THE DEVELOPMENT OF THE SIEVE ELEMENTS
OF PINUS PINEA

F. B. P. Wooding
Department of Biochemistry, University of Cambridge

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Summary. The fine structure found in the developing and mature secondary phloem of Pinus pinea is described. No longitudinal system of conduits in the sieve elements has been found at any stage in their differentiation. The endoplasmic reticulum undergoes a characteristic series of changes and possible functions are considered. The nature of the sieve connections and other specialised pores are discussed.

The controversy regarding the in vivo fine structure of mature phloem elements remains unresolved. Thaine (1964) has postulated discrete strands running between the sieve pores in which material is translocated. His thesis, supported by Preston (1963) and Parker (1965) is based on phase contrast observations of hand cut sections of living material. No evidence for such phloem strands has been found in mature phloem using the techniques of electron microscopy. Results from such methods indicate that the phloem element has a fibrillar lumen content, and that the sieve pores between individual elements are filled with the same sort of fibrillar material. Thaine and Bullas (1965) have suggested that the preparation of the mature tissue for electron microscopy would result in a disruption of the strands, however some indication of the strands or their precursors would be expected during development before the sieve element becomes functional.

Most of the electron microscopy has been carried out on Angiosperms, e.g. Buvat 1964, Esau 1964 on Cucurbitaceae, Bouck and Cronshaw 1965 on Pisum, Engleman 1965 on Impatiens, Evert and Murnanis 1965 on Tilia, Northcote and Wooding 1966 on Acer. Kolman has produced a series of papers dealing with a Gymnosperm Metasequoia (see review by Kolman, 1964) which indicated considerable differences in the phloem ultrastructure between angiosperms and gymnosperms. The present paper reports a study with the electron microscope of the developmental cytology of the secondary phloem of Pinus pinea, which differs considerably from the process as reported for Metasequoia.
Materials and Methods

**Autoradiography.** The terminal [2] in. of 2-year-old shoots of *Pinus pinea* were cut off under water and the shoot tips placed with the cut end in water (approximately 1 ml) containing antibiotics (polymixin 50 μg, streptomycin 100 μg, chloramphenicol 100 μg, celbenin 100 μg) to prevent growth of micro-organisms. [6-3H]D Glucose (200 μc) was added to the water, and the shoot was placed in the light and left for 3 hours.

Segments (1 to 2 mm) were then cut into a glutaraldehyde-based fixative consisting of 6.5% glutaraldehyde in a phosphate buffer (pH 7.2) containing 5% sucrose. After 1 hour the segments were washed with buffer and placed for a further hour in a 1% osmic acid solution buffered with veronal (pH 7.2) again containing 5% sucrose.

The segments were then washed in water, dehydrated in an alcohol series and embedded in Araldite. Sections were cut on a mechanical-advance microtome with glass knives, picked up on carbon-coated copper grids and stained with uranyl acetate (saturated solution in 50% alcohol) followed by lead hydroxide (MILLONG, 1961).

After staining the sections on the grids they were covered with a carbon film and coated with Ilford L4 emulsion by a modification of the centrifugal method of KOEHLER, MURLETHALER and FREY-WYSSLING (1962). The grids were then stored at room temperature for 2 or 3 months, developed in Kodak D 19 (1 min), washed and fixed with Hypam (Ilford) (diluted 1:4; 45 sec) and examined in a Siemens Elmiskop I at 80 kV.

**Fine-Structural Studies.** For examination of fine structure, stems were sectioned directly into the fixative without pre-incubation, and examined after staining.

**Results**

Details of the structure of the secondary cambium and phloem of the *Pinaceae* have been comprehensively dealt with in a monograph by SRIVASTAVA (1963), using the conventional techniques of light microscopy. SRIVASTAVA's terms e.g. albuminous cells for marginal ray cells with no starch in the plastids and with callose in the pores connecting with the sieve elements, will be used.

The secondary cambium cell of *Pinus pinea* is long, narrow and highly vacuolate with thin walls and a thin lining of cytoplasm containing the usual organelles (T.S.; Fig. 1). This cambium produces xylem derivatives toward the inside and phloem derivatives toward the outside. There is a radial sequence of development from the cambium in a centrifugal direction terminating in the crushed phloem derivatives, with lumen obliterated (Fig. 4). In the young stems used in this study there are about five or six cells between cambium and crushed phloem. Cells immediately adjacent to the crushed phloem are considered to be mature, e.g. sieve elements, which were active in translocation in vivo. Unfortunately no better criterion for recognition of a functional sieve element in an electron micrograph exists at present. The sieve areas of sieve elements adjacent to the crushed phloem are certainly the most