

WINTER ACTIVITY IN THE CAMBIUM OF *PINUS RADIATA*

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ABSTRACT

Light and electron microscope studies of cambium in young trees of *Pinus radiata* D. Don sampled during the few weeks following the winter solstice have shown that this tissue does not become completely dormant. Cell division is actively proceeding throughout this period in the fusiform and ray initials of the secondary meristem. Evidence suggests that no tracheids are being formed, and that cell divisions are actively involved in the production of phloem. The cytoplasm of developing tracheids is markedly different from that of young phloem cells, the former being involved mainly in wall thickening, the latter in production and storage of protein in addition to wall formation. *P. radiata* is different both in appearance and degree of activity during the winter, from *P. strobus* L. growing in the United States, and described by other authors. Preliminary work has suggested that New Zealand-grown *P. strobus* is similar in appearance and activity to that grown in USA. This work suggests that dormancy in the pines is as dependent on internal factors which vary among species as on climatic or day length factors.

INTRODUCTION

Several authors have published descriptions of winter cambium from a variety of *Pinus* species (Srivastava and O'Brien 1966; Alfieri and Evert 1968; Murmanis and Sachs 1969; Murmanis 1971). In each species the cambium was completely dormant; no cellular division or differentiation was taking place in the meristematic region. However, descriptions of the tissue in dormancy differ. Alfieri and Evert (1968) working on *P. banksiana* Lamb., *P. resinosa* Ait., and *P. strobus* demonstrated that latewood tracheids were fully thickened, phloem cells were fully differentiated and the cells comprising the cambial zone were completely undifferentiated. No intermediate stages in differentiation could be distinguished in their micrographs. Cambial activity recommenced at the end of March with phloem production, followed 6 weeks later by xylem formation.

Srivastava and O'Brien (1966) and Murmanis (1971) working with *P. strobus* observed complete dormancy, but in their samples differentiating phloem cells were present in addition to fully differentiated phloem and undifferentiated cambium. This

agreed with earlier observations reported by Strasburger (1891) and Abbe and Crafts (1939). These conflicting views of dormant cambium structure were only associated with that part of the cambium adjacent to the phloem; all workers observed completely differentiated latewood tracheids in direct contact with the cambium. The appearance of the tissue in all observations presumably remained the same throughout the winter months and/or the period of dormancy.

Dendrometer measurements of *P. radiata* growing in the Rotorua district have shown that the diameter of the trunk continues to increase at a slow rate during the whole of the winter (Jackson 1969). These observations are similar to those described by Shepherd (1967) on *P. radiata* grown in Victoria, Australia. The form that this growth takes with relation to the meristem has hitherto been unknown. The continuation of growth suggests that dormancy is never complete in this species in the central North Island of New Zealand where the major radiata pine plantations are located. The structure of winter cambium in *P. radiata* was studied in an attempt to explain this activity, and in the hope that it might provide some information about the nature of dormancy and the factors controlling this phenomenon.

MATERIALS AND METHODS

Sampling was commenced on 24 June 1970, and continued at approximately weekly intervals thereafter. A piece of tissue which included the bark, phloem, cambium, and about a year's growth of wood was removed from 5-yr-old trees growing in the Forest Research Institute nursery. The material was fixed in glutaraldehyde and osmic acid, then embedded in Epon 812 as described elsewhere (Barnett, in press). Sections for light and electron microscopy were cut using an LKB Ultratome 111 and examined with a Zeiss Photomicroscope 11 or a Philips EM300. Sections for electron microscopy were stained with uranyl acetate and lead citrate.

OBSERVATIONS

The cambium and its immediate derivatives are shown in Fig. 1, a bright field photomicrograph of the sample collected on 24 June. The undifferentiated cambium cells are arranged in radial files with 16 to 20 cells in each file. Phloem cells may be distinguished by their radial enlargement and thickened walls or, in the case of phloem parenchyma cells, by their osmiophilic contents. These contents are thought to be polyphenolic and/or lipoidal (Alferi and Evert 1968). In *P. strobus* Alferi and Evert observed a marked zonation of phloem parenchyma cells into tangential rows which they thought represented the borderline between early and late phloem. However, in the *P. radiata* trees described here the more random distribution of phloem parenchyma (Fig. 2) offers no such distinction between early and late phloem.

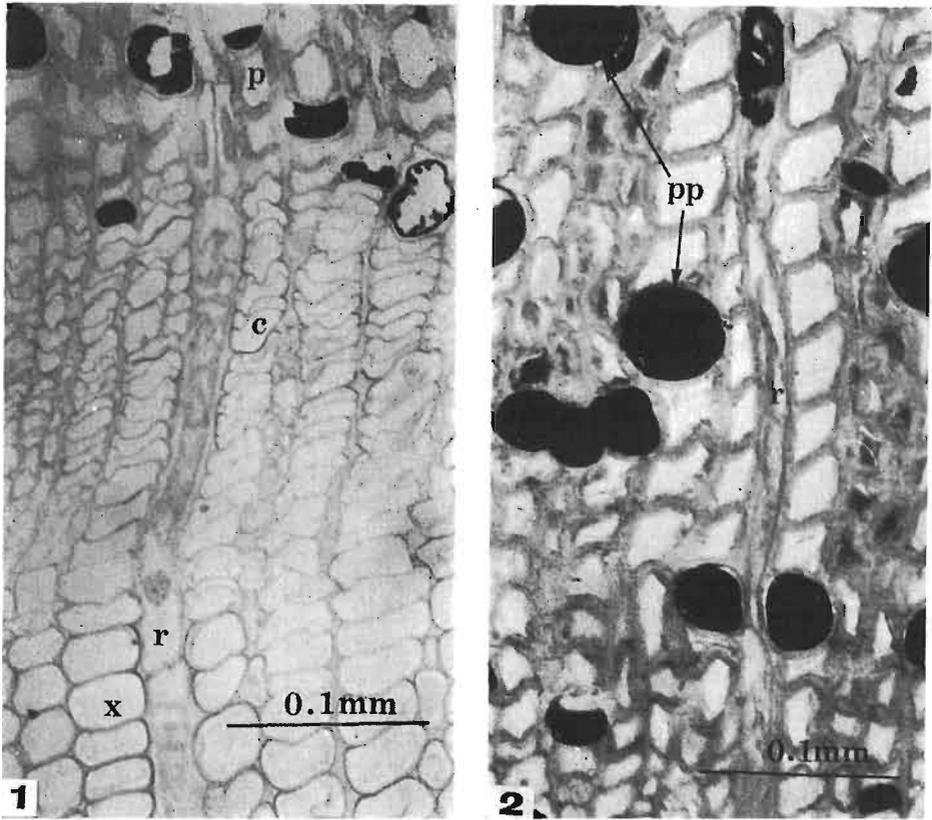


FIG. 1—Sample of 24 June. Transverse section (TS), through the cambial zone (c) of a 5-yr-old tree of *P. radiata*. Phloem cells (p) are distinguished by their large radial diameter and thickened walls. The young latewood tracheids in the xylem (x) have the dimensions of mature latewood cells, but as yet have unthickened walls. Rays (r) pass radially through the tissue at intervals. Bright field photomicrograph.

FIG. 2—Sample of 24 June. TS through the phloem. Phloem parenchyma cells (pp) are randomly distributed in contrast with these cells in *P. strobus* where they are arranged in tangential rows marking the early/late phloem boundary. Rays (r) pass for some distance into the phloem. Bright field photomicrograph.

The most surprising feature of the 24 June sample is the absence of the characteristic secondary thickening from those cells which were expected by this time to be fully differentiated latewood tracheids. This contrasts sharply with the appearance of latewood tracheids in the samples of *P. strobus* examined by the American workers. Interference contrast microscopy illustrates the absence of secondary wall much more convincingly (Fig. 3). The empty appearance of the fusiform cells of the cambium is a result of the cytoplasm in these cells being predominantly confined to a parietal zone by the large vacuole which occupies much of the cell volume. Ray parenchyma cells usually appear full of cytoplasm and are far less vacuolate. Fig. 3 shows that

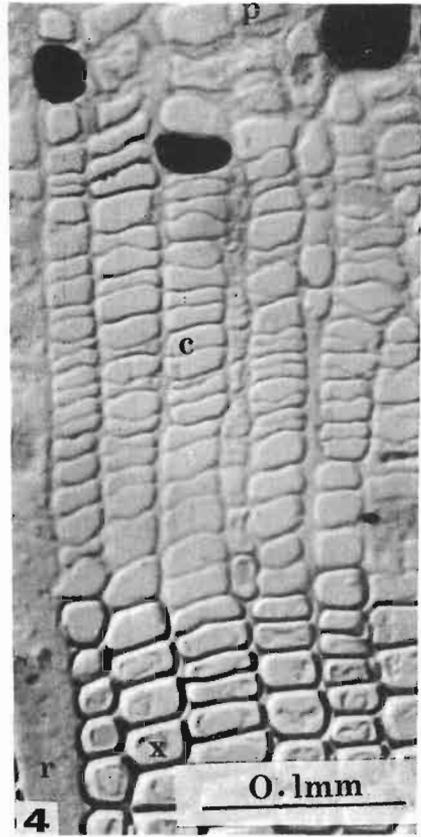
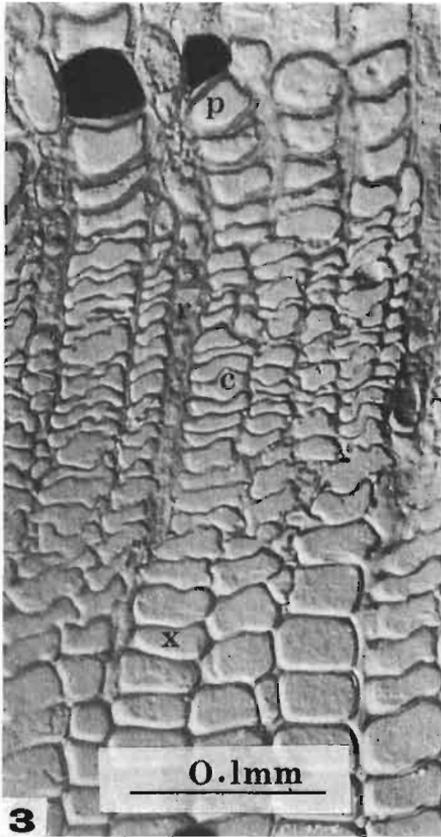


FIG. 3—Sample of 24 June. A similar area to that shown in Fig. 1 as seen using interference contrast microscopy. Cell walls are more sharply defined. Note the marked absence of secondary walls in the young latewood tracheids (x).

FIG. 4—Sample of 6 July. Secondary thickening is at a more advanced stage in the young latewood tracheids of this specimen than it was in the 24 June sample. For letter identifications see Fig. 1 caption.

most of the differentiating tracheids have some secondary wall, some have none, and none has the amount of thickening characteristic of fully developed latewood tracheids. Some of the cells near the cambium have the dimensions of latewood tracheids, suggesting that these are young latewood cells which merely need thickened walls to achieve maturity.

A sample taken 2 weeks later (6 July) is illustrated in Fig. 4. More secondary thickening has taken place near the cambium, and it may be that some of the cells which earlier appeared to be part of the cambial zone have acquired some secondary wall. A week later the young latewood tracheids appear almost complete and this appearance is maintained in the next two weekly samples. Fig. 5 illustrates the 27 July

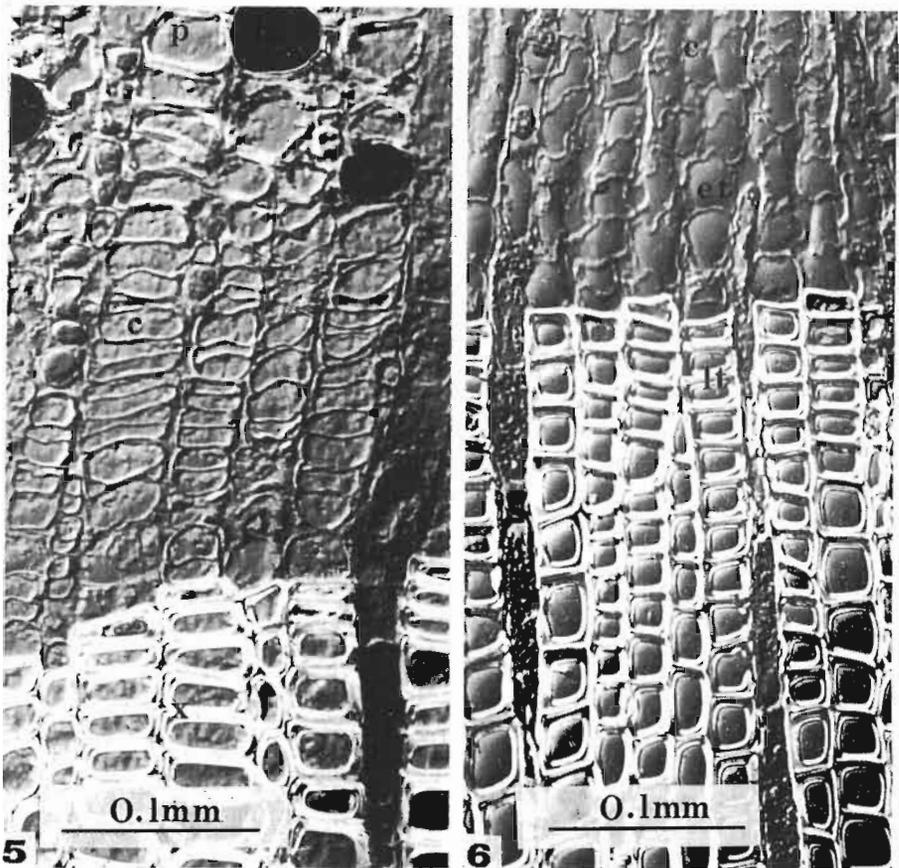


FIG. 5—Sample of 27 July. Secondary thickening in the latewood tracheids is now complete. The cambial zone is a little narrower than it was in the earlier specimens, probably because cells which earlier appeared to be part of the cambial zone have become latewood tracheids. For letter identifications see Fig. 1 caption.

FIG. 6—Sample of 3 August. Young earlywood cells (et) are beginning to differentiate in this specimen, although they have only primary wall. The latewood tracheids (lt) are now at a greater distance from the cambium (c) than they were before.

sample. The latewood present did not increase over the 2-week period, indicating that production of xylem cells by cell division was not occurring. If so, new cells were probably not being produced by the cambium for the production of xylem during the whole period when wall thickening was taking place in latewood. The absence of any sign of radial enlargement in the existing young tracheids provides additional evidence that the increase in stem diameter during winter is not due to xylem production. New wood formation commenced during the week following the sixth sampling, since the sample taken on the seventh occasion (3 August) contained a number of earlywood tracheids with, as yet, no secondary walls (Fig. 6).

Evidence for the formation of secondary wall in cells which appear to be part of the cambial zone in light microscope sections is provided by Fig. 7. This is a low magnification electron micrograph of the 24 June sample (see also Fig. 1). The fusiform

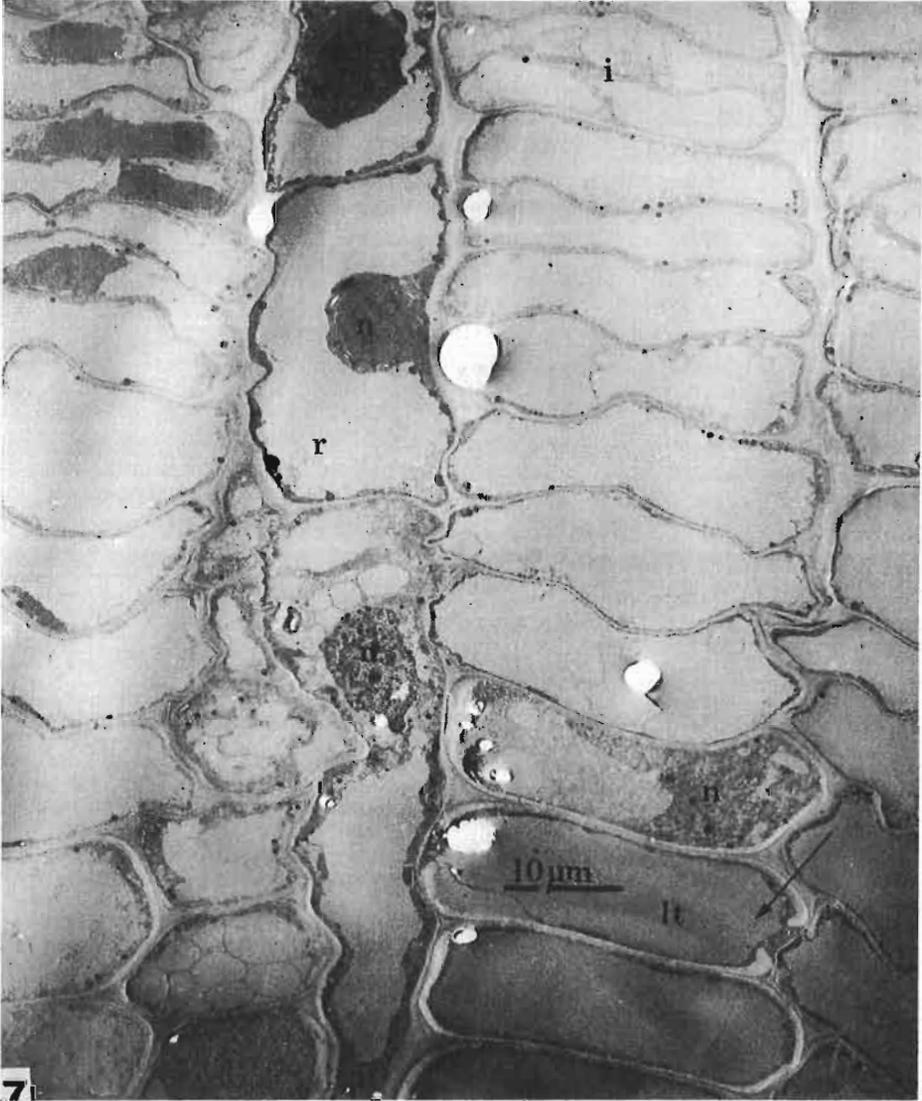


FIG. 7.—Sample of 24 June. Electron micrograph of the cambial zone. The initial (i) can be identified by the thin tangential wall which crosses it as the result of a recent division. It is possible using the electron microscope to see that each xylary derivative of the initial is at a slightly different stage of differentiation. The ninth cell removed from the initial (arrow) has reached the size of a mature latewood cell and its wall shows some secondary thickening. Cells between the initial and the ninth cell show a gradual increase in radial diameter and wall thickness. Nuclei (n) are visible in all of the ray cells (r) in this section and are occasionally seen in fusiform cells and young latewood tracheids (lt).

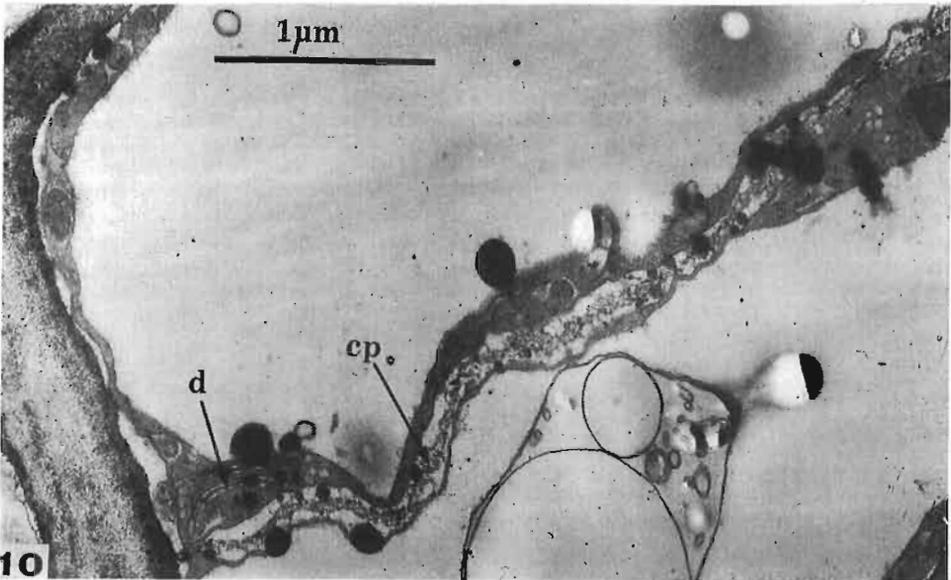
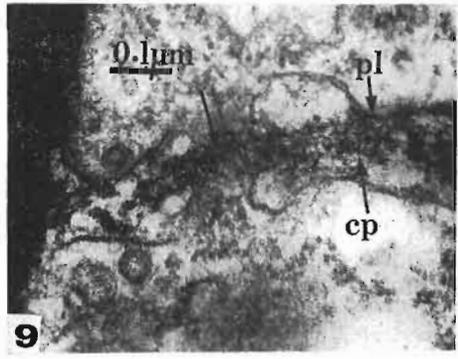
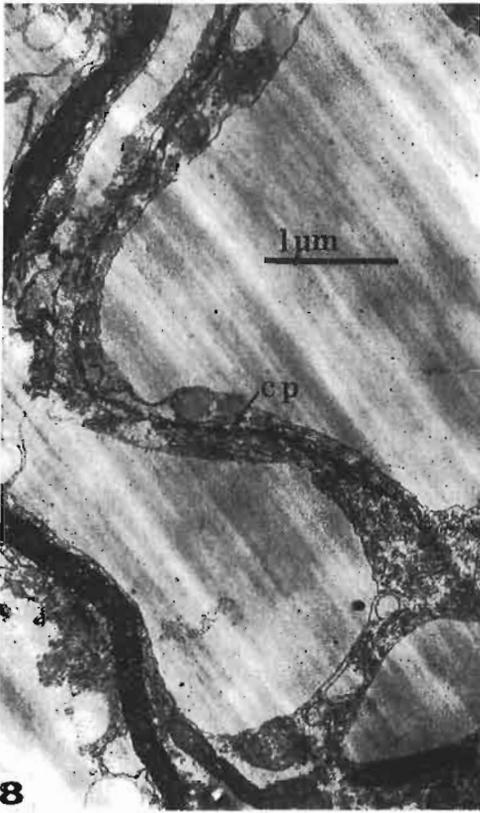
initial is easily detected by its thin tangential wall formed during the last periclinal division. Young xylem cells are grouped in fours as described by Mahmood (1968). A marked gradation in the radial diameter of the cells which will become tracheids is visible, the cells gradually enlarging as they are left further behind the initial. A gradual increase in the thickness of the cell wall accompanies the increase in radial diameter. Once secondary thickening has commenced it is unlikely that radial diameter can change significantly. The cell eight removed from the initial has probably reached its maximum size and its dimensions are certainly typical of a latewood tracheid seen in TS. The appearance of secondary wall so close to the fusiform initial contrasts with summer material which is actively producing large quantities of earlywood. In this case a cell may be as many as 16-20 cells removed from the initial before any noticeable secondary thickening occurs (Barnett, unpublished results).

The most striking feature of the 24 June sample was the observation that several cells on the phloem side of the cambium had a newly forming cell plate, suggesting that division was incomplete or continuing at the time of sampling. Fig. 8 shows a cell in which the newly forming plate is made up of regions of wall material separated from one another by membranes which enclose them in such a way that the cytoplasm of the daughter cells is continuous at several points. Higher magnification (Fig. 9) shows clearly that the plate is fragmentary at this stage, and the granular nature of the plate itself is visible. The new cell plate is not continuous in any way with that of the radial wall of the parent cell, but is merely apposed onto its surface. A later stage is illustrated in Fig. 10. Here the daughter cells are completely separated by the cell plate, although this still has a granular appearance, showing that formation is not complete. A dictyosome is present which is actively producing vesicles with osmiophilic contents. These are presumably cell plate precursors or enzymes. The activity of the dictyosomes and the presence of cell plates at various stages of completion strongly indicates that cell division was still taking place in the middle of the winter. Further evidence for this view was provided by the sample collected on 6 July in which

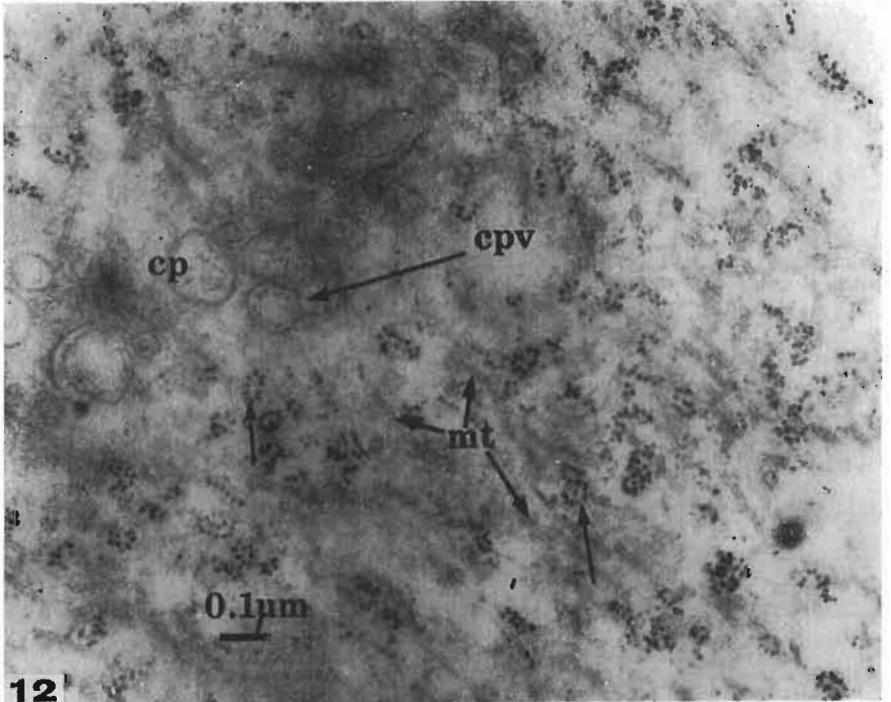
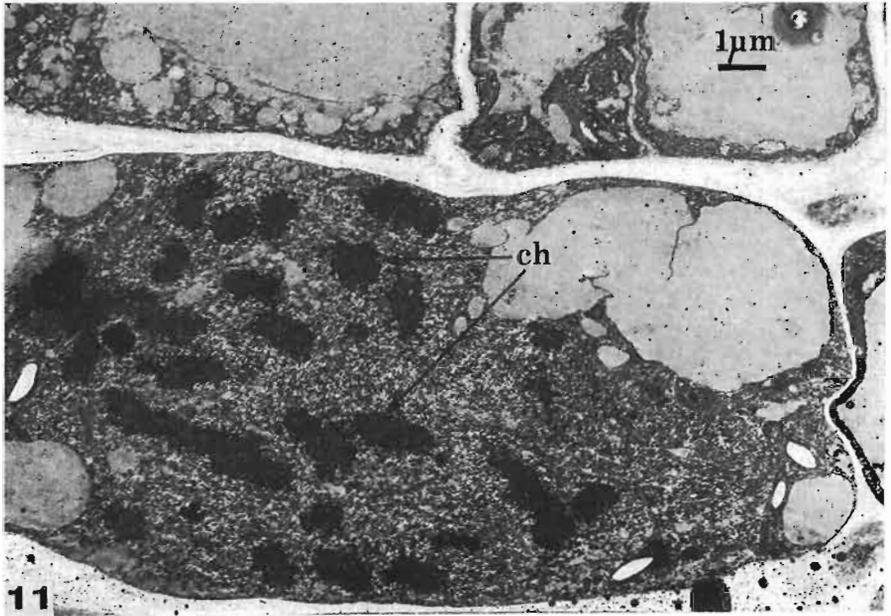
FIG. 8 (Opposite)—Sample of 24 June. A recently divided fusiform initial in which the tangential cell plate (cp) is as yet incomplete. Fragments of the cell plate are enclosed and separated from one another by membranes (unlabelled arrows).

FIG. 9 (Opposite)—Sample of 24 June. Higher magnification of a region of the cell plate in Fig. 8. The granular nature of the plate at this stage can be seen (cp). The plate is enclosed by the plasmalemmas (pl) of the daughter cells and in places the cytoplasm of the daughter cells are continuous through gaps in the structure (unlabelled arrow).

FIG. 10 (Opposite)—Sample of 24 June. A recently divided fusiform initial at a slightly later stage in cell plate formation than that shown in Figs. 8 and 9. The plate still has a granular appearance but is now continuous across the cell. A dictyosome (d) is actively producing vesicles which probably contain cell plate precursors.



several stages of cell division were observed. Fig. 11 shows a ray initial in which the chromatin material is condensed into chromosomes, no nuclear membrane is present and it is impossible to distinguish between the nucleoplasm and cytoplasm. This



appearance is typical of a dividing cell. Fig. 12 illustrates part of a dividing fusiform initial from the same sample. Microtubular elements of the spindle are on either side of the row of vesicles which will eventually coalesce and form the cell plate. There is much ribosomal activity, presumably associated with the formation of the plate, many of the ribosomes being grouped into polysomes, indicating that they are actively producing protein. These observations indicate that the cambium of *P. radiata* continues to produce new tissue through midwinter, but only towards the phloem since there is no evidence that new tracheids are being formed. A closer investigation of the cytoplasm of differentiating xylem and phloem cells supports this view.

Cytoplasm in developing latewood tracheids is seen in Figs. 13 and 14. In Fig. 13 the nucleus has been sectioned and contains a prominent nucleolus. Several dictyosomes actively producing vesicles are present in the cytoplasm as the main form of cytoplasmic activity. Some rough endoplasmic reticulum (ER) is found in these cells (Fig. 14) along with the usual cytoplasmic organelles—mitochondria, spherosomes, and free ribosomes. Plastids are of variable structure, although usually cigar-shaped as in Fig. 14; they contain a reticulate membrane system and often an osmiophilic inclusion of unknown composition. The activity of the dictyosomes in these cells supports the idea that these differentiating cells are engaged mainly in the production of secondary wall. It has been known for some time that these organelles are responsible in some species for the transport of hemicellulose and pectin precursors to the wall of the cell (Whaley and Mollenhauer 1963; Wooding 1968).

The appearance of the cytoplasm in the phloem is markedly different from that in the xylem. The cytoplasm of a differentiating phloem cell is illustrated in Fig. 15. The most striking feature of these cells is the large number of ribosomes which surround vacuoles in the cytoplasm. The vacuolar membranes often contain irregular regions thicker than the normal unit membrane. The contents of these vacuoles stain lightly with the techniques used here and resemble protein bodies seen in various tissues by other authors (e.g., Robards and Kidwai 1969; Klein and Pollock 1968). In maturing phloem cells large numbers of these protein bodies are often present. Their function is not understood, although it may be that the proteins stored in this way are enzymes which will be required for the formation of the sieve cell walls, and possibly the formation of sieve areas.

FIG. 11 (Opposite)—Sample of 6 July. A dividing ray initial. The chromatin material has condensed into chromosomes (ch), and it is impossible to distinguish between cytoplasm and nucleoplasm.

FIG. 12 (Opposite)—Sample of 6 July. A dividing fusiform cell in which the cell plate (cp) is at a very early stage of formation and consists entirely of small vesicles (cpv). Microtubular remains of the spindle can be seen (mt). Ribosomes are also present, often being grouped into polysomes (unlabelled arrows).

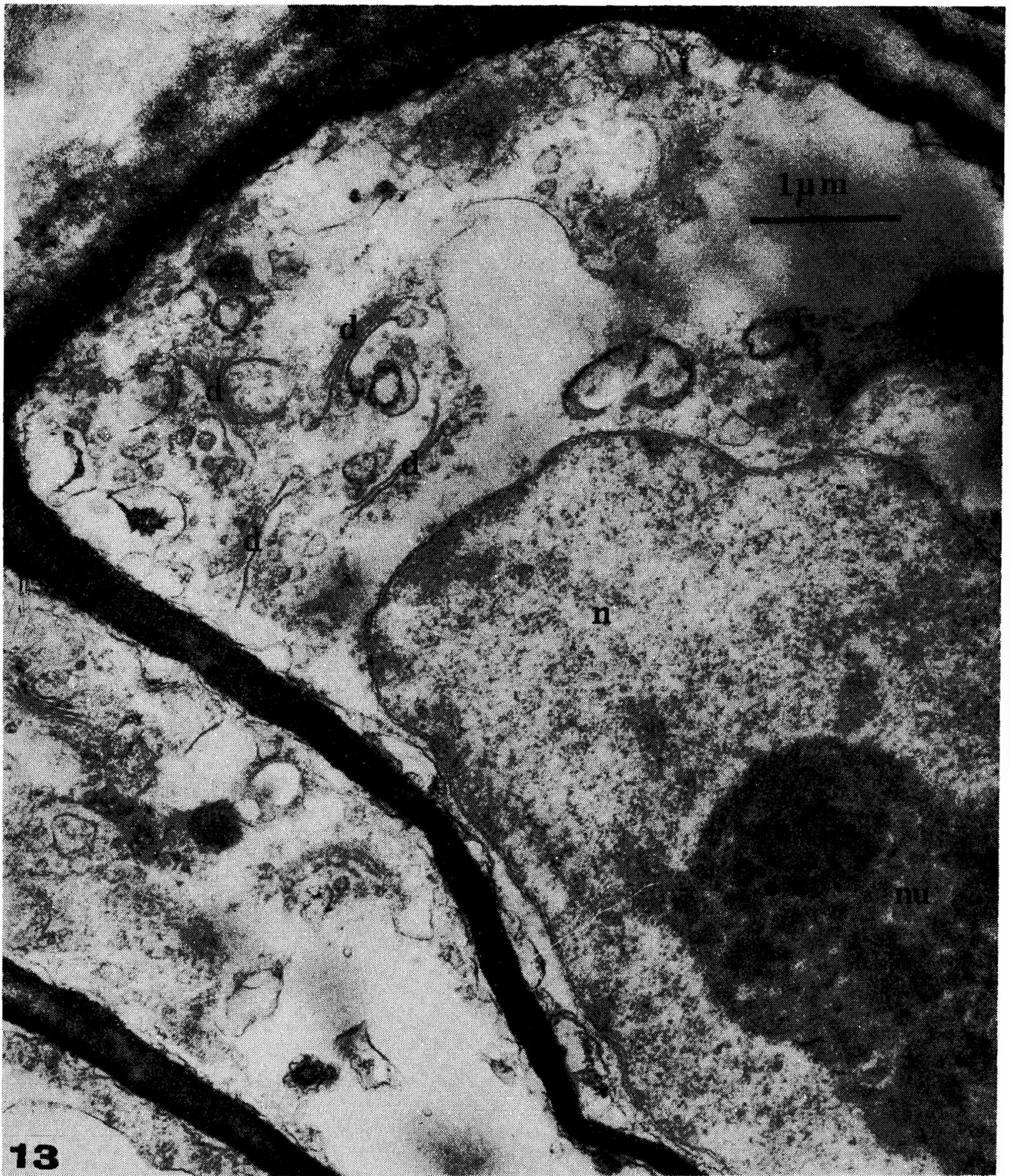
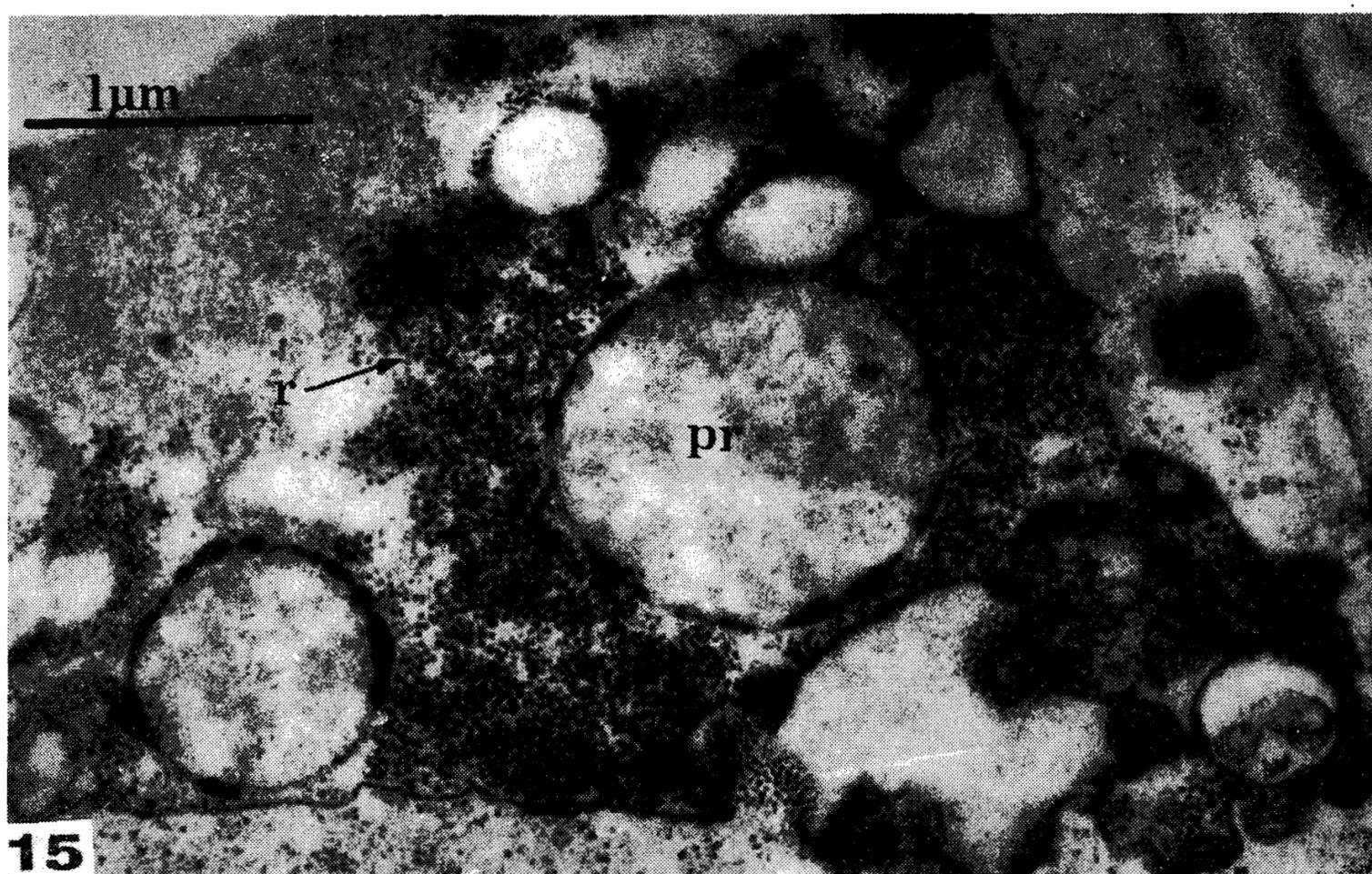
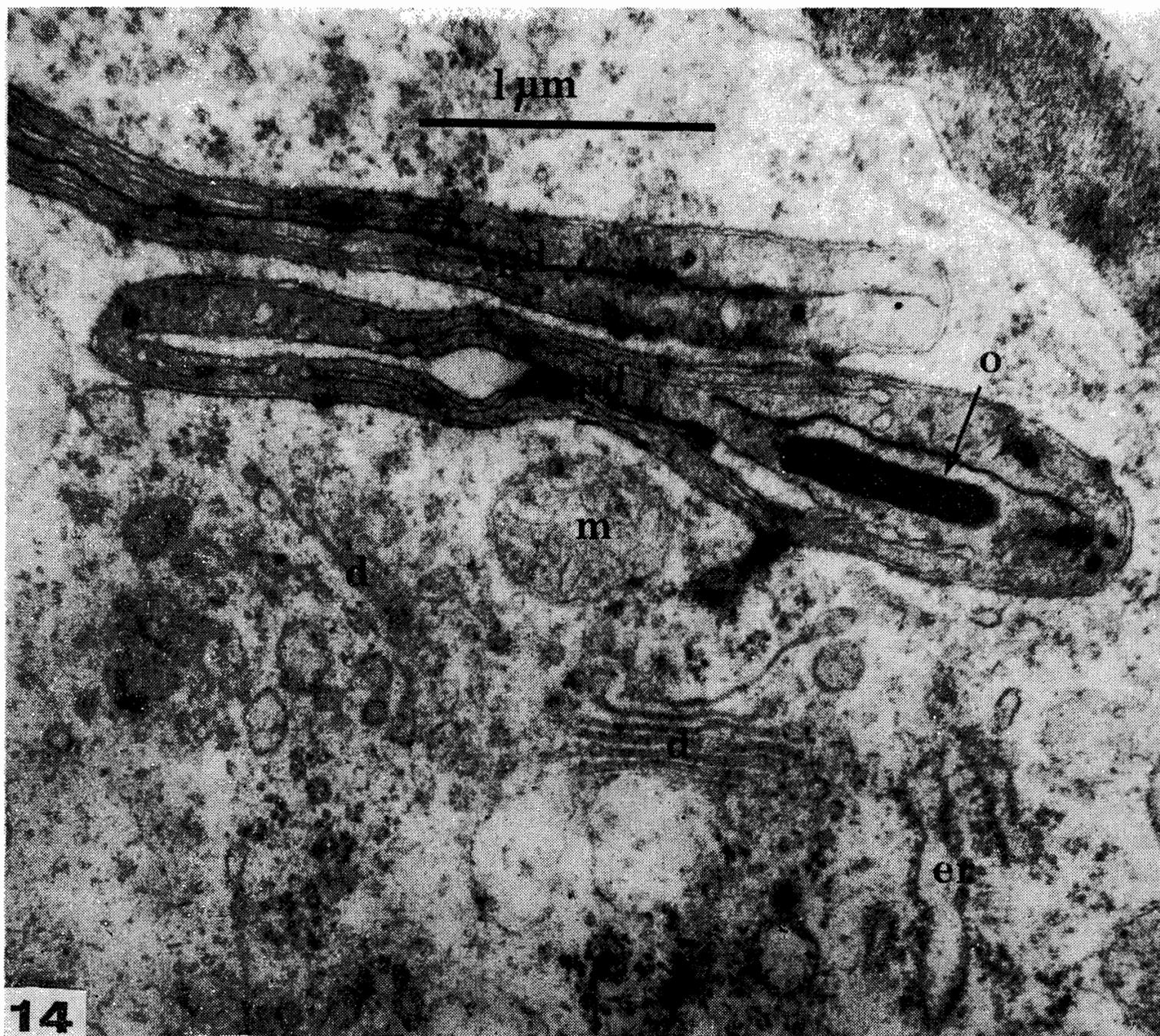


FIG. 13—Sample of 24 June. Part of a developing tracheid showing the nucleus (n) which contains a prominent nucleolus (nu). Several active dictyosomes (d) can be seen in this section.

FIG. 14 (Opposite)—Sample of 24 June. Part of the cytoplasm of a developing tracheid showing some of the structures typically found in these cells. Plastids (pd) with osmiophilic inclusions (o), mitochondria (m), endoplasmic reticulum (er) and dictyosomes (d) can all be seen in this micrograph.

FIG. 15 (Opposite)—Sample of 6 July. The cytoplasm of a differentiating phloem cell. Numerous ribosomes (r) surround vacuolar structures with lightly staining, probably proteinaceous contents (pr).



DISCUSSION

The work described above has shown that there are marked differences between the midwinter structure of the cambium and its immediate derivatives in *P. radiata* growing on the central volcanic plateau of the North Island of New Zealand, and that of these tissues in *P. strobus* and other *Pinus* species grown in the USA. The latter have been shown to be truly dormant for at least 3 months during the winter, with completed latewood tracheids abutting directly onto undifferentiated cambium cells on the xylem side. Fully differentiated, or dormant differentiating phloem cells adjoin undifferentiated cambium on the phloem side. In *P. radiata*, complete latewood tracheids were in contact with apparently undifferentiated cambium for only 2 weeks. Prior to this the walls of the young latewood tracheids were incompletely thickened and early-wood formation began only 2 weeks after the fully differentiated latewood tracheids were detected. Phloem cells have been shown to be highly active by virtue of their large numbers of ribosomes which appear to be actively storing protein in vacuoles in the differentiating phloem cells. Evidence for cell division in the samples taken during the winter is strong, although it seems likely that during the first 6 weeks after sampling was begun this division was devoted to phloem production.

These phenomena may be a result of the mildness of the winter months in Rotorua district. The 10-year mean minimum temperatures recorded at the Forest Research Institute for the months of April, May, June, and July are 8.0°C, 5.5°C, 3.3°C, and 2.8°C respectively. In 1970 these months produced mean minimum temperatures of 9.0°C, 5.3°C, 4.0°C, and 4.0°C, indicating that the winter of 1970 was typical in terms of temperature.

Digby and Wareing (1966) investigated the effects of growth hormones on cambial division and differentiation in *Populus robusta*. High gibberellic acid (GA) concentrations coupled with low indole-3-acetic acid (IAA) concentrations were found to favour phloem production, while high IAA concentrations combined with low GA concentrations favoured xylem production. Little is known about hormone levels in *P. radiata*, but similar physiological studies related to phloem and xylem production over the critical winter/spring period could prove invaluable for a deeper understanding of the processes described in this paper.

Shepherd (1967) suggested that cell division in *P. radiata* ceases in March or April and recommences about 4 months later in Australian-grown trees. He proposed that winter expansion was caused by cell enlargement or stem moisture variations. This is clearly not the case in Rotorua-grown *P. radiata* since winter tissue was found to contain actively dividing initials.

The *P. strobus* described by other workers clearly shows an annual growth cycle in the phloem, since the parenchyma cells are arranged in tangential rows. There are also cyclic changes in the morphology of the sieve cells which may be compared with the changes occurring in the xylem during the year. In *P. radiata*, however, it is difficult to distinguish "growth rings" in the phloem in this way as parenchyma cells are distributed randomly in this tissue. This might hint that the same cyclic pattern of growth of the phloem found in *P. strobus* might not occur in *P. radiata* and that the absence of a true dormancy period might explain this variation. Although xylem production in *P. radiata* is thought to stop for a time, it seems likely that this period

is very short, and does not affect secondary wall formation. It appears, in fact, that the thickened secondary walls of latewood tracheids in this species are added while these cells are stationary with respect to the cambial initial.

From the observations on *P. radiata* phloem it was suspected that *P. strobus* growing in mild areas might not exhibit the distinct annual zonation found in American samples. To test this theory samples were taken from both slow- and fast-growing trees of *P. strobus* also grown in the Rotorua district and prepared in the same way as the *radiata* samples. A preliminary study has shown that New Zealand-grown *P. strobus* has a similar phloem structure to the American-grown material. This raises the question as to whether *P. strobus* is in fact going through the same annual cycle as it does in its colder American environment, and is going through a period of true dormancy in spite of the milder climate in New Zealand. If this is so, then it suggests that one of the factors in the control of dormancy may be genetic, which has been selected for in the tree's native environment, and that this factor will override other factors such as day-length or climate. This may, of course, only apply where climatic factors, etc., are favourable for winter growth. Where conditions are harder than those of the tree's native environment, climatic factors may override genetic ones.

In view of this, it is tempting to speculate that *P. radiata* in its native environment has a similar anatomy and activity during the winter to the material described above. In order to test these theories, however, it will be necessary to investigate the effect of a severe winter climate on the growth of *P. radiata* and to determine whether *P. strobus* experiences complete dormancy in the relatively mild winter of the Rotorua district. This work is presently in its early stages, but should throw some light on the nature and causes of dormancy in gymnosperms. Continuation of sampling into the winter of 1971 has confirmed the observations on latewood formation described above; no extensive secondary thickening was present in young latewood tracheids during June.

CONCLUSIONS

It has been demonstrated that *Pinus radiata* does not experience full dormancy when grown in the Rotorua district of New Zealand. This is thought to be a result in part of the temperate winter climate in these areas, and in part of genetic factors. Cell division is actively occurring in this species throughout the year, although for a short period during the winter no new xylem cells are produced. At midwinter cambial activity produces only phloem cells. The cytoplasm in developing latewood tracheids is actively engaged in processes connected with wall formation, while the cytoplasm in young phloem cells is apparently involved in the production of large amounts of protein and the subsequent storage of this protein in spherical protein bodies. Further work along the lines described in this paper should provide information of value in solving some of the problems associated with dormancy in plants.

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