Fossil evidence of tylosis formation in Late Devonian plants

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Oldest fossil evidence of tylosis formation in plants

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Tyloses are swellings of parenchyma cells into adjacent water conducting cells that develop in vascular plants as part of the heartwood formation or specifically in response to embolism and pathogen infection. Here, we document tyloses in Late Devonian (ca. 360 Ma) *Callixylon* wood. This discovery suggests that already some of the earliest woody trees were capable of protecting their vascular system by occluding individual conducting cells.

Tyloses are protoplasmic inflations formed by parenchyma cells into the lumen of neighboring conducting cells [1, 2]. They extend through the pit pair and can completely fill the lumen of the conducting cell. The mechanisms underlying tylosis formation remain largely unresolved [e.g., 3, 4], but it is generally accepted that they represent a response to embolism and have a major role in blocking air-filled conducting cells. Moreover, tylosis formation is among the earliest processes in the Compartmentalization Of Decay In Trees (CODIT; [5]), which can delay the spreading of pathogens. Tyloses in fossil plants, therefore, provide direct insights into the evolution of one of the most important mechanisms with which plants protect their vascular system from biotic and abiotic stresses.

Structurally preserved fossils provide evidence that tylosis formation occurred in most lineages of vascular plants as early as the Mississippian (ca. 350 Ma) [6]. However, the oldest fossils believed to represent vascular plant conductive elements (tracheids) are dispersed microscopic tubes from the uppermost Silurian (ca. 430 Ma) [7], and the first record of secondary xylem (wood) comes from the Lower Devonian (410 Ma) [8-10]. By Middle Devonian times, a great many plants possessed wood [11] in which the arrangement of conducting cells and parenchyma could, in theory, have allowed the formation of tyloses. The development of larger and more complex plant bodies during the later Devonian, along with the evolution of leaves and more extensive root systems [12, 13], likely increased the risk of embolism in the vascular system. There is also evidence from this period of geologic time of the existence of various types of vascular plant-fungal interactions, possibly including wood decay [14, 15]. It is therefore surprising that no tyloses have been hitherto reported in plant fossils older than the Mississippian.

The lack of documented evidence of tyloses in early vascular plants has spurred us to specifically look for these structures in Devonian structurally preserved woods. We found that many early woods are fossilized in a manner not conducive to the preservation (in a recognizable form) of tenuous parenchymatous structures, including tyloses. Nevertheless, we discovered evidence of early stages of tylosis formation in one piece of pyritized wood from the Upper Devonian (Famennian, ca. 360 Ma) of Sandeel Bay, County Wexford, Ireland.

This wood consists of tracheids and small parenchymatous rays (Fig. 1); distinct growth ring-like zones of tracheids with reduced radial diameters are indicative of intermittent periods of less-favorable growth conditions (Fig 1a). The radial tracheid walls show groups of pits separated by unpitted areas, a feature typical of *Callixylon*, the wood of the archaeopteridalean progymnosperms ((Fig 1c) [16]; see methods section). Rays are 1–2 cells wide and 1–15 (usually less than 10) cells high in tangential section (Fig 1d), abundant, and each xylem tracheid is in contact with a significant number of ray parenchyma cells. Conspicuous bubble-like outgrowths of ray cells into the lumen of adjacent tracheids occur in one area of a longitudinal section (Fig 1e-k). Although they appear relatively dark compared to the surrounding parenchyma cells, we interpret these outgrowths as tyloses, rather than gum deposits, based on their regular outline, physical connection to the rays, and the presence also of several translucent examples (Fig 1k). The majority of the tyloses are spheroidal in shape (Fig 1e, f), sometimes with a constriction at the base that likely corresponds to their passage through the pits connecting rays and tracheids; others are more flattened (Fig 1h, i). Still others completely occlude the tracheid (Fig 1g). There are often several tyloses emerging from a single ray, sometimes visible along both sides of the ray in tangential sections (Fig 1j). No evidence of tylosis formation has been observed in any other structurally preserved plant fossil from the Sandeel Bay locality.

Archaeopteridales are a Middle–Late Devonian group of progymnosperms, extinct plants that had a gymnosperm-like wood but reproduced via spores [16]. Archaeopteridalean trees produced a significant amount of wood, formed a deep and complex root system, and had true leaves, making them the first trees with a “modern” vegetative body [12, 18]. Recent studies of their hydraulic properties suggest that archaeopteridalean wood had a conductivity comparable to that of extant conifers, with some plasticity provided by variations in the size of the conducting cells and rays [19, 20]. Like extant trees, archaeopteridaleans were susceptible to wood decaying fungi that invaded their vascular system [14]. By the Late Devonian, these trees were a major component of ecosystems worldwide, having been reported from a diversity of latitudes, from the paleotropics to the high latitudes of Gondwana [21]. This wide altitudinal and geographic range implies that the group was able to adaptate to very diverse growth conditions. The ability to protect the vascular system from biotic and abiotic stresses by occluding (some of) the conducting cells may, therefore, have been an important advantage. The depositional environment of the Sandeel Bay anatomically preserved plants has been interpreted as a cut-off channel chute of a meandering river near the coastline [22], within a dry tropical area [23]. Growth ring-like zones in the archaeopteridalean woods from this deposit suggest that the trees were exposed to periodic shifts from favorable to less favorable growth conditions. It is likely that there were episodes of drought governed by variations of the water table that could have caused embolism in certain regions of the wood and led to tylosis formation. The fact that most tracheids are not completely blocked indicates that the specimen depicts an early stage in this process.

This discovery illustrates how structurally preserved fossils can provide snapshots of plant physiology in deep time, and allow us to piece together episodes in the evolutionary history of key biological processes. Moreover, it provides further evidence of the antiquity of tylosis formation in plants, and suggests that the emergence of this capacity could have been linked to the evolution of larger plant bodies with a more complex organization. As soon as plants had evolved a water conducting system [11, 12], they had also created a gateway through which physical (i.e., embolism) and biological (i.e., pathogens) threats could spread. Early vascular plants were perhaps able to mitigate this drawback simply through their small size and the short life span of their structures. However, as soon as plants grew larger, an effective system of protection of the vascular system became essential [24], with tylose formation being an integral part of it.

Online Methods

Age of the fossil wood, sedimentological and floristic context

The specimen was collected in 2021 at Sandeel Bay on Hook Head peninsula, Wexford County, Ireland. Outcrops containing Late Devonian plant macrofossils in this area belong to the Harrylock Formation [25] (Supplementary Fig. 1a), which corresponds to fluvial sediments deposited in a proximal alluvial plain setting. Anatomically preserved specimens occur as pyritic permineralizations in grey-green mudstones, which also contain compressions, dispersed spores, and cuticles (Supplementary Figs. 1b, 2). The miospore assemblage at Sandeel Bay corresponds to the LL Miospore Biozone of latest Famennian age [25]. New findings in 2021 of *Retispora lepidophyta* (Kedo) Playford 1976 and *Vallatisporites pusillites* (Kedo) Dolby and Neves 1970 in the layers containing the macrofossils (Supplementary Fig. 2h, j) confirm their age as latest Famennian. Other permineralized plants recovered from the source deposit of the specimen with tyloses include axes of the lycopsid *Wexfordia hookense* (Matten) Klavins 2004 at different developmental stages [26, 27] (Supplementary Fig. 2a), as well as axes and wood with the typical *Callixylon* Zalessky 1911 anatomy of archaeopteridalean progymnosperms [16]. *Callixylon* was first reported from this locality by Klavins [22], who also described an anatomically preserved branch assigned to *Archaeopteris* cf. *hibernica*. Newly collected specimens from this locality include the first anatomically preserved *Archaeopteris* roots (Supplementary Fig. 2d-g). Finally, Klavins [22] reported 3 different types of pyritized seeds, which indicate the presence also of seed plants in the paleoenvironment. Paleosol layers at Sandeel Bay show traces of relatively deep and branched root systems that could belong to either *Archaeopteris* and/or seed plants.

Fossil preparation and observation

The fossil is approximately 17 cm long and 2–3 cm wide, and consists of 7 consecutive portions of a single axis. Selected axis portions were prepared at UMR AMAP in 2022 according to the following protocol. They were embedded in Epoxy (DBF, Escil), and transverse and longitudinal sections of 1–2 mm thickness were subsequently cut with an Isomet 1000 diamond saw and polished on both sides. To improve contrast, the section were then etched following a modified version of the protocol for pyrite permineralizations published by Stein and collaborators [28]. They were initially etched in 70% nitric acid (HNO3) heated to 70°C for 60–90 seconds. After neutralization in sodium hydroxide (NaOH) they were additionally etched in 37% hydrochlorydric acid (HCl) for a few seconds, neutralized, and then etched again in 5-10 % HCl before being rinsed in water and let to dry. Selected sections were mounted on glass slides and observed in reflected light. Due to the distortion of the wood, there was no good quality view of the radial pitting on the mounted sections. Photographs of the radial pitting (Fig. 1 c) were thus taken directly on the surface of fragment HH5-E after a fracture in the radial plane. All photographs were taken with a Keyence VX 7000 electronic microscope and the associated software. Composite figures were assembled in Adobe Photoshop 21.2.2. In slide numbers such as HH5-D-G1, the first part refers to the specimen number (5), the second to the part of the specimen (D), and the last to the section (G1). Letters r or v in photographs indicates the side of the wafer that was photographed (recto, bearing the number, or verso).

Systematic affinity of the wood

The systematic affinity of the specimen was determined based on wood anatomical traits in longitudinal and transverse sections. While no primary tissues are preserved, the presence of wood tracheids with groups of radial pits separated by unpitted areas on their radial walls is a distinctive feature of the fossil genus *Callixylon*, which represents the wood of archaeopteridalean progymnosperms [16]. Other woody plants discovered from the source layers of the specimen described here are lycopsids, which clearly differ from *Callixylon* in the radial pitting (Supplementary Fig. 2c). Seed plants, currently only evidenced from this locality based on the seeds described by Klavins [22], are not known to produce wood with unpitted areas on the radial walls. Species of *Callixylon* are distinguished based on differences in certain wood anatomical features, such as ray width, and the presence, abundance, and distribution of ray tracheids [29]. Rare ray cells with thicker walls and a squarish outline in the new specimen (e.g., Fig 1d) could correspond to ray tracheids. However, the preservation of the specimen prevents a confident assignment at the species level.

Interpretation of the outgrowths as tyloses

An alternative interpretation of the bubble–like outgrowths views them as partly coagulated (due to water loss) cell contents of the ray cells that have oozed out into adjacent tracheids as a result of the pyritization process. Strongly arguing against this interpretation are the facts that (1) similar structures have not been observed in any other pyrityzed wood from the Sandeel Bay site; and (2) the occurrence of outgrowths is limited to one particular region of the specimen that does not differ from the rest in regard to preservation. Small circular structures lacking a clear connection to parenchyma cells could also correspond to tyloses, but could also be gum or other organic residues.

Data availability.

All data that support the findings of this study are included in this published article and its supplementary information files. The fossil and associated slides are currently on loan at UMR AMP Montpellier and accessible under specimen number HH5 (for HookHead #5). This material and other fossils illustrated in the supplementary figures will ultimately be deposited in the Earth Science collections of the National Museum of Ireland, Dublin, once the study of the assemblage is completed.

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Author contributions

ALD and CJH initiated the research project. ALD, CP, and TD participated in the fieldwork during which the material was collected. MR prepared the sections. ALD and MR photographed the sections. ALD, CJH and MK analysed the data with inputs from TD and CP. CP analysed the sedimentological context and prepared the spores and cuticles illustrated in the supplementary figure. ALD and MK prepared the manuscript with contributions from all co-authors.

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Figure legends

Fig 1. Late Devonian *Callixylon* wood containing tyloses. a, transverse section, showing growth ring boundaries (white arrows) b, detail of transverse section with two rays. c, radial section, showing groups of pits separated by unpitted areas (white arrows) typical of *Callixylon*. d, longitudinal section of a ray containing what appear to be ray tracheids (black arrow). e, f, general views of area with abundant tyloses. g, tyloses entirely blocking conducting cell. h, ray with several small outgrowths (developing tyloses) into adjacent tracheid. i, small outgrowth from ray cell into tracheid. j, ray with tyloses on both sides. k, tylosis lacking dark content (translucent).

All images were selected from the direct observation under the microscope of preparations of a single fossil (#HH5): a, b: slide HH5-B-CT1r; c: HH5-E (surface of specimen); d-g, j, k: slide HH5-D-G1r; h: slide HH5-D-G1v .

Scale bars: a: 200 µm; b, e, f: 100 µm; c, g-k: 50 µm.

Legend: R: ray. Arrowheads: tyloses.