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Transfer Cells and Nematode Induced Giant Cells in *Helianthemum*

M. G. K. Jones and B. E. S. Gunning

Department of Developmental Biology, Research School of Biological Sciences, Australian National University, Canberra A.C.T., Australia

With 9 Figures

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Summary

The morphology of wall ingrowths in xylem and phloem transfer cells in *Helianthemum* is different. It is possible to use nematode infection to induce the formation of giant cells which abut both xylem and phloem elements to test whether ingrowth morphology is controlled by the solutes presumed to be transported across the plasmalemma of the cells. This experiment has been done and it is found that although wall ingrowths develop against both xylem and phloem, the giant cells exhibit only the ingrowth structure characteristic of xylem transfer cells.

1. Introduction

The specificity with which plants can shape the wall ingrowths of their transfer cells implies that relatively precise genetic programmes are expressed during transfer cell development. Rock rose (*Helianthemum* spp.) and Japanese millet (*Echinochloa utilis*) are the only plants in which it is indicated that two such genetic programmes are present: in both plants the wall ingrowths in transfer cells in different anatomical situations of the same plant have different morphologies (Gunning, Pate, and Green 1970, Zee and O'Brien 1971). In minor veins of *Helianthemum* leaves the wall ingrowths in companion cells ("A" type transfer cells) are flat plates or flanges, whereas in xylem parenchyma cells of the node they are anastomosing fingerlike projections. In *Echinochloa*, ingrowths in xylem transfer cells in the vascular tissue supplying the pedicel of the spikelet differ from those of the aleurone transfer cells.

Since the flange type of ingrowth seen in *Helianthemum* phloem tissue is widespread in xylem transfer cells in *Gramineae* and certain Dicotyledons while the fingerlike type seen in *Helianthemum* xylem tissue is common in

phloem (GUNNING and PATE 1974), it is clear that in seeking causal factors that might determine ingrowth morphology we must look elsewhere than a simple anatomical association between xylem and one type and between phloem and the other. The control of morphology might, however, be related to the solutes presumed to be transported by the cells. It is reasonable to suggest that solutes transported by xylem transfer cells in nodes and phloem transfer cells in leaves will be different.

The giant transfer cells induced in roots by root-knot nematodes (*Meloidogyne* spp.) (JONES and NORTHCOTE 1972) provide a convenient and anatomically simple situation in which to examinate the control of wall ingrowth morphology. Since one giant cell may abut both xylem and sieve elements, root-knot nematodes may be used as a tool to induce giant cell formation and hence to investigate whether ingrowth morphology is specific to a particular class of cell-to-cell junction, and to the solutes transported across it.

2. Materials and Methods

Cuttings of various varieties of *Helianthemum* were taken from gardens in Canberra and propagated in a greenhouse. The plants were then grown in soil infested with larvae of the root-knot nematode, *Meloidogyne javanica*, to select for varieties which were susceptible to this nematode. The nematode inoculum was kindly supplied by Dr. A. F. BIRD, C.S.I.R.O., Adelaide. Susceptible plants were characterised by the presence of galls on the roots, and three to four weeks after infection gelatinous sacs containing eggs were visible on the surface of galls, thus indicating that the nematodes had successfully completed their life cycle.

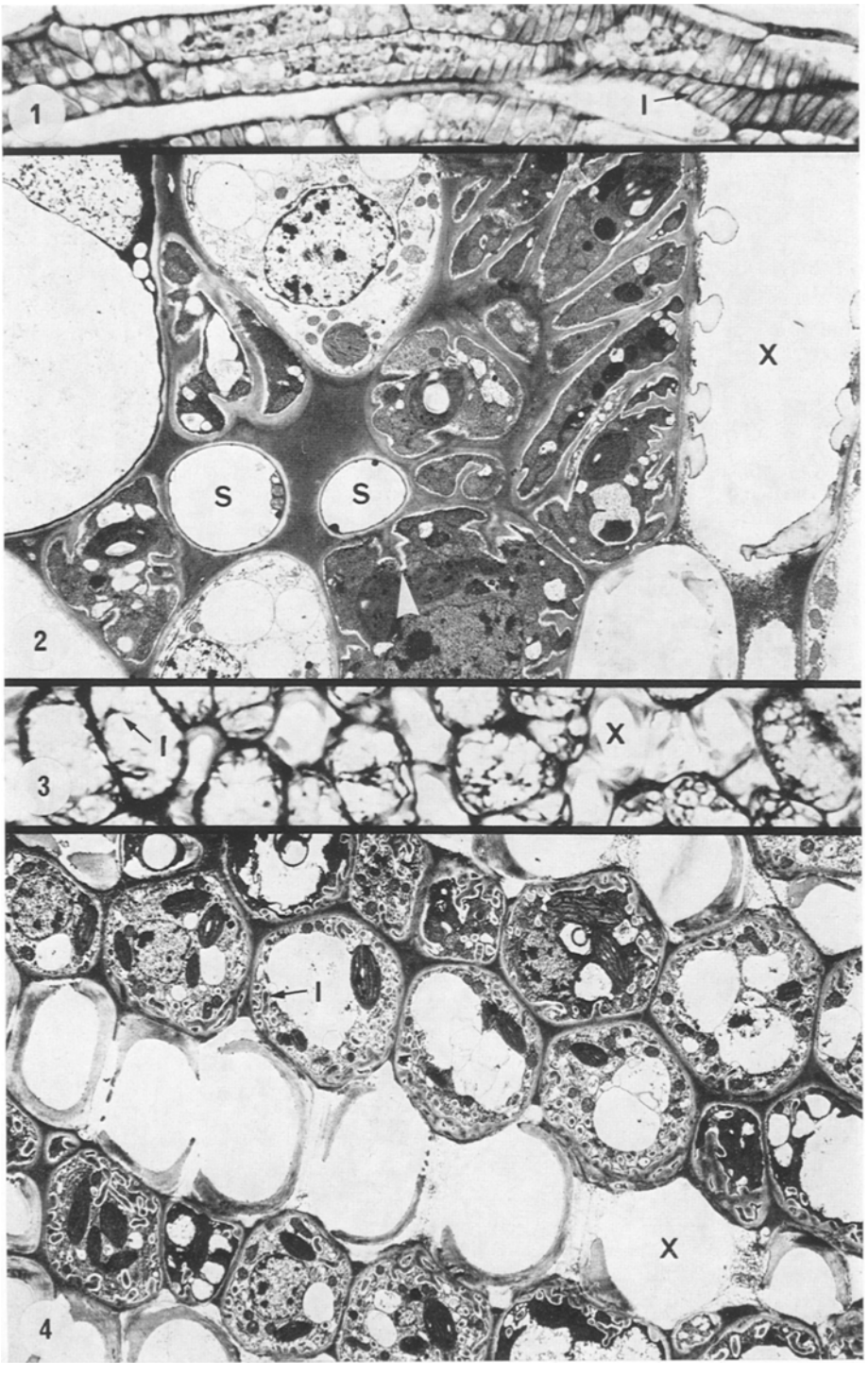
Samples of tissue from minor leaf veins, stem nodes and root galls, were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 7.2 followed by 2% osmium tetroxide in the same buffer, dehydrated in an acetone series, and embedded in Spurr's resin. Sections were cut, stained in 10% uranyl acetate in 50% ethanol, then Reynold's lead citrate, and examined in a JEOL JEM 100B electron microscope at 60 kV. For light microscopy, tissues were fixed in glutaraldehyde and embedded in glycol methacrylate (FEDER and O'BRIEN 1968).

Fig. 1. Light micrograph of a minor leaf vein. The wall ingrowths (*I*) are roughly perpendicular to the long axis of the transfer cells. ×1,200

Fig. 2. Electron micrograph of a minor leaf vein. The transfer cells are modified companion cells. They have dense cytoplasm, in contrast to that of the phloem parenchyma cells. The reticulate pattern of wall ingrowths is evident in the transfer cell which abuts the xylem element (*X*) and these contrast with the lignified secondary thickenings of the xylem element. In cross section the ingrowths may be indented, as indicated by the unlabelled arrow next to the sieve element (*S*). ×4,285

Fig. 3. Light micrograph of transfer cells of a node. Wall ingrowths (*I*) are evident in the transfer cells next to xylem elements (*X*). ×1,450

Fig. 4. The nodal transfer cells occur in tiers between rows of lignified xylem elements (*X*). Although the density of the cytoplasm varies from cell to cell, wall ingrowth (*I*) development is relatively uniform. ×3,555



Figs. 1–4

3. Results

In light microscope sections the wall ingrowths of transfer cells of minor leaf veins form a scalariform pattern with successive flanges oriented at a fairly constant oblique angle to the long axis of the cell (Fig. 1). At the ultrastructural level it is evident that the transfer cells are modified companion cells (Fig. 2). In glancing sections through the ingrowths "Y" shaped junctions between adjacent flanges occur frequently. In cross section, the plates do not appear to have such a smooth outline as in the light microscope, as the flanges are often indented (Fig. 2). Circular cross-sections of ingrowths are not observed in these sections. Although the pattern of flanges resembles the thickenings of differentiating xylem elements, a comment also made by Zee and O'Brien (1971) in respect of the transfer cells of the wheat spikelet, they can be distinguished by their lack of lignification. This difference is clearly seen in Fig. 2, where the companion cells with wall ingrowths may back onto xylem elements. The cytoplasm of the phloem transfer cells is much more electron dense than that of the phloem parenchyma cells; both cell types contain chloroplasts. There are many plasmodesmata between adjacent phloem transfer cells, and typical branched plasmodesmata between them and the sieve elements.

The nodal transfer cells are modified xylem parenchyma cells, and occur in tiers between xylem elements (Figs. 3 and 4). The wall ingrowths are finger-like, and circular cross-sections of ingrowths are frequent (Fig. 5). The transfer cells are inter-connected by frequent plasmodesmata, many of which contain median nodules and a number of branches. In the regions where these occur ingrowth development is reduced (Fig. 5). Within a tier of transfer cells, the cytoplasm of a few of the cells is more electron dense than the others, but this does not seem to affect ingrowth formation (Fig. 4). A few chloroplasts are present in the cells (Figs. 4 and 5).

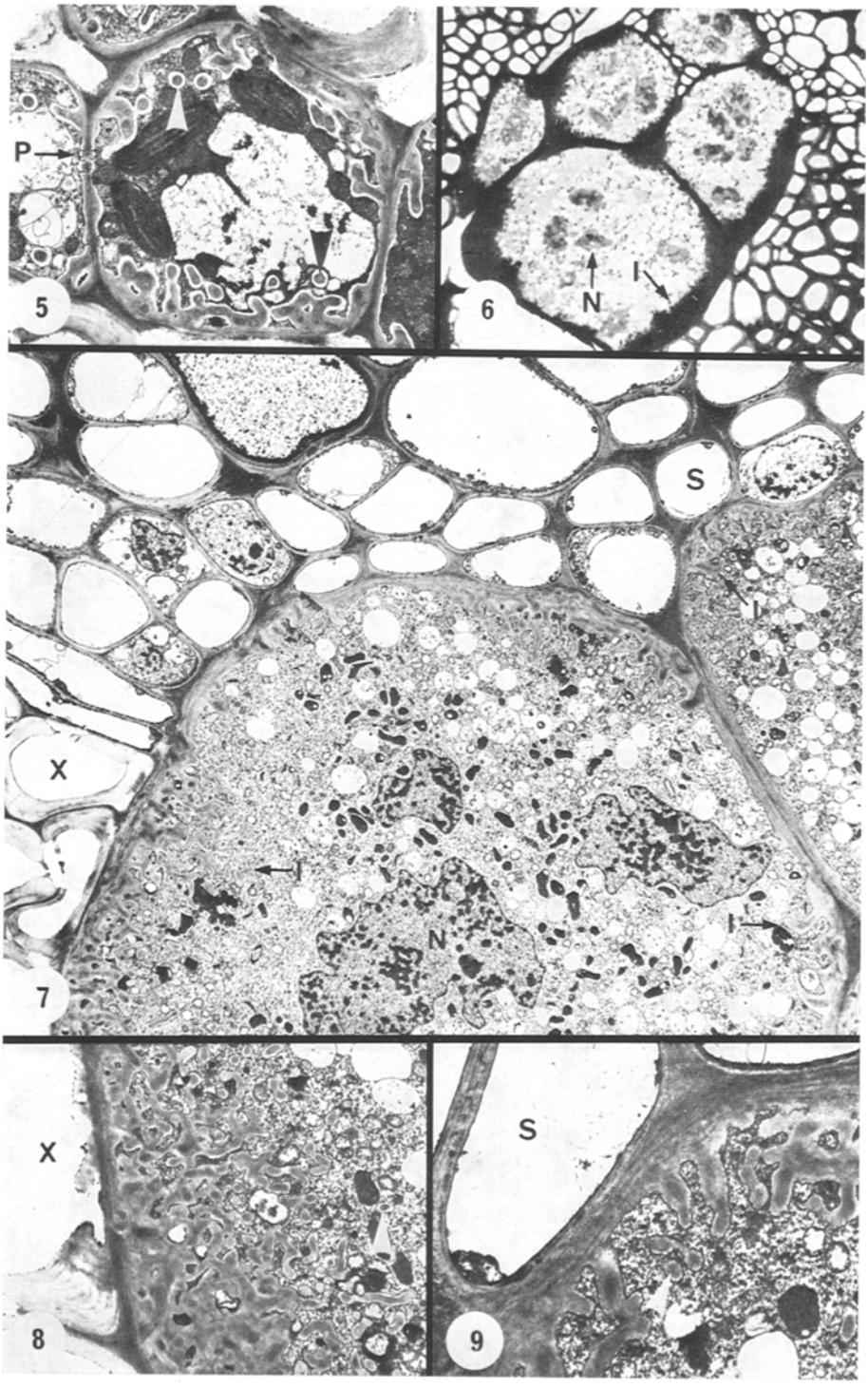
Fig. 5. Wall ingrowths of nodal transfer cells are finger-like, and frequently circular cross-section profiles are seen (unlabelled arrows). Plasmodesmata (*P*) are common between the tiers of transfer cells. ×5,490

Fig. 6. Light micrograph of giant cells in a root. The cells are filled with cytoplasm and small vacuoles, and are multinucleate (*N*). Ingrowths (*I*) occur on outer giant cell walls, and on walls between giant cells. ×400

Fig. 7. Part of giant cells which abut both xylem (*X*) and sieve elements (*S*). Wall ingrowths (*I*) are present at both of these locations and on walls between neighbouring giant cells. The large heterochromatic nuclei (*N*) are prominent. ×2,875

Fig. 8. Wall ingrowths in a giant cell next to a xylem element (*X*). Circular profiles of ingrowths (unlabelled arrow) are evident. ×4,665

Fig. 9. Wall ingrowths in a giant cell next to a sieve element (*S*). Circular profiles of ingrowths (unlabelled arrow) are evident. Ingrowths are not so extensive as next to xylem elements (Fig. 8). ×6,305



Figs. 5–9

In the roots, ingrowths are present on walls between neighbouring giant cells, and on giant cell walls which abut xylem and sieve elements (Figs. 6 and 7). In each case, the form of the ingrowths is the same as that of the xylem transfer cells of the node. The ingrowths are finger-like, and circular cross-sections are observed next to xylem (Fig. 8) and sieve elements (Fig. 9) and between neighbouring giant cells (Fig. 7). Typically in giant cells ingrowth development is more extensive on walls next to xylem elements and locally on walls between giant cells than it is on walls adjacent to sieve elements. As in other plants (Jones and Northcote 1972, Jones and Dropkin 1975), giant cells are multinucleate, and the cytoplasm is filled with small vacuoles, mitochondria and other organelles (Figs. 6 and 7).

4. Discussion

The morphology of ingrowths in transfer cells is within limits, species specific, so their formation involves the expression of a morphogenetic programme. In both *Helianthemum* and *Echinochloa* the genome evidently carries the information for more than one form of wall ingrowth. The failure of an individual giant cell to form ingrowths with the two different morphologies in *Helianthemum* suggests either that ingrowth morphology in xylem and phloem transfer cells is not controlled simply by the different solutes presumed to be crossing the plasma membrane where ingrowths form, or that one cell may only be able to express one of the two morphogenetic programmes at a time.

We may postulate that the number of carrier sites per unit area of plasmalemma for a given solute has a limiting value. Once this value has been reached at a particular location within the cell, one way to increase solute transport at that site is to increase the surface area of the plasmalemma. In transfer cells this increase in surface area is achieved by the formation of wall ingrowths. In addition to ensuring that the amplification in plasmalemma area is stable, the ingrowths, which are probably relatively porous, increase the local volume of the apoplast, and may provide geometries especially favourable for solute transport processes (Gunning and Pate 1974). The significance of the different forms of ingrowth in *Helianthemum* is puzzling. The finger-like ingrowths potentially allow the greater amplification in surface area of the plasmalemma, and are possibly more flexible and specific with regard to location in the cell than the flange-type ingrowths.

If it is not the solutes themselves which have saturated carrier sites at the plasmalemma and thus through a feedback system initiate the deposition of ingrowths, then wall ingrowth formation may require both saturated carrier sites at the plasmalemma plus particular levels of specific compounds within the cell. For example it is unlikely that the xylem transfer cells will have as high a content of free sugars as the phloem transfer cells, which are pre-

sumably mainly involved with collecting sucrose from the apoplast and loading it into the sieve tubes (GUNNING, PATE, MINCHIN, and MARKS 1974). Thus in *Helianthemum* high levels of sugars within the cytoplasm, coupled with saturated carrier sites at the plasmalemma, might be required to induce plate-like ingrowth formation, whereas similar saturated carrier sites at the plasmalemma coupled with higher levels of other metabolites such as amino acids might be required to induce the formation of finger-like ingrowths. Amino acid levels in giant cells are relatively high, but as a result of the intense metabolic activity of giant cells, the levels of free sugars may be reduced within the cytoplasm (OWENS and SPECHT 1966). We assume that sugars are the predominant solutes transported across the plasmalemma where wall ingrowths form in giant cells next to sieve elements. Here we have a situation where although the carriers for sugar may be saturated the level of sugars in the cytoplasm is nevertheless low while by contrast that of amino acids is high. In this way the xylem transfer cell-like morphology of the wall ingrowths in the giant cells can be rationalised, despite the anatomical and functional relationships that exist with the sieve elements.

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Authors' address: Dr. M. G. K. JONES, Department of Developmental Biology, Research School of Biological Sciences, Australian National University, Canberra A.C.T. 2601, Australia.

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