A SPECIAL TYPE OF TRACHEARY ELEMENT ASSOCIATED WITH "XYLEM DISCONTINUITY" IN THE FLORAL AXIS OF WHEAT

By S. Y. Zee* and T. P. O'Brien*

[*Manuscript received April* 22, 1970]

Summary

In wheat, the continuity of xylem between the pericarp of the caryopsis and the rachilla is interrupted by a core of thick-walled cells which have been characterized as a special type of tracheary element. We have provided evidence to show that the xylem discontinuity restricts the flow of material from the xylem in the rachilla to that in the pericarp.

I. Introduction

In comparison with the physiology, which has been the subject of extensive research, the histology of the spikelet of wheat has received scant attention. Detailed knowledge of the vascularization of the developing grain and glumes is lacking. Without such information it is difficult to interpret many of the relevant physiological observations that have been published.

Using new techniques for tissue preparation (Feder and O'Brien 1968), we have worked out in some detail the structure of the spikelet, especially in the attachment region between the ovary and the rachilla. First, we have found that at the region where the glumes, lemma, and palea are attached to the rachilla the xylem and phloem contain an abundance of transfer cells (Pate and Gunning 1969). Secondly, though strands of xylem occur in both the pericarp and rachilla, there is a region in the floral axis at the base of each floret where the continuity of the tracheary elements is interrupted for about 1 mm by a group of previously undescribed cells.

Details of the distribution and structure of the transfer cells will be given in a later paper. The present paper is concerned with the nature of the xylem discontinuity, and the morphological characteristics of these special cells.

II. Materials and Methods

Spikelets of wheat [*Triticum aestivum* L. cv. Heron] were obtained from plants grown in the glasshouse. The spikelets, at various stages of development after anthesis and with part of the rachis attached, were fixed in an 0·025M phosphate-buffered solution of glutaraldehyde and acrolein (4%+3%) overnight at 0°C. The tissues were dehydrated by passing them through methoxyethanol, ethanol, propanol, and butanol, and embedded in glycol methacrylate. Serial longitudinal and transverse sections, 1–2 μm thick, were cut with glass knives, stained with the periodic acid–Schiff's (PAS) reaction or toluidine blue O or with both stains, and examined by light microscopy. The methods are given in detail by Feder and O'Brien (1968).

* Botany Department, Monash University, Clayton, Vic. 3168.

For electron microscopy, segments of the floral axis were dissected out from the plant and fixed in phosphate-buffered 4% glutaraldehyde for 3 hr at room temperature. The segments were then washed in buffer and post-fixed in 2% OsO_4, dehydrated in alcohol, and embedded in a low-viscosity epoxy resin (Spurr 1969). Sections were doubly stained with uranyl acetate and lead citrate and viewed in an Hitachi HU11E electron microscope at 75kV.

III. Results and Discussion

Figure 1 is a diagram of a sagittal section of a wheat spikelet, showing two of the three florets. The attachment region between the rachilla and the base of each ovary is figured.

Serial transverse and longitudinal sections through the attachment region of such spikelets reveal that the tracheary elements of the pericarp bundle are not in direct continuity with those of the rachilla. Rather, the files of normal tracheary elements of the pericarp bundle and rachilla converge upon a core of thick-walled cells (Figs. 2 and 3; the location of these sections is shown on Fig. 1) situated within the tissues of the attachment region. The cells lie close together, forming a central

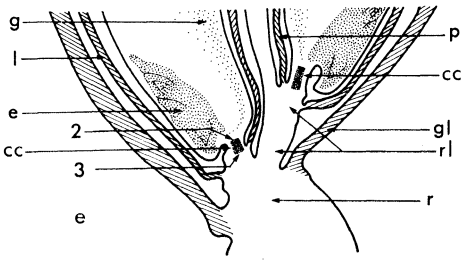


Fig. 1.—A sagittal section of a wheat spikelet showing the relation between the caryopses and the rachilla (*rl*) and rachis (*r*). The positions of the glumes (*gl*), lemmas (*l*), paleas (*p*), groove (*g*), embryo (*e*), and the central core of thick-walled cells (*cc*) are marked. The regions shown in Figures 2 and 3 are marked 2 and 3 respectively.

cylinder which is surrounded by numerous sieve elements and transfer cells (Fig. 4) resembling types A and B (Pate and Gunning 1969). This central core of thick-walled cells extends throughout the length of the attachment region and for some distance (up to 0·5 mm) into the base of the caryopsis at the bottom of the groove (or crease). Approximately at the level of the bottom of the coleorhiza, this tissue meets the vascular bundle of the pericarp (Fig. 2) that lies within the groove.

Although the sieve elements that surround this core of tissue appear to be continuous with those of the pericarp bundle, normal tracheary elements do not cross from the xylem of the rachilla to that of the pericarp. Preliminary studies upon three other species of grass (ryegrass, oats, and bromegrass), have revealed a comparable pattern, suggesting that the lack of xylem continuity between ovary and rachilla may be a common feature of grasses.

Fig. 4.—Transverse section through the middle of the attachment region. The central core contains thick-walled cells whose protoplasts vary from intact (cc_1) to necrotic (cc_2) to completely disintegrated (cc_3). Numerous sieve elements (*s*) and transfer cells (*t*) surround the core. Epoxy section, toluidine blue O stain.

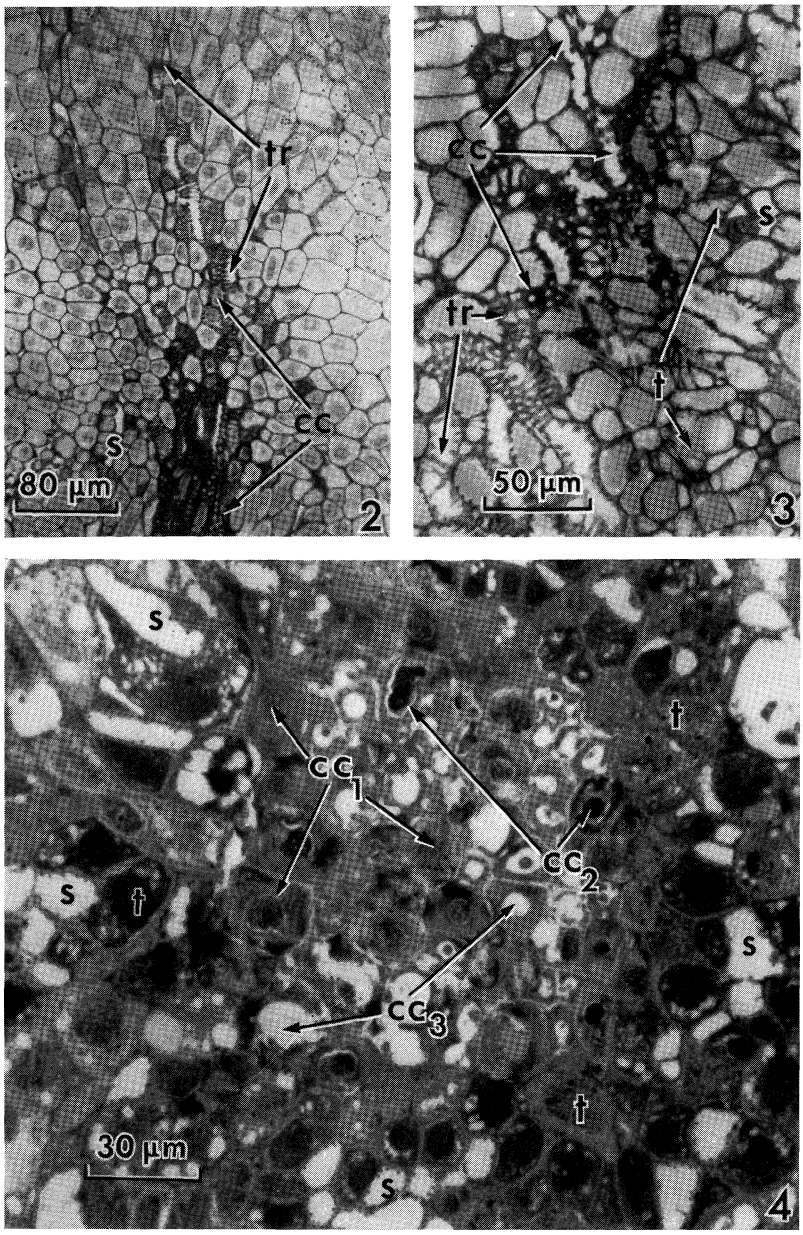
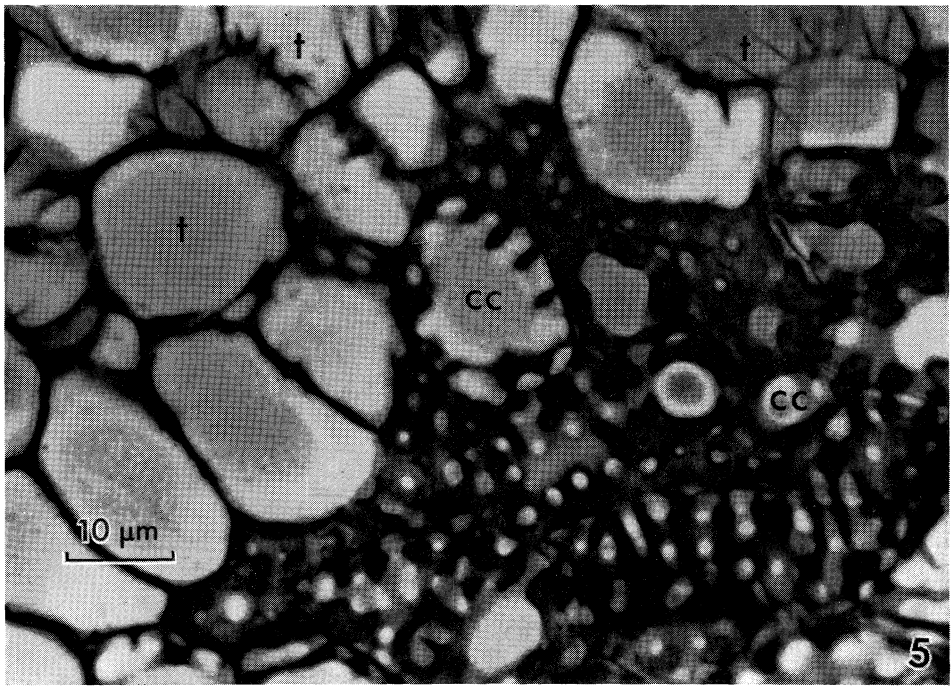


Fig. 2.—Longitudinal section through the junction of the vascular trace (*tr*) in the pericarp and the central core of thick-walled cells (*cc*; see also Fig. 1). *s*, sieve elements. Glycol methacrylate section, stained with PAS–toluidine blue O.

Fig. 3.—Longitudinal section through the junction between the vascular tissue (*tr*) in the rachilla and the core of thick-walled cells (*cc*). The location of this section in the spikelet is marked in Figure 1. *t*, transfer cells; *s*, sieve elements. Glycol methacrylate section, PAS–toluidine blue O stain.



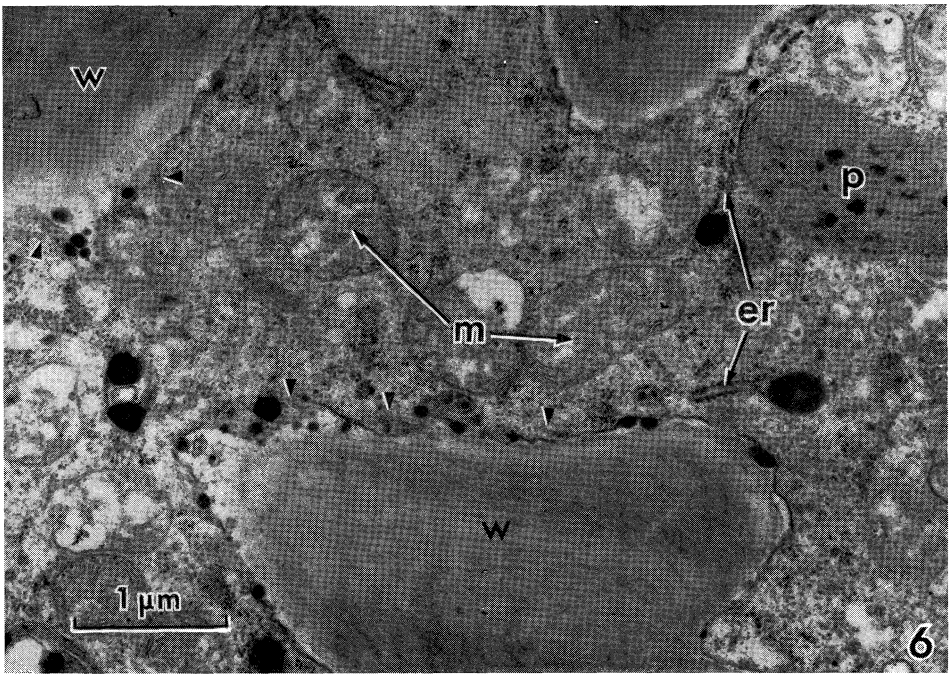


Fig. 5.—Light micrograph at high magnification of the thick-walled cells (*cc*) in the attachment region, seen in slightly oblique longitudinal section. Note the complex wall thickenings of the central core cells (*cc*) traversed by large pits. *t*, transfer cells. Glycol methacrylate section, PAS–toluidine blue O stain.

Fig. 6.—Portion of a thick-walled cell showing fine structural details of the cytoplasm. Arrowheads, microtubules; *er*, endoplasmic reticulum; *m*, mitochondrion; *p*, plastid; *w*, wall.

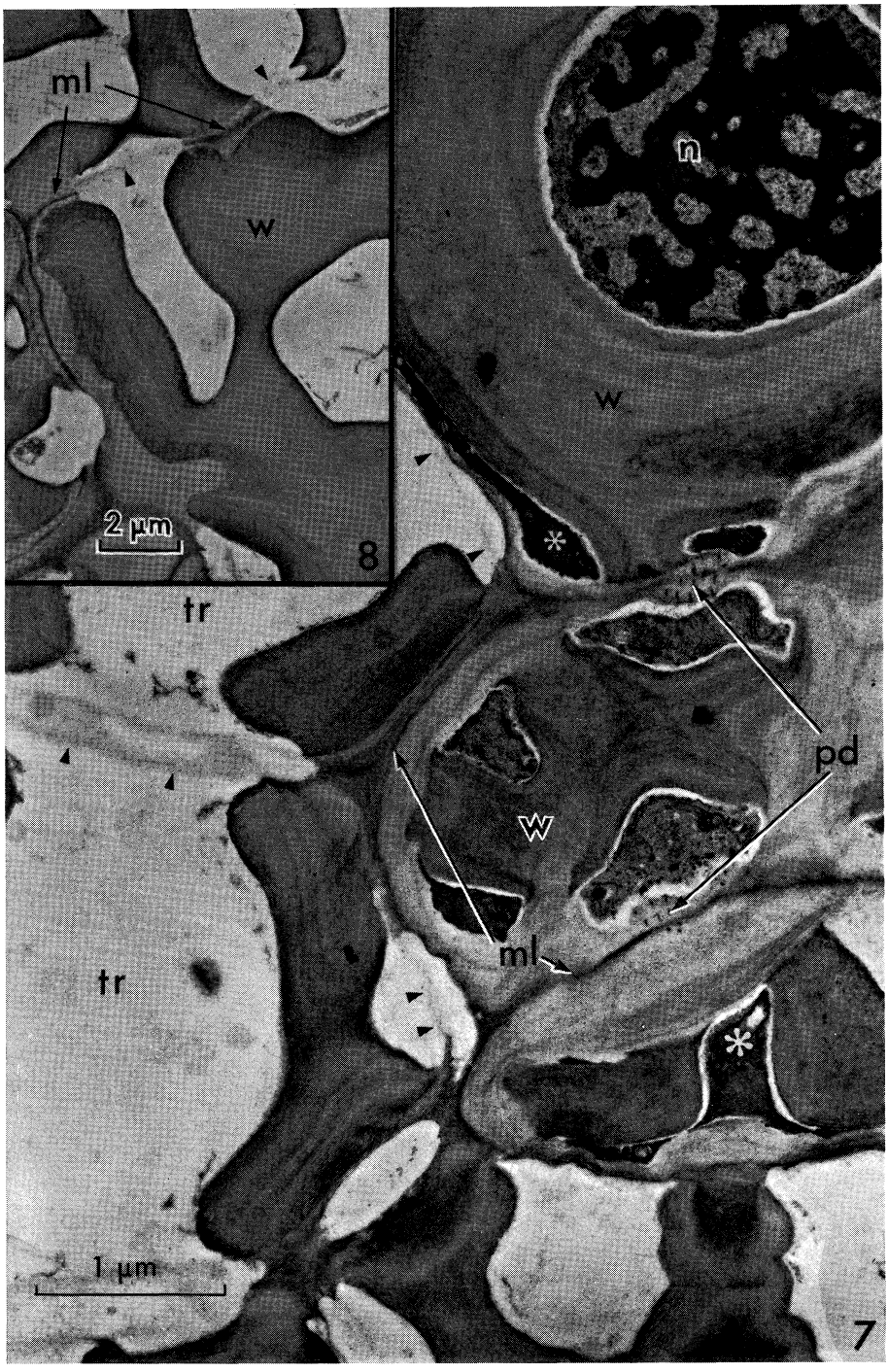
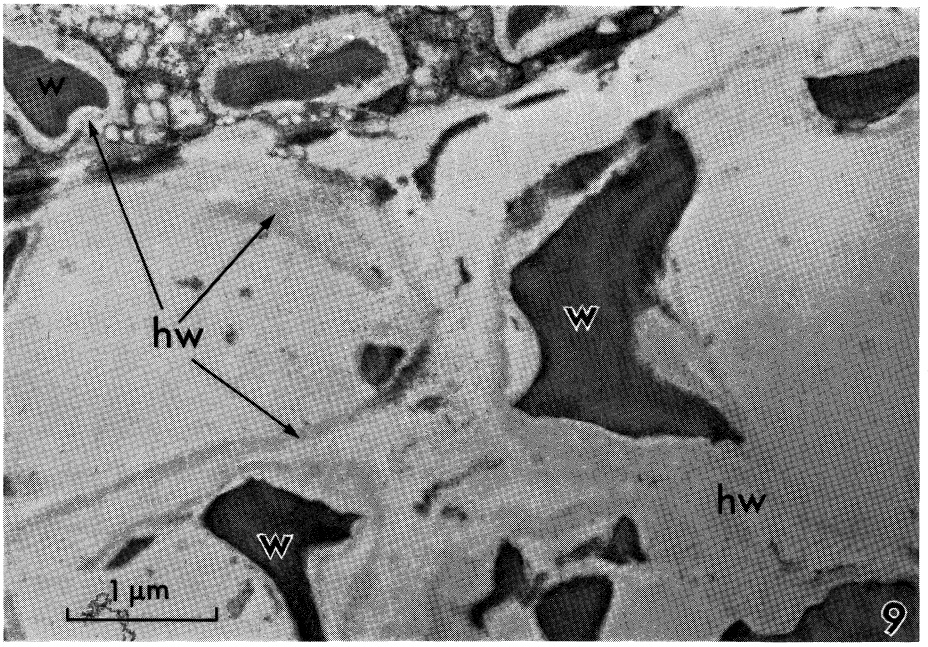
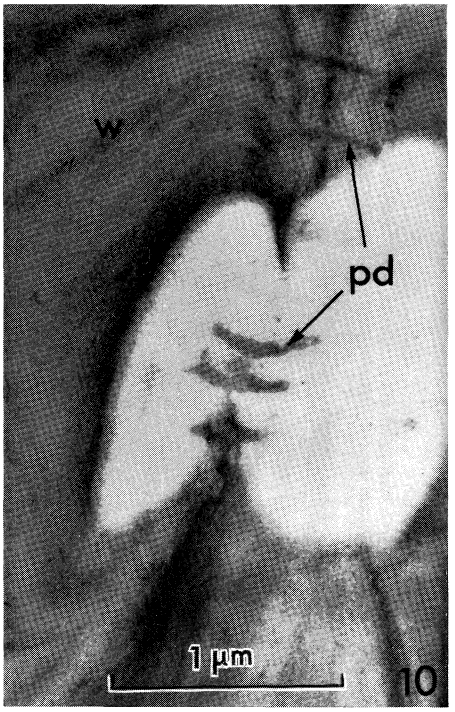


Fig. 7.—Transverse section of tracheary elements (*tr*) that abut parts of four thick-walled cells. Though the walls (*w*) of all thick-walled cells appear to be intact, the extremely electron-dense protoplasts (asterisks) of two of the cells are necrotic. Hydrolysed walls (arrowheads) are present in the tracheary elements. *ml*, middle lamella; *n*, nucleus; *pd*, plasmodesmata.

Fig. 8.—Transverse section of lignified thick-walled cells following protoplast destruction. Note the lamellae in the wall (*w*) and hydrolysed walls (arrowheads). *ml*, middle lamella.





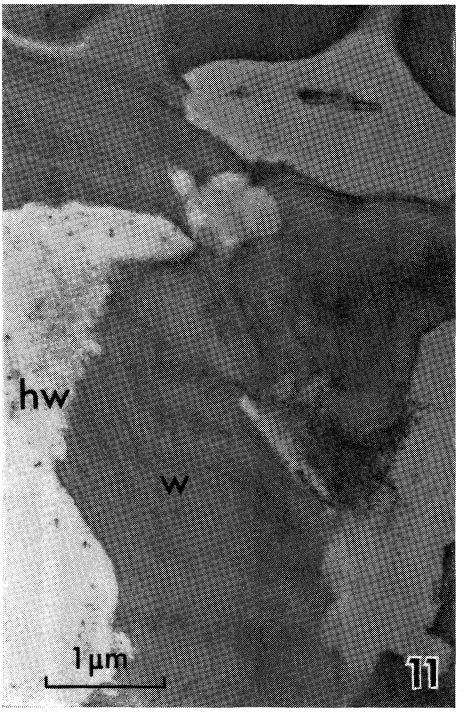


Fig. 9.—Thick-walled cells whose walls (*w*) have undergone extensive hydrolysis (*hw*). Note that in the upper cell the protoplast is reasonably intact but the cell membrane has been disrupted and the wall thickenings have undergone partial hydrolysis.

Fig. 10.—Electron micrograph of a pit between central core cells showing some remnants of plasmodesmata (*pd*) held in position within the hydrolysed wall. Note normal plasmodesmata underlying the wall thickenings. *w*, wall.

Fig. 11.—Another view of the walls (*w*) of the central core cells apparently undergoing hydrolysis. *hw*, hydrolysed wall.

These observations are supported by experiments tracing the flow of 1% solutions of acid fuchsin or ferric chloride which were introduced into the xylem by severing floral stalks immersed in these solutions. The position of the iron salt was rendered visible by soaking the tissues in a solution of sodium sulphide. Within minutes, these markers could be detected in the vascular bundles of the rachilla, glumes, lemmas, and paleas. Neither marker was ever detected in the tracheary elements of the pericarp, even when the inflorescences were allowed to transpire in the marker solutions for many hours. Dissection of such spikelets and free-hand sections showed that the markers piled up in the region of the floral axis that contains the core of thick-walled cells. These results suggest that this core of cells provides a barrier to the free-space transport of solutes from the rachilla to the developing ovary.*

It is difficult to classify the cells that make up this central core of tissue. In any one section, the cell walls of different cells provide a range of staining reactions with toluidine blue O (detectable as intensity differences in Fig. 4). Some stain green, suggesting that they are lignified, others stain orthochromatically, while others stain metachromatically (a deep reddish purple; O'Brien, Feder, and McCully 1964). The protoplasts also show a wide range of structural patterns. Some are intact, some necrotic, and others completely absent (Figs. 4, 5, 7, and 9). In the formation of normal tracheary elements, the lignification of the wall thickenings proceeds in concert with the differentiation of the protoplast (Pickett-Heaps 1968; O'Brien 1970). However, in these thick-walled cells, there appears to be no correlation between the staining reaction of the wall and the nature of the protoplast, for some cells that have lost their protoplasts have unlignified walls.

In intact cells viewed with the electron microscope, the cytoplasm reveals the usual array of organelles (nucleus with extensive chromatin aggregates, mitochondria, plastids, endoplasmic reticulum, dictyosomes) and a rather dense ground substance with numerous ribosomes (Fig. 6). Microtubules are abundant in the cell cortex, especially beneath that part of the cell membrane that lies beneath the wall thickenings. The cells have distinct primary walls separated by a middle lamella, and these wall layers are traversed by plasmodesmata at the pits where living cells abut one another (Fig. 7). Occasionally, plasmodesmata are also encountered beneath the wall thickenings (Fig. 10). The wall thickenings are distinctly lamellated (Figs. 6–11), and are deposited in a pattern that gives rise to bordered pits (Fig. 5). Since the ontogeny of these cells has yet to be followed, we are not sure if these wall thickenings are truly secondary walls or thickened primary walls. Their strong affinity for cationic dyes and their weak birefringence suggest that the thickenings have a low cellulose content.

O'Brien and Thimann (1967) and O'Brien (1970) have shown that in tracheary elements of both the primary and secondary xylem, areas of wall unprotected by lignin are attacked (presumably by polysaccharidases) during the closing stages of maturation of the element. All of the acidic polyuronides and PAS-positive poly-

* Strictly speaking, the region occupied by these cells should not be called a "xylem discontinuity" for there is no anatomical gap. We use the term here in a more physiological sense to indicate that the flow of the transpiration stream through this region is sharply reduced by these special cells.

saccharides are removed from the unlignified walls leaving a fibrillar, birefringent remnant that they called a "hydrolysed wall". Wherever thick-walled cells or tracheary elements have lost their protoplasts, hydrolysed primary walls and middle lamellae are evident (Figs. 7 and 8). O'Brien (1970) suggested that the hydrolysis of the non-cellulosic polysaccharides from the wall was a general phenomenon consequent upon the autolysis of the protoplast. It was envisaged that even a thick wall would be degraded if it was not adequately protected by lignification. The weak and variable lignification of the walls of these cells have provided unexpected evidence to support this view. Careful study of Figures 9 and 11 reveals that some of these thick walls have been degraded, in whole or in part, to hydrolysed walls, leaving just a fine fibrillar remnant identical to that found in the hydrolysed primary walls of Figures 7 and 8.

Two further observations deserve comment. We have repeatedly observed remnants of plasmodesmata trapped in hydrolysed walls (Fig. 10), a feature not recorded from the earlier work. In addition, the cell at the top of Figure 9 shows a relatively intact protoplast in a cell with partially hydrolysed wall thickenings. In the earlier paper (O'Brien 1970) wall hydrolysis was never encountered until the cell membrane degenerated. It appeared that in those cells (bean leaf xylem) the cell membrane was the last cytoplasmic membrane to degenerate. Figure 9 suggests that this sequence may not be general, for the cell membrane is very clearly damaged in Figure 9, though the cytoplasm is relatively intact.

In our minds, these thick-walled cells are best regarded as modified tracheary elements whose differentiation is abnormal. The central core of tissue thus comes to consist of a mixture of normal tracheary elements with properly lignified walls, and cells in which the normal sequence of lignification, protoplast degeneration, and wall hydrolysis has been disturbed to varying degrees. The net effect is that this central core contains unconnected islands of tracheary elements, separated to varying extents by incompletely differentiated tissue. The experiments with the dyes and iron salt indicate that the discontinuity in the files of normal tracheary elements severely limits solution flow from the rachilla to the pericarp bundle.

The full physiological significance of this reduced solution flow to the grain cannot yet be assessed. The occurrence of a similar structure in four grass species suggests that it may be general. The central core is ringed by sieve tubes and transfer cells—could the reduction in xylem flow at this point be a device to aid solute transfer to the sieve tubes?

IV. Acknowledgments

We wish to thank Mrs. Jane E. Lee for drawing Figure 1, Dr. A. T. Pugsley and Messrs. Dalgety and Sons for donations of seed, and our colleagues in the Botany Department for helpful criticisms on the manuscript. We also wish to thank Dr. Katherine Esau and Dr. V. Cheadle of the University of California at Santa Barbara for their help in classifying these cells. This work is supported by grants from the Australian Research Grants Committee and the Rural Credits Division of the Reserve Bank of Australia.

V. References

FEDER, N., and O'BRIEN, T. P. (1968).—Plant microtechnique: some principles and new methods. *Am. J. Bot.* **55**, 123–42.

O'BRIEN, T. P. (1970).—Further observations on hydrolysis of the cell wall in the xylem. *Protoplasma* **69**, 1–14.

O'BRIEN, T. P., FEDER, N., and MCCULLY, M. E. (1964).—Polychromatic staining of plant cell walls with toluidine blue O. *Protoplasma* **59**, 417–42.

O'BRIEN, T. P., and THIMANN, K. V. (1967).—Observations on the fine structure of the oat coleoptile. III. Correlated light and electron microscopy of the vascular tissues. *Protoplasma* **63**, 443–78.

PATE, J. S., and GUNNING, B. E. S. (1969).—Vascular transfer cells in angiosperm leaves: a taxonomic and morphological survey. *Protoplasma* **68**, 135–56.

PICKETT-HEAPS, J. D. (1968).—Xylem wall deposition. Radioautographic investigations using lignin precursors. *Protoplasma* **65**, 181–205.

SPURR, R. S. (1969).—A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**, 31–43.