Anatomy of Leaf Abscission Induced by Oak Wilt

David TeBeest, R. D. Durbin, and J. E. Kuntz

Research Assistant; USDA Laboratory Leader for Pioneering Research Laboratory and Professor of Plant Pathology; and Professor of Plant Pathology and Forestry; respectively, Department of Plant Pathology, University of Wisconsin, Madison 53706.

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ABSTRACT

Oak wilt-induced leaf abscission was compared to artificially induced and natural leaf abscission in mature and juvenile red oak trees. In infected trees, the abscission process was indistinguishable from natural deciduous leaf abscission, both of which resulted from lysis of cell walls in the separation layer. Abscission of debladed petioles from healthy juvenile oaks developed from a cell division layer not found in natural or disease-induced abscission. Leaf abscission was not induced in drought-killed or deep-girdled trees. The results suggest that oak wilt-induced leaf abscission may be physiologically similar to natural abscission.

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Leaf abscission has long been recognized as a characteristic symptom of oak wilt within the susceptible Erythrobalanus (red oak) subgenus. It is not known, however, when or how the abscission process is initiated during disease development, or whether it is anatomically similar to that of natural leaf abscission. Natural leaf abscission within this group is of two types: deciduous leaves which abscise in the autumn, and marcescent leaves which remain on the tree during the winter and abscise in the spring. Both the presence of deciduous leaves and the manner in which these leaves abscise appear to be species-dependent, even within the Erythrobalanus. For example, Hoshaw & Guard (5) stated that no anatomical changes occur in the fall with abscission in *Quercus palustris* or *Q. coccinea*. Berkeley (3), however, reported that deciduous leaf abscission occurred by the formation of a separation layer. He also found that this layer could develop by cell division followed by digestion or by digestion of pre-existing cell walls. Occasionally, the separation layer ceased forming and remained incomplete in the fall. Marvin (7) reported that although deciduous leaves of *Q. velutina* sometimes formed a separation layer, digestion was not involved.

Abscission of marcescent leaves has been reported to occur by formation of a periderm proximal to the separation layer (7), by both digestion of cell walls and breakage of the petiole at the separation layer (5), and by formation of a regular separation layer in which a layer of dividing cells becomes active within the petiole in the spring (3).

This paper presents an anatomical examination of leaf abscission in red oak, *Quercus rubra* L., effected either naturally or induced by infection with *Ceratocystis fagacearum* (Bretz) Hunt and by excision of leaf laminae.

MATERIALS AND METHODS.—Natural abscission was studied in stem petiole segments collected from September through November 1969, from juvenile (1 m tall) and mature trees growing in the field.

Disease-induced abscission was examined in leaves of greenhouse-grown, juvenile trees that were inoculated by means of a cone technique (9) with 2 ml of a conidial suspension (approx 7×10^6 conidia/ml) of *C. fagacearum*. The spores were obtained by filtration of potato-dextrose-broth cultures through two layers of Reeve Angel No. 202 filter paper and centrifugation (650 $\times$ *g* for 5 min). Leaves were collected daily from the trees as soon as discoloration was noticed, usually 2-3 weeks after inoculation, until they abscised readily as a result of handling during collection.

Abscission of debladed petioles was also studied. Leaf laminae of five trees 1 m tall were removed from the petioles 3 to 4 weeks after leaf emergence in the spring. The petioles were then collected daily until they abscised from the branches during collection of samples.

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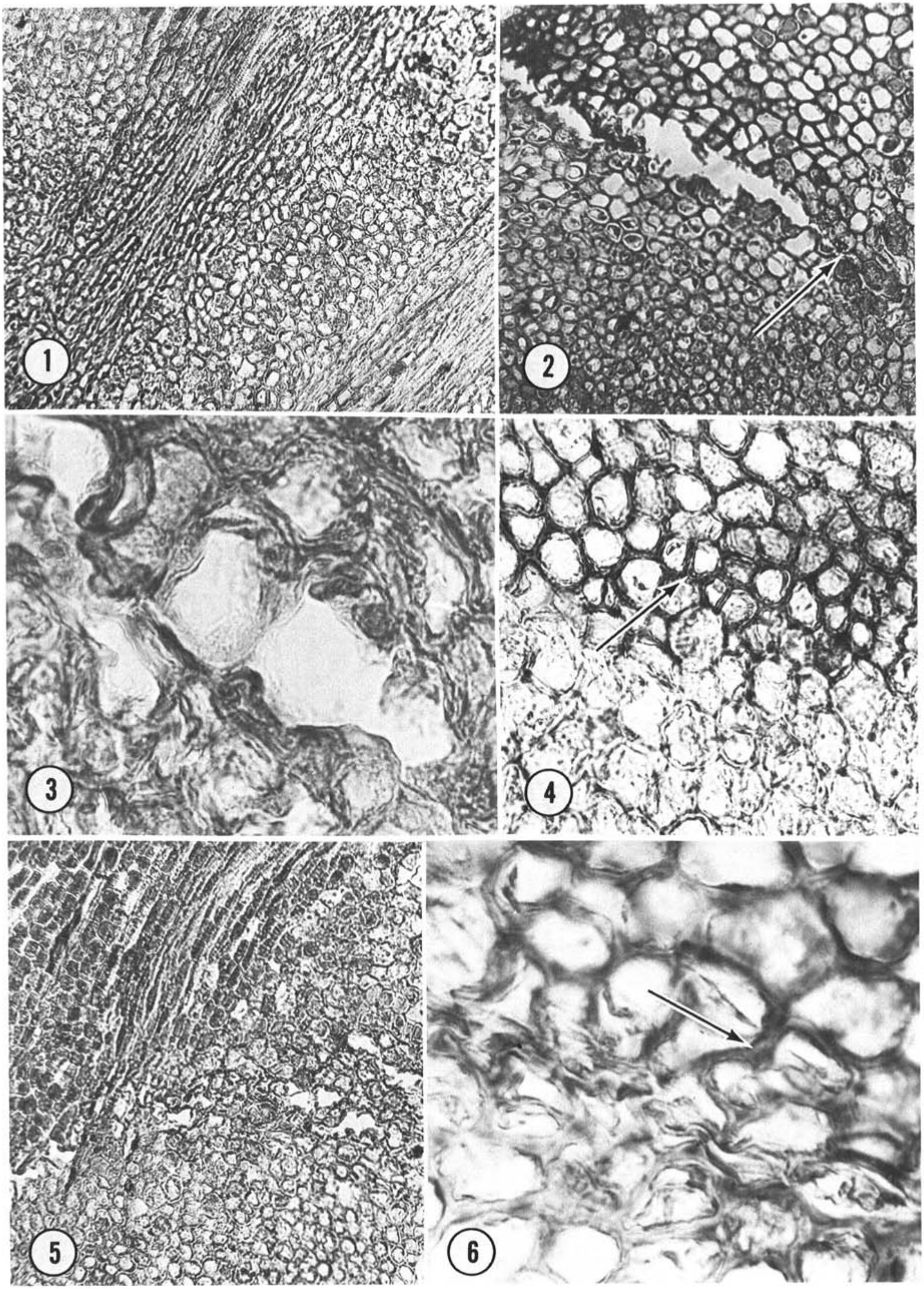
Fig. 1-6. Anatomical changes in leaves of *Quercus rubra* associated with abscission induced either naturally or by infection with *Ceratocystis fagacearum* or by deblading petioles. **1)** A longitudinal section of an immature abscission layer from a healthy tree illustrating the typical tiers of closely packed parenchyma cells that later become the separation layer. Stained with carbol-thionin (× 145). **2)** A large tear and lysis of walls of several cells (arrow) are visible in this longitudinal section of a petiole from a healthy juvenile tree. Stained with basic-fuchsin (× 145). **3)** Lysis of cell walls occurs in the abscission layer of petioles of healthy juvenile and mature trees. Stained with carbol-thionin (× 715). **4)** Lignification (arrow) of cell walls occurs distal to the abscission zone as the separation layer develops in petioles of healthy mature red oaks. Stained with basic-fuchsin (× 360). **5)** The separation layer is shown beginning to develop in the upper part of the abscission zone during natural abscission in mature *Q. rubra*. Stained with carbol-thionin (× 145). **6)** Disease-induced abscission results from lysis of cell walls in the separation layer. This dissolution results in cell walls lacking their normal rigidity (below arrow). Also evident is the lignification of cell walls (arrow) distal to the separation layer. Stained with basic-fuchsin (× 715).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Artificially induced abscission was attempted with juvenile trees (0.5 m tall) that either were girdled or from which water was completely withheld. The trees were girdled by cutting deeply into the stem near the soil line. Petioles were collected daily from girdled and drought-killed trees from the first day after treatment until 2 weeks after the trees had died.

Immediately after the samples were collected they were fixed and preserved in Formalin-acetic acid-alcohol (6). Petioles were sectioned longitudinally on a freezing microtome. The sections (10-20 μ thick) were stained with aqueous carbol-thionin or basic-fuchsin ammoniacol (4), dehydrated in an ethanol series, and mounted in Canada balsam. Photomicrographs were made using a Xenon lamp (X130/55X/150 W) as a light source and BG-12 and UG-1 filters.

Abeles' (1) terminology for describing abscission anatomy and processes are used in this report. He defines the abscission zone as the region through which separation occurs. It may consist of two layers: a cell division layer and a separation layer. The cell division layer is formed by the division of cells across the petiole. However, depending upon the species, it may not be found and only the separation layer may be present. The separation layer, itself, is the layer of cells through which separation actually occurs. Periderm may form after the leaf has abscised or, if present prior to abscission, it may form a layer of cells arising by division within the petiolar stump just proximal to the separation layer.

RESULTS.–The immature abscission zones of healthy red oaks consisted of many tiers of closely packed, ground parenchyma cells that were smaller in size than the cells either distal or proximal to the zone (Fig. 1). Leaves abscised from mature and juvenile, healthy red oaks through lysis of a layer of these cells without involvement of cell division (Fig. 2, 3). The time from fall coloration to leaf abscission was long; leaves yellowed by the end of September, reddened by mid-October, and began to fall in early November. The actual abscission process was much shorter, however, and extended over a period of ca. 5-7 days. The first noticeable change within the petiole was lignification of the cell walls in a distinct layer beginning in the petiole on the side opposite the bud and extending across the entire petiole immediately distal to the separation layer (Fig. 2, 4). Lignification of the cell walls ended abruptly distal to the separation layer, and did not occur proximal to the separation layer. Concurrent with lignification, tyloses began to develop in the xylem vessels distal to, within, and proximal to the abscission zone.

The development of the separation layer did not begin until after lignification had proceeded nearly across the petiole. The separation layer was found in the upper part of the abscission zone and developed through lysis of cell walls, starting on the side of the petiole opposite the bud and continuing until it extended through the entire petiole (Fig. 2, 5). Cells of the separation layer had walls that were uneven or broken, unlike adjacent intact cells, indicating that a lysogenous process had taken place. Leaves fell when lysis had weakened the petiole enough for it to be broken. At no time was lysis of vessels observed; they simply broke upon abscission. Periderm did not form below the separation layer of leaves of mature or juvenile trees, since cell division, indicative of periderm formation, was not observed.

The abscission of leaves showing oak wilt symptoms usually occurred 3-5 days after symptom appearance. We observed that leaves on any single tree generally took longer to abscise from the first branch that developed symptoms than from branches that developed symptoms later.

The abscission process induced by oak wilt was similar in anatomical development to that described for natural abscission. Lignification of the cells distal to the separation layer developed first on the side of the petiole opposite the bud and gradually extended across the entire width of the petiole (Fig. 6). It stopped abruptly at the separation layer, and was not found proximal to that layer. Tyloses had become extensive even before the development of leaf symptoms, unlike that which was found for natural abscission. The separation layer, as in natural abscission, was discontinuous, and developed around tracheal bundles. It developed by lysis of cell walls, first on the side of the petiole opposite the bud, and gradually across the entire petiole (Fig. 6). Lysis resulted in the loss of birefringence of cell walls, so that under polarized light the separation layer was evident as a darkened layer across the petiole (Fig. 7). The appearance of cell divisions below the separation layer in a single petiole stump indicated that the formation of periderm may have begun in that stump (Fig. 8). The dividing cells were found in a layer extending about halfway across the petiole below the separation layer. Lysis of cell walls in the separating layer was already complete across the entire width of the petiole.

Deblading the petioles of red oaks 3 weeks after leaf emergence caused abscission ca. 5-7 days later. Changes which occurred early within the abscission zone were: (i) the lignification of a layer of cells immediately distal to the future separation layer; and (ii) cell division in which two to five tiers of cells were involved (Fig. 9, 10). Cells that arose by division appeared to be the forerunners of the separation zone, as these cells appeared to lyse during formation of the separation layer (Fig. 10). The cell division layer was not found in the petioles either distal or proximal to the zone of lysis. As lysis of the separation layer neared completion, lignification was observed proximal to the separation layer. Tylosis filled the xylem vessels within and below the separation layer before lysis and abscission was complete.

Girdling and dehydration did not induce leaf abscission in juvenile red oak trees, nor did a separation layer develop.

DISCUSSION.–Leaves on mature and juvenile trees of *Q. rubra* naturally abscised in the fall. The abscission process involved lysis of cell walls within a separation layer, thus confirming the results of Berkeley (3). Leaf abscission induced by oak wilt was

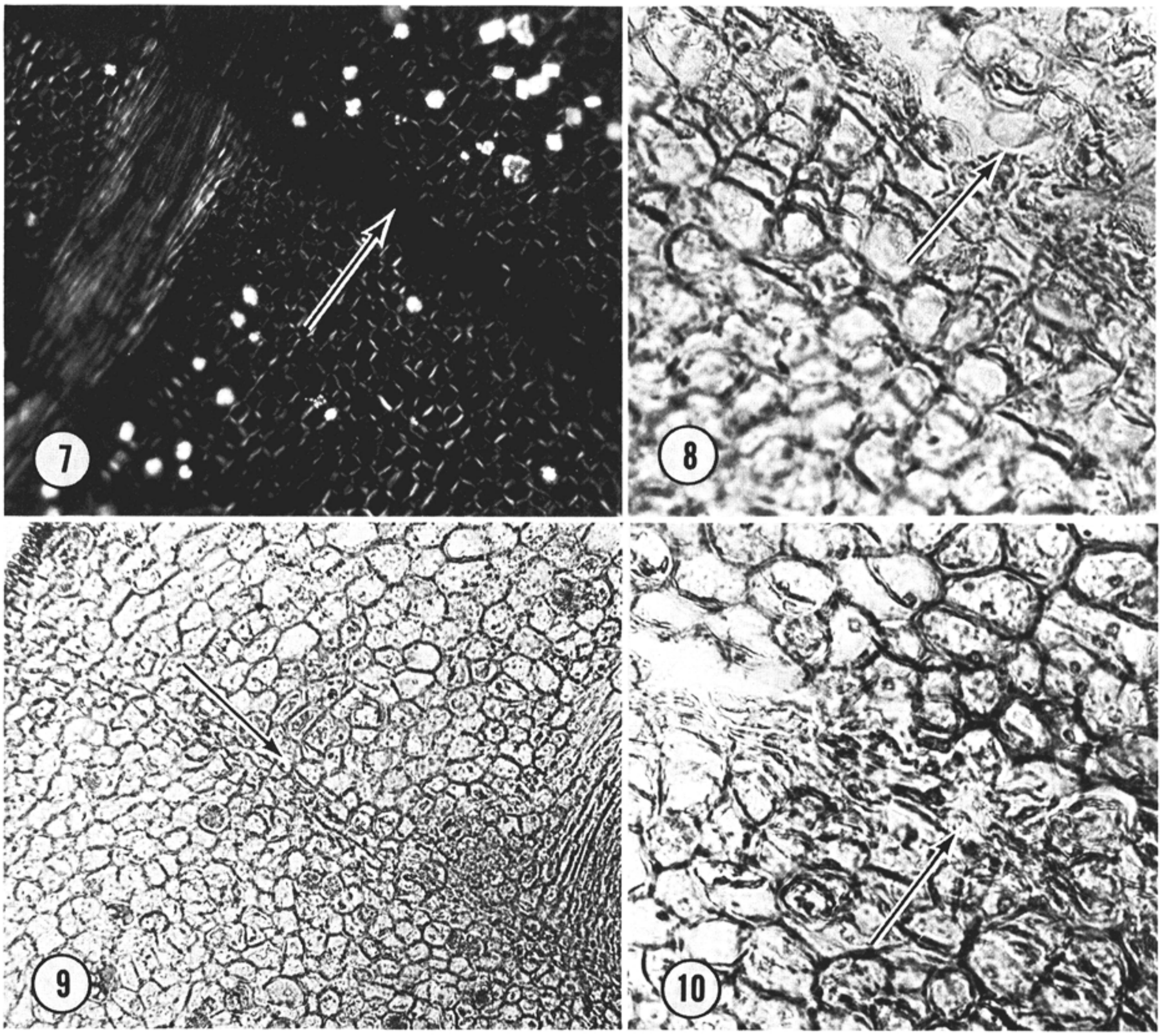

Fig. 7-10. 7) The cells of the separation layer (arrow) are not visible under polarized light. Lysis of the cell walls has resulted in the loss of birefringence (× 145). **8)** Periderm formation is visible below the separation layer of this petiole from an infected tree. This is the only petiole in which it was found. Lysis of cells is complete in the separation layer (arrow), and cell division has begun proximal to it. Stained with carbol-thionin (× 360). **9)** Cell divisions (arrow) occurred in the abscission zone of a debladed petiole prior to cell wall lysis and abscission. Stained with carbol-thionin (× 145). **10)** Lysis (arrow) of cell walls of the newly divided cells is shown in this section of a debladed petiole. Lignification of cell walls distal to the separation layer is also evident. Stained with basic-fuchsin (× 360).

indistinguishable from natural leaf abscission. Thus, disease-induced leaf abscission may be physiologically closely related to natural abscission processes, although the exact process by which abscission is initiated either naturally or by *C. fagacearum* is unknown. The similarity of these abscission processes suggests a relationship between oak wilt and natural senescence.

Conversely, leaf abscission induced by deblading petioles did not resemble natural or oak wilt-induced abscission. The separation layer of debladed petioles developed from a cell division layer not found in natural or disease-induced abscission. This points to the occurrence of a physiological mechanism responsible for debladed petiole abscission different from that induced by oak wilt.

Also, if girdling and dehydration approximate water stress caused by tyloses, then tylose formation is probably not the immediate cause of leaf abscission in oak wilt because these treatments failed to induce abscission. The effects of these water-stress-inducing treatments closely resemble the symptoms shown by leaves on sucker shoots from diseased trunks, sprouts from infected stumps, or other succulent immature tissues. Such leaves differ from those of the parent plant by failing to abscise after wilting (8). Thus, our results and those of Beckman et al. (2) strongly suggest that factors other than water stress are involved in symptom expression in oak wilt.

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