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5. Neither Briggs and Andrew (*2*) nor we find evidence of such radiation away from the planet's limb. Synchrotron radiation is preferentially emitted perpendicular to magnetic field lines, however, and for a dipole field viewed nearly pole on the regions where the field is perpendicular to the line of sight may appear projected on top of the planetary disk. The steep spectrum normally associated with such emission would explain its absence at 2 cm.
6. We computed the disk fluxes by plotting the directly measured interferometer visibilities against projected base line. Extrapolating these plots to zero base line yields the estimated total flux. Noise contributes about ±2 K to estimated uncertainty, calibration uncertainties about ±3 K.
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8. Another example of a change in opacity, this time at a much higher level in the atmosphere, is evident from photographs of the planet taken through a filter centered on the strong methane band at 890 Å. The appearance of Uranus has changed from that of an eccentric annulus with the darkest region near the subsolar point and the brightest region over the pole in 1976 to a uniformly illuminated disk in 1983 [B. A. Smith and H. J. Reitsema, in *Uranus and the Outer Planets*, G. E. Hunt, Ed. (Cambridge Univ. Press, New York, 1982), p. 173; B. A. Smith, personal communication].
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11. We thank K. Noll, I. de Pater, and S. Gulkis for helpful discussions and M. Klein for providing his unpublished compilation of microwave observation. This research was supported in part by NASA grants NGR33015141, 953614, NSG7320, and NGL 05-002-003 and NSF grant AST 79-11806A01. The Very Large Array is part of the National Radio Astronomy Observatory, which is operated by Associated Universities, Inc., under contract with the National Science Foundation.

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The Fine Structure of Fossil Plant Cell Walls

Abstract. *The cell walls of sieve elements in the primary phloem of the Carboniferous fern* Tubicaulis *contain structural features that morphologically resemble cellulose microfibrils in extant plants. This may be the oldest example of distinct fibrillar structures in the fossil record. The possible identity and significance of these features are discussed and their structure is compared with that of cell walls in other fossil and extant plants.*

Phloem is the food-conducting tissue in vascular plants, and conducting cells (sieve elements) in this tissue have become adapted for transport by the loss or change of many cytoplasmic elements and the development of pores (sieve pores) in end and side walls. There are two basic types of sieve elements: sieve tube members, which occur almost exclusively in angiosperms, and sieve cells, which are found in gymnosperms and vascular cryptogams (ferns and related plants). The sieve cell is considered to be less specialized because it has diverged the least from a typical parenchyma cell (*1*). This is especially true of sieve cells in the vascular cryptogams, which are relatively small in diameter and only slightly elongate, with horizontal to somewhat oblique end walls (*2*). The perforated regions (sieve areas) are similarly developed on both side and end walls—a feature considered to represent the more primitive state (*1*).

The material we examined consists of petioles of the fern *Tubicaulis stewartii* (*3*) found in coal balls (calcium carbonate permineralizations) from Berryville, Illinois (*4*). Coal balls are known for their exceptional preservation of cellular detail in fossil plants (*5*) and have been found in Carboniferous coal seams in North America and Europe. Work on the phloem anatomy of *T. stewartii* petioles (*6*) has revealed features of the sieve cell walls that appear morphologically similar to cellulosic microfibrils in the cell walls of extant plants (*7*). This discovery provides the opportunity to examine some aspects of cell wall organization and structure in a plant approximately 290 million years old (Upper Pennsylvanian).

The phloem tissue in *T. stewartii* is unusual among the known fossil ferns (*6, 8, 9*), since it consists of several types of cells arranged in a very specific manner (Fig. 1A). The adaxial phloem (the tissue on the side of the petiole facing the main axis) consists of sieve cells approximately 10 μm in diameter and phloem parenchyma. The abaxial phloem (away from the axis) contains a single row of large sieve cells approximately 90 μm in diameter and at least 2.5 mm long (arrows in Fig. 1A). Since the cells taper gradually, it is difficult to distinguish end walls from side walls.

Where two of these large cells are adjacent, the common wall is covered with crowded, circular thin areas about 7.5 μm in diameter. Since this diameter is comparable to that of sieve areas in extant plants (*1*), these circular areas are interpreted as being sieve areas. However, the resolution obtained with light microscopy is inadequate to show sieve pores in these areas, which is necessary to confirm their identification as sieve areas.

Preparation of the fossils for scanning electron microscopy required the removal of the mineral matrix by selective etching (*9*). Since the cell walls are delicate, they often collapse after etching (Fig. 1B). The sieve areas on the larger cells are conspicuous, and appear as large circular to oval depressions (Fig. 1, B and C). Each of these areas is surrounded by a border of wall material (Fig. 1C), believed to have resulted from the collapse of cell walls during etching, and consists of numerous randomly arranged fibrils less than 0.1 μm in diameter (Fig. 1D). Spaces about 0.1 μm in diameter are visible between the individual fibrils (Fig. 1D). Microfibrils in extant plants are generally around 10.0 nm in diameter (*7*). However, it is impossible to measure the fibrils in the fossil material with this level of accuracy since they cannot be sectioned for transmission electron microscopy. The size of the fibrils as determined with the scanning electron microscope is at best a rough estimate because of distortion due to tilting of the specimen and the added thickness of the gold coating. Although the chemical nature of these fibrils is unknown, it is unlikely that they represent calcium carbonate artifacts, since this substance would have been removed during etching.

The appearance of the wall areas is nearly identical to that of primary pit fields on the walls of parenchyma cells and developing sieve elements in extant plants (*7*). In sieve cells the primary pit fields develop into sieve areas by enlargement of the plasmodesmata-sized perforations to form sieve pores. These pores are often ringed with depositions of callose that appear early in development and generally remain at maturity (*1, 10*). In vascular cryptogams the sieve pores are often so small at maturity as to be almost indistinguishable from plasmodesmata (*2*). In addition, some vascular cryptogams do not deposit callose at any time during their development (*11*). Thus it is sometimes difficult to distinguish sieve elements and parenchyma cells, even in living plants. The problem is compounded in fossils by poor preservation, crushing of cells, and the inability to view the material in a functioning state.

The size and shape of the large cells in *Tubicaulis* phloem indicate that they represent sieve cells and not parenchyma cells, although the sieve areas more closely resemble primary pit fields than mature sieve areas. The fibrillar areas may represent primary pit fields in im-

mature sieve elements, in which case they would presumably develop further by enlargement of the sieve pores and possible deposition of callose cylinders. The observations suggest, however, that the circular regions are mature sieve areas. The small diameter of the sieve pores (within the size range of plasmodesmata) may simply reflect a relative lack of advancement in phloem anatomy in this particular fossil fern. Further evidence to support this assumption is the mature nature of the other tissues in the *Tubicaulis* petioles (Fig. 1A). For example, tracheids in the xylem exhibit well-preserved, multiseriate pitting. The other cells in the phloem, such as phloem parenchyma and small sieve elements, are also comparable in size and shape to cells from mature tissue. However, regardless of whether these circular areas represent primary pit fields or sieve areas, they provide evidence that the wall structure in this Carboniferous fern was nearly identical to that in extant plants.

There are no chemical data to prove that the fibril-like units are cellulosic (*12*). Research on the chemistry of fossil wood (*13, 14*), especially the recent study on the chemistry of cellular fractions in coal balls (*15*), indicates that cellulose is one of the first components of the cell wall to be lost during degradation of the plant. However, there is some indication that, after the initial loss of unaltered cellulose, the pattern and texture of fibrils can be preserved in the lignin within the cell wall (*14*). In this case the wall patterns observed would be biogenic. There also is evidence that the microfibrillar pattern can be preserved in the crystal configurations of tracheid walls, even in material as old as Permian (*16*) or Devonian (*17*). In view of the age of the present material, it is unlikely that any cellulose would remain in the cell walls, and, since sieve elements generally have only primary walls, lignin would not be expected to be present. Hatcher *et al.* (*15*), working with coal balls from central Illinois, showed that cellulose and lignin are not chemically recognizable in coal ball material. Perhaps the structure of the original cellulose fibrils was replicated by crystalline material (*18*). Despite the difficulties associated with chemical analysis of these structures, some fibrillar component of the cell wall is still morphologically recognizable in the present specimens.

The presence of structurally identifiable but chemically altered fibrils in a fossil plant 290 million years old underscores the fidelity of morphological preservation in coal balls and suggests that it may be possible to use fossil material to investigate the evolution of such basic biological phenomena as the organization of cell wall constituents.

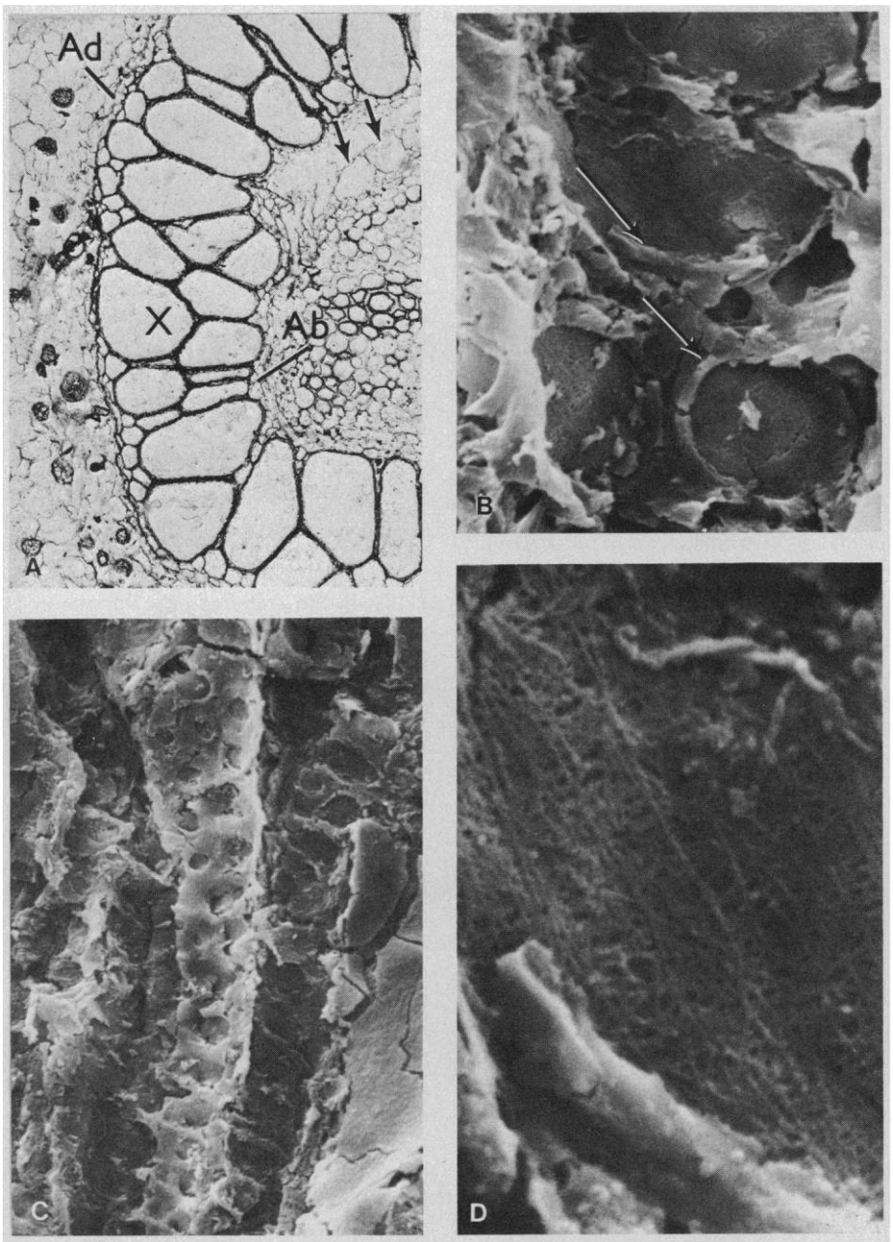

Fig. 1. (A) Cross section of part of a *T. stewartii* petiole, showing abaxial phloem (*Ab*) with large-diameter sieve cells (arrows), metaxylem tracheids (X), and adaxial phloem (*Ad*). Peel preparation (×60). (B) Wall of several large sieve elements, showing numerous, circular to oval sieve areas. Note cracking and collapse of cell wall at right (×1000). (C) Part of four sieve areas, showing their general shape and the border surrounding some of them (arrows) (×5000). (D) Wall of part of a single sieve area, showing random arrangement of fibrils and spaces between them. Part of the intervening wall (border) is visible below (×20,000).

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18. X-ray analysis with the scanning electron microscope has been performed on coal ball material prepared by selective etching. Results were equivocal, with a great deal of interference from surrounding $CaCO_3$ crystals. Since the sieve areas cannot be removed from the surrounding rock matrix, this technique has limited application.
19. Supported in part by NSF grants DEB-8001803 (T.N.T.) and DEB-7908607 (T.N.T. and E.L.S.), a grant-in-aid of research from Sigma Xi, an Ohio State University graduate school alumni research award, and a fellowship from the American Association of University Women Educational Foundation. We thank P. Van Faasen and G. L. Floyd for helpful comments.

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4-Nonylphenol in Sewage Sludge: Accumulation of Toxic Metabolites from Nonionic Surfactants

Abstract. *Anaerobically treated sewage sludge was found to contain extraordinarily high concentrations of 4-nonylphenol, a metabolite derived from nonionic surfactants of the nonylphenol polyethoxylate type. Concentrations in activated sewage sludge, in mixed primary and secondary sludge, and in aerobically stabilized sludge were substantially lower, suggesting that the formation of 4-nonylphenol is favored under mesophilic anaerobic conditions. Because 4-nonylphenol may be highly toxic to aquatic life, further research is needed on the fate of 4-nonylphenol after sludge is disposed of in the environment.*

4-Alkylphenol polyethoxylates (structure A in Fig. 1) are widely used nonionic surfactants. In 1982 their end-use market in the United States reached approximately 140,000 metric tons (*1*). Their degradation during aerobic treatment of wastewater by activated sludge leads to the formation of 4-alkylphenol mono- and diethoxylates (structure B in Fig. 1), which have been found as major refractory constituents of treated wastewater effluents (*2*) and river water (*3*). In addition, alkylphenol polyethoxy carboxylic acids have been detected in biologically treated domestic wastewater (*4*). This report concerns the degradation of the 4-alkylphenol ethoxylates during sludge treatment and, in particular, the high concentrations of the toxic compound 4-nonylphenol (structure C1 in Fig. 1) in anaerobically stabilized sewage sludge.

Sludge samples were collected from pipelines of mixed digesters during sludge transfer and extracted in a closed-loop steam distillation-extraction apparatus (*5*). Cyclohexane extracts were subjected to column chromatography on deactivated silica. The methylene chloride–eluted fractions were then analyzed by high-resolution capillary gas chromatography (Fig. 2A). Compounds were identified on the basis of comparisons with a commercial, technical grade 4-nonylphenol. Since 4-nonylphenol is manufactured from tripropylene and phenol (*6*), it contains many isomers with variously branched structures of the nonyl substituents. The different alkyl branching gives rise to several incompletely resolved peaks in the capillary gas chromatogram of commercial 4-nonylphenol (*2*, *5*). Major peaks in the gas chromatogram of the sludge extract (Fig. 2A) were assigned to isomeric 4-nonylphenols. In addition, 4-octylphenol and 4-decylphenols were detected.

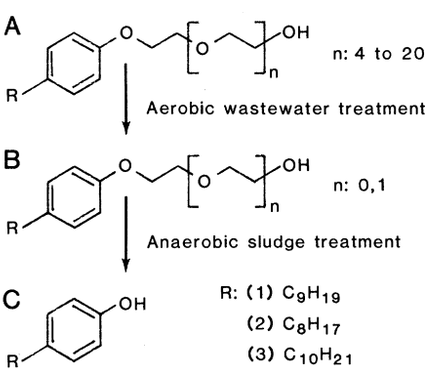

Fig. 1. Biological transformation of alkylphenol polyethoxylates (A) during wastewater and sludge treatment. Refractory metabolites are alkylphenol mono- and diethoxylates (B) and alkylphenol (C). Nonyl (1), octyl (2), and decyl (3) form the alkyl substituents (*R*).

Directly coupled gas chromatography–mass spectrometry provided the basis for identifying the alkylphenols. Molecular ions (mass/charge ratios, 206, 220, and 234) and base peaks (mostly 135, but also 107, 121, 149, and 163) were identical for corresponding peaks in the sludge sample and in the reference material. The mass spectra of 4-nonylphenols have been published elsewhere (*5*).

We also used high-performance liquid chromatography (HPLC) for further confirmation. Normal-phase HPLC [Lichrosorb-NH_2 column, hexane-isopropanol (9:1) eluent, 278-nm detection wavelength] allowed for a semipreparative separation of 2- and 4-nonylphenols from the standard nonylphenol. The major compounds in the sludge extracts coeluted with the 4-nonylphenols. Reversed-phase HPLC [Lichrosorb RP-8, methanol-water (3:1) eluent] provided a means for separating the alkyl homologues (structures C1, C2, and C3 in Fig. 1). Ultraviolet and infrared spectra of the sewage sludge extracts were identical to the corresponding spectra of the isolated 4-nonylphenols. Proton and carbon-13 nuclear magnetic resonance (NMR) spectra supported the identification of 4-nonylphenol: the aromatic proton signals (Fig. 2B) indicated 4-substitution of the nonylphenol.

For quantitative determinations, an internal standard (*n*-nonylbenzene) was added to the sludge extracts and concentrations were calculated from the sum of the electronically integrated areas of the 4-nonylphenol peaks in the chromatogram. Response factors were determined with technical grade 4-nonylphenol. Reproducibility was ±4 percent (four determinations) at 1.18 g per kilogram of dry matter. Analyses of samples spiked with 0.4, 0.9, and 1.3 g of 4-nonylphenol per kilogram of dry matter revealed recoveries of 105, 104, and 93 percent, respectively.

The concentration of 4-nonylphenol in 30 anaerobically stabilized sludge samples (Fig. 3A) ranged from 0.45 to 2.53 g/kg (mean, 1.01 ± 0.52 g/kg, or 4.7 mmol/kg). Eight samples taken from the same digester over 10 months (September 1982 to June 1983) contained 0.81 to 1.49 g/kg (mean, 1.18 ± 0.23 g/kg). 4-Nonylphenol concentrations in eight aerobically stabilized sludge samples were significantly lower [Fig. 3B; range, 0.08 to 0.5 g/kg; mean, 0.28 ± 0.15 g/kg; $t(36) = 3.91$, $P < 0.0005$]. Activated sludge and