also become compressed. Additionally, the phelloderm has crystal-containing layers that become sclerified after its formation (Arzee, Waisel & Liphschitz, 1969).

In addition to periclinal divisions, the phellogen also undergoes anticlinal divisions to compensate for the increasing diameter of the plant. As the plant grows and diameter increases, the production of secondary phellogen is initiated in the deeper layers of the plant. This is important because as the old tissue is pushed outward it is cut off from nutrients and is unable to survive. This causes a division of a living and dead tissues, which makes up the inner and outer bark. The inner bark consists of the living,

meristematic tissue, while the outer bark is dead and is known as the rhytidome. The

rhytidome serves as the primary protective layer against the external environment (Esau,

1977; Mauseth, 1988).

Objectives

Texas Red Oak, *Quercus buckleyi* Nixon & Dorr (Fagaceae), is a small to

medium sized tree with dark grey bark that is somewhat smooth and furrowed, and

produces thin, grayish or brownish twigs. While the development of protective tissues

has been investigated in several different *Quercus* species, no studies have been located

examining the production of protective tissue layers in *Q. buckleyi*.

The objectives of this study are:

1. To examine the protective tissues found in stems of *Q. buckleyi*

2. To observe cell division in the epidermal and subepidermal layers

3. To describe epidermal formation from its early stages to later replacement by the

    periderm

4. To describe the stages of periderm initiation and maturation

5. To compare protective tissue development in *Q. buckleyi* with other species

    previously studied.

Previous studies of the genus *Quercus* reveal that the phellogen in *Quercus suber*,

which produced in commercial cork, produces several millimeters of phellem as opposed

to other species, which typically only produce a few layers of phellem (Fahn, 1990).

Therefore, the bark of *Q. suber* is much thicker than stems of other plants. Another study

showed that in some *Quercus* species the periderm first develops beneath epidermal

hairs, which may serve as centers of periderm initiation (Arzee, Kamir, & Cohen, 1978).

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In addition, the first phellogen in *Q. suber* is thought to develop in the epidermis itself (Fahn, 1990). In some species of *Quercus* such as *Q. ithaburensis* and *Q. infectoria*, phellogens are only active for the production of the first phellem. After this, the phellogens activity may be linked with vascular cambium activity (Arzee, Kamir, & Cohen, 1978).

CHAPTER TWO

Materials and Methods

Specimen Collection

Specimens of Texas Red Oak, *Quercus buckleyi* Nixon & Dorr were collected in April 2011 and September 2011 in McLennan County, Texas. Several shoots were cut from the tree then placed in a 0.1 M sodium phosphate buffered 4% gluteraldehyde, 2% formaldehyde solution for preparation. The leaves were removed from the shoots and the shoots were then cut into 0.5 cm sections and separated in to five groups, each placed in a separate vial.

SEM Specimen Preparation

Once separated into 5 vials, specimens underwent primary fixation in a 0.1 M sodium phosphate buffered solution containing 3% gluteraldehyde and 2% formaldehyde at a pH of 7.0 for 2-4 hours at room temperature and were then placed under vacuum for 7 minutes to aid in the fixation process. After primary fixation, the specimens were stored in fixative in the refrigerator for later processing. The specimens were removed from the refrigerator and transferred into 0.1 M sodium phosphate buffer. Several sections from each vial of specimens were cross-sectioned and dehydrated by an increasing acetone series of 30%, 50%, 70%, and 100% acetone. Each increase in concentration of acetone was made after 10 minutes at the previous level.

After dehydration, the samples were critical point dried with liquid CO_2 in a Denton Vacuum DCP-1 critical point drying apparatus. Each group of samples (vials 1-5) was mounted on a large aluminum stub using double-sided tape, edged with silver

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colloidal paint. Stubs were then coated for 75 seconds in a Denton Vacuum Desk II gold

sputtterer. After coating, micrographs were taken using a JEOL JSM 5410 scanning

microscope. When not being viewed, samples were stored in a desiccator.

CHAPTER THREE

Results

Epidermis

The epidermis is the outermost layer of cells in the stem and is composed of a single layer of cells (Figure 1). In this early stage, epidermal cells are not undergoing periclinal or anticlinal division, which is evidenced by the fact that each individual epidermal cell has its own thickened outer wall covering above it. Additionally, there are no divisions occurring in the subepidermal layer, as the cells are compacted together.

Epidermal cells divide anticlinally to keep pace with an increase in stem diameter during early development (Figure 2, right arrow). Anticlinal divisions produce two daughter cells with a common outer cell wall. Figure 3 clearly shows an anticlinal division in the epidermal layer that created two daughter cells with the same outer cell wall. The dividing cells are significantly smaller than the cells to the left and right, but will grow in size until they are mature cells.

Periderm Initiation

Periclinal divisions in subepidermal cells likely mark the initiation of the periderm (Figure 2, left arrow; Figure 4). A thin wall can be seen between the two newly formed cells, which are smaller in size compared to the other cells in the same layer. Combined, the two daughter cells have the same height as each of the cells to the right and left of them. The periclinal divisions that initiate the periderm add layers of cells to the stem and contribute to the overall increase in stem diameter.

As the stem increases in diameter due mainly to vascular cambium activity and the production of secondary vascular tissues, cell divisions in the epidermis and subepidermis are not rapid enough to compensate. Therefore, in addition to cell division, cells also pull away from each other in the deeper layers allowing for intercellular spaces to be formed (Figure 5). These spaces help increase the diameter of the cell, while the epidermal and subepidermal layers continue to have anticlinal divisions in order to compensate for this increased diameter.

As the stem continues to grow and increase in size, the cells continue to divide in an anticlinal and periclinal fashion (Figure 6). The meristematic phellogen produces radial files of thick walled phellem. The row of dividing cells (indicated by arrows) reveal the meristematic layer of the periderm and the cells it produces. The inner of the two dividing cells is the phellogen, which produces phellem, the outer cell of the dividing cells. With time, each phellem cell will eventually undergo death of its cytoplasm and thickening of its cell wall, as it becomes a dead layer of cells cut off from nutrients and water. The inner phellogen cell will go on to produce more phellem cells until it too becomes inactive. Anticlinal divisions (Figure 6; right arrow) allows for the continual compensation for the increased diameter of the stem. No protective phelloderm layers inside the phellogen were observed in *Q. buckleyi.*
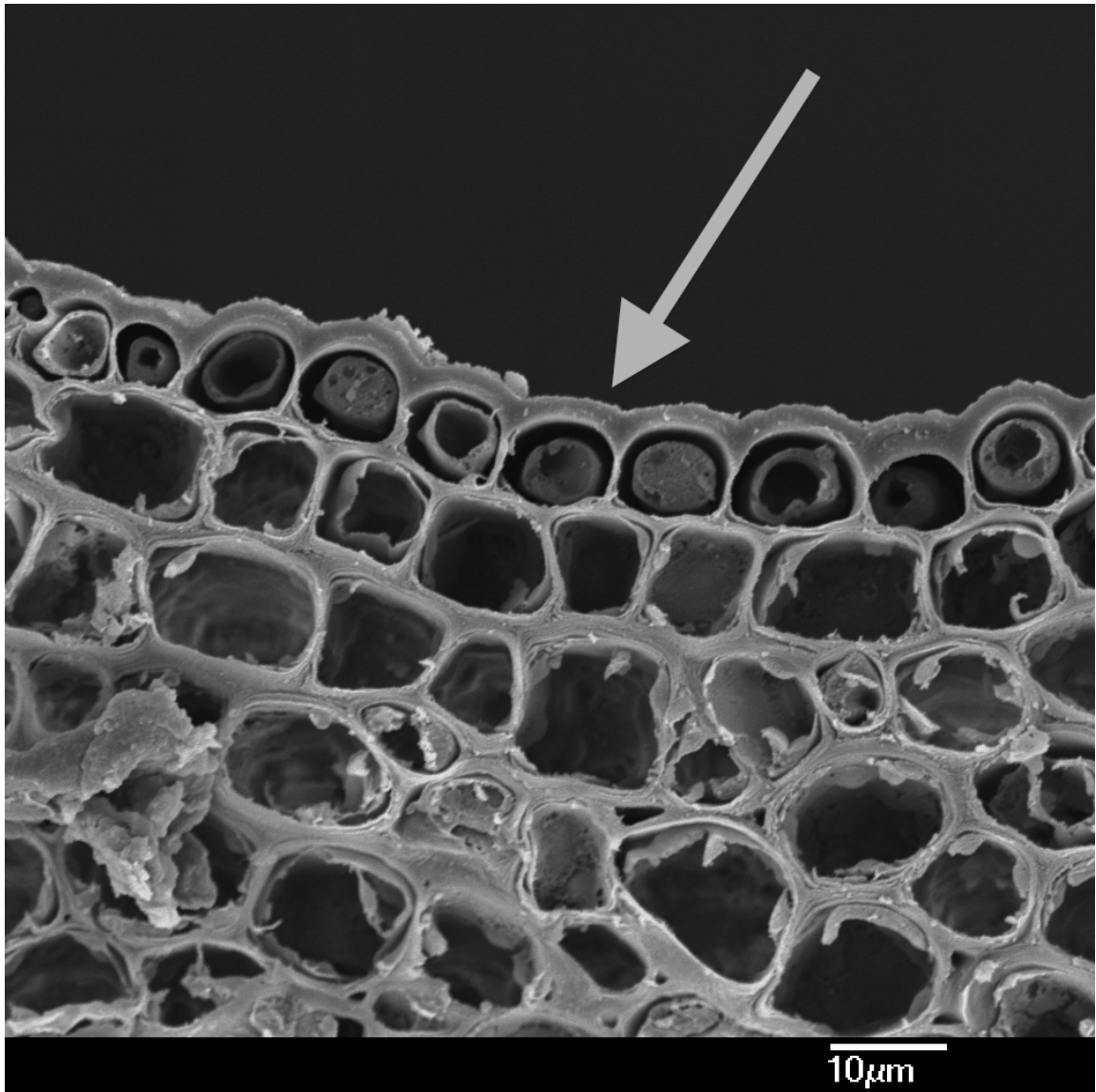
Figure 1 Early stage of stem development. The epidermis is composed of a single layer of rectangular cells with thickened outer wall. At this stage, no additional cell divisions in epidermal cells are evident. The subepidermal layer of cells is compact with no divisions.
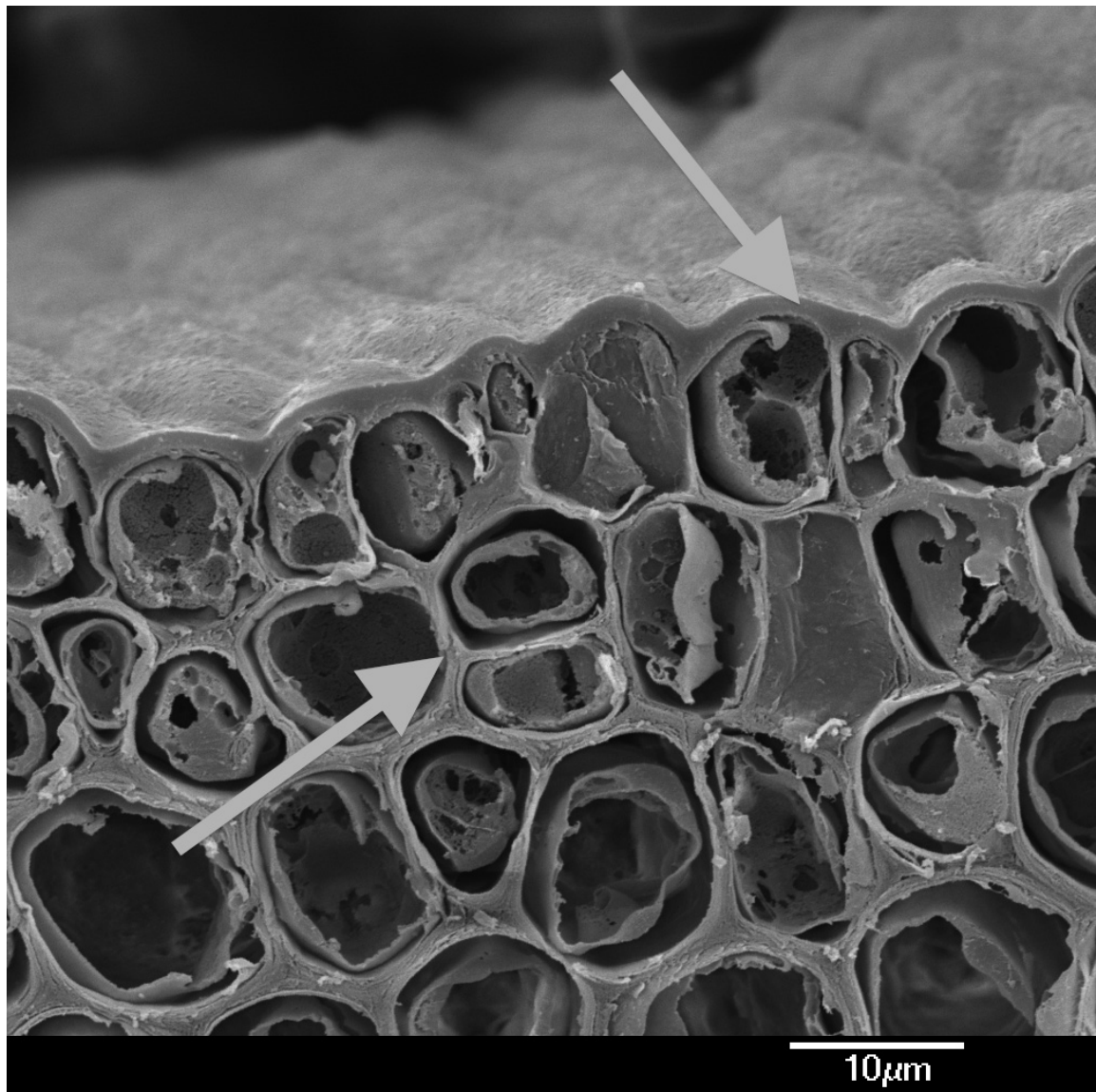
Figure 2 Epidermis and early periderm formation. Right arrow: Anticlinal division in the epidermal layer allows the epidermis to expand in diameter to keep pace with expanding stem diameter. Left arrow: Periclinal division in the subepidermal layer begins the formation of the periderm.
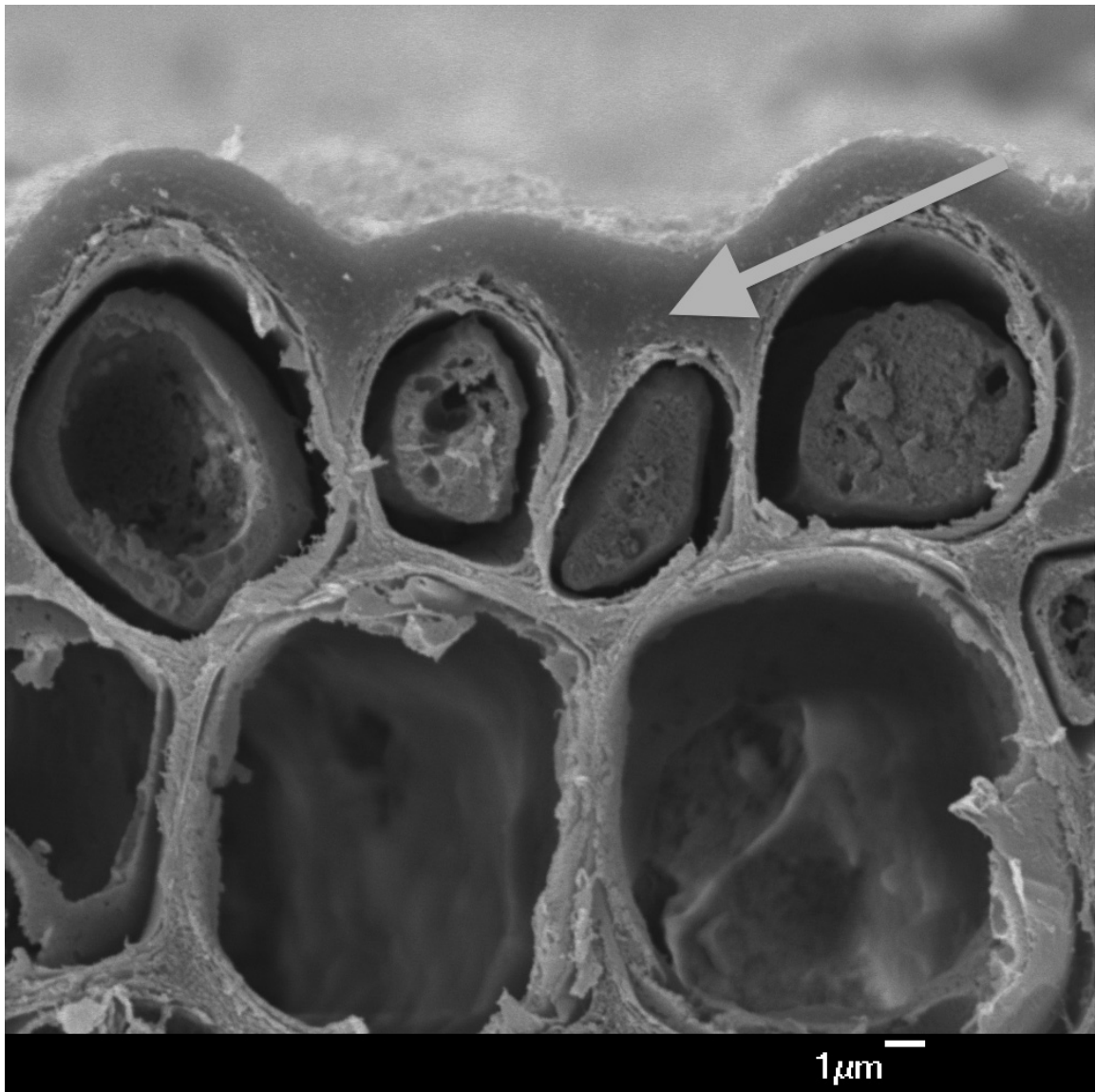
Figure 3 Anticlinal division in the epidermal layer (arrow). Division evidenced by small adjacent cells beneath the same thick outer wall covering. This type of division allows for expansion of the epidermal layer.
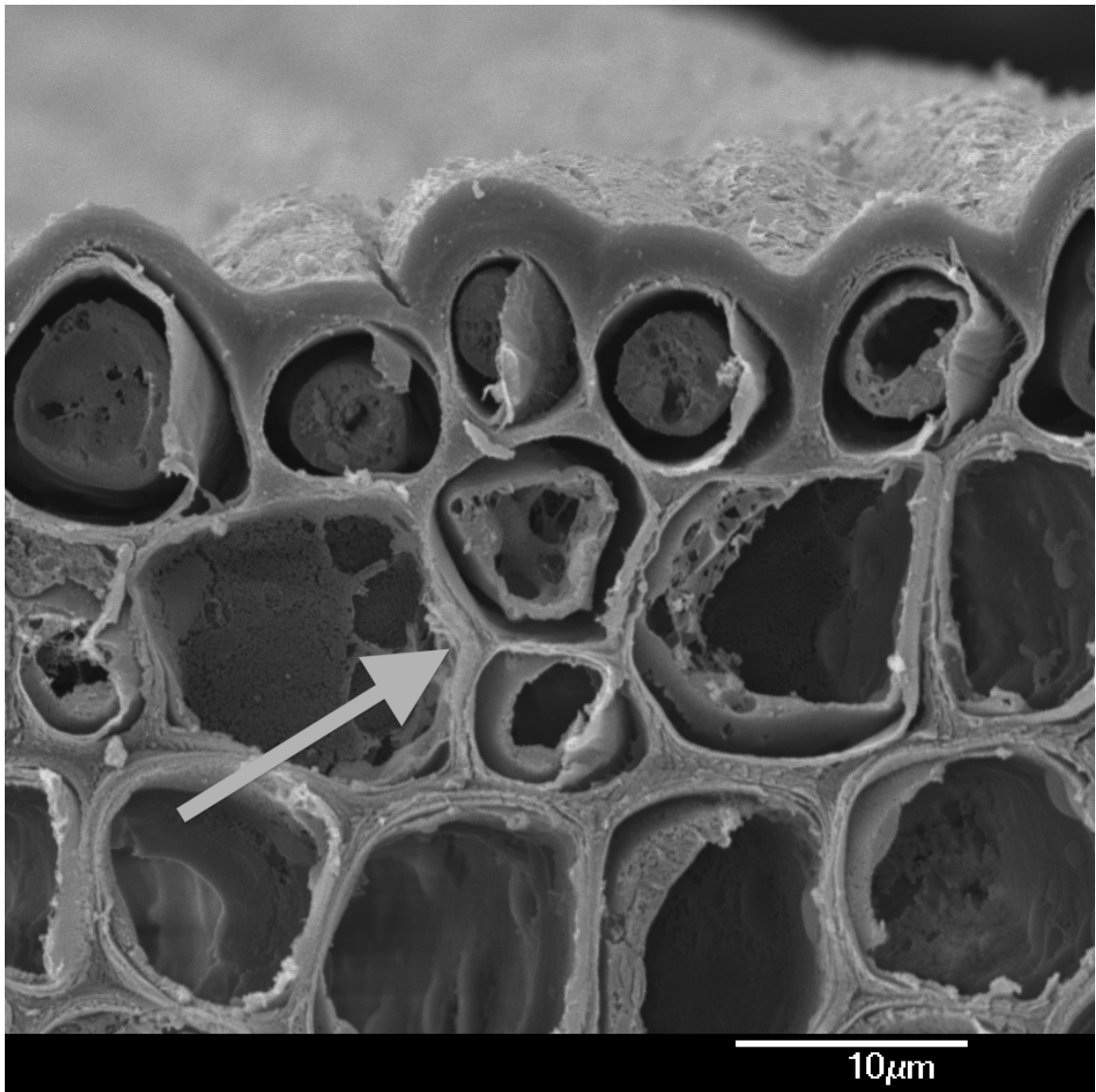
Figure 4 Periclinal division in the subepidermal layer (arrow). Division evidenced by thin cell wall between small cells and combined height similar to that of mature cells to left and right of dividing cells. This division signals the beginning of periderm formation.
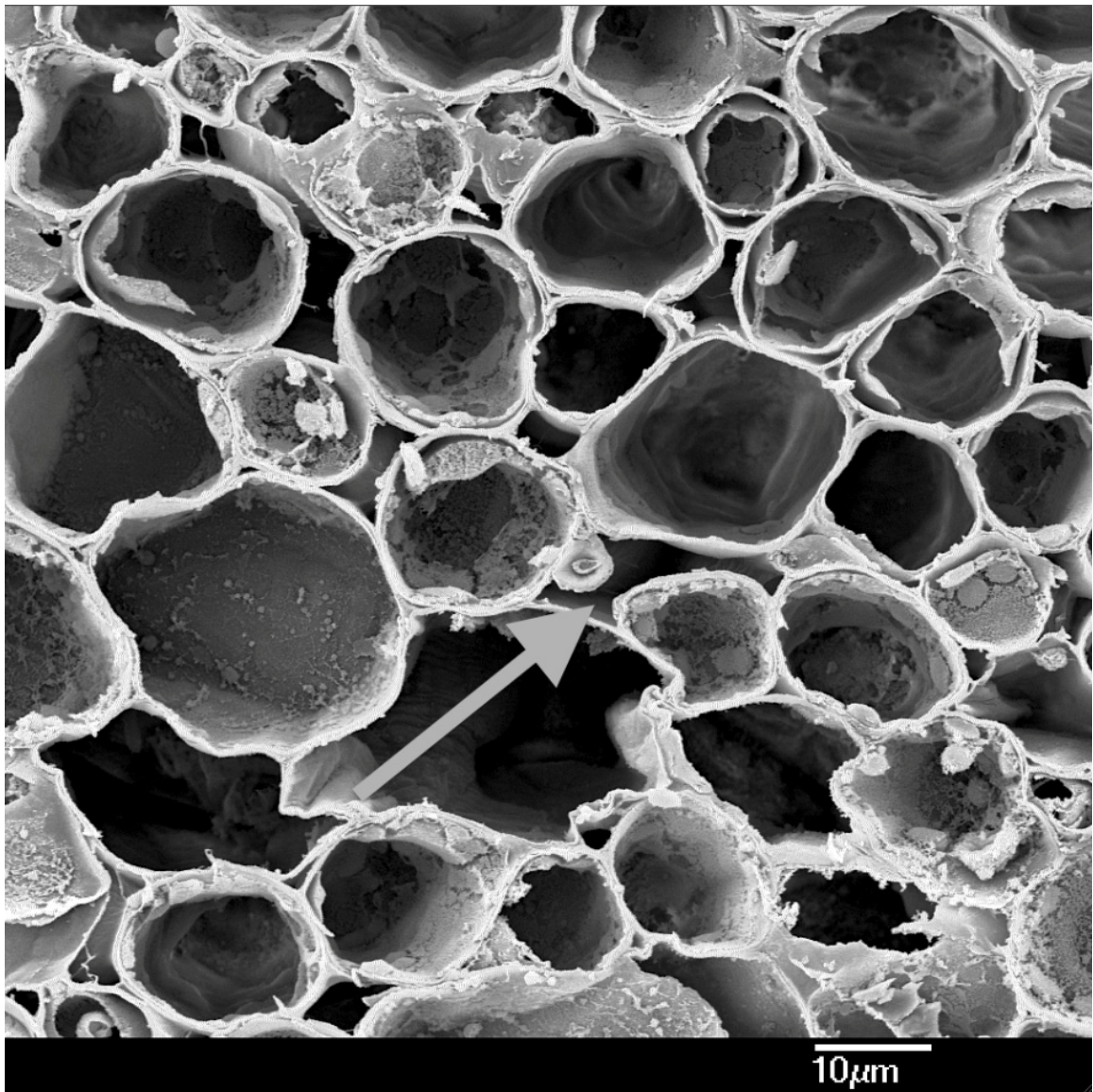
Figure 5 Spaces between cells in deeper layers of the plant, closer to the vascular cambium. Intercellular space allows the plant to increase in diameter in order to compensate for growing vascular cambium and secondary vascular tissues.
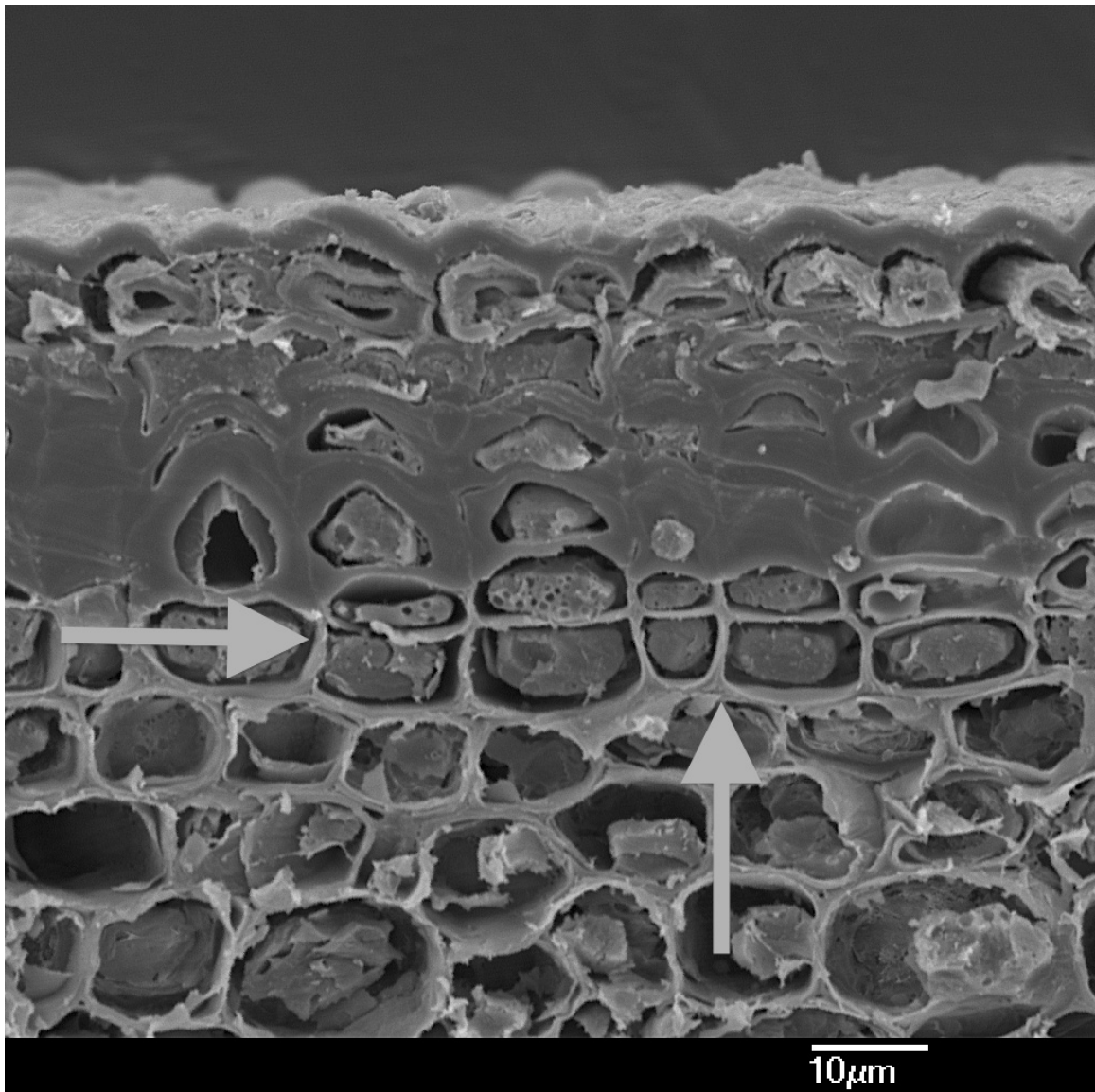
Figure 6 Formation of periderm (phellogen and phellem), evidenced by anticlinal and periclinal divisions in the meristematic phellogen (arrows). Left arrow: periclinal divisions add layers to the periderm. Right arrow: anticlinal divisions add cells to increase diameter of the phellogen.

Mature Periderm

With time the epidermis can no longer compensate for the increasing size of the stem and is eventually replaced by the mature periderm. In the beginning stages of the shedding of the epidermal layer (Figure 7), the thick upper covering of the epidermal cells begins to detach and split away from the phellem cells below it. In Figure 7, the right portion of the epidermis still fully attached to the phellem below it, while to the left the epidermis is gradually splitting away, exposing a gap between the periderm and older epidermal tissue. Eventually it cracks and peels away from the surface of the stem (Figure 8). In Figure 8, on the left the epidermis has pulled away from the periderm but not completely sloughed away, while on the right the epidermis has fully detached, exposing the mature periderm below. Figure 9 also shows the epidermal splitting (left arrow) along with a view of the mature periderm surface (above right arrow).

This mature periderm is the new outer protective layer of the plant. This mature periderm is characterized by cells organized in radial files (Figures 9, 10) due to the periclinal divisions of phellogen in the meristematic layer. At least 7 layers of phellem can been seen in Figure 9 (right arrow), as well as in Figure 10. Phellem cells maintain their radial arrangement even after the cytoplasm dies and the cell walls thicken (Figure 10, arrows). It is difficult to determine if the cells in the meristematic layer are still active, but at some point they will no longer be active and eventually this mature first periderm layer will be cracked away and replaced by a lower second periderm layer. As the plant continues to grow, additional layers of periderm will arise from the deeper layers of the plant, and will push outward causing the first periderm protective layer to crack away just as the epidermis did to make way for the first periderm.
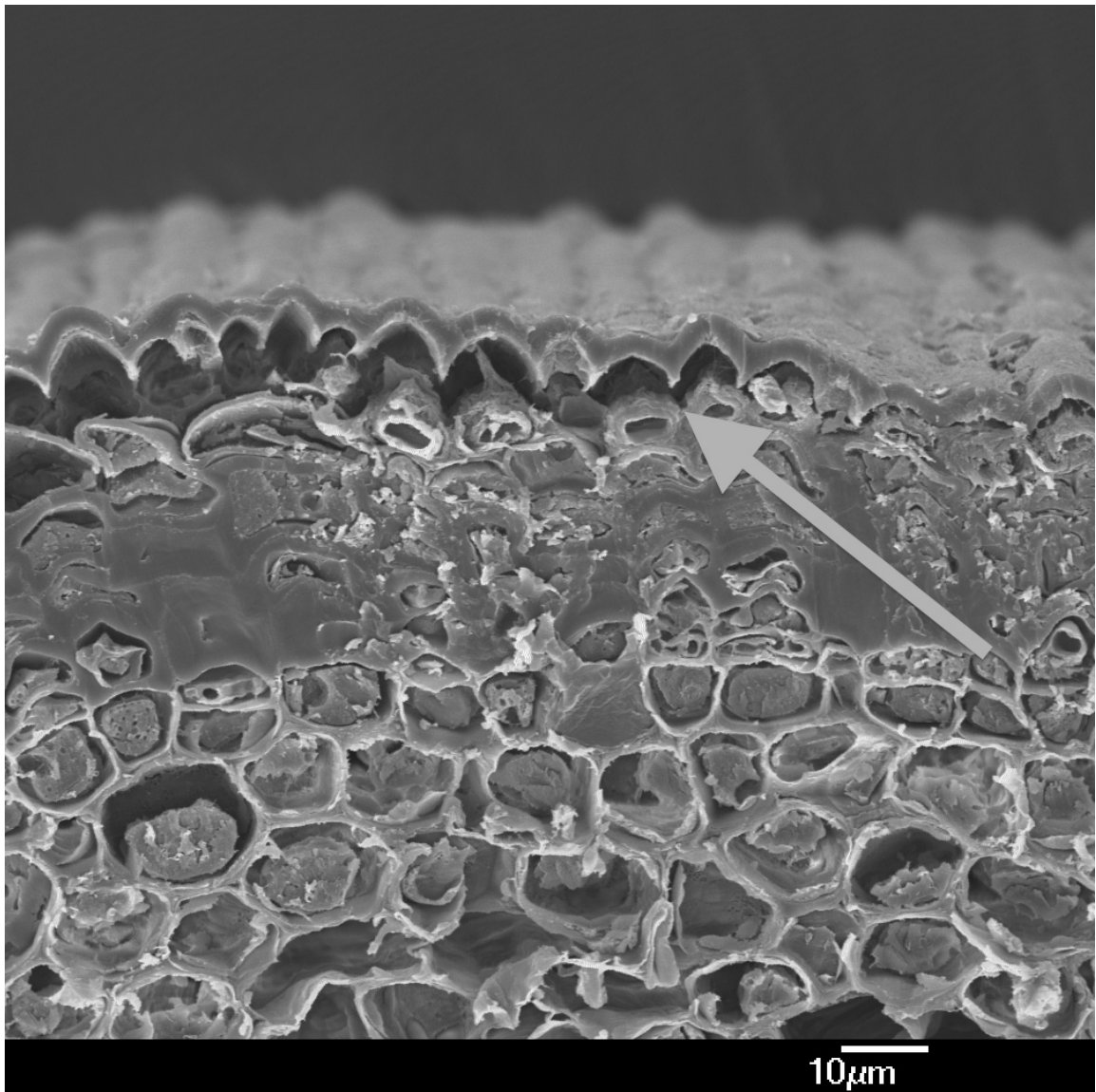
Figure 7 Initial splitting of epidermis from underlying tissues. As stem diameter increases, the outer epidermal wall (arrow) pulls away from the periderm forming a small gap between itself and the periderm.
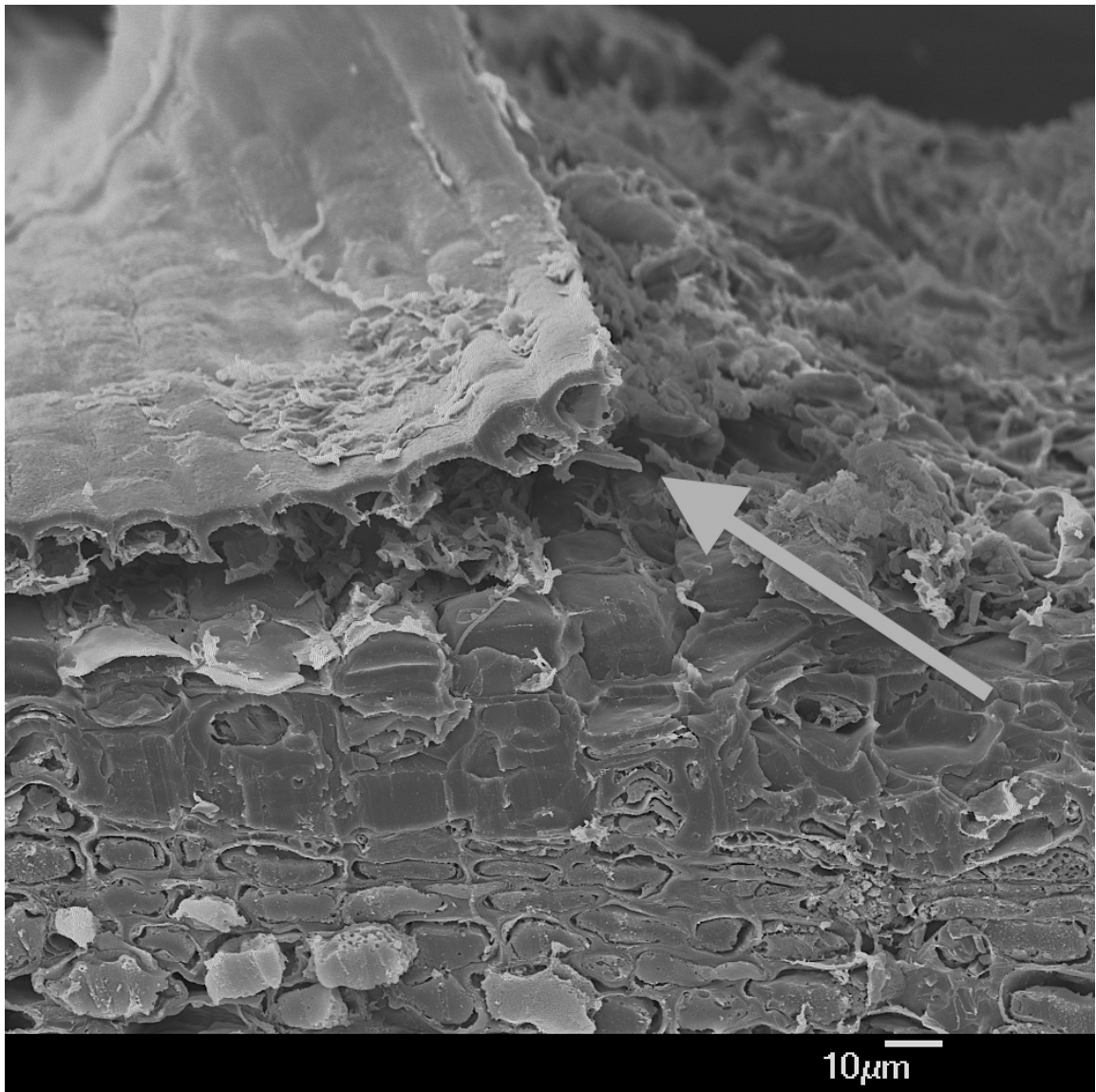
Figure 8 Epidermis pulling away and detaching from underlying periderm. Arrow indicates epidermis lifting off of the surface of the periderm. The area on the right side of the image shows the surface of mature periderm, the new outer protective layer.
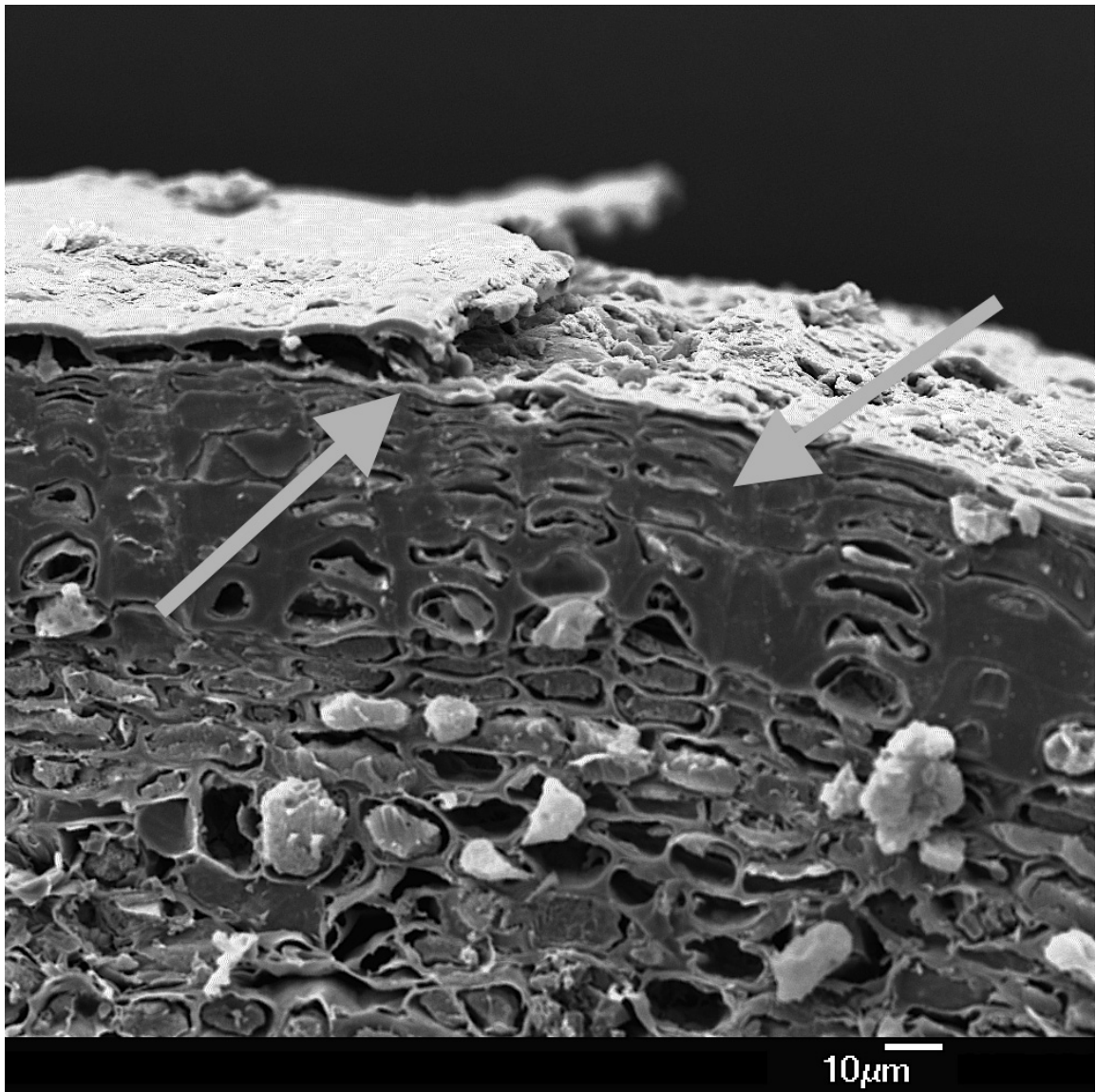
Figure 9 A later stage of periderm development with at least 7 layers of phellem. Left arrow: shows epidermis pulling away and detaching from underlying periderm. Right arrow: shows distinct radial files of phellem cells with thickened walls.
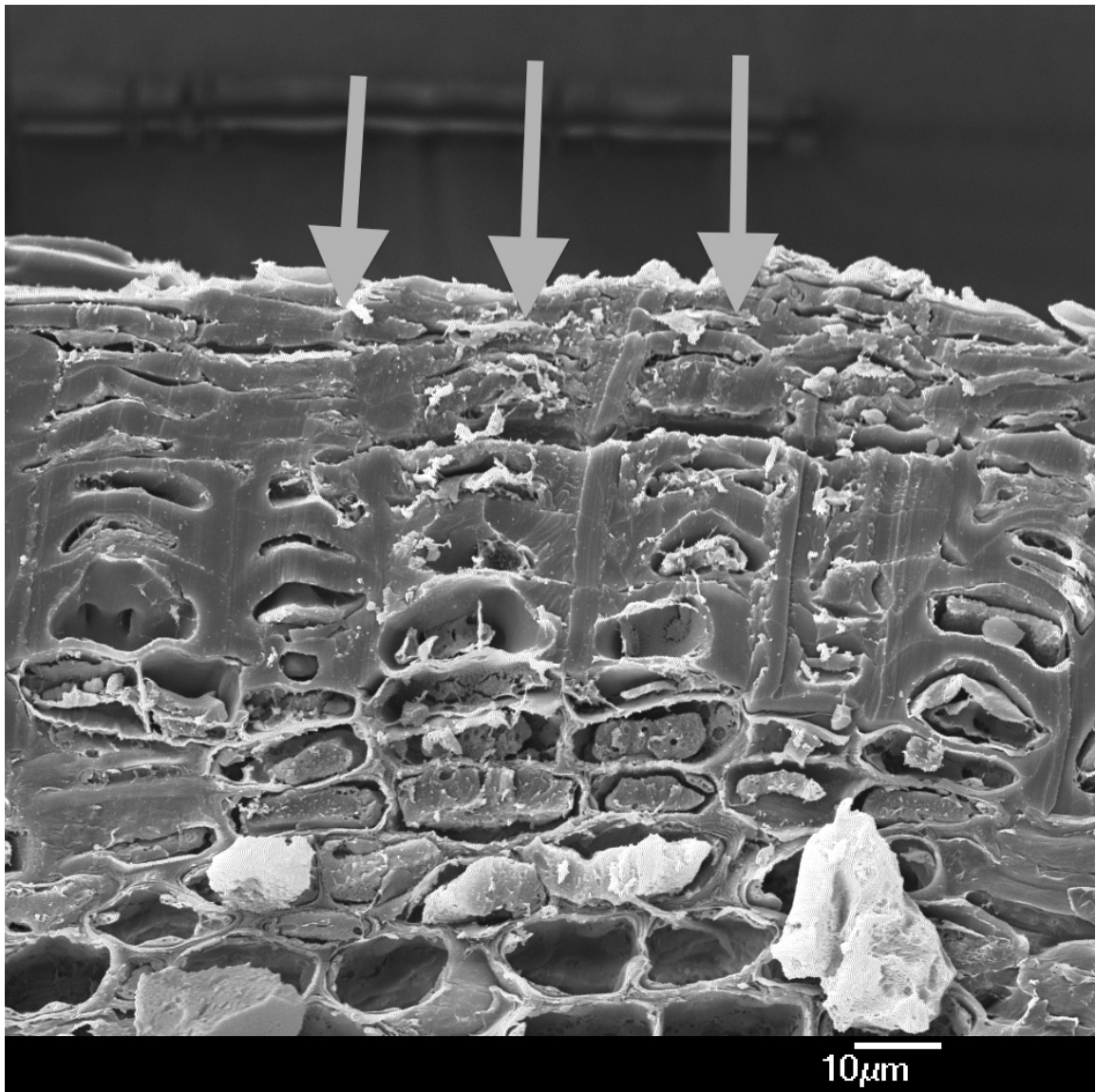
Figure 10 Mature periderm reveals radial files of cells with thickened cell walls and loss of cytoplasm. Arrows indicate separate columns of cells, formed by periclinal divisions in the meristematic phellogen layer. The original epidermis is no loner present.

CHAPTER FOUR

Discussion and Conclusions

The objectives of this study were to examine the protective tissues found in stems of *Q. buckleyi*, to observe cell division in the epidermal and subepidermal layers, to describe the process of epidermal replacement by the periderm, to describe the stages of periderm initiation and maturation, and to compare protective tissue development in *Q. buckleyi* with other species previously studied.

Epidermis and Periderm Initiation

Plants require a protection from environmental factors such as temperature, light, water shortage, and other damaging substances. The epidermis and periderm are that protection in the stems of plants. The epidermis offers a layer of protection during the earliest stages of development in the plant, and the periderm offers a layer of protection during the later stages of development, as the stem increases in diameter causing the epidermis to slough away. The periderm is thicker and serves as a more substantial protective layer than the epidermis.

The epidermis is the outer layer of cells with a thick outer wall. As the diameter of the plant increases due to the growth of the vascular cambium, the epidermis cells have a limited capacity to divide anticlinally to increase in circumference. The periderm is the protective tissue that forms below the epidermis. In some plants, the periderm arises out of the epidermis, but in *Q. buckleyi* this was not the case. Instead, the periderm appears to arise from the subepidermal layer, which is the layer of cells just below the epidermis. In plants, periderm first initiates in localized patches and then goes on to form a

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continuous layer. However, the specimens used in this study did not show a continuous layer of early periderm division, so I was not able to observe this aspect of periderm initiation. Additionally, periderm does not appear to initiate as early in development as some other plant species. For example, in *Ulmus alata*, the periderm initiates as soon as nodal growth is completed (Fowler, 2006). Another study done by Arzee, Liphschitz & Waisel on *Robinia pseudoacacia* demonstrated how environmental conditions can affect the timing of phellogen initiation and can also affect the specific site of initiation (Arzee, Liphschitz & Waisel, 1967). Although environmental factors were not studied in my investigation on *Q. buckleyi*, it is possible that environmental factors could have delayed periderm initiation in the early stages.

Previous studies have shown that the first periderm most often forms in the subepidermal layers, but at times initiates in the epidermal layer itself, or in the deeper layers in the primary phloem. For example, in *Robinia pseudoacacia* the first phellogen differentiates in the second or third layer beneath the epidermis (Arzee, Liphschitz & Waisel, 1967). In *Q. buckleyi*, the periclinal divisions that can be seen in the subepidermal layer during early stages of development provide evidence for subepidermal initiation of the periderm as opposed to epidermal initiation. If the periderm initiated in the epidermis, then there would not be a clear epidermal layer after the formation of the phellem because it would become a layer of thick walled cells in the radial files of phellem. If the periderm were to initiate in any layer of cells lower than the subepidermal layer then there would be more cells intact outside of the phellem during early development.

The circumstantial evidence of an intact epidermis, radial files of thick walled cells just below the epidermis, followed by a layer of meristematic dividing cells, shows that the periderm must arise out of the layer of cells just below the epidermis. As these cells divide, the outer daughter cell becomes a part of the phellem, and the inner cell continues to be the meristematic cell for future divisions. Phellem cells are easily distinguished by their thickened cell walls with no cytoplasm. In some plants there is a layer known as the phelloderm, but in *Q. buckleyi* there are no thin walled cells inside the meristematic layer of cells, meaning there is no phelloderm.

Comparison to Literature

In some species of *Quercus,* such as *Q. ithaburensis* and *Q. infectoria*, phellogens are only active for the production of the first phellem. After this, the phellogens activity may be linked with vascular cambium activity (Arzee, Kamir, & Cohen, 1978). This information is not easily extracted from the specimens in this study because only the early stages of periderm initiation and development were studied, so later phellogen activity could not be observed.

The nature of the phellem in *Q. buckleyi* seems to be somewhat similar in nature to the phellem found in *Q. suber*. In *Q. buckleyi,* the phellem cell walls are very thick, just as they are in *Q. suber*. In *Q. suber*, the phellem layer is several millimeters thick as opposed to only a few layers of cells (Eames & MacDaniels, 1947). While *Q. buckleyi* may not have a phellem layer that is quite this thick, the phellem in *Q. buckleyi* is definitely more than just a few layers thick. The thick walls of the phellem, coupled with the small spaces remaining from the cytoplasm, likely would make *Q. buckleyi* impervious and flexible like phellem in *Q. suber,* which is used for cork due to its

impervious and flexible nature (Eames & MacDaniels, 1947). In order to obtain this cork, the periderm is removed from the tree, and then the subsequent phellogen is allowed to continue producing. These subsequent layers of phellogen produce more rapidly than the first layer (Faun, A., 1990). One notable difference between *Q. buckleyi* and *Q. suber* is the location of periderm initiation in the stem. *Q. suber* shows a relationship between epidermal hairs and periderm development, whereas such a relationship was not observed in *Q. buckleyi*.

Recommendations for Future Study

In this study I was able to observe some aspects of epidermal growth and periderm initiation. However, there are still interesting questions to be answered. For example, it would be interesting to investigate the timing of initiation and the location of subsequent periderm formation. It would also be interesting to investigate these topics in other species of *Quercus* and continue comparing and contrasting the nature of protective tissues in each species.

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