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# MACRO-MICROSCOPIC ANATOMY: OBTAINING A COMPOSITE VIEW OF BARRIER ZONE FORMATION IN ACER SACCHARUM

by

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**Summary**

The technique for constructing a montage of large wood sections cut on a sliding microtome is discussed. Briefly, the technique involves photographing many serial micrographs in a pattern under a light microscope similar to the way flight lines are run in aerial photography. Assembly of the resulting overlapping photographs requires careful trimming. A composite of many low power micrographs allows for a resolution of the sample that could not be obtained from a single photograph. A barrier zone in sugar maple (*Acer saccharum*) is used as an example of a 'macro-microscopic' montage. The scope of the montage aids is understanding the condition of the tree before injury, the tree's developing response to this problem, and the consequences of its defense strategy.

## Macro-microscopic anatomy

'Macro-microscopic' montages are short stories written with pictures. They can be excellent aids in understanding and teaching anatomy when focused on a particular subject. The subject of this montage (Fig. 1) is the formation of a barrier zone due to wounding. It is a photographic reproduction of a larger montage composed of fifty individual photographs. The 18-µm-thick-section of sugar maple (*Acer saccharum*) it illustrates, was cut on a sliding microtome and stained with toluidine blue 0 (0.05% aqueous). The actual sample block measures approximately 1.3 cm × 1.0 cm. The finished montage measures 89 cm × 63 cm, having a scale of 3.2 cm (montage) = 0.5 mm (actual).

The stained section was photographed under low power using a light microscope and attached 35 mm camera. Overlapping photos were taken in rows (similar to the flight lines used in aerial photography) until the entire section was covered. The photos were then sequentially printed on resin coated paper and numbered on the back before development. The resulting photos were trimmed of their white borders.

Reassembly began first by rows. Each photo in a row was overlapped with its adjoining horizontal complement and attached to it using artist's tape. This type of tape does not strongly adhere to the surface of photos and leaves no residue upon removal. Once the rows were assembled, each was trimmed in the vertical plane of any drift caused by movement of the microscope stage. Each row was then overlapped with its adjoining complement and taped into place.

At this point, the outer edges of the temporarily completed montage were cropped using a T-square, a metal straightedge and razor. This allowed the montage to be molded into the final desired shape (rectangular). It was then centered on white art board and its outer edges traced in pencil. The pencilled lines were later used as a guide in gluing the photos to the board.

The montage was then taken off the art board and placed on a large piece of cardboard which served as a cutting surface. A metal straightedge was placed on each vertical overlap so that a single razor cut would eliminate the overlap and allow for the edges of rows to be butted flush. The same procedure was then followed in dealing with the horizontal overlap between photos in a row. The result was fifty loose 'puzzle pieces'. The back of each piece was sprayed with a special adhesive for mounting photos. They were pressed into place on the art board using the pre-

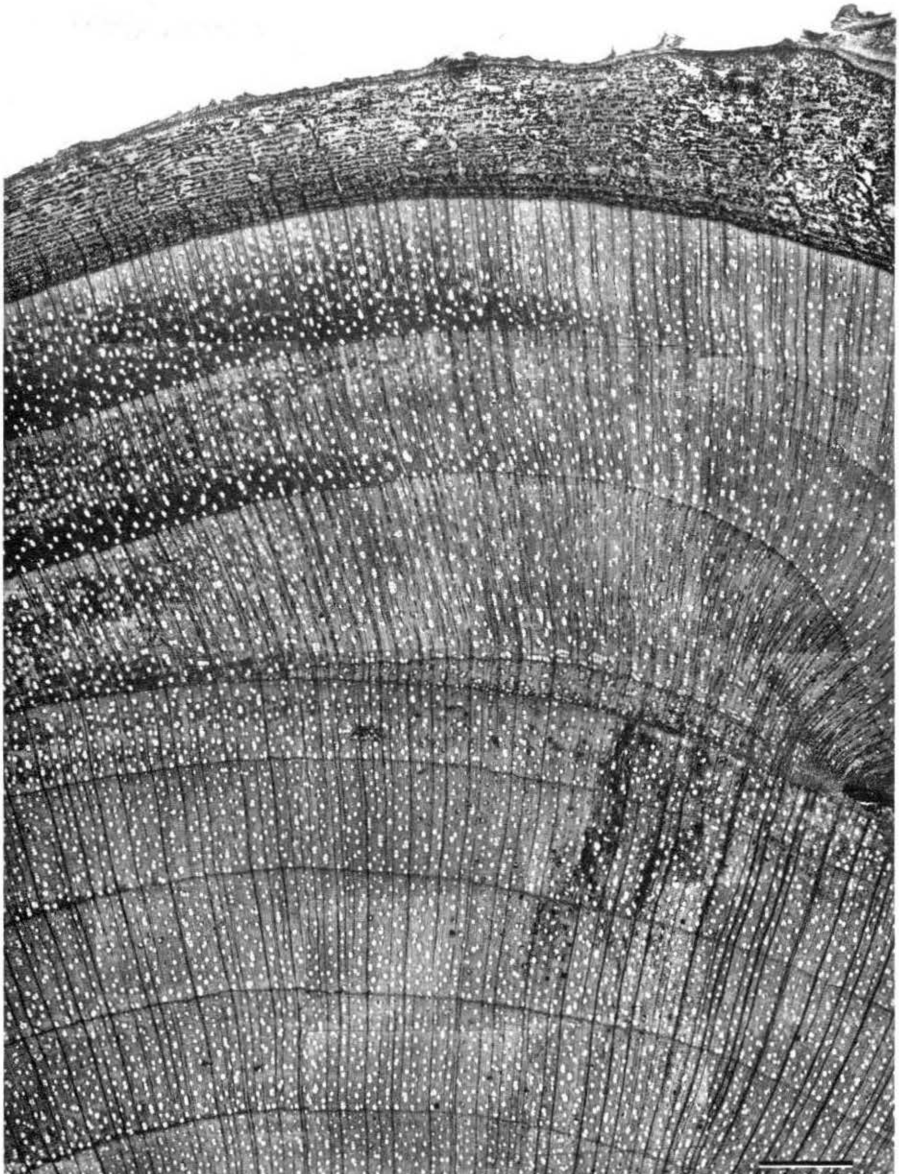


Fig. 1. Barrier zone in *Acer saccharum*. The figure is a composite of fifty individual photographs of a single 18 µm thick section. Also visible are bark and the growth rings formed prior and subsequent to wounding.

viously drawn pencil marks as a guide. It took approximately forty man-hours to go from sectioning the block to finishing the montage.

The extremely large size of the finished montage was an attempt to produce a detailed 'macro-microscopic' view of wound response in a tree that could be used for instructional purposes. This response follows the CODIT (Compartmentalisation Of Decay In Trees) Model as conceived by Dr. Alex L. Shigo (retired, U.S. Forest Service, Durham, N.H., U.S.A.). This model describes the four 'walls' that a tree builds to compartmentalise the spread of infection to as small an area as possible. A particular tree's ability to compartmentalise relates to its genetic disposition and the severity of the wound. The 'scope' of the montage also allows one to easily orient to its spatial position in the tree by the presence of the cambium and attached bark. The montage helps one to understand the condition of the tree before injury, the tree's developing response to the problem and the consequences of the defense strategy on subsequent growth.

The edge of the wound is visible on the right side of the montage, proximal to the start of the third growth ring from the cambium. A distinct attenuating band of tissues emanates from this wound site in response to the injury. These tissues are collectively referred to as the 'barrier zone', or 'Wall 4' of the CODIT Model. The barrier zone separates the wood present at the time of wounding from subsequent tissues formed by the cambium. (Stem cross sections of certain trees have revealed barrier zones encircling entire growth rings.) The wound to this tree can then be determined to have occurred three years prior to harvest. The time frame can be further narrowed to the early spring of that year as evidenced by a small amount of growth (relative to the rest of the ring) between the previous year's latewood and the barrier zone.

The cells of the barrier zone are irregularly shaped and thick-walled. Parenchyma cells, especially those found nearest to the wound site, are often darkly discoloured by the presence of wound gums. Ray parenchyma cells are hypertrophied in the area of barrier zone formation.

In an area of the section containing growth rings formed prior to barrier zone formation, a large proportion of the cells appear plugged. This area is known as 'Wall 1' of the CODIT Model and is proximal to the wound. These cells are plugged in resistance to the vertical spread of infection. A mirror image of this plugging in cells from the same cambial initial area, but formed subsequent to wounding, does not take place. The radially distal edge of Wall 1 is 'Wall 3' of the CODIT Model. It helps resist the lateral spread of infection. 'Wall 2', not present in this section, would resist the inward spread of infection toward the pith. It often follows a growth ring. There is no Wall 2 when a column of discoloration reaches the pith.

A period of increased growth often occurs in wood formed subsequent to wounding. This increase is in part due to an attempt by the tree to close over the wound as quickly as possible. This area of hypertrophic growth is often localised to an area proximal to the wound site. This is especially true in the case of a small wound, but will vary in and between trees of different species. Any external indication of the wound shown in this montage may already have been erased due to closure.

One final note of interest lies in imagining an increment borer coring the tree from which this section was taken. A core taken along a radius located just beyond the distal edge of the barrier zone would make it appear that the tree had just undergone three good years of growth. The only indication that this observation might be false would be a slightly skewed growth pattern beginning three years prior to coring.

A core taken on a radius through the barrier zone might lead the untrained eye to observe *not only* increased growth, but an extra year of growth! This extra year would be the barrier zone mistakenly identified as the latewood of a growth ring. The possibility of such an error would no doubt increase if the barrier zone had occurred in the centre of the growth ring. An understanding of the tissue changes in a barrier zone, the likelihood of discoloured wood behind it and increased growth ahead of it would lessen the chances of such a mistake.

**References**

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