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Abstract. The development of the leaf abscission zone in *Ginkgo biloba* trees and seedlings was studied. Leaves from *Ginkgo* seedlings were debladed to enhance anatomical changes in the abscission region. The development of an abscission layer was observed six days after deblading. The formation of the zone was not complete, but an abaxial to adaxial pattern of development was apparent. No evidence of separation was noted. Debladed *Ginkgo* petioles remained green and did not abscise during the six weeks period of study.

The development of an abscission layer was observed in leaves from a *Ginkgo* tree one week prior to separation. The zone occupied an area approximately eleven cells wide at the junction of the petiole and stem. Separation of the petiole occurred through an apparent dissolution of the middle lamella. The separation was observed in the distal region of the zone between the ninth and tenth cell layers.

The pattern of leaf abscission zone development is similar in *Ginkgo* trees and seedlings.

Abscission refers to the natural separation of any part of a plant. It is most noticeable as the means by which deciduous plants shed their leaves prior to dormancy. Other examples are the separation of flowers, fruit and bark. The process could also be considered a self-pruning device utilized for the removal of injured or senescent organs (1).

The abscission zone is a general term for the region at the base of the abscising organ through which separation eventually occurs. Separation denotes the physical separation of accessory organs from the main axis of the plant. Those cells involved in separation are referred to as the separation layer. Separation is usually within or distal to the tiers of cells formed during abscission.

The primary studies on abscission were anatomical. Von Mohl (10) reported an abscission layer where separation occurred. Further, he recognized two independent phases of the process: the separation of the organ and development of protective tissue.

This early work has led to investigations of the abscission zone and changes taking place in this region prior to separation. Plants such as *Coleus,* maple and oak have a structurally distinguishable zone where abscission is favored. Rubenstein and Leopold (12) reported *Gymnocladus* to have ninety zones in one compound leaf.

Yager (19) reported that the abscission zone consisted of twelve tiers of cells in tobacco flowers. He distinguished the cells of the separation layer as being smaller than surrounding cells. Yager

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inferred that separation cells do not develop in the same proportion as surrounding cells, thereby explaining their small size. Addicott (1) agreed, referring to the abscission layer as a zone of arrested development.

Facey (4) demonstrated that the leaf abscission zone of *Fraxinus* was twelve to sixteen cells thick and differentiated from cells on either side. She indicated that separation is preceded by cell division beginning in the proximal cells, proceeding distally through the entire zone. Further, lignification of abscission zone cells, with the exception of the narrow separation region, was observed several weeks before leaf-fall. Rubenstein and Leopold (12) proposed that cell division is not essential to abscission, but it does have an active role in the production of the protective scar.

The experiments of Scott *et al.* (15) suggested that the abscission zone might be a product of localized cell senescence brought about, in part, by water stresses due to tylose formation in the vascular elements. This evidence suggests a role for the specialized abscission layer in the process of organ separation. However, Gawadi and Avery (5) showed that cotton, poinsettia and pepper leaves fall without the development of an abscission zone. Based on this evidence they suggested that the term "abscission zone" be discontinued.

Separation has been reported to occur in one of three manners. Lee (8) described the dissolution of the middle lamella between primary walls as a factor leading directly to abscission. More recently Yager (19) has observed a similar situation in the abscission zone of tobacco flowers. Tison (17) observed a dissolution of the middle lamella and primary wall leaving a thin cellulose wall over the protoplast. Lloyd (9) also found this to be the case in *Mirabilis*. Hannig (6) reported a third type of separation resulting from a dissolution of entire layers of cells in two species. However, Lloyd (9) questioned the validity and occurrence of Hannig's observation. Observing one of the species Hannig used, Lloyd noted only a dissolution of the middle lamella and primary wall. More recently Webster (18) reported a similar type of separation in *Phaseolus*.

Rubenstein and Leopold (12) are of the opinion that anatomical changes during abscission vary somewhat from plant to plant. This was also noted by Pfeiffer (11) in 1928.

It was the purpose of this investigation to determine the general characteristics of abscission zone development in leaves of *Ginkgo biloba* L.

Materials and Methods

Ginkgo seeds were germinated in shallow trays lined with *Sphagnum*. Germinated seeds were potted and grown in the University of Northern Iowa greenhouse for one month prior to use.

The seedlings were maintained in the greenhouse during the experimental period. The latter part of this investigation involved *Ginkgo* trees adjacent to the Parkway Central Senior High School in St. Louis, Missouri.

Leaves of three two-month old seedlings in the greenhouse were debladed to enhance possible anatomical changes in the abscission region. Non-debladed seedlings were maintained under the same greenhouse conditions as controls. The seedlings were exposed to 15-20% relative humidity, 22° C and approximately 150 foot-candles of light intensity. The basal millimeter of the petiole proximal to the stem was considered to be the abscission zone. The abscission zone as well as some adjacent stem and petiole tissue was excised from different seedlings each day for six days. Excised sections were aspirated in 5 milliliters of Craf III fixative and stored for two weeks. The infiltration embedding procedures suggested by Sass (14) were followed. Ten micron longitudinal sections through the abscission zone and accompanying tissue were prepared on a rotary microtome and stained with safranin and fast green.

Other abscission regions were taken from *Ginkgo* trees grown under park conditions. The sections were hand-cut, prepared according to wet mount procedures and observed immediately.

Results and Discussion

Debladed petioles from *Ginkgo biloba* seedlings showed no macroscopic evidence of abscission at the end of three weeks. This is in contrast to debladed petioles of *Coleus* which abscise in four to five days (13). Debladed *Ginkgo* petioles remained green, upright and resisted pressure applied to the adaxial surface.

Microscopic analysis indicated that an abscission zone was lacking in non-debladed *Ginkgo* petioles (Fig. 1). Figure 2 shows the preliminary development of the *Coleus* leaf abscission zone twenty-four hours after debladng. The zone is noticeable as a transverse layer of smaller cells. These immature cells (1) were not characteristic of the *Ginkgo* leaf abscission zone.

Although zones were taken from *Ginkgo* seedlings over a six-day period, the most significant anatomical change appeared on the sixth day after debladng (Fig. 3). On the sixth day an abscission zone was visible on the abaxial side of the petiole. This is in agreement with the pattern of abscission zone development in debladed petioles of *Coleus*. The cells on the proximal side of the zone lacked nuclei. These cells also had greater accumulations of safranin in their cell walls as compared to surrounding cells. This indicates

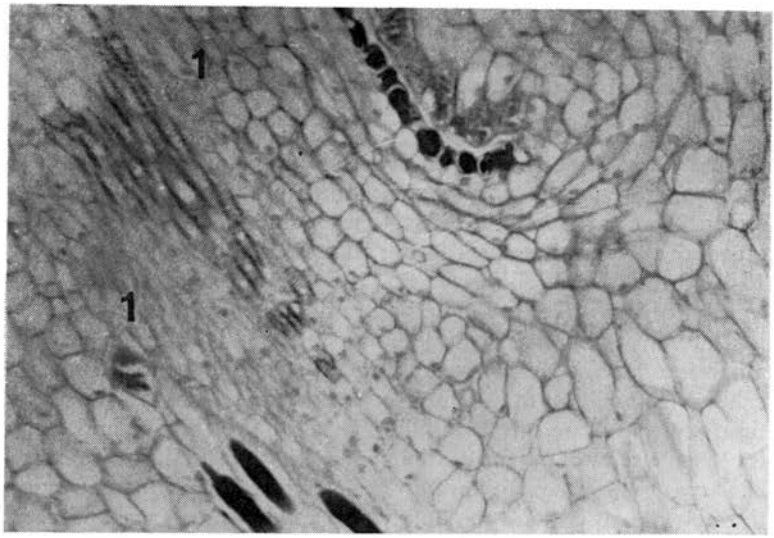

Figure 1. Abscission region in a non-debladed *Ginkgo* petiole. 1. petiole

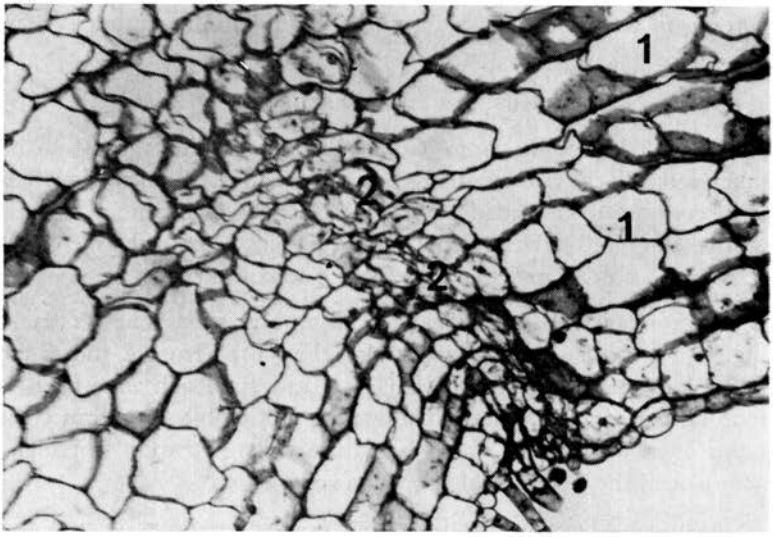

Figure 2. Coleus leaf abscission zone twenty-four hours after deblading. 1. petiole, 2. abscission layer

the possibility of lignin as a component of these cell walls. Lignified cell walls were observed in *Fraxinus* abscission zones several weeks before leaf-fall (4). The formation of an abscission zone in *Ginkgo* seedlings is incomplete six days after deblading.

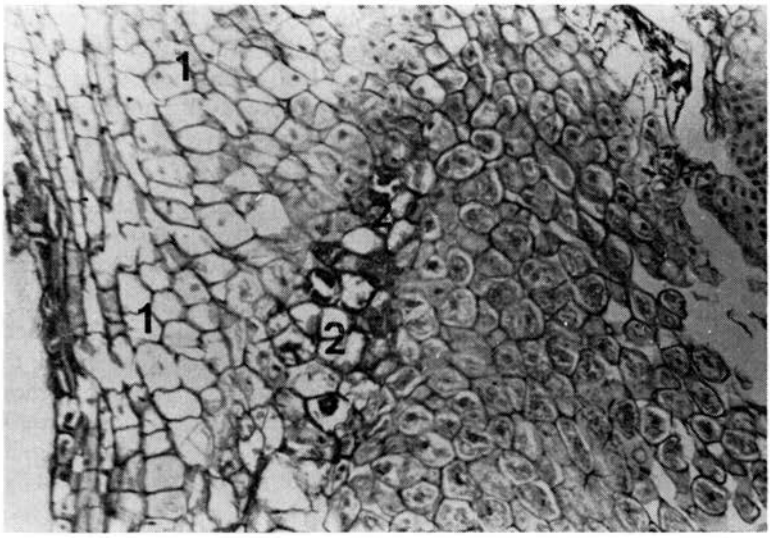

Figure 3. *Ginkgo* leaf abscission zone six days after deblading. 1. petiole, 2. abscission layer

Yager (19) reported the abscission zone in tobacco flowers to be twelve tiers of cells in width. Initially, the abscission zone in debladed petioles of *Ginkgo* seedlings was three to four cells wide on the abaxial side (Fig. 3). The width of the zone could be greater, however, since the formation of the abscission layer was probably incomplete.

A closer examination of the abscission region shows a difference in the cells on either side of the zone. The distal cells appeared to be vacuolated and lacking nuclei (Fig. 3). Proximal cells were nucleated and had a large number of inclusions or granules which might have been carbohydrates. This could have resulted from a movement of materials from distal to proximal cells as was observed by Scott and Leopold (16) in bean explants. They believed that increased metabolism resulting from cell division could serve as the driving force for the mobilization.

Facey (4) noted that cell division accompanied the development of the abscission zone in *Fraxinus*. She reported that the division began in proximal cells and spread distally through the zone. The irregular size and shape of proximal cells and the prominence of chromatin within their nuclei suggested the possibility of cell division in *Ginkgo*. Some of these cells appeared to be in telophase; however, no clearly defined mitotic figures were observed in the abscission zones during the six-day period. It is also possible that those cells thought to be in telophase could, conceivably, have been

binucleate cells. This was observed by Webster (18) in *Phaseolus* after treatment with ethylene. She explained the bi-nucleate condition as a result of mitotic divisions which were not followed by cytokinesis; or as a result of the dissolution of existing middle lamellae and walls between adjacent cells during ethylene treatment.

The development of the abscission zone in *Fraxinus* petioles was noted several weeks before leaf-fall (4). This also seems to be true of *Ginkgo* seedlings since the abscission zone was obvious by the sixth day after deblading, but separation requires longer than three weeks.

Webster (18) and Scott *et al.* (15) have suggested that lack of water due to tylose plugs in vascular elements contributed to stress on distal cells. This could hasten senescence and influence abscission. Tylose was not evident in the tracheids of *Ginkgo* seedlings. The fact that the petioles were green and not abscising is strong evidence that water transport was not interrupted.

Hormones have been shown to be a factor in retention of debladed petioles. Kuster (7) noted quite early that different amounts of leaf blade would serve to prevent leaf abscission in *Coleus*. The *Ginkgo* petioles were green, suggesting healthy tissue capable of production of hormones which could help retain the organ.

No zone of separation was noted. Apparently, physiological changes involving the middle lamella and primary cell wall that lead to separation are initiated later. These changes are probably correlated with induced senescence of the debladed petioles.

Petioles from a *Ginkgo* tree were examined for abscission zone development one-week prior to separation. All the petioles examined were found to have abscission zones at various stages of development. Figure 4 is a representation of the *Ginkgo* abscission zone which begins to form on the abaxial side of the petiole at the junction of the petiole and stem. This is in agreement with the initial development of the abscission layer in *Ginkgo* seedlings (Fig. 3).

The completed abscission zone was recognized as a layer approximately eleven cells wide. This was similar to the size of the abscission layer observed in tobacco flowers (19). Facey (4) reported a similar situation in *Fraxinus* leaves. The proximal part of the Ginkgo abscission zone was composed of five layers of non-nucleated cells. The cell walls in this layer were brown and easily differentated from cells on either side. Since a stain was not applied to these sections it was impossible to determine the components of the brown cell walls. It is possible that these cells were lignified. This possibility was also observed in *Ginkgo* seedlings (Fig. 3).

The cells on the stem side of the abscission layer were green, nucleated and seemed to have more inclusions than their counterparts in the distal petiole. This was also observed in petioles taken

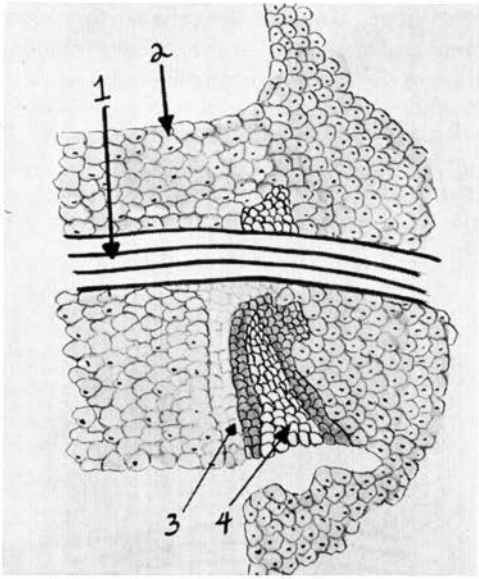

Figure 4. *Ginkgo* leaf abscission zone one week prior to separation. 1. vascular tissue, 2. petiole, 3. distal section of developing abscission zone, 4. proximal section of developing abscission zone

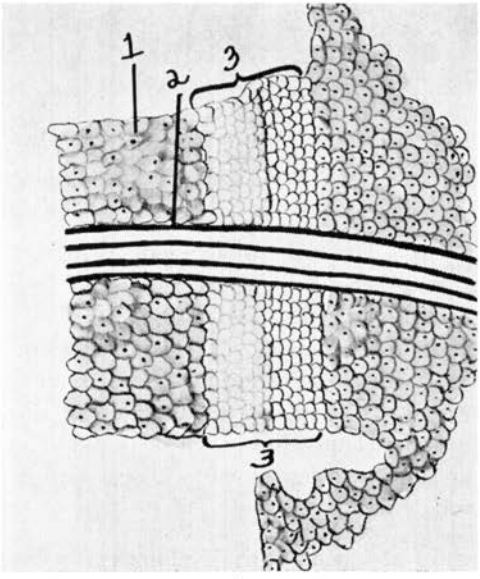

Figure 5. Completed *Ginkgo* leaf abscission layer. 1. petiole, 2. vascular tissue, 3. abscission layer

from *Ginkgo* seedlings. Many of the cells on the petiole side of the zone were green and contained nuclei. These characteristics stood in direct contrast to the cells of the abscission layer.

Most of the leaves abscised on November 4, 1969. All the petioles examined on that day showed a complete abscission layer (Fig. 5). Separation was observed to occur through the apparently suberized section of the abscission layer between the ninth and tenth

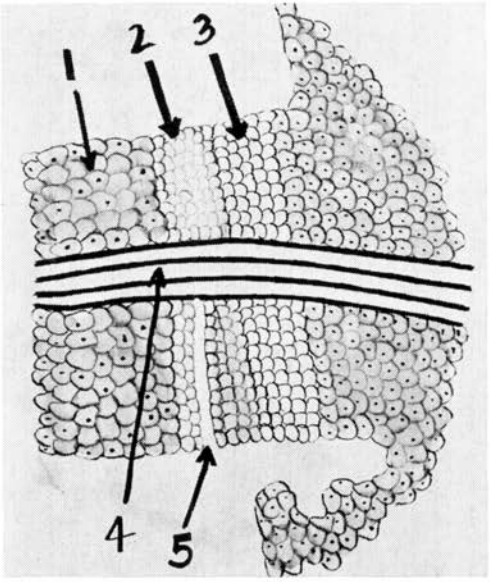

Figure 6. Completed Ginkgo leaf abscission layer showing separation layer. 1. petiole, 2. distal part of abscission zone, 3. proxmal part of abscission zone, 4. vascular tissue, 5. separation layer

cell layers (Fig. 6). The separation region is in the distal part of the abscission zone. Distal areas of separation were reported in *Fraxinus* (4), *Nicotiana* (19) and *Gossypium* (2). The mode of separation followed an abaxial to adaxial pattern. This pattern could have been influenced by the method of sectioning. The cells of the separation layer were not disrupted indicating that the act of separation results from a dissolution of middle lamellae between separation cells. This is in agreement with patterns of separation in *Brassica* (3) and *Mirabilis* (9).

The pattern of abscission zone development is similar in *Ginkgo biloba* trees and seedlings. The evidence suggests that the abscission zone has a direct relationship to the separation of *Ginkgo* leaves.

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