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Xylem vessel regeneration in carnation in response to infection by Fusarium oxysporum f.sp. dianthi

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Among numerous structural changes which occur in a host in response to infection by vascular wilt fungi, new xylem vessels can be formed to replace those which have been damaged by the fungus or blocked by gels or tyloses. This was documented in the 1950's for wilt disease of hop (Talboys, 1958) and oak (Schoeneweiss, 1959).

In the course of a comparative light-microscopic investigation of infection by *Fusarium oxysporum* Schlect. f.sp. *dianthi* (Prill. et Del.) Snyd & Hans. in two carnation cultivars (Harling, 1983), we also noticed the production of new xylem but its mode of development was unlike that of hop and oak and has not been previously reported in response to a wilt disease. We are reporting it here for this reason although formation of new xylem was one of a range of structural responses concerned with resistance to infection in carnation which will be described in detail elsewhere.

Rooted carnation cuttings (5 wk old) of the susceptible cv. Red Baron and the resistant cv. Carrier 929 were potted into John Innes No. 1 compost and established for 3 wk prior to inoculation in a glasshouse at a mean temperature of 22 °C (range 19-24 °C). Cuttings were inoculated with a spore suspension of *F. oxysporum* f.sp. *dianthi,* race 2, produced in shake culture and adjusted with water to contain 2 x 10^6 spores ml^{-1}. Inoculation was made directly into the stem. A reservoir made from a piece of plastic tubing was attached to the stem between leaf pairs 7 and 8 (counting from soil level upwards) then filled with inoculum (ca 1.5 ml). Using a scalpel blade, the stem was wounded to penetrate the xylem below the surface of the inoculum which was subsequently taken up into the xylem. Control plants were treated similarly using sterile distilled water.

Following inoculation, plants were maintained in a glasshouse at a mean temperature of 22 °C (range 17-29 °C). Up to 24 days after inoculation, three inoculated plants and one control from each of the two cultivars were sampled at intervals and stem tissue excised and processed for light microscopy. Stem tissue was excised from the internode immediately above that which received the inoculation wound, in order to avoid tissue with wound reactions (Moreau et al., 1978). After fixing and dehydrating, tissue was embedded in glycol methacrylate, sectioned at 1-3 μm and sections stained in toluidine blue (Harling, 1983).

Stems of control plants showed no wound response at the height sampled; typical control stem tissues are shown in transverse section in Fig. 1A. In the inoculated resis-

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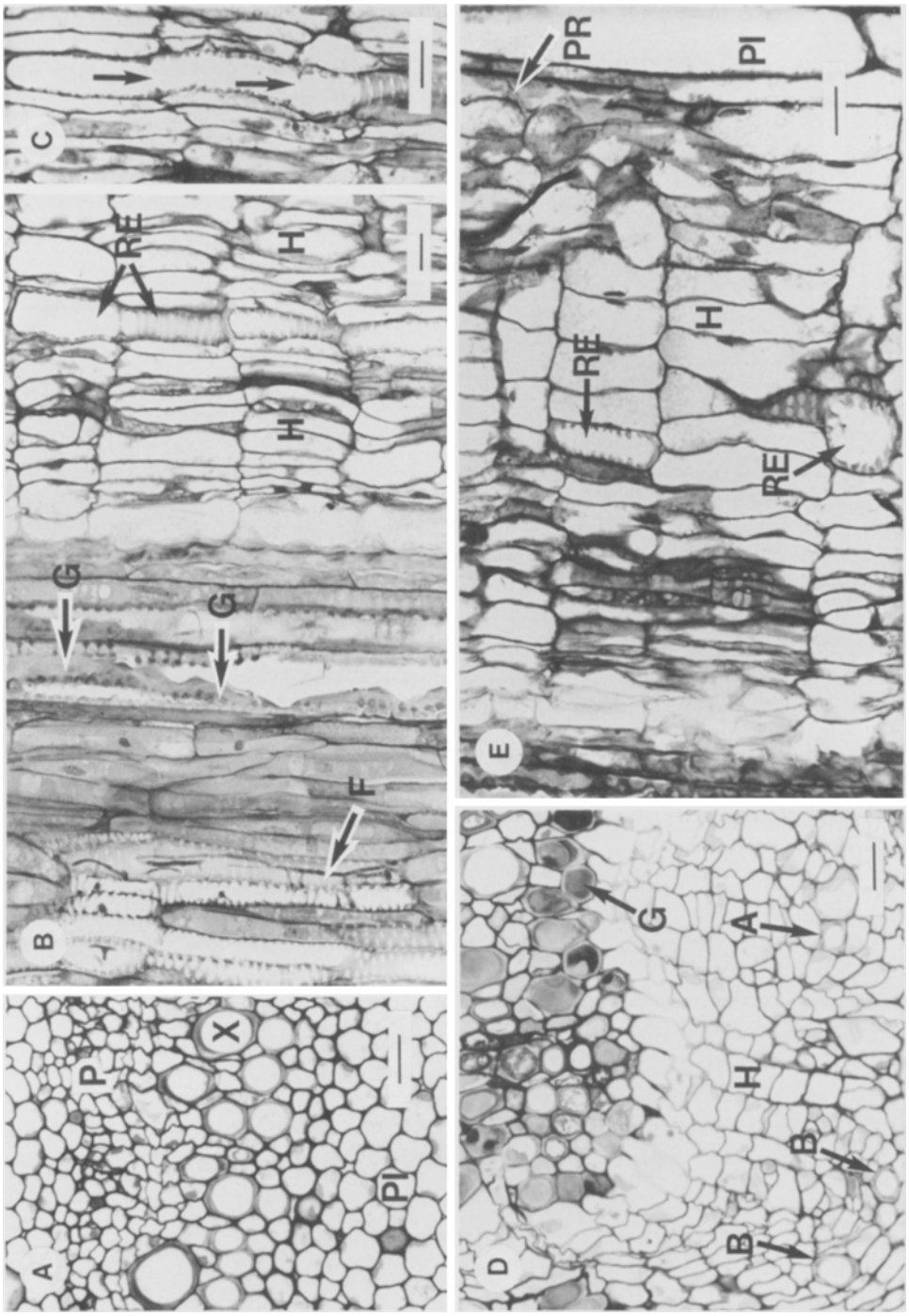
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Fig. 1. Transverse (TS) and radial longitudinal sections (LS) of carnation stems, cv. Carrier 929. Bars = 20 μm.
A. TS of vascular tissue from control stem. P = phloem; X = xylem; PI = pith.
B. LS at 20 days after inoculation with *F. oxysporum* f.sp. *dianthi* showing xylem element regeneration from hyperplastic xylem parenchyma. In the middle of a mass of hyperplastic xylem parenchyma (H, occupying the right half of the photograph), a single column of these cells has differentiated into elements (RE). These elements run parallel to existing xylem vessels which are occluded by gels (G) or invaded by fungal hyphae (F).
C. LS at 20 days after inoculation, showing vessel formation by dissolution of end walls (arrows) in regenerated xylem elements.
D. TS at 20 days after inoculation, showing hyperplastic xylem parenchyma (H) containing a possible regenerated xylem element (arrow A), and elements (arrows B) that may be regenerated or have become isolated from existing primary xylem by the hyperplastic tissue. Many of the existing xylem vessels are occluded by gels (G).
E. LS at 20 days after inoculation, showing location of regenerated xylem elements (RE). The position of the protoxylem (indicated by the spiral thickenings, PR) confirms differentiation of xylem parenchyma (H) not pith cells (PI).

tant cv. Carrier 929, hyperplasia of the xylem parenchyma was a frequently-observed response to infection, first noticed in plants sampled 10 days after inoculation. Hyperplasia is commonly seen in other hosts infected with vascular wilt fungi (Beckman, 1964). Wedges of hyperplastic tissue adjacent to infected vessels continued to increase in size and became quite extensive by 20 days after inoculation. Longitudinal sections through these regions of hyperplastic xylem parenchyma at 20 days showed scattered single columns of parenchyma cells which had differentiated into vessel elements (Fig. 1B). In some, dissolution of the end walls had occurred to create vessels (Fig. 1C).

Since regenerated elements were first observed in the longitudinal sections, the transverse sections which had already been cut were studied again to see if they were visible in similar areas. Fig. 1D indicates what is probably one of these regenerated xylem elements in transverse section as judged by its size and shape, and position in the file of hyperplastic cells. However, the evidence for regeneration in transverse sections is less clear because of possible confusion with previously differentiated elements separated from the rest of the primary xylem by hyperplasia of adjoining parenchyma cells (Fig. 1D). Regenerated vessels ran parallel to existing vessels that were invaded by fungus or occluded by gels (Fig. 1B). Regeneration was not seen in the susceptible cv. Red Baron.

Regeneration of vascular tissue can be readily induced in several dicotyledonous species by mechanically wounding the stems or roots, for example in *Coleus* (Sinnott and Bloch, 1945) and *Pisum* (Robbertse and McCully, 1979). A wound which severs the vascular tissue in the stem or root will cause a differentiation of the parenchyma cells of the cortex or pith to form new vascular tissue to bridge the gap.

We believe that xylem vessel regeneration in infected carnation is unique for three reasons. Firstly, the stimulus for differentiation has arisen from infection and not wounding as in the examples above; control stems sectioned at the same height showed no response to the inoculation wound. Blockage of the xylem through vessel gels and destruction by the fungus seems to have the same effect as mechanical severance.

Secondly, regeneration occurred from hyperplastic *xylem parenchyma* as opposed to cortex or pith cells which usually undergo differentiation in other species, e.g. *Coleus*. Fig. 1E shows that hyperplasia and regeneration have taken place within the xylem and not the pith because of the position of the protoxylem. Finally, production of new xylem has arisen not from the vascular cambium but by actual *regeneration* from parenchyma cells, rather than merely by renewed or continued activity of the vascular cambium as occurs in wilt diseases of hop (Talboys, 1958) and oak (Schoeneweiss, 1959) and thus, developmentally, the new xylem in carnation is fundamentally different to the new xylem in these species.

Regenerated xylem in the cv. Carrier 929 may create an alternative pathway of water transport to compensate for that lost by occlusion or destruction due to disease. Thus it may contribute to the resistance of this cv. against *Fusarium* wilt in a similar way to the production of extra xylem by the cambium in hop and oak except that, in carnation, the extra xylem is produced in a different manner.

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Samenvatting

Xyleemvatregeneratie bij anjers als reactie op Fusarium verwelkingsziekte

Vatbare en resistente anjerstekken werden via een stengelwond geïnoculeerd met *Fusarium oxysporum* f.sp. *dianthi*. Als reactie op de infectie werd in stengels van resistente anjers hyperplastisch xyleemparenchym gevormd waarin door regeneratie nieuwe xyleemvaten ontstonden. De regeneratie vond evenwijdig aan bestaande xyleemvaten plaats, die verstopt waren met gels of beschadigd waren door de schimmel.

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