*Annals of Botany* **110**: 1083–1098, 2012
doi:10.1093/aob/mcs186, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

INVITED REVIEW

# Collenchyma: a versatile mechanical tissue with dynamic cell walls

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Received: 29 April 2012 Returned for revision: 11 June 2012 Accepted: 6 July 2012 Published electronically: 29 August 2012

• *Background* Collenchyma has remained in the shadow of commercially exploited mechanical tissues such as wood and fibres, and therefore has received little attention since it was first described. However, collenchyma is highly dynamic, especially compared with sclerenchyma. It is the main supporting tissue of growing organs with walls thickening during and after elongation. In older organs, collenchyma may become more rigid due to changes in cell wall composition or may undergo sclerification through lignification of newly deposited cell wall material. While much is known about the systematic and organographic distribution of collenchyma, there is rather less information regarding the molecular architecture and properties of its cell walls.
• *Scope and conclusions* This review summarizes several aspects that have not previously been extensively discussed including the origin of the term 'collenchyma' and the history of its typology. As the cell walls of collenchyma largely determine the dynamic characteristics of this tissue, I summarize the current state of knowledge regarding their structure and molecular composition. Unfortunately, to date, detailed studies specifically focusing on collenchyma cell walls have not been undertaken. However, generating a more detailed understanding of the structural and compositional modifications associated with the transition from plastic to elastic collenchyma cell wall properties is likely to provide significant insights into how specific configurations of cell wall polymers result in specific functional properties. This approach, focusing on architecture and functional properties, is likely to provide improved clarity on the controversial definition of collenchyma.

**Key words:** Collenchyma, histology, plant anatomy, mechanical tissue, plant cell wall, primary and secondary cell walls, plant biomechanics.

## INTRODUCTION

The emergence of mechanical tissues was a key innovation in the evolution of land plants and a prerequisite for the appearance of large terrestrial species. By the middle Devonian, many plant species developed a hypodermal sterome consisting of heavily thickened sclerenchyma cells (Rowe and Speck, 2004). Biomechanical investigations indicated that the sterome significantly contributed to the stiffness of stems and allowed them to reach great heights and evolve diverse branched architectures compared with plants with turgor-based support systems (Rowe and Speck, 2004). While sclerenchyma tissues confer rigidity and tensile and shear strength to many plant organs (Niklas, 1992; Jarvis, 2007), their properties are incapable of supporting growing plant organs which undergo extensive turgor-driven elongation. Indeed, sclerified tissues generally consist of dead cells with non-extensible rigid cell walls which are unable to undergo mitotic divisions. In small slowly growing plant organs, turgor pressure generated in parenchyma cells may provide sufficient support, but many plant stems grow fast and are fragile, and therefore they cannot fully rely on turgor pressure for support. Partly because they are non-sclerified and only minimally lignified, young plant tissues are preferentially selected by grazing animals and plant bugs. For this reason, supporting tissues in these regions should not be terminally differentiated but capable of undergoing wound healing or tissue regeneration. Moreover, as secondary growth increases the diameter of stems, the ability to transdifferentiate and initiate periderm formation is an additional advantage. Finally, young above-ground organs are photosynthetic and the reinforcing tissues should ideally be as translucent as possible to enable light to reach the chloroplasts in tissues deeper in the plant. To meet most of the above-mentioned requirements, and to provide support without preventing cell elongation, many plants – eudicotyledons in particular – develop collenchyma: a mechanical tissue composed of elongated cells with thick flexible and translucent cell walls and with protoplasts capable of resuming meristematic activity.

In this review, I describe the remarkable origin of the term 'collenchyma' and discuss some of the controversies associated with the description of this tissue. In contrast to sclerified mechanical tissues such as wood and fibres, which are economically important raw materials, collenchyma tissues have received little attention. Consequently, a clear definition of collenchyma has never been given. It is not surprising that this has resulted in some confusion with respect to its designation in certain cases. However, it needs to be highlighted that one of the factors that makes collenchyma so unique is also the reason why it is difficult to define: the dynamic nature of its cell walls.

## HISTORY

Several textbooks (e.g. Esau, 1965; Fahn, 1990) report that 'collenchyma' is derived from the Greek word 'κόλλα', meaning glue and referring to the thick, glistening appearance of unstained collenchyma cell walls. Although this explanation seems perfectly acceptable, confusion exists because the first use of 'collenchyma' was by Link (1837) who used it to describe the sticky substance on *Bletia* (Orchidaceae, monocots) pollen. Two years later, in an anatomical survey of Cactaceae (eudicots), Schleiden (1839) criticized Link's (1837) excessive nomenclature and stated mockingly that the term 'collenchyma' could have more easily been used to describe elongated sub-epidermal cells with unevenly thickened cells. Although Schleiden (1839) himself used 'äussere Rindenlage' or 'Zellen der äussere Rindenschicht' rather than 'collenchyma', the term seems to have stuck as a way to describe elongated and thickened sub-epidermal cells similarly to currently accepted usage. Others such as Meyen (1830) used 'prosenchyma' to describe elongated cells with tapering ends, without distinguishing between vascular/ground tissue and even between sclerenchyma-like and collenchyma-like tissues. Common usage of 'collenchyma' can perhaps be attributed to Harting (1844) as he repetitively used 'collenchyma' *sensu* Schleiden in his anatomical survey of annual dicotyledonous angiosperms. French and English translations of his work soon followed (Giltay, 1882), spreading the new definition or appropriation of 'collenchyma'. That collenchyma was not in common use in the mid-19th century is perhaps suggested by von Mohl (1844) who described collenchyma tissues as 'jelly-like subepidermal cells' adding parenthetically 'the so-called collenchyma cells'. By the end of the 19th century, the term 'collenchyma' was incorporated in some prominent and influential plant anatomy text books and publications (e.g. Sachs, 1868; de Bary, 1877; Ambronn, 1881; Giltay 1882; van Tieghem, 1886–1888) and became more widely accepted.

## GENERAL MORPHOLOGY AND ONTOGENY

The three most characteristic morphological features of collenchyma are (i) their axially elongated cells; (2) their cell wall thickenings; and (3) their living protoplasts (Fig. 1A–D). During elongation, collenchyma cells do not divide as much as the surrounding parenchyma cells, which explains their prosenchymatic nature. However, cell size and shape still can vary from short isodiametric and prismatic cells to long, fibre-like cells with tapering ends. The latter may even reach lengths of up to 2·5 mm in *Heracleum sphondylium* (Apiaceae, eudicots) (Majumdar and Preston, 1941). In some cases, transverse divisions take place after or during elongation, and the resulting daughter cells often remain together enclosed by a shared cell wall derived from the mother cell, giving it the appearance of a septate fibre with non-thickened cross walls (Fig. 1D). Nonetheless, collenchyma shares more morphological and physical characteristics with parenchyma tissues, and therefore intermediate types are not uncommon. The similarities between both tissues even led several researchers to categorize collenchyma as thick-walled parenchyma (e.g. de Bary, 1877). Collenchyma and parenchyma cell walls both have the ability to stretch and/or grow during differentiation, but in the case of collenchyma the walls thicken throughout elongation and often post-elongation (Jarvis, 2007). Cell wall material is generally not distributed equally so that most collenchyma cells have irregular thickenings (see Histological typology). Similarly to parenchyma, collenchyma cells have living protoplasts, essential for controlling the hydration state of the cell wall, but also to enable transdifferentiation and cell wall thickening and modification. Many textbooks (e.g. Esau, 1965; Fahn, 1990) mention that chloroplasts are present in collenchyma, but in typical collenchyma tissue with a clear mechanical function, chloroplasts are rarely found (Evert, 2006). However, to allow photosynthesis, collenchyma cell walls are generally translucent, enabling light to be transmitted to the chloroplasts in tissues below.

Controversy remains regarding the ontogeny of collenchyma as it has been the focus of very few studies (Ambronn, 1881; van Wisselingh, 1882; Esau, 1936; Majumdar, 1941). According to Esau (1936), collenchyma of celery (*Apium graveolens*, Apiaceae, eudicots) originates in the ground meristem close to or against the protoderm. Periclinal divisions initially predominate but are soon followed by anticlinal longitudinal sections. As divisions rapidly follow each other, cells enlarge only moderately, appearing smaller than the surrounding ground meristem cells. The rapid succession of divisions generally prevents the formation of intercellular spaces, which are numerous in the ground tissue at that stage. Ambronn (1881), on the other hand, observed that in the Apiaceae collenchyma and vascular tissue arise from the same procambium strand, while in most other families both tissues arise independently from each other. van Wisselingh (1882), who did not study Apiaceae, never found a common origin for collenchyma and vascular tissue in any of the species he studied (including *Aucuba*, *Euonymus* and *Lamium*). A later investigation of *Heracleum* (Majumdar, 1941) failed to provide further clarity as it was reported that the inner parts of each collenchyma strand are derived in the earliest stages from the same meristem as the vascular bundles, whereas the outer parts are derived from the ground meristem. It needs to be noted that Esau (1936), Majumdar (1941) and Ambronn (1881) did not study the same species, and variation at species level may occur. Esau (1936) also studied the ontogeny and origin of collenchyma tissue associated with the vascular bundles in celery and showed that they are composed of phloem parenchyma cells that have enlarged and thickened their cell walls subsequent to the obliteration of sieve tubes and companion cells (Esau, 1936).

## SYSTEMATIC AND ORGANOGRAPHIC DISTRIBUTION IN THE PLANT

### *Position in the plant*

Collenchyma is a supporting tissue characteristic of the growing organs of many herbaceous and woody plants, and it is also found in stems and leaves of mature herbaceous plants, including those that are only slightly modified by secondary growth. Although the localization of collenchyma has been described by many authors, only Duchaigne (1955) proposed a typology which has been adopted here (Table 1).

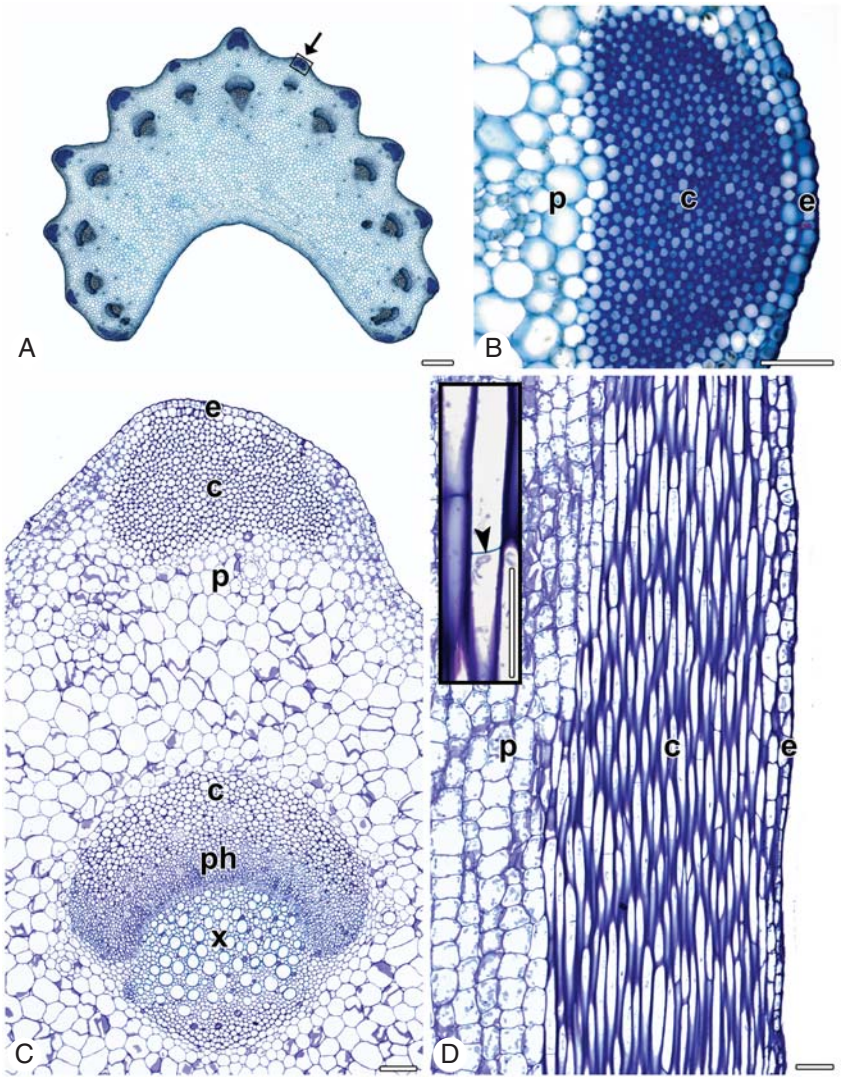


FIG. 1. General morphology of celery collenchyma (*Apium graveolens*, eudicot, Apiaceae). (A) Transverse vibratome-cut section of a fresh petiole triple-stained with acridine red, chrysoidine and astra blue showing collenchyma strands in prominent abaxial ribs. Vascular bundles are positioned opposite the collenchyma strands. (B) Detail of a collenchyma strand indicated in A. (C) Transverse section of a resin-embedded petiole stained with toluidine blue showing that collenchyma accompanies the vascular bundles at the phloem side. Note that dehydration, which is required for resin embedding, resulted in a decreased thickness of the collenchyma cell walls. (D) Longitudinal resin section stained with toluidine blue showing elongated collenchyma cells and isodiametric ground parenchyma and epidermis cells. Note that most collenchyma cells are septate with thin cross-walls (inset, arrowhead). Abbreviations: c, collenchyma; p, parenchyma; e, epidermis; ph, phloem; x, xylem. Scale bars: (A) = 500 μm; B–D, D inset) = 100 μm.

In stems and petioles, collenchyma typically occurs in a peripheral position and can be found immediately beneath the epidermis or separated from it by one to several layers of parenchyma. If collenchyma is located adjacent to the epidermis, its inner tangential walls may be collenchymatously thickened, or in some cases all epidermal walls may develop thickenings. It is not uncommon to observe one cell layer of collenchyma under the epidermis where only the cell wall facing the epidermis is thickened. Collenchyma can occur as a continuous peripheral layer (Fig. 2A), but may occasionally be interspersed by intercellular space-rich and/or parenchymatous tissues opposite stomata. In other cases, collenchyma is organized in discrete axial strands, and in stems or petioles with protruding ribs it is usually well developed in the ribs (Fig. 2B). Apart from its peripheral distribution, collenchyma is sometimes associated with vascular bundles (fascicular collenchyma), occurring at the phloem side (supracribral) (Fig. 2C), xylem side (infraxylary) or surrounding the vascular bundle completely (circumfascicular). Whereas most researchers recognize both peripheral and fascicular collenchyma, Esau (1965) suggested that only cells in a peripheral location in the plant should be called collenchyma. As mentioned earlier, celery collenchyma bundle caps differ in their ontogeny (Esau, 1936). Other observations such as differences in their biomechanical properties (Esau, 1936) and response to boron deficiency (Spurr, 1957) led Esau (1936) to conclude that this tissue should be referred to as 'collenchymatous', a term she also suggested to apply to any parenchyma in a

TABLE 1. *Distribution and histological types of collenchyma*

| Position of collenchyma in plant stems and petioles |
|---|
| 1. Peripheral collenchyma ('collenchyme périphérique ou cortical', Duchaigne, 1955): immediately beneath the epidermis or separated from it by one or more layers of parenchyma. |
| (*a*) Continuous collenchyma ('cylindre continu', Duchaigne, 1955): occurring as a continuous layer (although parenchymatous interruptions can occur below the stomata). |
| (*b*) Strand collenchyma ('cordon distinct', Duchaigne, 1955): occurring as axial strands separated from one another by parenchyma, often in externally visible stem ridges. |
| 2. Fascicular collenchyma* ('collenchyme profound ou fasciculaire', Duchaigne, 1955; 'perivascular collenchyma', Metcalfe, 1979). |
| (*a*) Supracribral ('supralibériens', Duchaigne, 1955): bordering the vascular bundle at the phloem pole. |
| (*b*) Infraxylary ('infralignieux', Duchaigne, 1955): bordering the vascular bundle at the xylem pole. |
| (*c*) Circumfascicular ('circumfasciculaire', Duchaigne, 1955): completely surrounding the vascular bundle. |
| **Histological types of collenchyma** |
| 1. Angular collenchyma (Esau, 1965; Metcalfe, 1979; Mauseth, 1988; Fahn, 1990) (syn. 'Eckencollenchym', Müller 1890; 'collenchyme angulaire', Duchaigne, 1955): extra wall material is deposited on the vertical walls where cells meet. |
| 2. Tangential collenchyma (Metcalfe, 1979) (syn. 'Plattencollenchym', Müller 1890; 'collenchyme tangentiel', Duchaigne, 1955; 'lamellar collenchyma', Esau, 1965; Mauseth, 1988; Fahn, 1990): thickenings mainly located on the inner and outer tangential cell walls. |
| 3. Annular collenchymas† (Metcalfe, 1979; Mauseth, 1988) (syn. 'collenchyma annulaire', Duchaigne, 1955): uniformly thickened cell walls. |
| 4. Lacunar collenchyma (Esau, 1965; Mauseth, 1988; Fahn, 1990) ('Lückencollenchym', Müller, 1890; 'Lacunate collenchyma', Metcalfe, 1979): walls facing the intercellular spaces are thickened. |
| 5. Collenchymatous thickenings (Esau, 1936, 1965): collenchyma-like cell wall thickenings which cannot be categorized in the four types mentioned above [e.g. thickened radial cell walls of sub-epidermal cells in *Mamillaria magnimamma* (Mauseth, 1988) or epidermal cell walls with thickened inner tangential walls]. By using this term it is implied neither that the cells are prosenchymatous, nor that they contribute to the mechanical support of the organs in which they occur. |

* Some authors do not recognize perivascular collenchyma and refer to this tissue as 'collenchymatous tissue'.

† The distinction between angular and annular collenchyma is often difficult, especially when massive thickening occurs, causing the lumen to lose its angular appearance.

non-peripheral position resembling collenchyma (Esau, 1965). Although this logic might be acceptable, few have adopted it. Therefore, I refer to the collenchymatous bundle caps as collenchyma as they are composed of elongated cells with collenchymatous thickenings.

In the lamina, collenchyma occurs in the ribs associated with the major veins where it can be found under the epidermis or as a cap at the phloem side of the vascular bundle, and/or along the leaf margins. Some leaves, such as these of *Robinia pseudoacacia* (Fabaceae, eudicots), have the ability to move due to the presence of joint-like thickenings at the base of the petiole. These structures, called pulvini, can contain a central perivascular collenchyma ring surrounded by cortical motor cells that swell asymmetrically to bend the petiole (Moysset and Simon, 1991). Sclerenchyma is also often replaced by collenchyma at the transition from blade to sheath in grass leaves (Dayanandan *et al.*, 1976, 1977; Paiva and Machado, 2003; Evert, 2006).

Collenchyma has been reported in roots (Kroemer, 1903; Bäsecke, 1908; von Alten, 1909; Turner, 1934; van Fleet, 1946) and, although this appears anomalous as roots are unlikely to require the type of support that collenchyma offers, von Guttenberg (1940) and van Fleet (1950) highlighted that collenchyma was especially apparent in aerial roots.

### *Systematic distribution*

Collenchyma is most commonly observed in eudicots (for an exhaustive overview, I refer to Metcalfe and Chalk, 1950, 1979). Interestingly, collenchyma is absent in stems and/or leaves of many of the ferns and monocots (grasses, including cereals) that develop sclerenchyma early (Falkenberg, 1876; Giltay, 1882; Metcalfe and Chalk, 1979). Tissues with similar properties, either in appearance or in function, have been reported to occur in representatives of other plant groups. However, some of these tissues have not been studied in detail and, while they may share some characteristics, it is unclear if they are homologous to collenchyma described from angiosperms. In bryophytes, collenchyma-like cells have been reported in *Dendroligotrichum* (Polytrichaceae, mosses) (Scheirer, 1977), and *Physcomitrium collenchymatum* (Funariaceae, mosses) was categorized on the basis of the collenchymatous nature of the exothecial cells of its capsules (Gier, 1955). However, Crum and Anderson (1964) did not observe these thickenings in a more mature sample and concluded that they are an unimportant expression of development. Rolleri and Prada (2007) observed collenchyma tissue in the lycophyte *Isoëtes* (Isoetaceae, lycophytes) but only provided drawings and no photographic evidence. In ferns, collenchyma has only been infrequently reported. Russow (1872) reported collenchyma in the petiole of the eusporangiate fern *Marattia* (Marattiaceae), but this observation was not complemented with drawings. In *Equisetum* (Equisetaceae), strengthening tissue under the ridges has been described as (annular) collenchyma by some authors (e.g. Hauke, 1963; Brown, 1976), while others referred to it as sclerenchyma (e.g. de Bary, 1877; Ogura, 1972; Johnson, 1933; Sørensen *et al.*, 2008). To avoid confusion some preferred more neutral terms such as hypodermis (Brown, 1976), hypodermal sterome (Gierlinger *et al.*, 2008) or strengthening tissue (Spatz *et al.*, 1998; Speck *et al.*, 1998). In the more advanced leptosporangiate ferns, collenchymatous tissues have been observed in *Asplenium rutifolium* (Aspleniaceae) (O. Leroux *et al.*, unpubl. res.). This fern contains annular collenchymatous tissues, which, at maturity, sclerify and become impregnated with brown phenolic compounds. Chaerle and Viane (2004) described false veins in *Asplenium* (Aspleniaceae), composed of moderately thickened annular collenchyma cells. Nayar and Bajpai (1970) and Nayar (1965) reported collenchyma-like thickenings in the corners of wing cells in the prothalli of *Hypodematium crenatum* (Hypodematiaceae) and drynarioid (Polypodiaceae) ferns, respectively. Unfortunately, neither study provided photographic evidence so their observations are questionable. Moreover, some reports, including Alston (1956) who stated that collenchyma is well developed in the black stipes of Adiantaceae, incorrectly

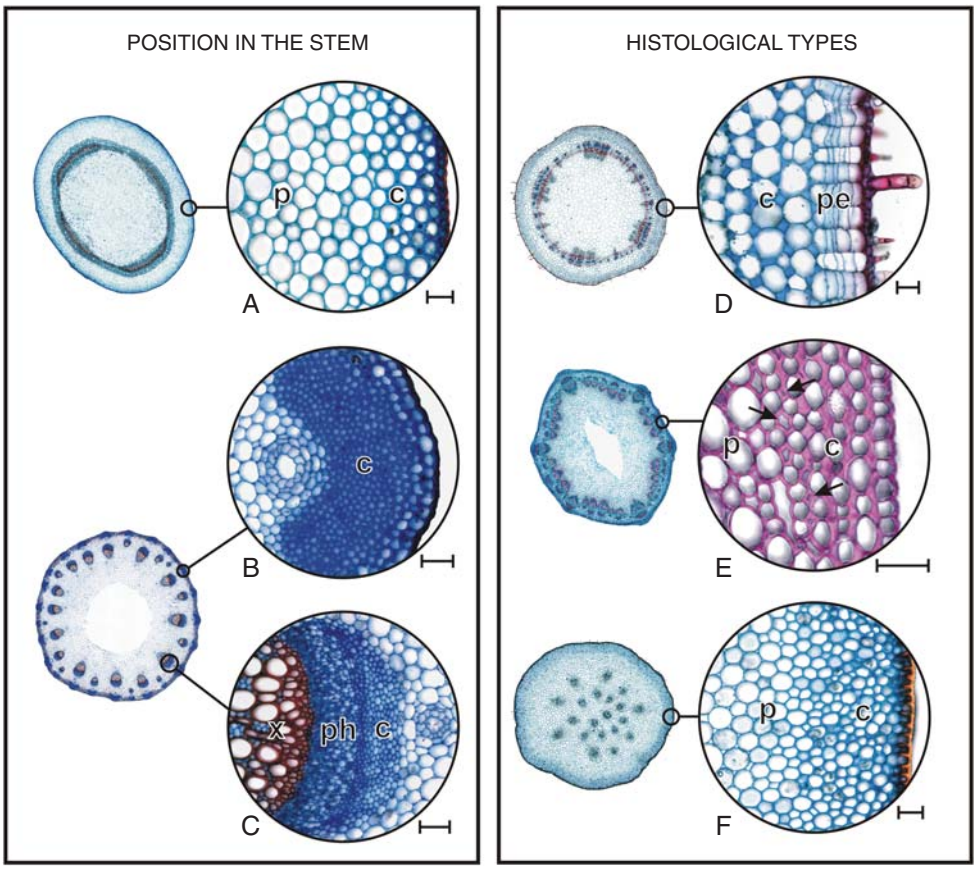


FIG. 2. Collenchyma diversity: position in the stem (A–C) and histological types (D–F). Vibratome sections triple-stained with acridine red, chrysoidine and astra blue. (A) *Coprosma repens* (Rubiaceae, eudicots) with a continuous peripheral layer of collenchyma. (B, C) *Levisticum officinale* (Apiaceae, eudicots) with collenchyma in the ribs (B) and at the phloem side of the vascular bundles (C). (D) Angular collenchyma in *Plectranthus fruticosus* (Lamiaceae, eudicots). Note the sub-epidermal periderm tissue. (E) Intermediate type between tangential and lacunar collenchyma in *Geranium sobolifolium* (Geraniaceae, eudicots). Note the many intercellular spaces (arrows). (F) *Peperomia* sp. (Piperaceae, basal angiosperms) with annular collenchyma. Abbreviations: c, collenchyma; p, parenchyma; pe, periderm; ph, phloem. Scale bars = 50 μm.

attributed collenchyma to ferns. In Gymnosperms, collenchyma cells have been reported in the leaves of *Chigua restrepoi* (Zamiaceae) (Stevenson *et al.*, 1996) and *Abies grandis* (Pinaceae) (Larsen, 1927). Although some reports were supported with microphotographs, most are unclear. It is yet to be determined if these collenchyma-like tissues in non-flowering plants are homologous or analogous to the collenchyma tissues commonly found in flowering plants.

## HISTOLOGICAL TYPOLOGY

Since the late 19th century collenchyma tissues have received more attention and several typologies have been presented. As different names were often applied to the same tissue type, I have adopted the typology of Metcalfe (1979) and mention some alternative names reported in other publications (Müller, 1890; Duchaigne, 1955) or in commonly used plant anatomy text books in which collenchyma is extensively discussed (Esau, 1965; Mauseth, 1988; Fahn, 1990; Table 1).

In the section on the history of the term collenchyma I mentioned that by the end of the 19th century, 'collenchyma' was included in most plant anatomy text books. However, in Sachs' popular 'Lehrbuch der Botanik'(Sachs, 1870), 'collenchyma' was mentioned solely in the figure legend for a drawing of a transverse section through a *Begonia* (Begoniaceae, eudicots) petiole, reporting that 'collenchyma, adjoining the epidermis, consists of cell thickenings where three cells adjoin each other'. In later editions Sachs promoted this description to the main text, further explaining that collenchyma cells are prosenchymatous, but different types were not distinguished. Vesque (1876) defined 'typical' collenchyma as a prosenchymatous tissue devoid of intercellular spaces with thickenings most apparent in the cell corners. He described this type as 'convex' collenchyma and distinguished it from 'concave' collenchyma, with the latter type giving the lumen a rather rounded appearance. de Bary (1877) described collenchyma in more detail, as a specialized type of thick-walled parenchyma, reproducing Sachs' image of *Begonia* collenchyma. Although he reported similar patterns to Vesque (1876), he did not distinguish different types. Haberlandt (1879) proposed a different typology by discriminating 'provisorisches collenchymgewebe' from 'dauercollenchym', with the latter consisting of cells with thickenings mainly located in the cell corners, and the first with all walls moderately thickened. In a comprehensive study, Giltay (1882) reported many different patterns of thickening in collenchyma tissues, but did not

propose a typology. Instead, he highlighted that collenchyma displays a natural gradient of shape and form towards both parenchyma and sclerenchyma.

The first exhaustive overview of the different types of collenchyma was published by Müller (1890). He distinguished several types based on the pattern of cellular thickening: 'Eckencollenchym' (angular collenchyma) with more pronounced wall thickening in the cell corners; 'Lückencollenchym' (lacunar or lacunate collenchyma) with only that portion of the wall thickened which borders intercellular spaces; 'Bastcollenchym' ('bast collenchyma'), characterized by cells grouped in sub-epidermal strands with no intercellular spaces and cells thickened all around; 'Knorpelcollenchym' ('cartilage collenchyma'), with walls thickened strongly all around and with a sharply distinguishable inner lamella giving the tissue the appearance of a transverse section of cartilage, with separate tubes imbedded in a homogeneous matrix; 'Plattencollenchym' (tangential, plate or lamellar collenchyma), with the thickenings on the tangential walls; 'Metacollenchym', formed very late in the differentiation of organs, by the obliteration of primary phloem and xylem cells (called 'keratenchym' by Wigand, 1863) and; finally, a type which resembles sclerenchyma in shape, 'Protosclerenchym', a transitionary collenchymatous phase prior to the development of sclerenchyma (cf. Haberlandt's 'Provisorisches Collenchym'). Later, Duchaigne (1955) simplified earlier classifications by recognizing only the three types of collenchyma which are still distinguished in most contemporary plant anatomy text books (e.g. Esau, 1965; Fahn, 1990; Mauseth, 1988; Dickison, 2000; Beck, 2005; Evert, 2006). The first type, 'angular collenchyma' ('collenchyme angulaire') (Figs 2D and 3A), is the common, classical type of collenchyma where the cell corners appear more heavily thickened. This type is seen most as sub-epidermal tissue in many stems and petioles of herbaceous dicots. The second type, 'tangential collenchyma' ('collenchyme tangential') (Figs 2E and 3B), also known as lamellar or plate collenchyma, is characterized by thickening of the inner and outer tangential cell walls. I favour the term 'tangential collenchyma' as it best suits the actual distribution of the thickenings and avoids confusion with the lamellar structure of collenchyma cell walls (see Cell wall structure). Finally, the third type, 'annular collenchyma' ('collenchyme annulaire') (Figs 2F and 3C), is distinguished by having uniformly thickened walls. Although these types show clear-cut differences, in reality there appears to be a continuum, and separation of these types is not always clear. For example, the distinction between angular and annular collenchyma is often difficult, especially when massive thickening occurs causing the lumen to lose its angular appearance (Fig. 2F). Therefore, some authors (Esau, 1965; Fahn, 1990; Beck, 2005) do not recognize this type. Several textbooks also distinguish lacunar (or lacunate) collenchyma (Müller's 'Lückencollenchym') (Fig. 3D) when thickened cell walls occur adjacent to intercellular spaces (Esau, 1965; Mauseth, 1988; Dickison, 2000; Beck, 2005; Evert, 2006). Duchaigne (1955) and Fahn (1990) did not distinguish this type, as they state that intercellular spaces often occur in other collenchyma types. Therefore, intermediate forms occur where, for example, tangential collenchyma can be lacunate (Fig. 2E), and these are often found at the interface with parenchyma tissues. As mentioned in the previous section, the term 'collenchymatous tissue' was introduced by Esau (1936) to describe the bundle caps composed of collenchyma-like cells. Although this terminology is not adopted by many authors, it is still applicable to parenchyma resembling collenchyma in any location in the plant, e.g. collenchymatous thickenings occurring in epidermal and secretory cells (Evert, 2006). Often parenchymatous cell types can have thickened walls which can be referred to as 'collenchymatous thickenings'. By using this term, it is implied neither that the cells are prosenchymatous nor that they contribute to the mechanical support of the organs in which they occur.

In tissues lacking intercellular spaces, cell wall material is often accumulated in the cell junctions and, therefore, they have frequently been mistakenly referred to as collenchyma. During the formation of intercellular spaces these accumulations are generally reorganized as filamentous or wart-like protrusions, and have been named intercellular pectic protuberances (Carlquist, 1956; Potgieter and Van Wyck, 1992; Leroux *et al.*, 2007). Carlquist (1956) observed such structures in the intercellular spaces of the peripheral collenchyma of *Fitchia speciosa* (Asteraceae, eudicots), explaining that some intercellular spaces were occluded by 'centrifugal extrusion of pectic materials'. These tissues with large, occluded intercellular spaces resemble, but may not be referred to as, collenchyma.

## COLLENCHYMA: A PRIMARY OR SECONDARY CELL WALL?

Collenchyma cell walls are generally described as being primary walls which they resemble in properties and composition. However, the terms 'primary wall' and 'secondary wall' have been employed in several fundamentally different senses, often designating different structures or cell wall layers. Jarvis (2007) pointed out that collenchyma does not fit comfortably in most definitions as it is unclear how much of the thickening is deposited after cells have ceased elongation. Kerr and Bailey (1934) described a terminology based on morphology, reserving the term 'primary cell wall' for the original wall of the cell which is formed in the meristematic region after cytokinesis, and the 'secondary cell wall' for all subsequent layers deposited during differentiation. According to these definitions, collenchyma cell walls are secondary. This terminology was based on investigations of tracheids and fibres, and did not consider cell walls such as those of collenchyma which increase simultaneously in surface area and in thickness during the growth of young tissues. Wardrop *et al.* (1979) proposed an alternative concept and recognized a primary cell wall, in the sense of a meristematic one, a growing cell wall, present during elongation, and a secondary cell wall, representing the wall material deposited after surface expansion has ceased. In this case, a part of the collenchyma cell wall is secondary. A more widely adopted concept (Fry, 2008) defines a primary cell wall as 'a wall in which microfibrils were laid down while it was still capable of growing in area', and secondary cell walls as being composed of 'any additional microfibrils deposited after the cell has stopped growing'. This means that all growing cell walls, including collenchyma cell walls, are primary. However, it is not clear if collenchyma

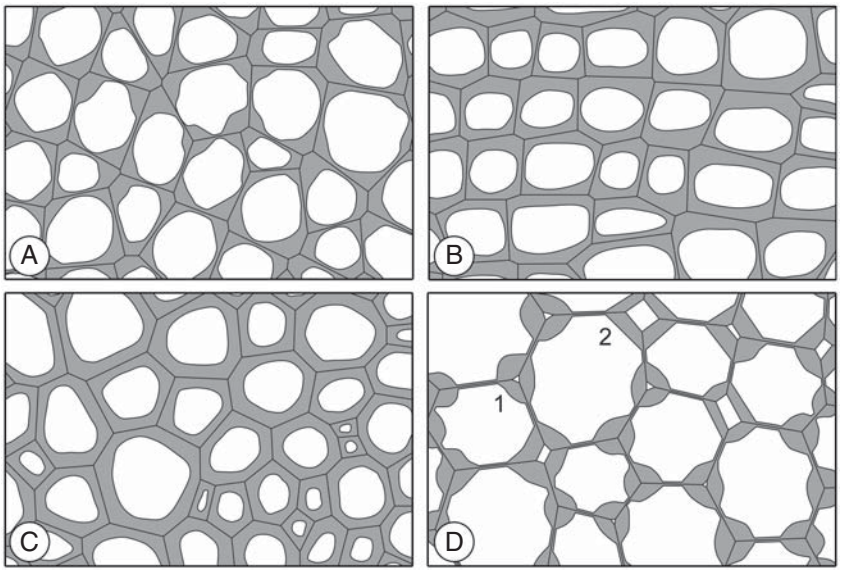


FIG. 3. Schematic drawings of the most common types of collenchyma. (A) Angular collenchyma. (B) Tangential collenchyma. (C) Annular collenchyma. (D) Lacunar collenchyma. This type often occurs as an intermediate type with angular and lamellar collenchyma, in which the size of the intercellular spaces can vary from minute spaces (1) to large cavities surrounded by collenchymatous walls (2).

cell walls lose their capability to grow in area after cell elongation has ceased and, how much, if any, cell wall material is deposited after termination of cell wall expansion. Therefore, a part of the collenchyma cell wall might be referred to as secondary. Moreover, this concept is problematic when describing thickened xylem cell walls. Lignified cell walls of protoxylem elements, organized in ring or helix patterns, are deposited during elongation and should therefore be called primary, whereas the lignified metaxylem thickenings are deposited when elongation has ceased and hence should be referred to as secondary. The latter problem can be solved by defining cell walls in terms of their extensibility (Lee *et al.*, 2011) – with primary cell walls being extensible and secondary cell walls being non-extensible – and allowing application of this concept either locally or over the entire cell surface. For example, the cell walls between the rings or helix structure of protoxylem elements are extensible and should be referred to as primary, whereas the locally lignified cell wall layers are non-extensible and therefore should be called secondary. Nonetheless, the confusion with regards to the nature (i.e. primary and/or secondary) of collenchyma cell walls remains as there is no clear view on the architecture and properties of the collenchyma cell wall layers that have been deposited after elongation has ceased.

Regardless of which concept is preferred, one needs to bear in mind that cell walls are complex biomaterials. While many concepts attempt to define boundaries, in reality there is more of a gradient of architectures and properties between primary and secondary cell walls (Lee *et al.*, 2011).

## CELL WALL STRUCTURE

To the best of my knowledge, Giltay (1882) was the first to report the lamellation of collenchymatous cell walls, and Anderson (1927) the first to have documented it. The latter researcher also suggested that pectin-rich and cellulose-poor lamellae alternated with pectin-poor and cellulose-rich lamellae. While some researchers (Majumdar, 1941; Majumdar and Preston, 1941) presented similar results for the collenchyma cell walls in *Heracleum sphondyllum* (Apiaceae, eudicots), others, including Preston and Duckworth (1946), reported a uniform distribution of cellulose in the collenchyma cell walls of *Petasites vulgaris* (Asteraceae, eudicots). It was not until the development of transmission electron microscopy and its subsequent use in biological disciplines in the mid 1950s that detailed studies were possible, and, after investigating the ultrastructure of collenchyma cell walls, Beer and Setterfield (1958) and Roland (1964, 1965, 1966) reported that the cellulose microfibril orientation in collenchyma cell walls was predominantly longitudinal. However, both Beer and Setterfield (1958) and Roelofsen (1951, 1958, 1965) found that, in elongating cell walls such as these of collenchyma tissue the microfibrils adjacent to the cell membrane had a more or less transverse orientation. These observations led to the 'multi-net growth' hypothesis (Roelofsen and Houwing, 1953; Roelofsen, 1959), which states that microfibrils, deposited transversely (or as transverse helices with a flat shallow pitch; Lloyd, 2011) adjacent to the cell membrane, change in orientation to a degree depending on the extent and polarity of wall growth. As well as disagreement on the distribution of cellulose and pectins in each of the lamellae, some reported that the lamellae were continuous (Majumdar and Preston, 1941) while others believed they were discontinuous (Beer and Setterfield, 1958; Roland, 1965) as extra layers appeared to arise at the corners inside and outside continuous lamellae. These controversies led Chafe (1970) to undertake a comparative study of different types of collenchyma, some of which had previously been studied, such as celery and *Petasites*. Instead of providing improved clarity, he observed that the transverse orientation of fibrils that appeared to be

restricted to the innermost layer in earlier works occurred throughout the cell wall, alternating with lamellae in which the orientation was longitudinal, the so-called crossed polylamellated wall. In addition, he showed that the lamellae were continuous. Roland *et al.* (1975, 1977) later mentioned that the observations that served as a basis for the multi-net growth hypothesis were made on macerated material (partial removal of matrix components). The cell walls in which the crossed polylamellation was shown, on the other hand, had either been stained with histochemical dyes or were investigated by applying shadow-casting techniques. The removal of matrix material probably disrupted the orientation of the microfibrils and may have caused the differences in observations. The crossed-polylamellated cell walls in collenchyma (Wardrop, 1969; Chafe, 1970; Wardrop *et al.*, 1979) challenged the multi-net growth hypothesis. Moreover, Roland *et al.* (1975) showed that orientation of microfibrils near the cell membrane could be either transverse or parallel to the long axis of the cell. These observations led to the 'ordered fibril hypothesis' (Roland *et al.*, 1975) in which microfibrils are considered to be deposited in alternating transverse and longitudinal orientations (Lloyd, 2011). During elongation, previously formed layers decrease in thickness due to stretching (Chafe and Wardrop, 1972). In fact, the crossed-polylamellated structure represents helices of shallow and steep pitch, respectively, as shown by the results obtained by Vian *et al.* (1993) (Fig. 4A, B). The pattern of deposition might thus not alternate discontinuously between left-hand oblique and right-hand oblique, but change more progressively, with the angle of each layer being regularly offset from its predecessor. During cell expansion or elongation this helicoidal organization of the expanding wall may become partly dispersed. When extension of collenchyma cells has ceased, the helicoidal pattern of deposition continues to thicken the wall (Vian *et al.*, 1993) and the transitional strata may become clearly observable, resulting in a cell wall structure consisting of consecutive bow-shaped arcs (Fig. 4B). In some cases these transitional layers are very thin and only a criss-crossed polylamellated pattern can be observed.

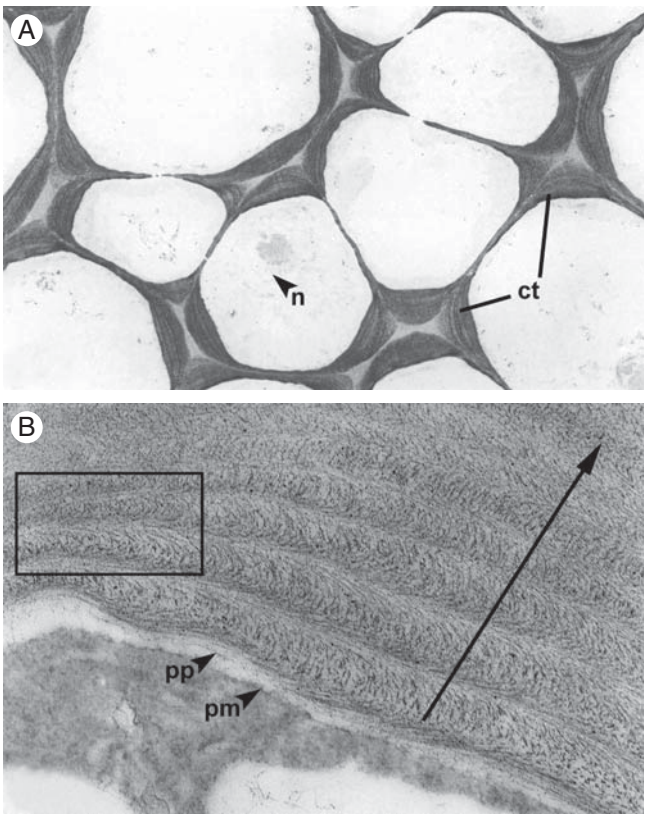


FIG. 4. Structure of celery collenchyma cell walls revealed by transmission electron microscopy. (A) Transverse section of collenchyma tissue treated with dimethylsulfoxide (DMSO) to extract matrix polysaccharides showing unevenly thickened cell walls. (B) Detail of a thickened cell wall showing its lamellated structure. While the helicoidal pattern is obvious near the plasma membrane, it is dispersed in the outward direction (arrow). Note the lateral thinning of the lamellae (squared area). Abbreviations: ct, collenchyma thickenings; n, nucleus; pm: plasma membrane; pp, periplasmatic space. Reproduced with minor modifications from Vian *et al.* (1993) with permission from The University of Chicago Press.

Some authors reported that the outermost layer of collenchyma cell walls shows a more distorted and random orientation of microfibrils (Wardrop, 1956*a*, *b*, 1969; Roland, 1965; Deshpande, 1976*a*, *b*, *c*; Matar and Catesson, 1988) and suggested that this layer represents the cell wall formed during cytokinesis. Furthermore, Beer and Setterfield (1958) observed a distinct inner layer in which the cellulose microfibrils have a transverse orientation. They suggested that this layer is deposited after cell elongation has ceased and therefore might represent a thin secondary cell wall.

## CELL WALL COMPOSITION

### *Early studies focused on the heterogeneity in collenchyma cell wall composition*

Apart from observations that collenchyma cell walls swell in water, most early researchers including de Bary (1877) and Giltay (1882) did not mention the chemical nature of collenchyma cell walls. After performing only a few histochemical tests, Ambronn (1881) concluded that they were composed of cellulose and lacked lignins. As late as the early 1900s, plant anatomy books (e.g. Haberland, 1914) only specified cellulose as the main constituent of collenchyma cell walls. However, Giltay (1882) mentioned that some researchers, including Harting, Mulder and Schacht, reported that collenchyma cells were not entirely composed of cellulose. Vesque, on the other hand, suggested that cellulose in older collenchyma walls is modified into a gum-like substance (Giltay, 1882).

To the best of my knowledge, Anderson (1927) was the first to report that collenchyma cell walls contain pectins in addition to cellulose. He suggested that the wall consists of alternating and closely packed cellulose and pectin lamellae. After retting (a process employing the action of moist and decay-producing bacteria to dissolve cellular components) and treatment with chromic acid to remove pectic substances, Majumdar and Preston (1941) concluded that pectin-rich lamellae alternated with cellulose-rich lamellae. This compositional heterogeneity was later affirmed (Beer and Setterfield, 1958; Roland, 1964, 1965, 1966). Deshpande (1976*b*) found that pectinase treatment of *Curcurbita* petiole collenchyma caused cells to separate at the middle lamellae and layers of

microfibrils to separate within the cell walls, which led him to suggest that pectins serve as 'glue' between adjacent cells as well as lamellae. However, in the collenchyma walls of *Petasites vulgaris*, cellulose was found to be distributed evenly within the cell wall, while pectin was found in alternating lamellae (Preston and Duckworth, 1946). To obtain more insight into the distribution of pectins in collenchyma cell walls, Chafe (1970) stained collenchyma of different species with pectin-specific dyes and showed that the distribution of pectic substances within the collenchymatous cell wall was relatively continuous in some species, but associated with specific lamellae in other species. In the latter case, he found the pectins in the lamellae with microfibrils in longitudinal orientation. The lack of a pronounced heterogeneity in pectin distribution in some species led Chafe and Wardrop (1972) to suggest that the lamellate appearance of the walls could be an optical effect caused by differences in microfibrillar orientation.

Majumdar and Preston (1941) reported that the inner layer of collenchyma cell walls in *Heracleum* is chemically distinct, being composed of cellulose only. Later, Preston (1952) mentioned that histochemical tests failed to provide evidence for the presence of lignins, pectins, cellulose and lipids in this layer. To date, no detailed study has been undertaken to confirm or complete these observations.

### *Recent studies provided more insight into the molecular composition of collenchyma cell walls*

Since the 1990s more detailed compositional analyses of the cell walls of specific tissues have been undertaken and also provided greater insight into the molecular composition of collenchyma. This was largely made possible by the increased use and number of available cell wall-directed monoclonal antibodies and carbohydrate-binding modules (CBMs; Biosupplies Australia, CarboSource Services, Complex Carbohydrate Research Center, University of Georgia, USA and PlantProbes, University of Leeds, UK) which have facilitated improved knowledge of *in situ* cell wall composition (Hervé *et al.*, 2011; Lee *et al.*, 2011). Unfortunately, to date, no detailed immunocytochemical study specifically focused on collenchyma cell walls has been undertaken. However, as collenchyma occurs in the stems of many dicots such as tobacco, which has been included in detailed analyses of cell wall composition, some data are available and are summarized below and shown in Fig. 5. Details of the molecular composition of collenchyma walls obtained by methods other than immunocytochemistry are also discussed.

Collenchyma walls have a similar composition to primary cell walls (Jarvis, 1992). These cell walls surround growing cells and are made up of cellulose microfibrils embedded in a hydrated matrix of complex polysaccharides classified as hemicelluloses and pectins (Cosgrove, 2005). Hemicelluloses are cellulose-binding polysaccharides, tethering cellulose microfibrils together in order to form a strong network. Pectins, on the other hand, are complex polysaccharides forming hydrated gels that could affect the physical properties of the cell wall. They are also important factors for controlling wall porosity and wall thickness and they are the main component of the middle lamella (Albersheim *et al.*, 2010). In addition to these polysaccharides, cell walls also contain small amounts of structural proteins. Secondary cell walls, which are thick and rigid, contain larger proportions of hemicelluloses, lower amounts of pectins, and are generally lignified (Albersheim *et al.*, 2010).

*Cellulose.* Cellulose was one of the first components reported to be present in collenchyma cell walls (Giltay, 1882). It is found in the form of linear insoluble microfibrils occurring in highly ordered crystalline, semi-ordered para-crystalline and disordered amorphous states (O'Sullivan, 1997). Using $^{13}C$ nuclear magnetic resonance (NMR) spectroscopy on living tissues, Jarvis and Apperley (1990) found high amounts (in comparison with cotton, wood and other secondary cell walls) of amorphous (possible crystallite-surface) cellulose relative to cellulose I in celery collenchyma. Recently, the repertoire of cell wall-directed probes has been extended by the development of CBMs obtained from microbial plant cell wall hydrolases (Boraston *et al.*, 2004; Shoseyov *et al.*, 2006) (Fig. 5A). The variation in binding patterns of some cellulose-directed CBMs (Blake *et al.*, 2006) showed that cellulose chains in collenchymatous cell walls in celery petioles do not form highly ordered crystalline structures, as CBM17, binding to internal regions of amorphous cellulose, labelled the collenchymatous thickenings, especially after enzymatic removal of pectic homogalacturonan.

*Pectins.* Pectic polysaccharides are abundant in primary cell walls and include homogalacturonans, rhamnogalacturonans, xylogalacturonans, galactans, arabinans and arabinogalactans (Harholt *et al.*, 2010). Pectic polymers display variation in terms of both glycosyl structure and polysaccharide modifications, such as methyl-esterification and acetylation, and these may vary within tissues and even single cell walls and are often developmentally regulated (Albersheim *et al.*, 2010). Jones *et al.* (1997) showed that JIM5 and JIM7, binding to pectic homogalacturonan with low and high degrees of methyl-esterification, respectively, bound to collenchymatous thickenings in tomato petioles, with JIM5 displaying a stronger binding. JIM5 also labelled collenchyma in elderberry (*Sambucus*, Adoxaceae, eudicots) (Fig. 5C) and tobacco (Fig. 5G). Pectins with low degrees of methyl-esterification have the ability to form gels (Willats *et al.*, 2001). For instance, in parenchyma, high-esterified pectins are generally present throughout the cell wall, while low-esterified homogalacturonans are generally found in the middle lamellae where they can participate in calcium cross-linking and gel formation (Albersheim *et al.*, 2010). In a study estimating the polymer rigidity of growing and non-growing celery collenchyma walls through *in vivo* solid-state NMR, Fenwick *et al.* (1997) showed a decrease in the proportion of methyl-esterified pectin in the collenchyma cell walls when growth ceased. If the pectin matrix, in which the layers of cellulose microfibrils are embedded, are rich in methyl-esterified pectins, the latter may facilitate a degree of shear between the lamellae and enable elongation. Jarvis (1992) reported that pectic polysaccharides might control the thickness of collenchyma cell walls by tethering the lamellae in its walls. He also suggested that the cell wall pectins in the collenchymatous thickenings form a gel continuous with that of the middle lamellae, preventing the layers from delaminating.

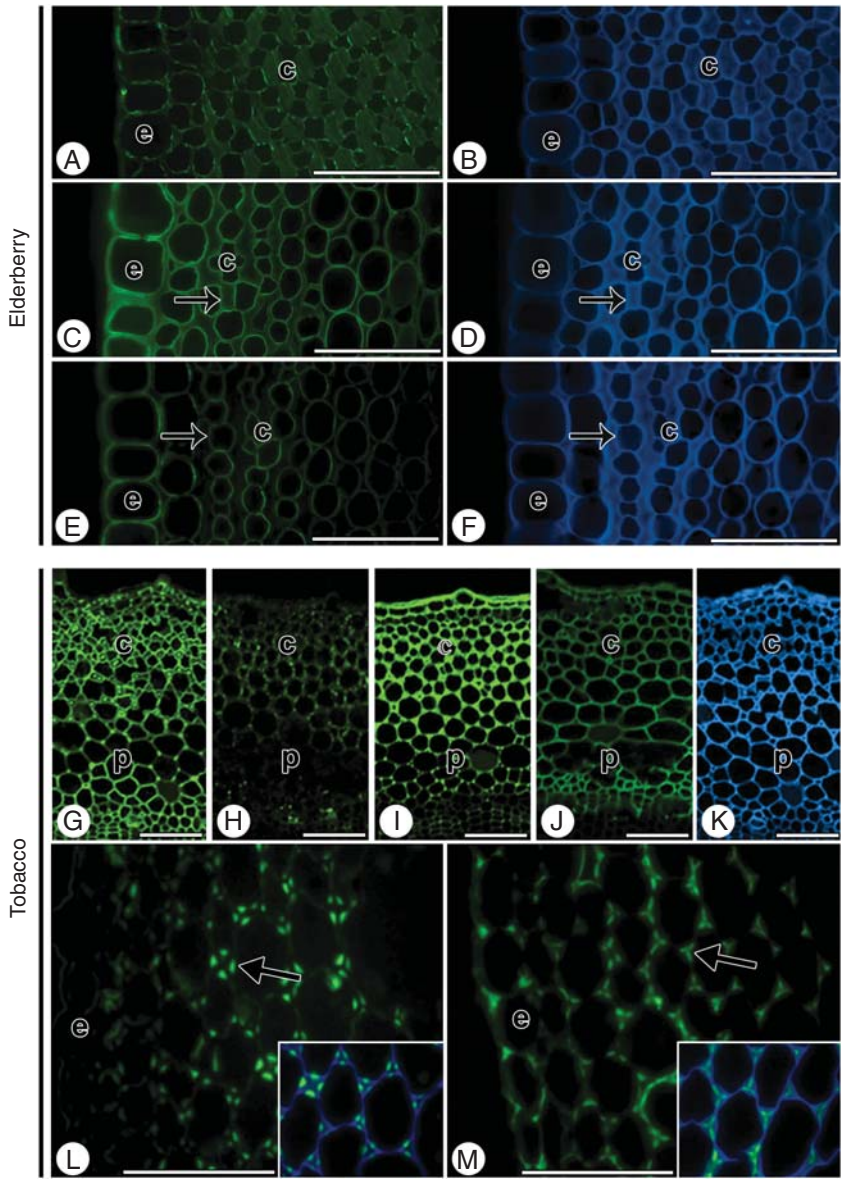


FIG. 5. Indirect immunolabelling of cell wall polysaccharides in collenchyma of elderberry (*Sambucus nigra*, Adoxaceae, eudicots) and tobacco (*Nicotiana tabacum*, Solanaceae, eudicots) with monoclonal antibodies and carbohydrate-binding modules (CBMs). (A) CBM3a, targeting crystalline cellulose, binds strongly to the collenchyma cell walls. (B) Equivalent section to (A) stained with Calcofluor White to show the full extent of cell walls. (C) Binding of the pectic homogalacturonan antibody JIM5 to all cell walls. Note stronger labelling of the inner cell wall layers of the collenchyma tissue (arrow). (D) Equivalent section to (C) stained with Calcofluor White to show the full extent of cell walls. (E) LM5, directed against pectic galactan, labels the inner layer of the collenchyma cell walls (arrow). (F) Equivalent section to (E) stained with Calcofluor White to show the full extent of cell walls. (G) Section immunolabelled with the pectic homogalacturonan-directed probe JIM5. (H, I) Weak recognition of the xyloglucan LM15 epitope (H) and its increased detection after pectate lyase pre-treatment (I). (J) Section immunolabelled with the pectic arabinan probe LM6 after pectate lyase pre-treatment. (K) Calcofluor White staining of equivalent section to (G–J) showing the full extent of cell walls. (L, M) The anti-xylan monoclonal antibodies LM10 and LM11 bind to the inner regions (arrow) of cell wall thickenings and to the outer regions (arrow) of the cell wall near the cell junctions, respectively (combined with Calcofluor White fluorescence in the insets). Abbreviations: e, epidermis; c, collenchyma; p, parenchyma. Scale bars: (A–K) = 100 μm; (L, M) = 10 μm. (A–F) are reproduced from unpublished results with kind permission of P. Knox (University of Leeds, UK); (G–K) are reproduced from Marcus *et al.* (2008), and (L, M) are reproduced from Hervé *et al.* (2009) with permission from John Wiley and Sons.

In contrast to the abundance of pectic homogalacturonan throughout collenchyma cell walls of tomato petioles, LM5, an antibody recognizing (1 → 4)-β-D-galactan which occurs as side chains on pectic rhamnogalacturonan-I (RG-I), was specifically detected in the inner cell wall layer of collenchyma (Jones *et al.*, 1997), as shown for elderberry in Fig. 5E (*Sambucus*, Adoxaceae, eudicots). It is possible that this layer corresponds to the distinct inner layer observed in collenchyma walls by Majumdar and Preston (1941), Preston (1952) and Beer and Setterfield (1958). The side chains of RG-I also include arabinan polymers. Weak labelling of collenchyma cell walls by the LM6 antibody, which recognizes pectic (1 → 5)-β-D-arabinan, was shown in tobacco by Marcus *et al.* (2008) (Fig. 5J). Willats *et al.* (2001) suggested that

the occurrence of RG-I and its structural variants may be related to mechanical properties. For instance, the appearance of galactan is correlated with an increase in firmness of pea cotyledons (McCartney *et al.*, 2000). McCartney *et al.* (2003) found that the incorporation of the pectic (1 → 4)-β-D-galactan epitope in cell walls preceded the main phase of cell elongation in arabidopsis roots. Moreover, galactans are enriched in primary cell walls of elongating cells of potato stolons (Bush *et al.* 2001) as well as in elongating carrot suspension cells (Willats *et al.*, 1999). Arend (2008) showed that in tension wood fibres the distribution of the LM5 epitope is restricted to a narrow cell wall area between the gelatinous G-layer and the secondary cell wall, further strengthening the view that galactan-rich pectins may play an important role in mechanically stressed tissues. Pectic galactans are also known to be some of the most flexible cell wall polymers (Ha *et al.*, 1996), and the presence of these polymers decreases the ability of pectin molecules to cross-link and form a coherent gel network (Hwang and Kokini, 1991). Jones *et al.* (2003) showed that enzymic treatments with arabinase prevented either stomatal opening or closing, suggesting that removal of arabinans, also occurring as side chains on RG-I, might induce stiffening of the cell wall by preventing the formation of calcium-mediated interactions between homogalacturonan domains (Harholt *et al.*, 2010). However, at present, there is no clear view on the function of these arabinan side chains. Other studies showed that they might have important functions in relation to cell wall hydration (Moore *et al.*, 2008).

Spurr (1957) showed that the amount of boron in celery has a pronounced effect on the cell wall thickness. In boron-deficient plants the cell walls of different tissues showed contrasting responses. While cortical and phloem parenchyma cell walls were thicker, collenchyma cell walls became markedly thinner. This effect was not (or not entirely) caused by swelling as Spurr (1957) showed that boron-deficient celery collenchyma cell walls contained fewer lamellae. An early symptom of boron deficiency in flowering plants is the formation of primary walls with abnormal morphology and mechanical properties causing stems to become more rigid, sometimes referred to as the 'cracked-stem' symptom (Purvis and Ruprecht, 1937). Kaneko *et al.* (1997) showed that borate diesters covalently cross-link rhamnogalacturonan-II (RG-II) dimers. RG-II is a structurally complex pectic polysaccharide present in the primary walls of lycophytes, ferns, gymnosperms and angiosperms, and its cross-linking is required for the formation of a three-dimensional pectic network *in muro* (O'Neill *et al.*, 2004). This network contributes to the mechanical properties of the primary wall and is required for normal plant growth and development (O'Neill *et al.*, 2004). The changes in wall properties that result from decreased borate cross-linking of pectin may have led to some of the symptoms reported by Spurr (1957).

Since pectins are hydrophilic, collenchyma cell walls are rich in water. The amount of water may reach 60 %, based on fresh weight (Cohn, 1892). Dehydration of collenchyma generally results in shrinkage, especially in the radial direction. By comparing vibratome sections, which are made without performing chemical fixation or dehydration, and sections of resin-embedded samples, which are chemically fixed and dehydrated, a notable shrinking of collenchyma cell walls can be observed in the latter (Fig. 1B, C).

*Hemicelluloses.* Hemicelluloses include xyloglucans, xylans, mannans and glucomannans, and their most important biological role is their contribution to strengthening the cell wall by interaction with cellulose (Scheller and Ulvskov, 2010). Although cellulose and pectins have long been seen as the sole constituents of collenchymatous cell walls, Preston (1952) reported that collenchyma cell walls also contain hemicelluloses. In the collenchyma of *Petasites*, Roelofsen (1959) detected that the cell walls were composed of 45 % pectin and 35 % hemicelluloses, meaning that cellulose only accounts for a maximum of 20 % of the wall polysaccharides. Xyloglucan is one of the most abundant hemicelluloses found in primary cell walls of non-graminaceous flowering plants and is proposed to have a functional role in tethering cellulose microfibrils. Moreover, there is evidence suggesting that hemicelluloses can be linked to pectins (Popper and Fry, 2008) and therefore might take part in a complex mechanism for the tethering and spacing of cellulose microfibrils. The LM15 monoclonal antibody, binding to the XXXG-motif of xyloglucan, labelled tobacco collenchyma cell walls, especially the inner cell wall layers adjacent to the middle lamella. However, this distribution pattern was only observed after pectate lyase pre-treatment (Marcus *et al.*, 2008) (Fig. 5H–I). In young celery petioles, xyloglucan endotransglycosylase action was found to be particularly high in the thick-walled collenchyma cells (Vissenberg *et al.*, 2000). As collenchyma tissues support the stem while its cells are still elongating, the increased XET action suggests that endotransglycosylase/hydrolase-mediated wall modification may contribute to the structural integrity of the plant body and/or that it plays an important role during cell expansion.

Although xylans are one of the major hemicelluloses found in most secondary cell walls (Carpita and Gibeaut, 1993; Harris, 2005), they have been detected in collenchyma cell walls, especially after enzymatic removal of pectic homogalacturonan (Hervé *et al.*, 2009). Hervé *et al.* (2009) showed that the xylan probes CBM15 and LM11 bound to the collenchymatous cell thickenings in tobacco stems, while LM10 labelled the complementary inner cell wall regions (Fig. 5L–M). The occurrence of xylans in thickened primary cell walls of collenchyma and the epidermis, and not in all primary cell walls, may indicate some similarities in the thickening processes of primary and secondary cell walls.

Mannans are another group of hemicellulosic polysaccharides associated with both storage and structural properties of cell walls (Scheller and Ulvskov, 2010). They are proposed to cross-link cellulose by means of hydrogen bonds, acting in similar ways to other hemicelluloses. Recently, mannan epitopes have been unmasked in collenchyma cell walls of tobacco after removal of pectic homogalacturonan (Marcus *et al.*, 2010). Although a link between pectins and mannans has not been proven, it is possible that this association may be implicated in cell wall structure and/or modification.

Even though the knowledge of the *in situ* distribution of cell wall polymers in collenchyma is fragmentary, the above examples serve to illustrate that much is to be discovered in future studies focused on collenchyma.

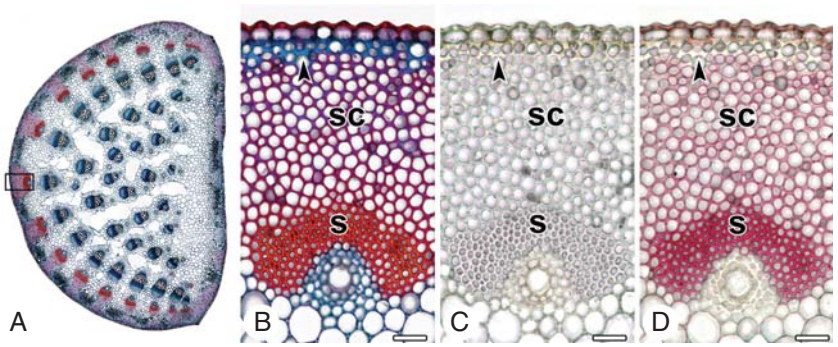


FIG. 6. Sclerified collenchyma tissue in the petiole of *Eryngium campestre* (Apiaceae, eudicots). (A) Vibratome section triple-stained with acridine red, chrysoidine and astra blue showing gross anatomy. (B) Detail of (A) showing sub-epidermal sclerified collenchyma (sc) and sclerenchyma (s). (C) Unstained vibratome section. (D) Vibratome section stained with phloroglucinol/HCl to indicate lignins (red). Only the inner layer of the collenchyma cell walls is lignified. Note the glistening nature of the non-lignified collenchyma cell walls under the epidermis (arrowheads). Abbreviations: s, sclerenchyma; sc, sclerified collenchyma. Scale bars = 50 μm.

## SCLERIFICATION AND PERIDERM FORMATION

Although collenchyma tissues are especially suited to provide support to young plant organs, they may also serve as the main supporting tissue in stems that are only moderately strengthened by secondary growth or in herbaceous stems that lack such growth altogether. In some (flowering) plant species belonging to the Acanthaceae, Apiaceae, Bignoniaceae, Fabaceae, Lamiaceae, Piperaceae and Polemoniaceae, peripheral collenchyma tissues are known to undergo sclerification by deposition of lignified secondary cell walls after elongation has ceased (e.g. Born, 1886; Went, 1924; Lemesle, 1929; Nobécourt and Papier, 1951; Duchaigne, 1953, 1954*a*, *b*, 1955; Roland, 1965; Wardrop, 1969; Calvin and Null, 1977) (Fig. 6). While most of the researchers listed above agree that sclerification implies the addition of extra lignified cell wall layers, much controversy remains when it comes to what extent, if at all, the original collenchyma wall is lignified. Some report that the original collenchyma wall remains non-lignified (Nobécourt and Papier, 1951), while others believe that it becomes lignified or that the wall is reabsorbed (Went, 1924). Duchaigne (1953, 1955) reports a double transformation process in which secondary cell wall material is deposited in a centripetal direction while the collenchyma wall is modified through deposition of new material and/or substitution in a transfugal direction. The addition of secondary cell wall material reduces the size of the cell lumen giving the cell the appearance of a thick-walled sclerenchyma cell. Roland (1965) and Wardrop (1969) both confirmed the double transformation system of Duchaigne (1953), which makes lignified collenchyma indistinguishable from sclerenchyma. A reduction in thickness of the original collenchyma cell walls does not necessarily imply that parts of it become lignified, as sclerification could cause the original collenchyma cell walls to shrink as a result of dehydration. Moreover, the controversy regarding whether or not the original collenchyma cell wall undergoes lignification should at least take into account that variation may exist between species. For comparison, when considering sclerenchyma tissues, in some xylem elements lignin is first deposited in the middle lamellae, sometimes even before elongation has ceased, while in other sclerified cells, lignin is not present in the primary cell walls (Evert, 2006). Both Wardrop (1969) and Calvin and Null (1977) reported that collenchyma tissues at the petiole base of carrots did not sclerify while higher up in the petiole sclerification was observed. Wardrop (1969) concluded that growth mainly took place in this region, but, as pointed out by Calvin and Null (1977), carrot leaves can change their angle with respect to the horizontal, and these adjustments might be facilitated by collenchyma tissues at the petiole base.

The cases mentioned above are examples of mature collenchyma undergoing sclerification. However, in some cases, collenchyma could represent sclerenchyma in which collenchyma is just a temporal developmental phase. Slowness of maturation relative to initiation and early differentiation is not uncommon in biological systems. This could explain why such tissues are present in some ferns species such as *Asplenium theciferum* (O. Leroux *et al.* unpubl. res.). In the latter case, cell walls may thicken as long as elongation takes place. The collenchymatous nature of these walls enables more light to reach the chloroplasts of the underlaying cortical tissue, and differences in growth rate might also enable unfurling of fern croziers. Once elongation is completed, the lamina takes over photosynthesis and the petiole can increase its stiffness through sclerification of some of its tissues, a process often accompanied by the impregnation of cell walls with dark phenolic compounds. The collenchymatous tissue shown to replace sclerenchyma at the transition from blade to sheath in grass leaves (Dayanandan *et al.*, 1976, 1977; Paiva and Machado, 2003; Evert, 2006) is another example of non-lignified sclerenchyma mimicking the properties of collenchyma in an early developmental phase.

Although collenchyma is the main supporting tissue in growing stems, it is also often implicated in the secondary growth process. At first, collenchyma cells enlarge in order to keep up with the increasing diameter of stems during extensive secondary growth. During this process, the cell walls become thinner. It is not clear if this thinning results from removal of wall components or from wall stretching and dehydration (Esau, 1965; Evert, 2006). When stretching has reached its limit, the phellogen initiates in a single cell layer of the collenchyma through periclinal divisions. While most divisions are periclinal, periodic anticlinal longitudinal

divisions are necessary to keep up with the increase in circumference of the stem. It needs to be noted that the phellogen can be initiated in any living, and thus potentially meristematic, tissue (Evert, 2006). Similar structures are initiated when collenchyma responds to injuries through formation of a wound-periderm.

## FUNCTIONAL ROLE

Although collenchyma was one of the first tissues that intrigued botanists because of its mechanical role in growth and development (Ambronn, 1881), few studies have focused on its biomechanical properties (Ambronn, 1881; Esau, 1936). By the end of the 19th century it became clear that plants react to gradually increasing strains by developing more mechanical tissue (for a review, see Hibbard, 1907). Many experiments were carried out to investigate the impact of mechanical stress on plant growth and development, and the term 'thigmomorphogenesis' was later coined by Jaffe (1973) to describe the response of plants to mechanical stimulation. Venning (1949) and Walker (1957) studied the effect of wind on celery collenchyma and found that while the number of collenchyma bundles remained unchanged, larger areas of collenchyma with heavier cell wall thickenings developed at an early stage. Walker (1960) measured the length of collenchyma cells in *Datura stramonium* (Solanaceae, eudicots) and found that mechanical stimulation decreased their size and thus inhibited elongation. He also examined if etiolation resulted in reutilization of collenchyma cell wall material and showed a reduction of wall thickening, which suggested that collenchyma wall material might be reabsorbed as a respiratory substrate during etiolation. A number of detailed studies have been performed on isolated collenchyma strands, and their tensile strength has been estimated by measuring the weight required to break them. Ambronn (1881) measured the breaking stresses of isolated mechanical tissues of different species and found that collenchyma and sclerenchyma could support 10–12 and 10–15 kg $mm^{-2}$, respectively. Collenchyma was shown to undergo plastic deformations at relatively low stresses compared with fibres, which regained their original length after tensile stresses that were up to 18 times higher (Ambronn, 1881). Esau (1936) reported that the average tensile breaking stress for collenchyma of celery was roughly 5·7 times higher than that of the primary xylem elements in the same region, but also showed that variation in breaking stress was correlated with the age of the tissue. The latter aspect was also shown by Jaccard and Pilet (1975) who reported that collenchyma exhibits less strain as it ages, with a decrease of the plastic to elastic strain ratios. These experiments indicate that young collenchyma tissues are characterized by their great tensile strength and plasticity, whereas sclerenchyma combines great tensile strengths with elasticity. The plastic deformation of young collenchyma walls at relatively low stress levels is important as most of the elongation occurs after collenchyma cells have started to thicken their walls. In some plant organs that do not develop sclerenchyma or secondary growth, collenchyma often persists as the main mechanical tissue, and, as mentioned above, their walls become more elastic. Some collenchyma tissues sclerify and modify their walls in such a way that they are able to withstand tensile and compressive components of bending stresses (Jarvis, 2007). In organs that undergo secondary growth, the xylem becomes the main supporting tissue and therefore the mechanical properties of collenchyma become less important.

Flexibility of collenchymatous tissues is an advantage for any plant part that is subjected to mechanical stress that would result in damaging its tissues. For example, the collenchymatous tissue found at the sheath–leaf blade transition area of the grass *Panicum maximum* (Paiva and Machado, 2003) enables greater flexibility, reducing the risk of ruptures. Likewise, the central position of the collenchyma in some pulvini (Moysset and Simon, 1991) facilitates bending and prevents damage to the vascular tissue.

## FUTURE PERSPECTIVES

Although there is a general consensus that collenchyma is a mechanical tissue supporting growing organs, there remains some controversy about its precise definition. Collenchyma cell walls thicken during elongation, but also after cell expansion has ceased, and therefore they do not fit comfortably in the definitions of either primary or secondary cell walls. Moreover, as their shape varies from short prismatic-like to elongated cells with tapering ends and some tissues undergo sclerification, efforts for sharp distinction of collenchyma may lead to partial overlap with other tissues. Regardless of its definition, many questions pertaining to collenchyma cell walls remain and these should be addressed by employing techniques that have become possible or more easily accessible in recent years. The distribution patterns of some cell wall glycan epitopes discussed in this review indicate that immunocytochemistry has significant potential to contribute to knowledge of collenchyma cell wall architecture. Is the distribution of glycan polymers uniform or restricted to specific lamellae? To what extent are polymers and their structural variants associated with specific developmental stages of collenchyma cell walls? How are the cell walls remodelled during and after cell elongation? Detailed analysis of the chemical composition of collenchyma cell walls will also provide increased insight into the structural–functional relationships of cell wall architectures, especially when its biomechanical properties are the subject of concurrent investigations. Which architectural modifications are associated with the transition from plastic to elastic properties? As mechanical properties are determined not only by collenchyma cell wall architecture, but also by the shape and arrangement of cells in the tissue, a combinatorial approach is preferred, correlating structural and compositional information with biomechanical properties, at both the tissue and cell level.

## ACKNOWLEDGEMENTS

This work was supported by an IRCSET-EMPOWER postdoctoral fellowship grant at the National University of Ireland, Galway (NUI Galway) as well as by the Systematics Research Fund (The Linnean Society of London) awarded to the author. The NUI Galway James Hardiman Library provided support through its interlibrary loan service, handling many requests for literature quickly and efficiently. I thank Z. A. Popper for critically reading the manuscript.

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