Collenchyma and sclerenchyma in *Ampelopsis brevipedunculata* tendrils

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Summary

Vines like *Ampelopsis brevipedunculata* (vitaceae) produce tendrils which are motile and flex in a circular motion, seeking solid supports. Then, when a support is found they adhere firmly by coiling. To achieve these two functions tendrils use a cellular and developmental mechanism which involves microscopic alteration of cell wall composition and physical properties. Immature tendrils are flexible because their supporting tissues include abundant collenchyma and un-lignified primary cell walls, which allow considerable movement. Mature tendrils acquire large amounts of lignified tissue, become inflexible and decay resistant. This lignified tissue retains cell contents and is composed of fibers not tracheids.

Key words: *Ampelopsis brevipedunculata,* tendril, lignin, collenchyma, fiber.

Introduction

The gross anatomy and physiology of tendrils has been extensively but intermittently studied since the nineteenth century (von Sachs, 1887; Darwin, 1891, 1911; Jaffe and Galston, 1968 and many others). Tendril research continues mainly as molecular (Boss and Thomas, 2000; Hofer *et al.*, 2009) and physiological (Falkenstein *et al.*, 1991) experimentation. However, cellular and histochemical papers are much less abundant in the scientific literature (Lisk, 1924; Meloche *et al.*, 2007; Bowling and Vaughn, 2009)

The most commonly studied system is tendrils of the cucurbitaceae (Dastur and Kapaida, 1931) but these have a significantly different anatomy to tendrils of vitaceae (Bowling and Vaughn, 2009) including the subject of our research; *Ampelopsis brevipedunculata* (Porcelain Berry). This difference takes the form of an uneven distribution of vascular bundles and linear/concave appearance in transverse sections rather than the circular cross section of vitaceae tendrils.

Tendrils (Figure 1A) are a common feature of the vitaceae including *A. brevipedunculata*. This vine is native to northeast Asia and has been introduced to many countries for use as a landscaping plant. After introduction to the United States in the 1870, *A. brevipedunculata* escaped cultivation, and became invasive in many regions of the United States (Young, 2005). It is a weed which climbs at the expense of desirable trees and makes these trees vulnerable to wind damage by increasing wind-drag but making no contribution to stem strength. This causes increased storm damage and occasionally tree fall. A strong and resistant tendril is an essential organ for *A. brevipedunculata's* exploiting life strategy.

Materials and Methods

Common chemical reagents were purchased from VWR International (Bridgeport, New Jersey). Phloroglucinol (JT Baker JTU024-5) was used at 2% $^{w}/_{v}$ in 33% $^{v}/_{v}$ methanol and 33% $^{v}/_{v}$ HCl to stain for lignin, the brittle component of mature secondary cell walls.

For the polychromatic stain Toluidine Blue O (TBO, O'Brian *et al.*, 2004) a solution of the solid (JT Baker JTW143-3) was dissolved in 50 mg/mL in 50 mM citric acid buffer pH 4. Environmental samples of tendrils from live material colonizing vegetation near Whale Pond Brook (West Long

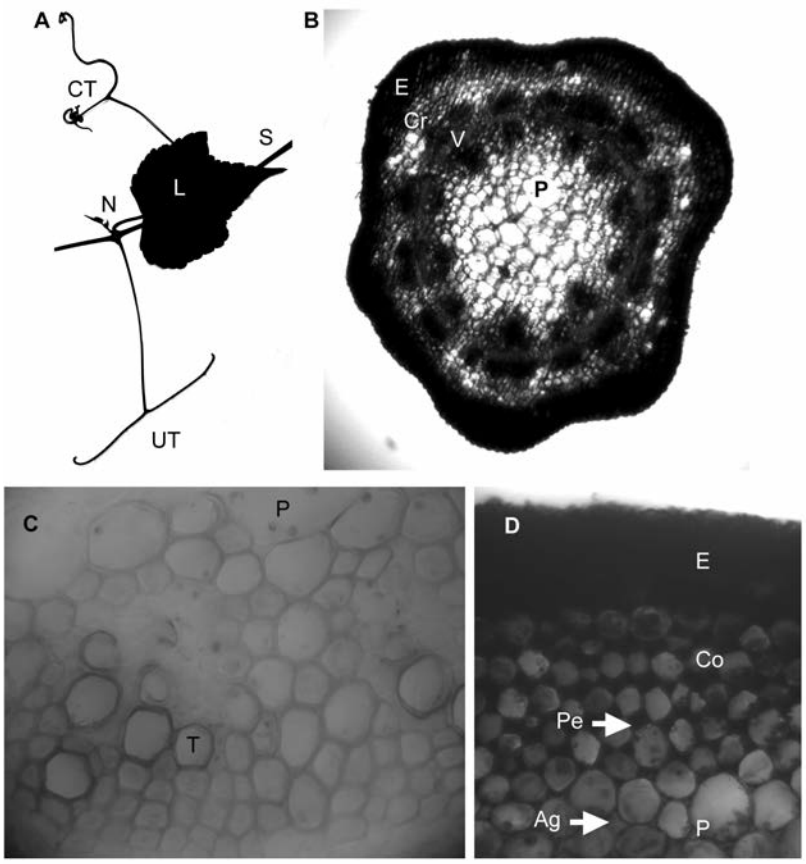

Figure 1. (A) Drawing of immature A. brevipedunculata tendrils. An untouched tendril (UT) reaching for support, coiling tendril (CT), node (N), stem (S) and leaf (L) are shown. Adapted from a photograph of the study material. (B) Immature tendril section showing even distribution of vascular bundles. The multilayered epidermis (E), vascular bundles (V), cortex (Cr) and pith (P) are shown. TBO stained transverse section x100. (C) Thin walled tracheids (T) which stain poorly for lignin. Phloroglucinol stained transverse section x400. (D) Sub epidermal collenchyma (Co) with air gaps filled by pectin (Pe) which appears a roughly rectangular and intensely stained shapes. Parenchyma air gaps are shown (Ag). TBO stained transverse section x400.

Branch, New Jersey) were harvested using a razor blade. These included uncoiled and roughly linear tendrils reaching for support, and tendrils which had coiled on a support but were still green. This live plant material was placed in a styrofoam clamp (Carrington, 2004) and sectioned by hand. The clamp and blade was lubricated with water and sections stored in water before staining. Sections approximately 50 µL were obtained.

For staining with phloroglucinol, sections were placed in a drop of water on a microscope slide and excess water was blotted away with a tissue. A drop of phloroglucinol stain was added then a cover slip applied before the material was observed under bright field using a Nikon YS2-H microscope. For TBO staining sections were placed in a drop of water on a microscope slide and approximately 2 µL stain solution added using an automatic pipette. Dilute stain was mixed using the plastic tip then removed with a tissue. Sections were repeatedly washed with a drop of water and a cover slip applied then the material was observed under bright field as above. Revealing slides were sealed with transparent varnish (Nutra Nail®) and photographed the next day.

Results

Sections of immature untouched tendrils show a multicellular epidermis and a regular arrangement of vascular bundles (Figure 1B). Phloroglucinol staining is very poor and only thin walled tracheids were seen to stain (Figure 1C). Helical thickening of primary cell walls is visible in some sections (data not shown). A conspicuous feature of young tendrils is sub-epidermal collenchyma, which was revealed by TBO staining (Figure 1D). This tissue has stained pectin in place of intercellular air gaps, gluing cells together in a strong but flexible layer.

Mature coiled tendrils which have attached to a solid support (Figure 2A) have a very different histochemistry. They react strongly with phloroglucinol to show thickened layers of cells which superficially resemble stem wood, surrounded by smaller isolated groups of lignified cells (Figure 2B). These cells have thicker walls than immature tendrils (Figure 1C and 2C) but are not water-conducting tracheids because TBO staining shows that almost all of them retain cell contents (Figure 2D). Only a small proportion of

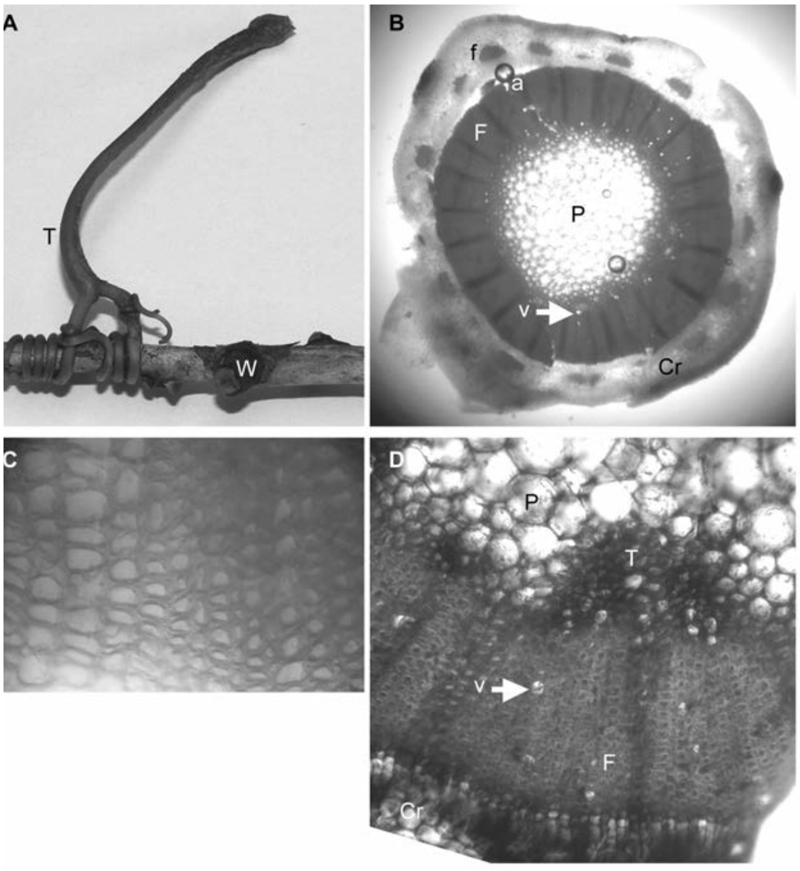

Figure 2. (A) Drawing of one mature A. brevipedunculata tendril (T) coiled around a Salix × pendulina Wenderoth (Weeping Willow) branch (W). Adapted from a photograph of the study material. (B) Mature tendril section showing peripheral bundles of fibers (f) and concentric layers of fibers (F). Occassional water conducting vessels (v) and air bubble (a) artifacts are seen. The cortex (Cr) and pith (P) are shown. Phloroglucinol stained transverse section x100. (C) Phloroglucinol stained fibers with thickened cell walls. Transverse section x 400. (D) BO stained tendril showing fibers with clearly stained cell contents (F). This organ shows occasional vessels (v). Parenchyma pith (P), tracheids (T) and cortex (Cr). Transverse section x 400.

hollow vessel elements are seen (Figure 2B and 2D).

Discussion

Conventional explanations of tendril function include an asymmetric anatomy and differential cell expansion by cells of the cortex and pith, causing curvature around a line or shallow curve of vascular bundles (Jaffe and Galston, 1968). This explanation uses plants of the cucurbitaceae as chief examples (Dastur and Kapaida, 1931). Our results show detailed anatomy of *A. brevipedunculata* tendrils, which do not support this simple view. Tendrils of this species have a symmetrical anatomy in transverse section in both immature and mature states

The supporting tissues in immature *A. brevipedunculata* tendrils are primary xylem and collenchyma which retains flexibility and allows the sweeping movements typical of tendrils (Darwin, 1911). When a support has been grasped, tendrils mature rapidly and the supporting tissue develops large amounts of inflexible fibers. These fibers are easy to confuse with tracheids but fiber cell contents are seen with TBO staining, very different to tracheids and vessel elements which must mature as hollow cells in order to conduct water. Vessel elements are seen, but are a very small proportion of mature tendril cells. Lignified organs have the advantage that they can continue functioning after cell death and tendrils which have lost all tissues except lignified fibrous material, such as epidermis and cortex, still function as adhering organs. Research on the mechanism of *A. brevipedunculata* curvature is continuing.

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