SHORT COMMUNICATION

Tyloses and the Maintenance of Transpiration

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During a study of transpiration and embolism-formation in petioles of sunflower, tyloses were frequently observed in early metaxylem vessels. Tyloses were confined to the inner ends of the xylem arcs, remote from the phloem. Vessels in this position are especially vulnerable to embolism. All stages of the invasion of vessel lumens by xylem parenchyma cells were observed, from the early protuberance of a cell through a pit to the complete occlusion of the lumen by one to several cells. The lumen space not occupied by tyloses was seen both filled with xylem sap, or embolized and gas-filled. Thus, during the early stages of tylosis formation the vessel remained active in carrying the transpiration stream. Thin-walled vessels of the protoxylem or early metaxylem were not tylosed, but were squashed and disappeared. These observations are interpreted as evidence that vessels vulnerable to embolism are decommissioned and replaced by parenchyma tissue, while new and less vulnerable vessels are added to the xylem arcs at the cambial side. It is proposed that tylosis formation is triggered by the frequent embolization of the vulnerable vessels to give, ultimately, an incompressible tissue. Then tyloses would be necessary to preserve the tissue pressure which expresses water to refill embolisms in the remaining vessels, and maintain transpiration, as explained by the compensating pressure theory of water transport.

Key words: Compensating pressure theory, embolisms, starch sheath, tissue pressure, transpiration, tyloses, vessel diameter.

INTRODUCTION

The invasion of the dead lumens of tracheary elements by living parenchyma cells (formation of tyloses) is a well known response to infection by pathogens and to wounding (Riou et al., 1995). It is a very common natural occurrence in the inner annual rings of trees, and marks the beginning of the transition from sapwood to heartwood (Esau, 1965; Strasburger, 1965; Fahn, 1977). It is often accompanied or followed by the formation of gums and tannins (Chattaway, 1949; Strasburger, 1965) which add to the strength and durability of the composite polymer of mature heartwood. The story of the discovery and anonymous publication of tyloses by Baroness Hermine von Reichenbach (Anonymous, 1845) was pieced together by Zimmermann (1979, 1983) who gives a succinct account of the considerable literature that treated them in the intervening years. The whole thrust of this literature emphasizes the terminal nature of the tylosed xylem as a protective barrier of dead tissue, contrasted with the active xylem which contains living parenchyma and rays and conductive tracheary elements.

I was thus unprepared to find, in a recent study of transpiration and embolisms (Canny, 1997a, b), tyloses being formed in the early metaxylem vessels of young, undamaged, uninfected, sunflower leaves. This paper reports details of these findings, shows how these tylosed vessels become indistinguishable from ground parenchyma, and proposes that their formation is part of an active strategy of vessel renewal and elimination which maintains rapid water flow.

MATERIALS AND METHODS

Full details of the experimental procedures are given in Canny (1997a, b). In summary, sunflower plants (Helianthus annuus L.) were glasshouse-grown, in pots under natural illumination supplemented by high intensity lights morning and evening. There were two groups, one for each experiment. Plants of the first group were 4 weeks old with about 12 expanded leaves, and the experiment ran from 1100 to 1600 h on a short spring day with abundant water supply to the pots. The second group were 7 weeks old with about 30 expanded leaves, and the experiment ran from 0900 to 1900 h on a midsummer day. Plants were liberally watered before the experiment, but not during the day of the experiment. The leaves referred to were numbered upward from the node above the cotyledons, and, to distinguish the stage of their development will be referred to as leaf ‘x’ of 12, or of 30. Thus leaf 7 of 30 is one of the oldest leaves on a large plant, and leaf 7 of 12 is one of the younger leaves on a small plant. Vascular tissues midway along the petioles and midribs were studied by freezing intact or recently-severed leaves in liquid N\textsubscript{2}, and examining the frozen tissues in the cryo-scanning electron microscope. Transverse faces of the frozen organs were planed flat with a cryo-microtome at $-80$ °C, etched briefly at $-90$ °C in the column of the microscope, and coated with aluminium as explained in Huang et al. (1994). They were examined at 7 kV, and
Fig. 1. Transverse views of sunflower petioles. B–F are planed, frozen faces of snap-frozen fresh tissue viewed in the cryo-scanning electron microscope. Position of the phloem is downwards. A, Thick hand-section of fixed petiole, showing the three large vascular strands containing radial arcs of xylem vessels (phloem is on the outer side of each strand). Single-layered starch sheaths lie next to each strand on both the inner and outer margins at the positions indicated by arrowheads. Iodine stain, reversed contrast. Bar = 0.5 mm. B, Vessels of a vascular strand which were full of sap when frozen appear black (v). Ground parenchyma cells (p) are distinguished by their grey colour produced by solutes sequestered during freezing. One vessel (arrowhead) is embolized. Immature vessel elements (x) arise from the cambium on the phloem side. Leaf 6 of 12,
micrographs recorded on Kodak T-Max 120 rollfilm developed in T-Max developer.

The location of starch in hand cut sections of fresh or fixed tissue was studied by light microscopy after staining with iodine (0.5% I₂ in 2% aqueous KI).

RESULTS

There were three large vascular strands and several small ones in the petiole (Fig. 1A). All the observed tyloses were formed in the large strands, whose general form is shown in transverse section in Fig. 1B. In each strand there were six to 12 radial arcs of vessels diverging slightly inwards, each composed of four to eight vessels. In the frozen preparations the parenchyma cells appear pale grey (high electron emissivity) because they contain abundant solutes. These solutes are sequestered from ice during freezing into narrow sheets that appear as characteristic line-patterns in the transverse face. The vessels that contained sap on freezing appeared black (low electron emissivity) with few white lines or spots because their ice contains few solutes. Their thickened walls of hydrated cellulose were also black. Vessels that were embolized when frozen were empty of sap, but were often filled with stray pieces of wall material which had collected in the cavity during planing. Immature vessel elements formed from the cambium were often found at the outer, phloem end of the arcs (Fig. 1B). They can be distinguished from parenchyma cells by their size and position and from the conducting vessels by their solute-rich contents, apparent in the images as solute lines (usually in a different pattern from those of the parenchyma). They contain high concentrations of potassium (Canny, 1995a).

Tyloses were found in all ages of leaf from 10 of 12 to 7 of 30, in petioles and midribs, on the inner ends of the radial arcs. The image that first alerted me to their presence is shown in Fig. 1C, where two parenchyma cells protrude into a vessel lumen, which is otherwise filled with sap. Once it was appreciated that tylosed vessels occurred in this position, many other stages of their formation became apparent. A fully developed tylosed vessel is shown in Fig. 1D, where three cells fill the whole lumen, and no room is left for sap or gas space. The thickened walls are still clearly apparent. Intermediate stages of partially tylosed vessels are shown in Figs 1E, F, and 2A.

Although fully tylosed vessels transport no water, some vessels in which tyloses were developing were still carrying streams of transpiration water. The evidence for this is that they displayed the two states characteristic of an active vessel, viz. the non-tylosed space could be either full of sap (Figs 1E and 2A) or contain gas (Fig. 2B and E). As shown in Canny (1997a, h), active vessels alternate constantly between these two states as they are embolized by evaporative stress and refilled by reverse osmosis.

Beyond the inner end of the radial arcs tylosed vessels became increasingly difficult to recognize. The wall thickenings of the original vessel became obscure (Fig. 2C). When the thickened walls were no longer distinguishable it was impossible to tell parenchyma cells that had formed as tyloses from those that were part of the original ground parenchyma. In this region vessels were seen which had become greatly flattened (Fig. 2D). These were always small vessels with thin walls compared with the walls of the tylosed vessels. All stages of this flattening were seen from slightly elliptical to almost linear. From their sizes, these flattened vessels were the early-formed metaxylem and protoxylem elements. Like the active, and partly-tylosed vessels, these flattened vessels were seen both filled with sap and embolized.

The vessels in which tyloses formed were those identified in Canny (1997a) as being the most vulnerable to cavitation during the first evaporative stress of the day. In petiole samples during the morning, embolisms were conspicuously concentrated in the inner part of the arcs of vessels (Fig. 2E). After the refilling mechanism was activated in the afternoon, and embolisms were reduced to a minimum, the remaining embolized vessels were more randomly distributed along the arcs (Fig. 2F).

In petioles where secondary vessels had formed from the cambium the new population of vessels on the phloem side contained many small vessels with diameters in the range 10 to 30 μm mixed in with large vessels (30 to 70 μm) (Figs 1A and 2E).

DISCUSSION

Klein (1923) had, even at this early date, developed a fairly full understanding of how, where, and under what conditions tyloses formed. He was well aware that they were not confined to woody tissues, but also formed in herbaceous stems in response to wounding. He demonstrated by elegant experiments that they were caused not by wounding, nor by cessation of transpiration, nor by a richer supply of oxygen when the vessels filled with air, but by the replacement of the water phase in a conducting vessel with a gas phase in a non-conducting vessel. He also showed that if the vessels became too dry under the gas phase, tyloses did not develop. It was necessary that the gas in the vessels remain damp so that the xylem parenchyma cells had sufficient turgor to invade the lumen through the pits. His emphasis on what produced the gas phase to initiate tylosis formation was on both the artificial act of wounding, and on the natural loss of conduction in older rings of the wood. He was satisfied that
Fig. 2. Transverse planed frozen faces of sunflower petioles (A, B, D and E) and midribs (C and F) viewed in the cryo-scanning electron microscope. Direction of the phloem is downwards. A, Inner ends of vessel arcs showing several stages of tylosis formation. Those at $t_1$, $t_2$ have still some sap flowing in them; those at $t_3$, $t_4$ are filled with tylosis cells; those at $t_5$, $t_6$ have become almost indistinguishable from the ground parenchyma. One vessel on the right is embolized (e). Leaf 18 of 30, sampled at 1300 h. Bar = 50 µm. B, The central vessel contains a tylosis and is also embolized. Leaf 9 of 30, sampled at 1900 h. Bar = 10 µm. C, The lower arc of vessels ends in three tylosed vessels (*) which are merging...
the tyloses formed by artificial and natural causes were identical.

From Klein’s conclusions we may be confident that the stimulus to tylose formation in the early metaxylem of the sunflower leaves was the presence of a gas phase produced in them by embolism. It is surely significant that the tyloses form in just those vessels at the inner end of the arcs which are most liable to embolisms under the initial evaporative stress of each day’s transpiration. These inner vessels were also identified by Robb et al. (1979) in infected petioles of Chrysanthemum as containing tyloses.

When we ask what it is about these inner vessels which makes them especially vulnerable to embolism, we must turn to the hypothesis of refilling developed in Canny (1997b). This proposes that the osmotically-generated tissue pressure in the petiole forces water by reverse osmosis out of parenchyma cells into the empty spaces of embolized vessels. A primary source of this tissue pressure is the phloem (Canny, 1995b); a secondary, regulatable source is the starch sheath which adjusts to increased evaporative demand by hydrolysing starch to sugar and increasing the available osmotic pressure (Canny, 1997b). According to this hypothesis, the vulnerability of the inner metaxylem vessels is due to their remoteness from the phloem, whose pressure is especially important early in the day. Later, as the starch sheath develops increasing pressure, this operates from the positions of the sheath on both the inner and the outer sides of the vascular strands, and increases the refilling rate in the inner vessels. As the cambium adds more vessels radially outwards and the inner vessels become more remote from the phloem, they would spend longer each day with gas rather than water contents, and the tylosis formation would be stimulated. On this view, the strand is decommissioning its inner vessels, and adding more on the outside, maintaining a fairly constant number of active vessels in each arc. The other act of decommissioning observed—the squashing of the thin-walled protoxylem and early metaxylem vessels until they vanish altogether, is a simpler means of achieving the same end.

The importance of this act of decommissioning vulnerable vessels is also explained by this hypothesis. The maintenance of a positive tissue pressure in the petiole depends on minimizing the volume of gas space, because in such spaces the pressure is dissipated by compressing the gas. An embolized vessel is such a gas space, and so a potential danger to the whole pressurizing, refilling process. Tylosis formation turns the embolized-vessel gas space into incompressible ground parenchyma, and preserves the tissue pressure. Furthermore, gas spaces in the tissue are alternative destinations for the parenchyma-derived water which might be used for repairing embolisms. Occluding such spaces conserves this water.

It is also notable, that while the inner vessels, which are small diameter vessels of the protoxylem and early metaxylem, are put out of action by squashing and tylosing, more small vessels are produced on the phloem side of the strand by the cambium, mixed in with large vessels. As was stressed in Canny (1997b), the significance of a proportion of small vessels in a block of xylem is that they spend less time in the embolized state. Because of their surface/volume relation, they both empty faster on embolization and fill faster on refilling than wider vessels. Therefore, at times of high evaporative demand and rapid embolization it is the small vessels which provide continuity of flow, until the protective mechanisms of increased pressure are activated, and full forward flow is restored for long periods in the wider vessels. In the experiments of Canny (1997a, b) this restoration of flow in the majority of vessels was achieved by mid afternoon.

The observation of tylosis formation in early metaxylem has therefore revealed another two levels of regulation in the maintenance of water flow during transpiration: a regulation to maintain pressure by preserving incompressible tissue space and a regulation to supply a population of small vessels to replace those lost in maintaining the pressure.

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LITERATURE CITED


