Ultrastructural Changes of Secondary Phloem Cells in the Cambium Annual Activities of *Taxodium Ascendens*

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***Abstract*—** *Tree bark is an important part of tree trunk and it derives from the activities of cambium. More studies were focused on the xylem cells development of trees at the ultrastructural level and not on phloem cells. To explore the ultrastructural changes of all kinds of secondary phloem cells in the cambium annual activities of Taxodium ascendens, and to study the mechanism of its wood formation. The samples of Taxodium ascendens were collected in different growth periods. The ultrastructural changes of all kinds of cells in the secondary phloem of Taxodium ascendens and the process of wood formation were observed by transmission electron microscope. The Results show as follows:*

The sieve cells began to differentiate in late March and their cell walls thickened continuously during the periods of April to June. At the end of August, part of the sieve cells entered programmed death, with nucleus pyknosis, chromatin agglomerated, nucleoli disappeared, and the nucleoplasm decreased. At the same time, the size of the nucleus became smaller, and when the sieve cells stopped functioning, the protoplasts died. The whole cell was squeezed and deformed. Phloem parenchyma cells began to differentiate in mid-April. In the early stage of cambium activity (from mid-April to early May), the resin in the parenchyma cells was distributed in the form of large droplets. At the peak of cambium activity (from the end of May to the end of June), the resin was dispersed in the cells as fog, and the resin droplets were evenly distributed along the cell wall during the dormant period. In mid-April, newly differentiated young ray parenchyma cells were observed in the phloem near the cambium. From mid-late April to early June, the young phloem ray cells developed to mature and remained in this state until the middle of November. After that, the cells went into programmed death, and the cells contained only a large number of oil droplets and empty vesicles. The development and maturation of phloem fibers occur in all periods. Phloem fibers can be watched in any period of phloem development. The proportion of mature and developing phloem fibers differs slightly in each period.

The active period of cambium cells lasted from the beginning of March to the end of November. Newly differentiated sieve cells could be observed in March, but newly differentiated phloem parenchyma cells and ray parenchyma cells were not observed until the middle of April. Therefore, the sieve cells differentiated earliest, while the phloem parenchyma cells and ray parenchyma cells were about half a month later than the sieve cells. During the period from the beginning of phloem differentiation to the end of cambium activity, the division and differentiation of secondary phloem cells can be observed continuously.

Keywords*— *Taxodium ascendens, Secondary phloem, Sieve cell, Parenchyma cell, Phloem fiber, Ultrastructure.

I. INTRODUCTION

The secondary phloem derived from the vascular cambium of trees is responsible for the synthesis and storage of organic matter and the transport and distribution of photosynthates (Esau K,1969). At present, there have been a large number of studies on the phloem development of hardwood phloem, with a comprehensive summary of all kinds of cells in the phloem of hardwood (Derry *et al*. 1967; Zhao 2012), while most of the studies on softwood are focused on xylem (Rossi et al. 2016; Pattathil et *al*. 2016; Zhang *et al*. 2018; Zheng *et al*. 2022) and cambium (Rossi *et al*. 2006; Prislan *et al*. 2016; Myśkow *et al*. 2019), there are few systematic reports on the structure and development of all kinds of cells in the phloem of softwood.. The phloem of softwood is usually composed of axial sieve cells, phloem fibers, phloem parenchyma cells, and radial phloem ray parenchyma cells (Abbe *et al*. 1939). In different developmental stages, the morphology, contents, and composition of all kinds

of cells are different (Gričar *et al*. 2017). For more than half a century, scholars have carried out a large number of phloem development studies on various tree species and combined them with climatic factors to explore (Gričar *et al.* 2007; Luis *et al*. 2007; 2011; Dié *et al*. 2012; Salmon et al. 2019). However, most studies focus on the seasonal growth pattern and growth of phloem (Cardoso et al. 2019; Kopanina *et al*. 2022; Ohse *et al*. 2022). The studies on the seasonal changes of various cell morphology of softwood phloem are mainly focused on the microscopic layer (Mullendore 2010; Prislan *et al*. 2013; Baba *et al*. 2019). At present, the ultrastructural layer studies on phloem are mainly focused on sieve cells (Knoblauch *et al*. 2018; Prislan *et al*. 2018), the study of other phloem cells is not reported comprehensively. The resolution of modern electron microscopy has gone deep into the most basic structural units of wood cells (Singh *et al*. 2001; Fromm *et al*. 2003; Chukhchin *et al*. 2020). Using electronic microscope to explore the process of wood formation will be the research trend in the future.

Taxodium ascendens is a successful fast-growing timber tree species introduced and cultivated in the Yangtze River in China. In this paper, the ultrastructural changes of sieve cells, phloem parenchyma cells, phloem ray parenchyma cells, and phloem fibers were observed by transmission electron microscope, to obtain more information about the seasonal activity of cambium and secondary phloem changes in gymnosperms. The purpose of this paper is to enrich the biological knowledge of the growth and development of coniferous wood, combine the formation of phloem and xylem, and connect with the overall growth of trees. This not only helps to consolidate the theory of cambium development but also helps to provide a basis for practical problems such as forest management measures and regulation of wood and partial bark growth. It also provides a scientific basis for the cultivation of *Taxodium ascendens* plantations, wood material improvement, and forest resource utilization, and contributes to the efficient utilization of bark resources.

II. MATERIAL AND METHODS

In this study, five normally grown trees were meticulously selected from the forest of *Taxodium ascendens* near South Lake in Wuhan, Hubei Province of China. Samples of 2-3-year-old branches were cut from the middle part of the crown of *Taxodium ascendens* trees, which were twenty years old and their heights are about 20-meter in tall.

Samples are taken every 3 days from March to June 2019, every 7 days from July to October 2019, and every two weeks from November 2019 to March 2020. When sampling, select the 2-3-year-old branches in the middle and upper part of the crown, which are about 3-4 m above the ground and grow well, and then cut the middle of them into several small sections of 0.5-1cm. Afterwards, fix them with glutaraldehyde and vacuum treatment to make them completely fixed, and pay attention to avoid the branches of reaction wood. Detailed records were maintained for each sampling site, including the longitude and latitude, altitude, sampling date, and weather conditions, to facilitate further analysis.

Samples were softened with 40% hydrofluoric acid (HF) to allow free cutting with double-sided blades. Then fixed at 4℃ for 5-6 hours, rinsed with phosphate buffer solution (PBS) 3 times for 20 minutes each time, and then fixed for 4 hours. Then rinse with pH7.2 PBS 3 times, followed by gradient alcohol dehydration, propylene oxide replacement, Epon812 resin embedding, slicing with LKB-V ultramicrotome, lead citrate-uranium acetate staining, transmission electron microscope observation. The images obtained were collated and analyzed by Photoshop software, and the phloem ray morphological characteristics of sieve cells, phloem fibers, phloem parenchyma cells, and radial system of the axial system were described in detail, and the changes in the characteristics of secondary phloem during the branch activity were summarized.

III. RESULTS

3.1 Ultrastructural changes of phloem sieve cells in the annual activities of cambium

The sieve element in the phloem of gymnosperms is sieve cells, and they are of the same shape, a single narrow spindle-shaped cell, usually rectangular in cross-section and tapering at the end. Granular or crystalline inclusions dispersed in the cytoplasm were observed in the sieve element of *Taxodium ascendens*. In this experiment, the changes in ultrastructure and wall structure of sieve cells were observed by making ultrathin sections and transmission electron microscope.

During the period of cambium recovery activity on March 18, a small number of functional sieve cells that survived the previous year could be seen in the phloem; most of them were nonfunctional sieve cells, and high-density crystalline P plastids could be seen in the sieve cells (Figs. 1A, 1B). The newly differentiated young sieve cells were observed in the materials collected on March 26, indicating that the vascular cambium started activity and differentiated into the phloem. The nucleus of the young sieve cells was not obvious, and a large number of dispersed cytoplasm could be seen in the cells, the sieve cells showed vacuolization, and the nucleus began to be visible, but the size was small. Like the primordial cells that produced them, the protoplasts of immature functional sieve cells have visible nuclei, vacuoles, and other normal organelles (Fig. 1C). On

April 11, cambium entered the active phase and cytoplasmic content significantly increased. A small number of mitochondria can be seen along the cell wall (Fig. 1D). The obvious nucleus can be seen in the material on April 22 (Fig. 1E). At the end of May, S plastids were observed for the first time near the nucleus of the sieve cells (Fig. 1F). On June 5, the diameter of the sieve cell began to expand, the developing plastids could be observed in the cytoplasm, the number of plastids also increased, and the nucleus was darker. Long oval S plastids were observed, S plastids contained spherical osmiophilic bodies, and P plastids began to condense to a small extent. At this time, *Taxodium ascendens* was in the active stage, and the transfer and transport of assimilation products on the sieve cells had reached an active peak. The cell wall began to thicken (Figs. 1G, 1H). On July 10, it was observed that the black osmiophilic substance condensed into a sphere in some sieve elements (Fig. 1I).

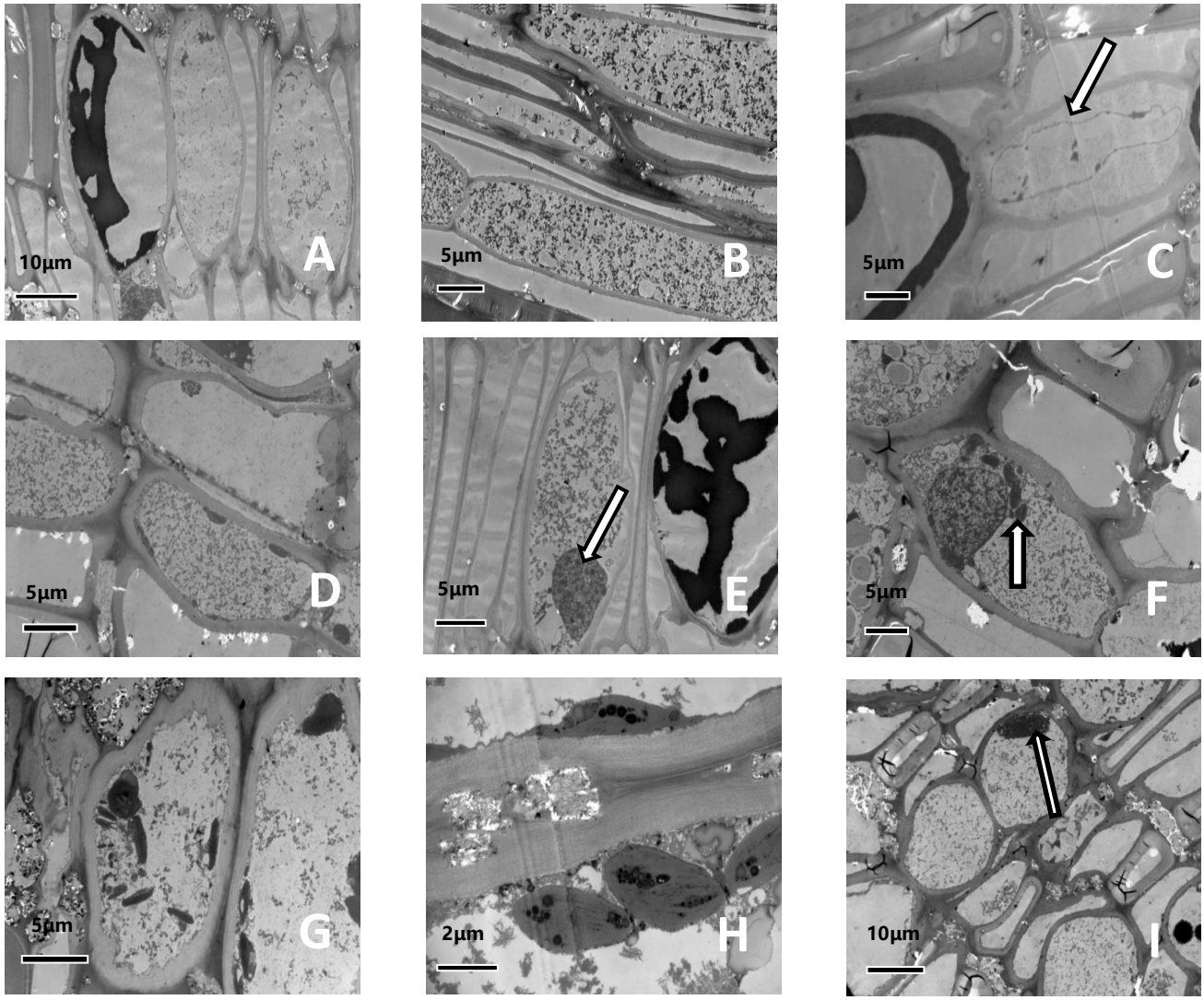

FIGURE 1: Ultrastructural changes of sieve cells during the recovery active and active period of cambium

A: Cross section of the material on March 18, a small number of functional sieve cells survived in the previous year, most of them are nonfunctional sieve cells. B: March 18 material diameter section. C: March 26 material, new division and differentiation of sieve cells. D: April 11 cross section, increased cytoplasmic concentration. E: April 22 material, mature nucleus developed in the sieve cell (shown by the arrow). F: S plastids appeared near the mature nucleus on May 31. G: Cross section of the material on June 5, a large number of S plastids can be seen. H: On the radial section of the material, S plastids are enlarged, and osmiophilic bodies can be seen in the cytoplasm. I: On the cross section of July 10, black osmiophilic substances are observed to condense into spheres in some sieve molecules (shown by the arrow).

In the late active phase (August 13), the chromatin and nucleoplasm disappeared to almost invisibility, and some of the sieve plastids collapsed and released osmiophilic substances (Fig. 2A). On August 27, it was observed that ribosomes, microtubules,

microfilaments, Golgi apparatus, and tonoplast disintegrated to disappear, with only one small pyknotic nucleus, obvious thickening of the cell wall, and obvious layered structure of the cell wall, it can be distinguished from the inner layer (S3), middle layer (S2), and outer layer (S3). A small amount of cytoplasm is distributed on the edge of the cell, which is mainly composed of the plasma membrane, a small number of mitochondria and sieve plastids (Fig. 2B). On October 22, *Taxodium ascendens* began to enter the dormant period, only a small amount of functional phloem could be seen in the phloem, and most of the sieve cells developed into dead cells with only transport and support function, that is, the nonfunctional sieve cells were obviously delaminated and the cell wall was thicker (Fig. 2C). On November 19, few functional sieve cells were seen. During this period, most of the nonfunctional sieve cells were compressed by phloem parenchyma cells and swollen stone cells, and the cambium was in a dormant phase (Fig. 2D). The secondary phloem still has a very small number of functional sieve cells to maintain the transport function until the following spring, ensuring the transport of assimilates in the overwintering stage.

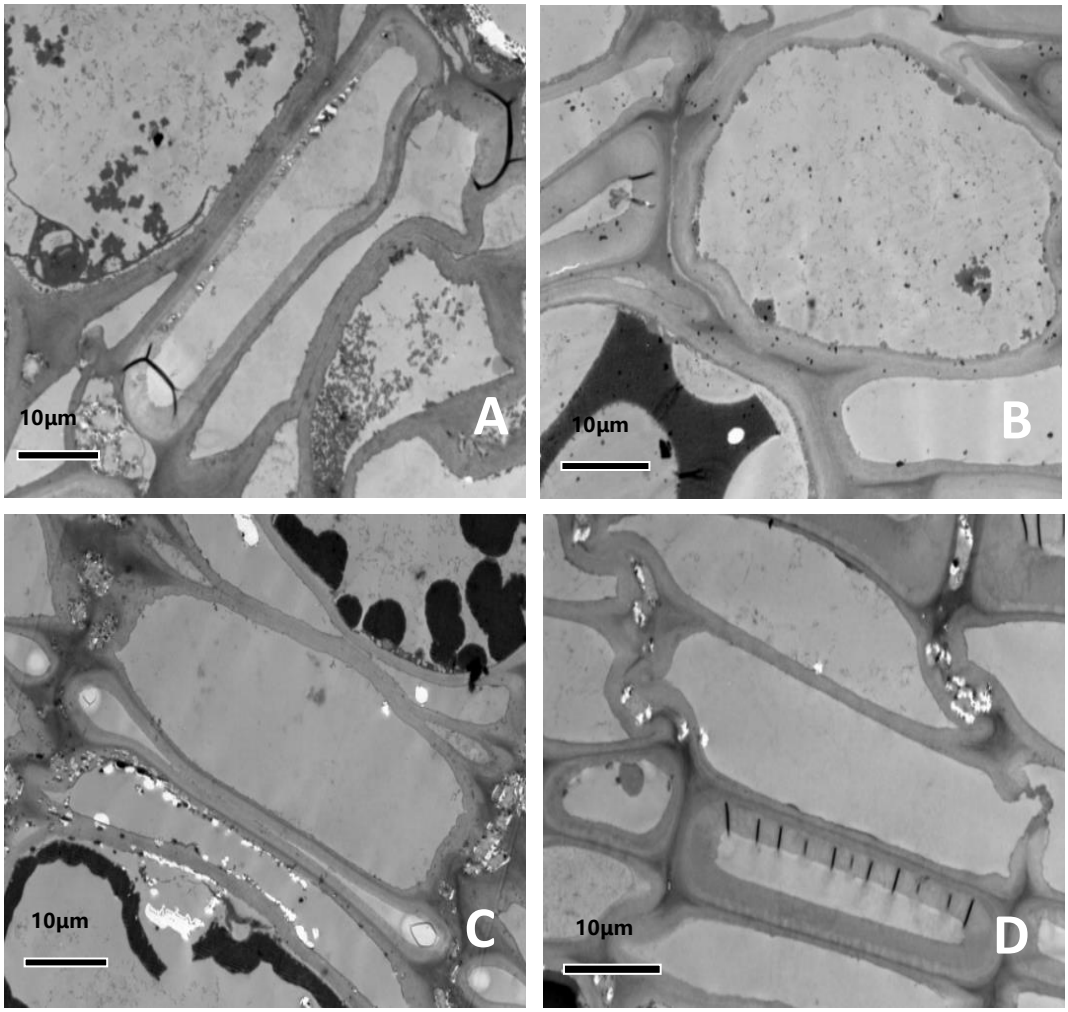

FIGURE 2: Ultrastructural changes of sieve cells during the late active and dormant period of cambium

A: In the cross section of the material on August 13, the chromatin and nucleoplasm disappeared to almost invisible, and some of the sieve intracellular plastids collapsed and release osmiophilic substances. B: On August 27, the ribosome, microtubule, microfilament, Golgi apparatus and tonoplast basically disintegrated to disappear, with only one small suspected pyknotic nucleus, obvious thickening of the cell wall and obvious layered structure of cell wall. C: In the cross section of the material on October 22, only a small amount of functional phloem can be seen in the phloem, and most of the sieve cells develop into dead cells with only dredging and supporting functions, that is nonfunctional sieve cells, with obvious delamination of nonfunctional cell wall and thicker cell wall. D: On November 19, most of the materials are nonfunctional sieve cells

3.2 Ultrastructural changes of phloem parenchyma cells in the annual activities of cambium

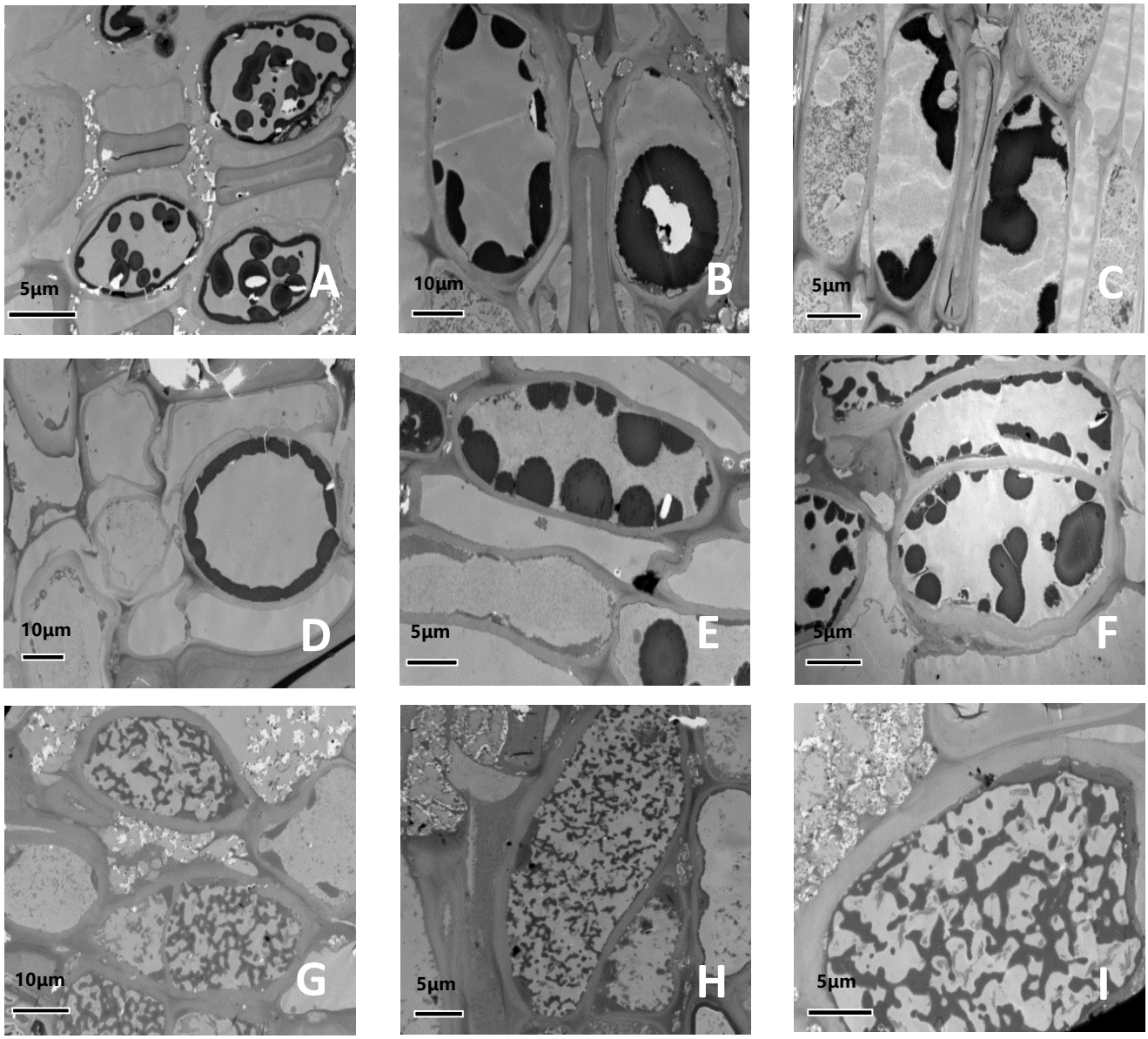

FIGURE 3: Ultrastructural changes of parenchyma cells during the recovery active and active period of cambium

A: On March 13 the material cross section, phloem parenchyma cells are in dormancy, there are small droplets inside the cells, and some resin droplets are homogeneous along the wall. B: Dark resin in phloem parenchyma cells in the cross section of material on 18 March appears droplet like. C: On March 26 the material was largely maintained. D: Cross section of the material on April 11, newly differentiated phloem parenchyma cells were observed, and the intracellular resin was evenly distributed along the cell wall in a candidate shape. E: Small droplets of resin on the inner wall of the cell reaggregate on April 22. F: 17 May material observed droplets dispersing intracellularly. G: Cross section of material May 31, showing dispersion in small droplets. H: Material cross section on June 1 with droplets almost misty highly dispersed. I: 26 June resin droplets condensed to a small extent

On March 13, the cambium of *Taxodium ascendens* was in the dormant period, and part of the resin distribution along the inner wall of the cell could be observed (Fig. 3A). On March 18, *Taxodium ascendens* entered the cambium recovery period, and the dark resin in the phloem parenchyma cells showed a droplet shape (Fig. 3B), and gradually dispersed to the cell wall in late March (Fig. 3C). On April 11, the newly differentiated phloem parenchyma cells were observed, the cell wall was very thin, and the intracellular resin was uniformly distributed along the cell wall (Fig. 3D). On April 22, the resin droplets on the inner wall of the cell gathered again and then gradually dispersed into the cell (Fig. 3E). The resin in the phloem parenchyma of *Taxodium ascendens* was highly dispersed from May to June, and the division and differentiation were more active (Figs. 3F,

3G). Almost foggy and uniformly distributed in the cell fluid was observed on June 5, but no resin droplets were observed (Fig. 3H). At this time, the cambium activity was in the active period, and the cambium differentiation rate reached the peak, and then the cambium differentiation rate decreased gradually. A small condensation of resin droplets was observed on the material on June 26 (Fig. 3I). On July 10, the resin droplets condensed into clusters (Figs. 4A, 4B, 4C). By September 11, the resin was in the shape of large droplets, accounting for most of the intracellular volume, and a small number of starch granules were observed (Fig. 4D), and the cambium activity entered the end of the active period. In early January, the resin was redispersed, and in mid-November, the resin was again dispersed as a droplet to the inner wall of the cell, and starch granules could be seen along the cell wall (Figs. 4E, 4F). After that, the resin in the phloem parenchyma cells remained in this state through the winter, until the following spring, when the cambium resumed activity and the resin droplets gathered again in the cell (Fig. 3A).

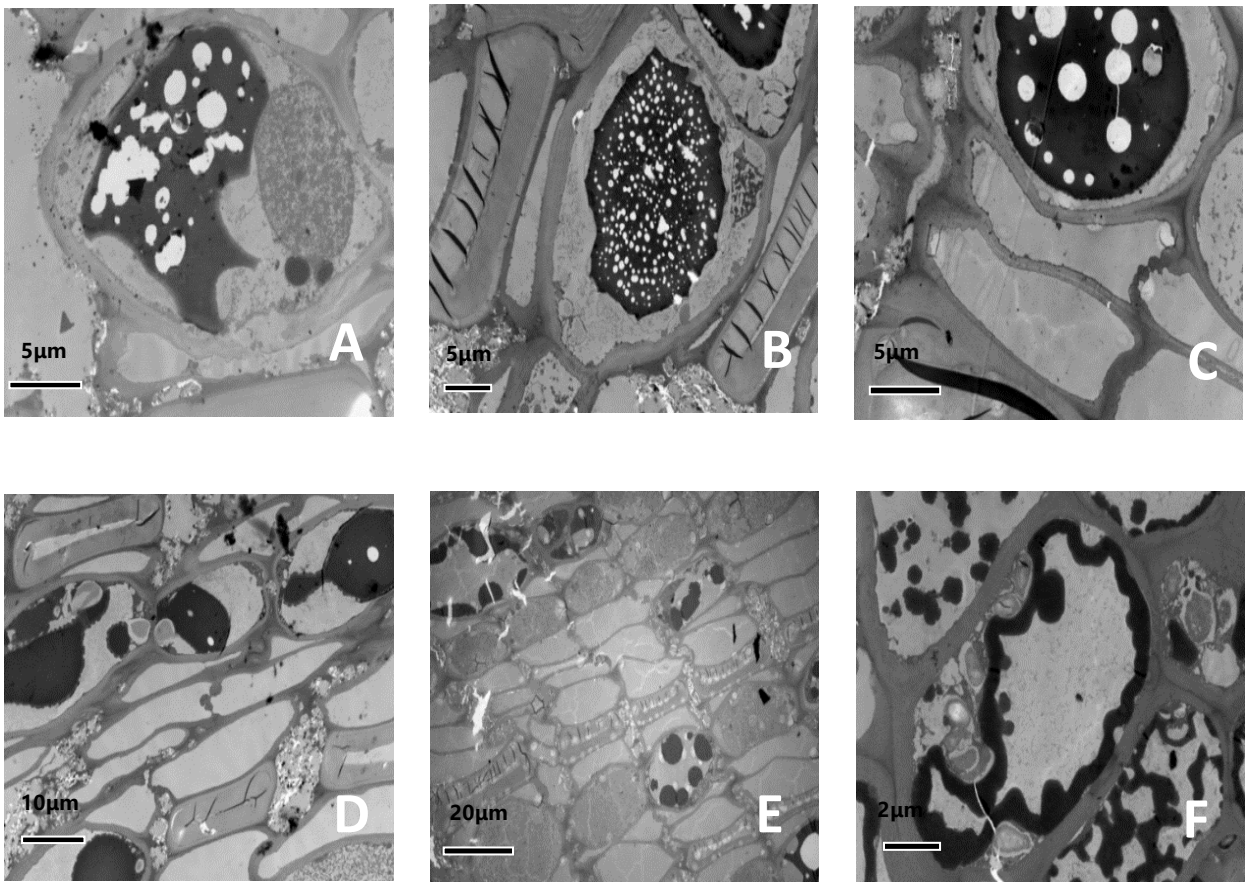

FIGURE 4: Ultrastructural changes of parenchyma cells during the late active and dormant period of cambium

A-C: In July and August materials, resin droplets condensed into agglomerates. D: September 11 materials, resin droplets are large droplets, and a small number of starch particles can be seen. D-F: From October 22 to November 19, the resin was dispersed again as a small drop to the inner wall of the cell, and the number of starch granules along the cell wall was significantly more than that of the material in mid-September

3.3 Ultrastructural changes of phloem ray parenchyma cells in the annual activities of cambium

On March 18, the cambium had not yet begun to differentiate into the phloem, and mature phloem ray parenchyma cells were observed in the phloem, with mature nuclei, a small number of multivesicular bodies and less cytoplasm, most of which were distributed along the inner wall of the cells, the cell wall is thicker (Fig. 5A). The young ray parenchyma cells were still not observed on March 26, the nucleus of mature phloem ray parenchyma cells was smaller than that on March 18, and the number of starch granules decreased. It is speculated that the cambium becomes active during this period, and nutrients are needed to meet the needs of cell division and differentiation (Fig. 5B). On April 11, newly differentiated young ray parenchyma cells were observed in the phloem near the cambium; the tangential cell wall was very thin, the cells showed high vacuolization, and vesicles could be seen near the inner wall of the cells. At the same time, the deposition of lignin in the cell wall was observed (Figs. 5C, 5D). On April 18, large nuclei were seen in the cells, nucleoli were clearly visible, multivesicular bodies appeared near the nuclei, and there were no inclusions in the vesicles (Fig. 5E). From early April to mid-May, most of the cell

cavity was occupied by vacuoles (Fig. 5F). On May 31, the number of cell inclusions increased, the large vacuoles had been degraded, and abundant small vacuoles could still be observed (Fig. 5G). By June 5, multivesicular bodies had become smaller, dark substances were deposited inside, and the cambium was active (Fig. 5H). Since then, ray parenchyma cells have maintained the state of larger nuclei and more multivesicular bodies (Fig. 5I). The situation lasted until mid-September.

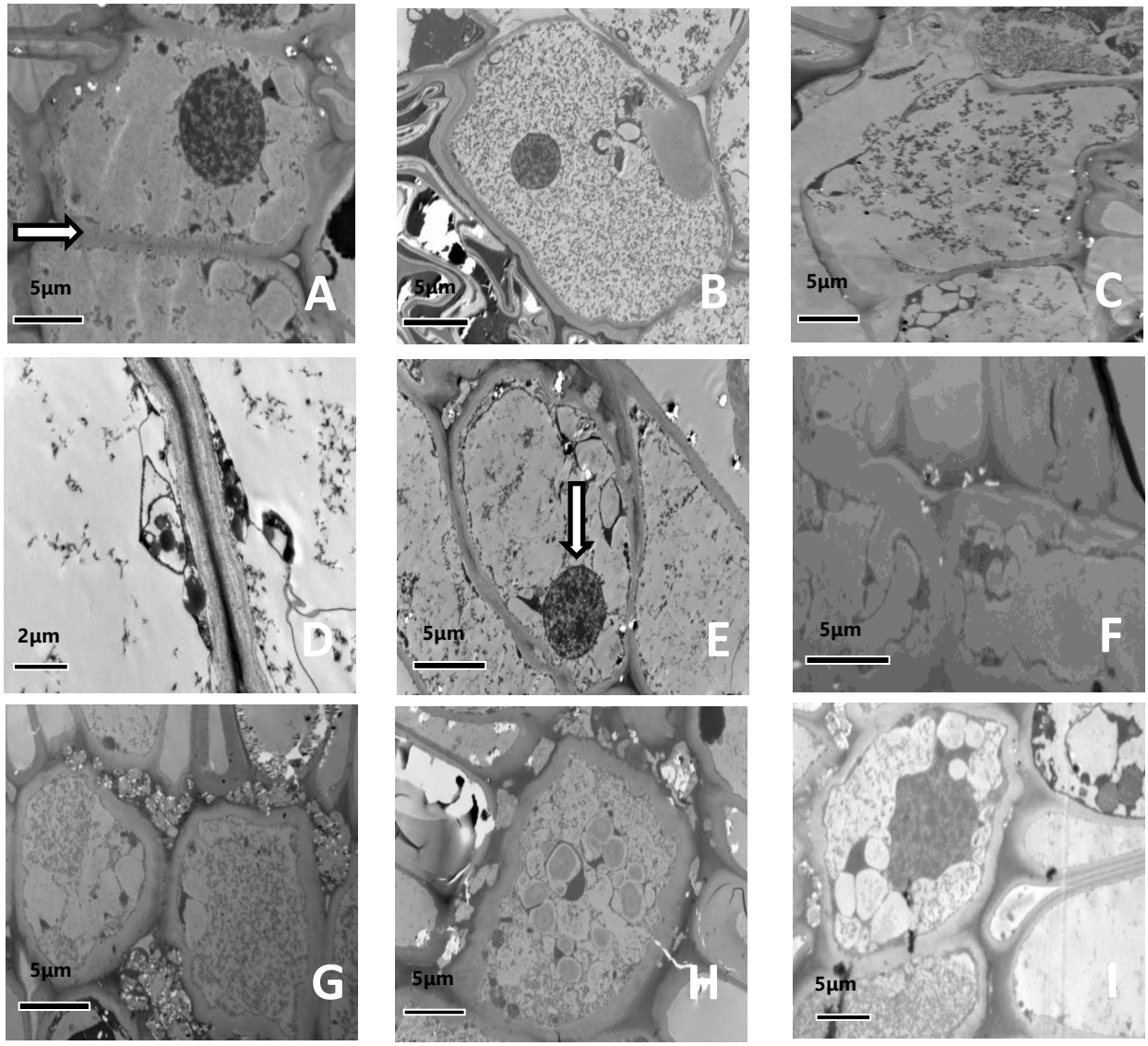

FIGURE 5: Ultrastructural changes of ray parenchyma cells during the recovery active and active period of cambium

A-I: Cross section. A: Mature phloem ray parenchyma cells were observed in the phloem on March 18, with mature nuclei, a small amount of multivesicular bodies, less cytoplasm, mostly distributed along the inner wall and thicker cell wall (shown by the arrow). B: The nuclear volume of mature phloem ray parenchyma cells on March 26 was smaller than that on March 18, and the number of starch granules decreased. C: Young ray parenchyma cells were observed on April 11, indicating that ray parenchyma cells began to differentiate, during which the cell wall was extremely thin and contained large vacuoles. D: On April 11, Golgi apparatus and vesicles were observed near the cell wall. E: On April 18, the nucleus of developing phloem ray parenchyma cells became significantly larger (shown by the arrow). F: On May 17, the cells were almost occupied by vacuoles G: On May 31, the number of cell inclusions increased, the large vacuoles had been degraded, and abundant small vacuoles could still be observed, and the small vacuoles remained free. H: On June 5, the multivesicular bodies became smaller and dark substances were deposited inside. I: On July 10, the nucleus was clearly visible, occupying most of the cells

On September 11, it was observed that the fusiform ray primitive cells still maintained the state of division and differentiation, indicating that the vascular cambium was still active. At the same time, secondary lysosomes and residual undigested bodies after digestion were observed, representing the transformation of ray parenchyma cells from a mature state to an aging state (Figs. 6A, 6B), but cell division and differentiation continued. The division and differentiation of ray parenchyma cells could still be observed in mid-October (Fig. 6C). On November 19, it was observed that some of the chromatin condensed in the nucleus, and the nuclear DNA was degraded and broken, finally forming a dense apoptotic body surrounded by the membrane. The apoptotic cells maintained the integrity of the cell membrane; there was no cell inclusion overflow; and the cells were undergoing programmed death (Fig. 6D). On December 6, it was observed that the tonoplast ruptured and the apoptotic bodies in the nucleus overflowed and dispersed into the cytoplasm (Fig. 6E). At the beginning of January, most of the ray parenchyma cells in the dormant phase of cambium activity had become dead cells, and there were only a large number of oil droplets and empty vesicles in the cells (Fig. 6F).

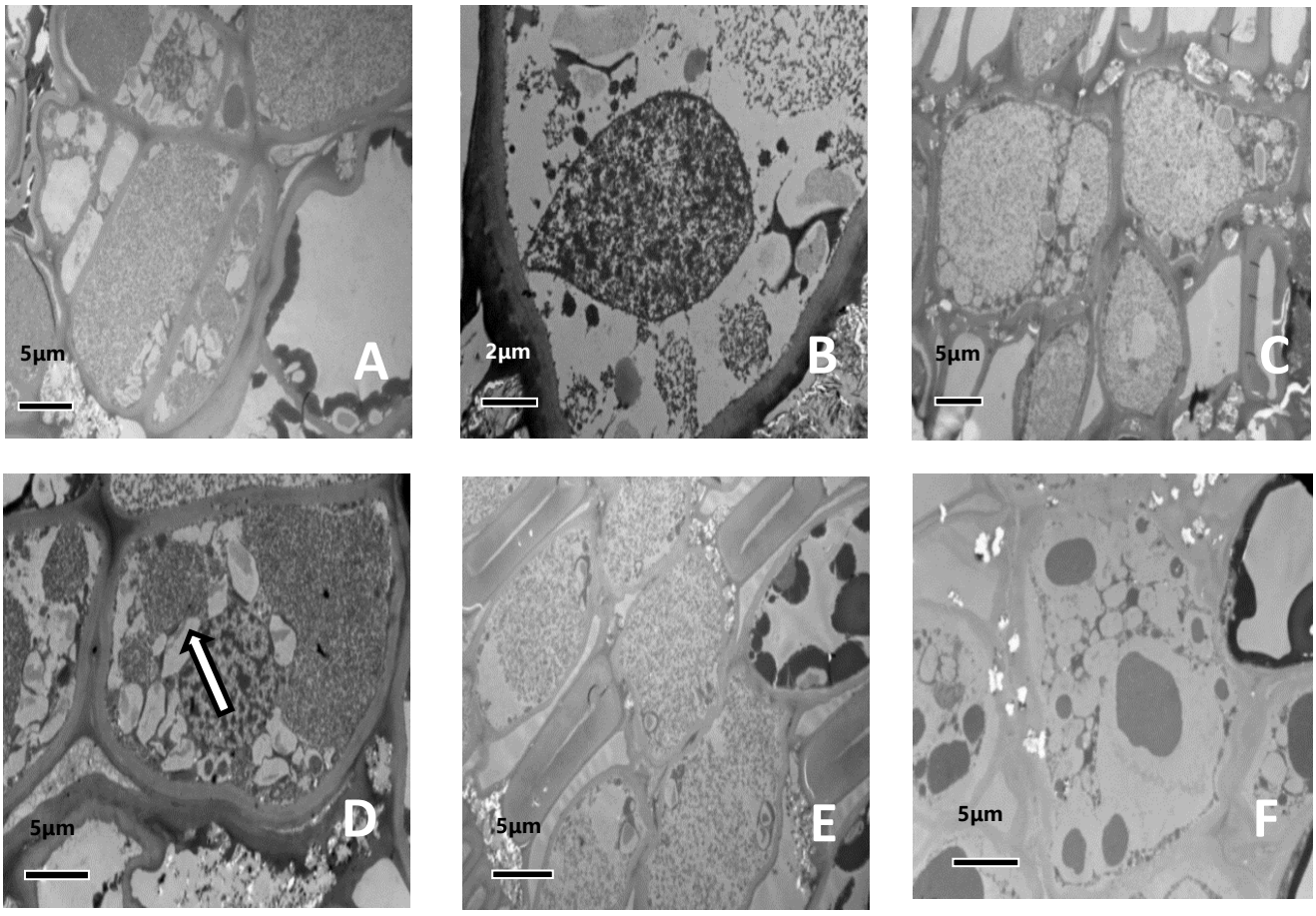

FIGURE 6: Ultrastructural changes of ray parenchyma cells during the late active and dormant period of cambium

A-F: Cross section. A: The fusiform ray primitive cells were still in the state of division and differentiation, indicating that the vascular cambium was still active. B: The enlarged version of the phloem ray cells of the material on September 11, during this period, the organelles were obviously visible, and lysosomes and residual corpuscles were observed. C: The division of phloem ray cells could still be seen on October 22. D: On November 19, the chromatin concentration in part of the ray nucleus (shown by the arrow) was observed, and the nuclear DNA was degraded and broken and finally formed dense apoptotic bodies surrounded by the membrane. E: On December 6, the tonoplast ruptured, the apoptotic bodies in the nucleus spilled and dispersed into the cytoplasm. F: The dead ray parenchyma cells were observed on January 6, which contained only a large number of oil droplets and empty vesicles

3.4 Ultrastructural changes of phloem fibers in the annual activities of cambium

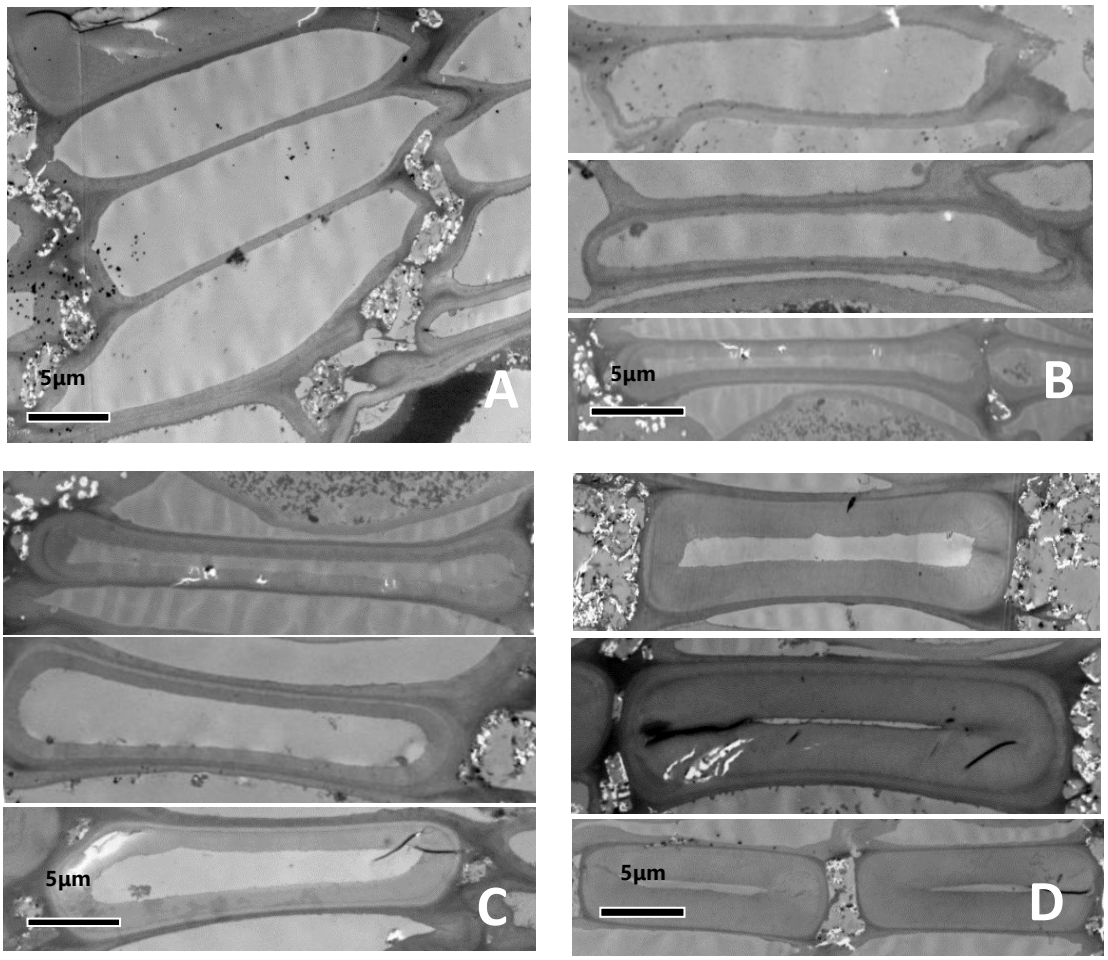

FIGURE 7: Ultrastructural changes of phloem fibers during different periods of cambium activity

A: Nonfunctional sieve cell with just programmed death, the cell has no inclusion and is spindle-shaped. B: Nonfunctional sieve cell-phloem fiber transition period, during which the primary wall thickens. C: Phloem fiber development stage, the secondary wall thickens greatly. D: Phloem fiber mature stage, phloem fiber is basically mature, with different shapes, the common characteristic is that the cell cavity is very small

During the active period of the cambium, that is, from March to November, some of the nonfunctional sieve cells were thickened and transformed into immature phloem fiber cells. At the same time, the dense arrangement of mature phloem fiber cells was observed near the periderm. The development and maturation of phloem fibers occur in all stages. Phloem fibers can be seen in any stage of phloem development. The proportion of mature and developing phloem fibers differs slightly in each stage. The sign of the transition from sieve cells to phloem fiber is that the radial wall begins to thicken, which is much larger than the thickness of the tangential wall.

In this paper, according to the cell wall development stage, the development of phloem cells is divided into three stages: (1) the nonfunctional sieve cell-phloem fiber transition period; (2) the phloem fiber development period; (3) the phloem fiber maturity period. The nonfunctional sieve cells with just programmed death are still spindle-shaped and have no inclusions (Fig. 7A). In the nonfunctional sieve cell-phloem fiber transition period, the cells gradually flattened and the cell wall thickened, which was mainly the intercellular layer thickening; the secondary wall thickening was not obvious in this period (Fig. 7B). During the development of phloem fibers, the morphology of fiber cells was slender, and the delamination of the intercellular layer and secondary wall was gradually obvious. The cells extended radially and contracted in the tangential direction, and the radial width and tangential wall width were about 1:6. Most of them were extruded and deformed by functional sieve cells and phloem parenchyma cells; the secondary wall continued to thicken, the layering of the secondary wall and intercellular layer became obvious gradually, and the cell lumen became smaller until it developed into the mature fiber. During this period, the ratio of the intercellular layer thickness to the secondary wall thickness was about 1:4 (Fig. 7C). At the mature period of phloem

fiber, the cell wall thickness is up to 2μm. The ratio of intercellular layer thickness to secondary wall thickness was about 1:6, the cell lumen was very small, and the morphological difference was great (Fig. 7D).

IV. DISCUSSION

The phloem of *Taxodium ascendens* consists of sieve cells, phloem fibers, phloem ray parenchyma cells, and phloem parenchyma cells. From the cross section, it can be seen that there is an obvious "stratification" phenomenon in the secondary phloem. Sieve cell molecules, phloem fibers, and parenchyma cells are arranged alternately in tangential bands, and the ultrastructure and composition of various molecules are different in different developmental stages. In the Wuhan area, the active phase of cambium cells lasted from the beginning of March to the end of November, and the newly differentiated sieve cells were observed in the materials on March 26. However, the newly differentiated phloem parenchyma cells and ray parenchyma cells, that is, the earliest differentiation of sieve cells, were observed on April 11, that is, in the middle of April. The appearance of S plastids in the sieve cells runs through the whole active phase, which can be regarded as a sign of the beginning and end of the active phase. The time of division and differentiation of phloem parenchyma cells and ray parenchyma cells was about half a month later than that of sieve cells.

A small number of functional sieve cells were found in *Taxodium ascendens* all year round. It is speculated that some of the sieve cells formed at the end of the last long season remained mature through the winter and became the earliest functional sieve cells in the next spring, until the new sieve cells began to differentiate, at which point they lost their contents and enter into programmed death. The resin droplets of phloem parenchyma cells were highly dispersed in the active phase and uniformly distributed along the cell wall during the dormant period. The nucleus and abundant cytoplasm of sieve cells, phloem parenchyma cells, and phloem ray cells were all observed in the active phase. In the late active phase, the cytoplasm of sieve cells began to condense, the contents of the cells decreased sharply, and a large number of substances were consumed. On the contrary, ray parenchyma cells accumulated a large number of inclusions in the late active phase, and it was not until the middle of dormancy that oil droplets and starch grains in some ray parenchyma cells were consumed. It can be inferred that most of the ray parenchyma cells can survive for multiple growing seasons and store nutrients during the dormant period.

Climatic factors have a great influence on the partial formation of cambium and phloem. Wuhan belongs to the temperate zone, and the activity of tree cambium has obvious periodicity. Some studies have shown that the division peak and differentiation rate of cambium cells are affected by the average rainfall in the rainy season. Rainfall has a great influence on the division and differentiation of cambium, while temperature has little effect on it (Konrad *et al*. 2019). In this experiment, it was found that the phloem of *Taxodium ascendens* in Wuhan began to split in mid-late April. In this experiment, it was found that the phloem of *Taxodium ascendens* began to divide and differentiate earlier in 2019, and the newly differentiated young sieve cells could be observed in late March. Combined with climatic factors, it was speculated that it was caused by intensive precipitation from early March to the end of April in the Wuhan area; the cambium activity was advanced and phloem cells differentiated earlier.

At present, the understanding of phloem structure, whether in ontogeny or phylogeny, is difficult to achieve in-depth and comprehensive understanding of the xylem, especially at the ultrastructural level. The main reasons are as follows: (1) There are many kinds of cells in the phloem, and the arrangement is more complex; (2) Some or all of the phloem is easy to destroy and disappear during senescence; (3) They change during ontogeny and are not as easy to understand as the xylem; (4) The protoplasts of sieve elements are very sensitive; the cell walls of all kinds of parenchyma cells are very fragile and sensitive; the contents are complex; and the cells vary in length. Therefore, the original state is often changed in the process of fixation, especially in the radial section and tangential section, and the images under a different electron microscope are often obtained because of different fixing methods and slice angles.

V. CONCLUSION

1) The newly differentiated young sieve cells were observed in late March. The cell walls of the newly differentiated young sieve cells were thickening during the developmental period (April to June), and S plastids could be observed in June. Some sieve cells went into programmed death at the end of August. In the process of sieve cell programming, P plastids condensed, and the nuclear changes were very obvious. The nucleus in the sieve cells showed pyknotic degeneration, chromatin aggregates, nucleoli disappeared, and nucleoplasm decreased. At the same time, along with the gradual reduction of nuclear volume, the sieve cells reach their functional stage when they stop functioning and become phloem sieve cells without dredging function. The protoplasts also died, and finally, the whole cell was squeezed and deformed.
2) The phloem parenchyma cells of *Taxodium ascendens* belong to secretory cells, which contain a lot of resin. Phloem parenchyma cells began to divide and differentiate in mid-April. At the initial stage of cambium activity, that is, from mid-April to early May, the resin was distributed in large droplets, and at the peak of cambium activity (from the end of May to

the end of June). The resin was highly dispersed in the cell, and then the resin droplets gathered. During the dormant period, resin droplets were evenly distributed along the inner wall of the cells.

3) In mid-April, newly differentiated young ray parenchyma cells were observed in the phloem near the cambium. From mid-late April to early June, the young phloem ray cells developed to mature and remained in this state until the middle of November. After that, the cells entered programmed death, and most of the ray parenchyma cells died in dormancy in December, and there were only a large number of oil droplets and empty vesicles in the cells.

4) The development and maturation of phloem fibers occur in all periods. Phloem fibers can be seen in any period of phloem development. The proportion of mature and developing phloem fibers differs slightly in each period. The development of phloem fiber can be divided into three periods: the transition period between nonfunctional sieve cells and phloem fiber, the phloem fiber development period, and the phloem fiber maturity period. In the nonfunctional sieve cell-phloem fiber transition period, the sieve cell is extruded and the intercellular layer is thickened; at the phloem fiber development stage, the secondary wall is greatly thickened; at the phloem fiber mature stage, the mature phloem fiber cell lumen is very small.

AUTHOR CONTRIBUTIONS

Conceptualization, Y. X; Methodology, Validation, Formal analysis, Y. X and H.W; Investigation, H.W., and J.T.; resources, H.L.; data curation,Y.X. and H L; writing—original draft preparation, H.W. and Y.X; writing—review and editing, supervision and funding acquisition, Y.X.; project administration and visualization, Y.X and H L.; All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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