Ultrastructure of the haustorial interface and apoplastic continuum between host and the root hemiparasite *Olax phyllanthi* (Labill.) R. Br. (*Olacaceae*)

J. Kuo¹, *, J. S. Pate², and N. J. Davidson²

¹Electron Microscopy Centre and ²Department of Botany, The University of Western Australia, Nedlands, Western Australia

Received October 25, 1988
Accepted February 24, 1989

**Summary.** Structural features of haustorial interface parenchyma of the root hemiparasite *Olax phyllanthi* are described. Walls contacting host xylem are thickened non-uniformly with polysaccharides, not lignin, and show only a thin protective wall layer when abutting pits in walls of host xylem vessels or tracheids. Lateral walls of interface parenchyma exhibit an expanded middle layer of open fibrillar appearance, sometimes with, but mostly lacking adjoining layers of dense wall material. Free ribosomes and rough endoplasmic reticulum are prominent and occasional wall ingrowths present. Experiments involving transpirational feeding of the apoplastic tracers lanthanum nitrate or uranyl acetate to host roots cut below haustorial connections, indicate effective apoplastic transfer from host to parasite root via the haustorium. Deposits of the tracers suggest a major pathway for water flow through host xylem pits, across the thin protective wall layer, and thence into the haustorium via the electron-opaque regions of the terminal and lateral walls of the contact parenchyma. Graniferous tracheary elements and walls of parenchyma cells of the body of the haustorium appear to participate in tracer flow as do walls of cortical cells, stele parenchyma and xylem conducting elements of the parasite root, suggesting that both vascular and non-vascular routes are involved in extracytoplasmic transfer of xylem sap from host to parasite. The Casparian strip of the endodermis and the suberin lamella of the exodermis of the *Olax* root act as barriers to flow within the system.

**Keywords:** Root hemiparasite; *Olax*; Host-parasite interface ultrastructure; A poplastic tracers; Transport pathway; Water: solute uptake.

**Introduction**

The ultrastructure of haustoria of phanerogamic parasites has been well documented for a number of genera, notably *Arceuthobium* (Srivastava and Esau 1969, Alosi and Calvin 1985, Sadik et al. 1986), *Viscum* (Smith and Gledhill 1983), *Orobanche* (Dörr and Kollmann 1975), *Castilleja* (Dobbin and Kuijt 1973), *Phthirusa* (Dobbin and Kuijt 1974 a, b), *Boschniakia* (Kuijt and Toth 1985), *Striga* (Mallaburn and Stewart 1987), *Cuscuta* (Dörr 1987) *Korthalsella* (Coetzee and Fineran 1987, Fineran 1987), and a number of root hemiparasites belonging to the *Loranthaceae*, *Santalaceae*, and *Olacaceae* (see Fineran 1985, 1987; Fineran et al. 1987). Members of the latter three families are unusual in possessing graniferous tracheary elements in their haustoria, and the possible function of these highly distinctive cells has been recently reviewed (Fineran 1985). The above studies have generally reported that the vast majority of cellular contacts achieved by angiosperm parasites at their haustorial interfaces with host xylem involve parenchyma as opposed to terminating tracheids or vessels members (e.g., see Kuijt 1964, 1977; Kuijt and Toth 1976; Lamont 1983), implying that direct flow between partners via vessels and tracheary elements may be of limited magnitude. These findings prompted the present tracer investigation into possible apoplastic pathways for haustorial transfer of xylem derived water and nutrients from host to parasite root in *O. phyllanthi*. The study follows upon the elegant demonstration by Coetzee and Fineran (1987) of an apoplastic continuum at the haustorial junction between the dwarf mistletoe *Korthalsella lindsayi* and its host.

**Materials and methods**

Mature haustoria of *O. phyllanthi* were collected from a range of common host species in coastal heath near Albany and Denmark, SW Australia, in spring (September/October 1987 and 1988). Following fixation in 2.5% glutaraldehyde (0.025 M phosphate buffer,
pH 7.0), haustorial tissue for light microscopy was dehydrated and embedded in glycol methacrylate (see O'Brien and McCully 1981). Blocks were sectioned at 2 μm thickness with glass knives, using a Sorvall microtome with block orientations in planes transverse to a host root (longitudinally through the attached haustorium) or transverse to parasite fine roots bearing haustoria. Sections were subjected to any one of the following staining procedures: (1) 0.05% toluidine blue O (G. T. Gurr, London, U.K.) pH 4.2 for phenol and pectin; (2) 1% amido black 10 B (G. T. Gurr) in 7% acetic acid for protein; (3) saturated Sudan black (B. D. H. Poole, U.K.) in 70% ethanol for cutin and lipid; (4) periodic acid-Schiff (PAS) reagent for polysaccharides; (5) PAS counterstained with toluidine blue, amido black or Sudan black. Unstained sections were mounted in paraffin oil for studies of autofluorescence using a Carl Zeiss epifluorescence microscope.

Glutaraldehyde-fixed tissue was prepared for transmission electron microscopy by postfixation in 1% OsO₄ in 0.025 M phosphate (pH 7.0) at room temperature for two hours, dehydration in an acetone series, followed by embedding in Spurr’s resin (Spurr 1969). Sectioning was on a Reichert Ultracut microtome using glass or diamond knives. Sections were stained in 1% aqueous uranyl acetate followed by lead citrate (Reynolds 1963), and examined and photographed using a Siemens 102 or Philips 410 electron microscope at 80 kV.

Scanning electron microscopy of glutaraldehyde-fixed material involved dehydration in graded acetone, drying in a critical point dryer and examination in a Philips 505 Scanning Electron Microscope.

Feeding of apoplastic tracers to host: parasite partnership was conducted in situ in the field in coastal sand heath at Denmark, W. Australia. Putative hosts growing on steeply sloping ground were selected at dawn (October 1987) and excavations made down slope under the main roots of the host at 30-40 cm depth. Each time a parasitized root was encountered, it was very carefully exposed in such a manner as to retain intact the whole root and shoot of the host plant and any parts of the root system of the parasite connecting the exposed haustorium or haustoria to the parent shoot of Olax. At some time between 9.00 and 10.30 h host roots were cut 10 cm below each attached haustorium (Fig. 1), and again immediately in a vial of water 8 cm below the haustorium to prevent embolisms in the host xylem. Once measurable transpirational uptake of water from the vial was observed (10.30-11.30 h), the cut end of the shoot was transferred for 4 h to either 1% aqueous uranyl acetate or 2.5% buffered glutaraldehyde for 2-4 h, and subsequently dehydrated in an acetone series and embedded in Spurr’s resin, with or without postfixation in OsO₄. Thin sections were then examined for electron dense deposits of lanthanum or uranium at high and low resolution transmission electron microscopy. A total of seven host: parasite haustorial junctions were labelled in the above manner, the host involved being Acacia littorea (Mimosaceae), Scaveola nitida (Goodeniaceae), and Bossiaea linophylla (Fabaceae).

Results

General anatomy of haustorium and fine roots of O. phyllanthi

The haustorium of Olax is a relatively simple structure developed laterally on fine roots of the extensive root system of the parasite (Fig. 1). Attachment to the host root surface is by means of an adhesive appressorial disc. A penetrating sucker (Fig. 2) develops secondarily from the center of this disc and, on reaching the cambium, expands laterally around part of the host stele (Fig. 2). Each mature haustorium exhibits a continuous system of tracheids extending from the interface with host xylem, through the sucker into a core of graniferous tracheary elements (see Fineran et al. 1987) and thence, via an interrupted nongraniferous zone of xylem elements, to the stele of the parent parasite root (Fig. 2). There is no evidence of a phloem connection with the hosts. Fine roots of the parasite bearing haustoria (Figs. 3 and 5) show a well demarcated stele and cortex, cortical deposits of starch, well differentiated sieve elements and xylem elements, an endodermis with well suberized Casparian thickenings (Fig. 4) and an exodermis (Fig. 5, Ex) with strongly suberized walls (Fig. 5) immediately underlying a collapsed epidermal layer.

Fig. 1. Diagrammatic representation of experimental procedure for feeding electron dense tracer solutions to host plants parasitized by Olax phyllanthi. HR Host root, H haustorium, AP appressorial disc of haustorium, PR principle lateral, PR₁ fine lateral root of parasite bearing a haustorium

Figs. 2-5. Materials embedded in glycol methacrylate and sections stained in PAS/toluidine blue

Fig. 2. Transverse section of root of Scaveola nitida at midpoint of attachment of a haustorium of O. phyllanthi. HS host stele, S sucker (endophytic tissues) of haustorium, CL collapsed layer, VC core of tracheary elements in center of sucker, J interrupted zone of vascular tissue connecting VC to the parent root (PR) of the parasite. Scale marker = 5 mm.

Fig. 3. Transverse section of fine root of O. phyllanthi (see Fig. 1, PR₁) showing central stele (St) surrounded by several layers of cortical cells (Ct) with prominent starch reserves. Scale marker = 5 mm.

Fig. 4. Detailed anatomy of outer part of stele of root shown in Fig. 3. Note endodermis (En) with Casparian bands ( ), starch (Sh) in cortex, sieve elements (S), and xylem elements (X) in vascular tissue. Scale marker = 500 μm.

Fig. 5. Outer region of root shown in Fig. 3 to show suberin impregnated exodermis ( Ex) external to cortex (Ct) and immediately inside a collapsed epidermis (Ep). Scale marker = 500 μm.