Phytoprotection

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L'histopathologie de la fusariose du vinaigrier (*Rhus typhina*) causée par le *Fusarium oxysporum* f. sp. *callistephi* race 3. III. Réactions cellulaires et tissulaires chez l'hôte

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Résumé de l'article

Diverses réactions cellulaires ont été observées chez des plants de vinaigrier inoculés par le *Fusarium oxysporum* f. sp. *callistephi*. Ces effets ont été notés au moyen de la microscopie photonique et électronique à transmission et par des tests cytochimiques. Ainsi, on a observé la formation de nombreux canaux de latex dans le liber et de thylles dans les éléments de vaisseaux, des épaississements ou autres modifications des parois cellulaires, des cloisons adventives dans certaines cellules et de l'hyperplasie et de l'hypertrophie cellulaires dans certains tissus. Les parois des thylles se sont marquées pour la pectine et la cellulose, sauf les couches lucides apparemment subérisées présentes dans plusieurs de ces thylles. Ces couches étaient également présentes dans les cellules de la couche médullaire ainsi que dans plusieurs cellules de parenchyme et même des fibres. Des barrières de protection se sont formées et elles étaient composées de cellules de parenchyme avec des parois épaissies et à contenu de forte densité et de fibres avec des parois additionnelles dites gélatineuses. Des altérations pariétales prononcées, reliées à de la matière opaque, se trouvaient dans ces cellules ou dans les parois avoisinantes. Plusieurs particules interreliées occupaient certaines cellules infectées pourvues de seulement quelques traces de cytoplasme altéré. Tel qu'observée en microscopie photonique, une zone de prolifération de cellules plus ou moins bien différenciées était visible à la marge extérieure du xylème. Du côté de la moelle et de la couche médullaire, une multiplication accrue de cellules provenant de ces tissus a également été notée. Ces masses tissulaires inusitées avaient découpé le xylème, formant avec celui-ci un genre de muraille et émergeant en monticules sur la tige.

Histopathology of Fusarium wilt of staghorn sumac (*Rhus typhina*) caused by *Fusarium oxysporum* f. sp. *callistephi* race 3. III. Host cell and tissue reactions

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Various cell reactions occurred in staghorn sumac plants inoculated with *Fusarium oxysporum* f. sp. *callistephi*. Light and transmission electron microscopy observations and results of cytochemical tests showed: 1) increased laticifers and latex production in the phloem; 2) tylosis formation; 3) host cell wall modifications, including appositions or other cell wall thickenings; and 4) unusual cross wall formation in some cells, and cell hypertrophy and hyperplasia. Tylosis walls labelled for pectin and cellulose and many displayed inner suberin-like layers. These layers were also noted in cells of the medullary sheath and in many cells with dense content and thickened walls in the barrier zones that had formed. These zones also contained fibres with newly-formed gelatinous-like layers. In the vicinity of these cells, host cell walls were frequently altered, associated with opaque matter. Many small particles present in chains also occurred in some of these cells, which contained only remnants of host cytoplasm. Light microscopy observations showed that pronounced tissue proliferation and aberrant cells occurred in the outer xylem in the infected plants. Unusual neoplasmic tissue also formed from cells surrounding the pith and medullary sheath, and it spanned directly across the pre-existing xylem tissue and burst as large mounds on the stems.

Keywords: Barrier zone formation, host cell wall modifications, labelling for cellulose and pectin, neoplastic tissue, suberization, tylosis formation.

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Diverses réactions cellulaires ont été observées chez des plants de vinaigrier inoculés par le *Fusarium oxysporum* f. sp. *callistephi*. Ces effets ont été notés au moyen de la microscopie photonique et électronique à transmission et par des tests cytochimiques. Ainsi, on a observé la formation de nombreux canaux de latex dans le liber et de thylles dans les éléments de vaisseaux, des épaississements ou autres modifications des parois cellulaires, des cloisons adventives dans certaines cellules et de l'hyperplasie et de l'hypertrophie cellulaires dans certains tissus. Les parois des thylles se sont marquées pour la pectine et la cellulose, sauf les couches lucides apparemment subérisées présentes dans plusieurs de ces thylles. Ces couches étaient également présentes dans les cellules de la couche médullaire ainsi que dans plusieurs cellules de parenchyme et même des fibres. Des barrières de protection se sont formées et elles étaient composées de cellules de parenchyme avec des parois épaissies et à contenu de forte densité et de fibres avec des parois additionnelles dites gélatineuses. Des altérations pariétales prononcées, reliées à de la matière opaque, se trouvaient dans ces cellules ou dans les parois avoisinantes. Plusieurs particules interreliées occupaient certaines cellules infectées pourvues de seulement quelques traces de cytoplasme altéré. Tel qu'observée en microscopie photonique, une zone de prolifération de cellules plus ou moins bien différenciées était visible à la marge extérieure du xylème. Du côté de la moelle et de la couche médullaire, une multiplication accrue de cellules provenant de ces tissus a également été notée. Ces masses tissulaires inusitées avaient découpé le xylème, formant avec celui-ci un genre de muraille et émergeant en monticules sur la tige.

Mots clés : Barrières de protection, formation de thylles, marquage pour cellulose et pectine, modifications pariétales, subérinisation, tissus néoplasmiques.

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INTRODUCTION

A disease affecting staghorn sumac (*Rhus typhina* L.), caused by *Fusarium oxysporum* f. sp. *dianthi* (Prillieux & Delacroix) Snyder & Hansen, was first observed in the region of Québec (QC, Canada) in 1993. Observations of aspects concerning the mode of plant colonization by the pathogen, and brief mentions of possible cell reactions were reported in Ouellette *et al.* (2005a, b). Host cell walls and content were altered in manners similar to those observed in the other diseases studied (Baayen *et al.* 1996; Ouellette and Rioux 1992, 1993; Rioux and Biggs 1994; Rioux and Ouellette 1991). As mentioned in these works, barrier zones are formed, compartmenting areas of the invaded xylem in elm trees and non-hosts inoculated with *Ophiostoma novo-ulmi* Brasier and in resistant carnation (*Dianthus caryophyllus* L.). A characteristic of these tissues is a thickening and strong lignification of the native walls and deposition of additional wall layers, including a suberin-like layer. In fibres, the inner thick layers deposited are similar to the so-called gelatinous layer and may also contain a suberin-like layer (Ouellette 1981a, b). Vessel secondary walls in certain cases can also be more opaque and thickened (Ouellette 1981a).

In addition to cell and wall modifications analogous to those related to compartmentalization of invaded xylem in these other hosts, a number of peculiar host reactions occurred in infected staghorn sumac. Some were related to the presence of latex in newly differentiated vessel elements, which appeared to influence infection. In this work, further examples of the role these reactions may play in the pathogenesis of this type of disease are reported.

MATERIALS AND METHODS

Most of the study material was from inoculated samples collected 2, 4, or 8 d after inoculation and 5 to 10 cm from the inoculation point, and in two instances 57 d after inoculation at the tip of streaks in the invaded xylem. Two of the present illustrations are from natural infection and are specified in the figure captions. Only the plants from which the *Fusarium* pathogen was isolated or re-isolated in pure cultures, or its absence in non-infected (H_2O-injected) controls, were used for light or transmission electron microscopy examination.

Healthy, approximately 1 m high staghorn sumac plants, growing in open or partly shaded areas (Quebec City region, QC, Canada) were used for inoculations. These were made by cutting with a sterilized scalpel across a drop of inoculum (approximately 1 x 10^6 conidia mL^{-1}, obtained from a culture on PDA), placed on bark of shoots that were previously surface sterilized with 70% ethanol. In one site, in 1994, 12 plants were inoculated with one of two isolates obtained in 1993, and four plants were injected with water. In 1997, five plants were inoculated in each of two sites with the 1993 isolates and another isolate obtained from a different location.

The 1993 samples were double fixed with 2.5% glutaraldehyde for 2 h under a slight vacuum, and with 1% osmium tetroxide at 4°C for 1 h at room temperature. The other samples were first fixed in a 2.5% mixture of glutaraldehyde and paraformaldehyde and then with reduced osmium (Tamaki and Yamashina 1994). Embedding was in Epon 812.

For light microscopy (LM) observations, sections 0.5 to 1.0 µm thick were cut from the Epon-embedded material using a glass knife, mounted on slides, and stained with a commercial solution of toluidine blue and basic fuchsin. Three or four contiguous sections from each sample were examined with a Polyvar (Reichert-Jung, Vienna, Austria) microscope.

For transmission electron microscopy (TEM) observations, ultrathin sections (straw colour), made with a Reichert Ultracut II, were stained with uranyl acetate and lead citrate before examination with a Philips 300 TEM at 80 kV. At least two samples from each sampling date and ordinarily two sections per sample (five when including those used for cytochemical tests) were examined.

For localizing cellulose, a purified exoglucanase (kindly provided by Dr. Colette Breuil, University of British Columbia, Vancouver, BC, Canada) was complexed to colloidal gold as previously described (Ouellette *et al.* 1995), using a gold particle size of approximately 15 nm (Frens 1973). Briefly, sections were floated for 5 min on a drop of PBS and 0.02% PEG (20 000) at pH 6.0 and then incubated for 30 min on a drop of the complex. Specificity was assessed by the following control tests: 1) incubation with exoglucanase-gold complex previously saturated with β-1, 4-D-glucan from barley at the rate of 1 mg mL^{-1}; and 2) incubation with the non-complexed enzyme followed by incubation with the gold-complexed one.

For localizing pectin compounds, a monoclonal antibody (denoted JIM 5 and kindly provided by Dr. R. Keith, Long Ashton Research Institute, UK) was used as previously described (Rioux *et al.* 1998).

RESULTS

Tylosis characteristics

Tylosis formation was common in staghorn sumac in control as in inoculated plants, very early and later after inoculation, even in much altered reacting tissue in the outer xylem. These tyloses were generally formed in vessel elements having well developed secondary walls, but often from small parenchyma cells in which secondary walls along the vessel lumen side were seemingly lacking, except in small localized areas (see below). Indeed, the native membranes of half-bordered pits were appreciably long and less opaque than the confluent compound middle lamella of adjoining parenchyma cells (Fig. 1a).

Tylosis wall layers, particularly the outer one, and material surrounding them generally labelled for pectin (Fig. 1b), even when they were very thin and compressed between vessel walls or other tyloses, whereas their inner wall layers were strongly labelled for cellulose (Fig. 1c). A thin, additional lucent layer

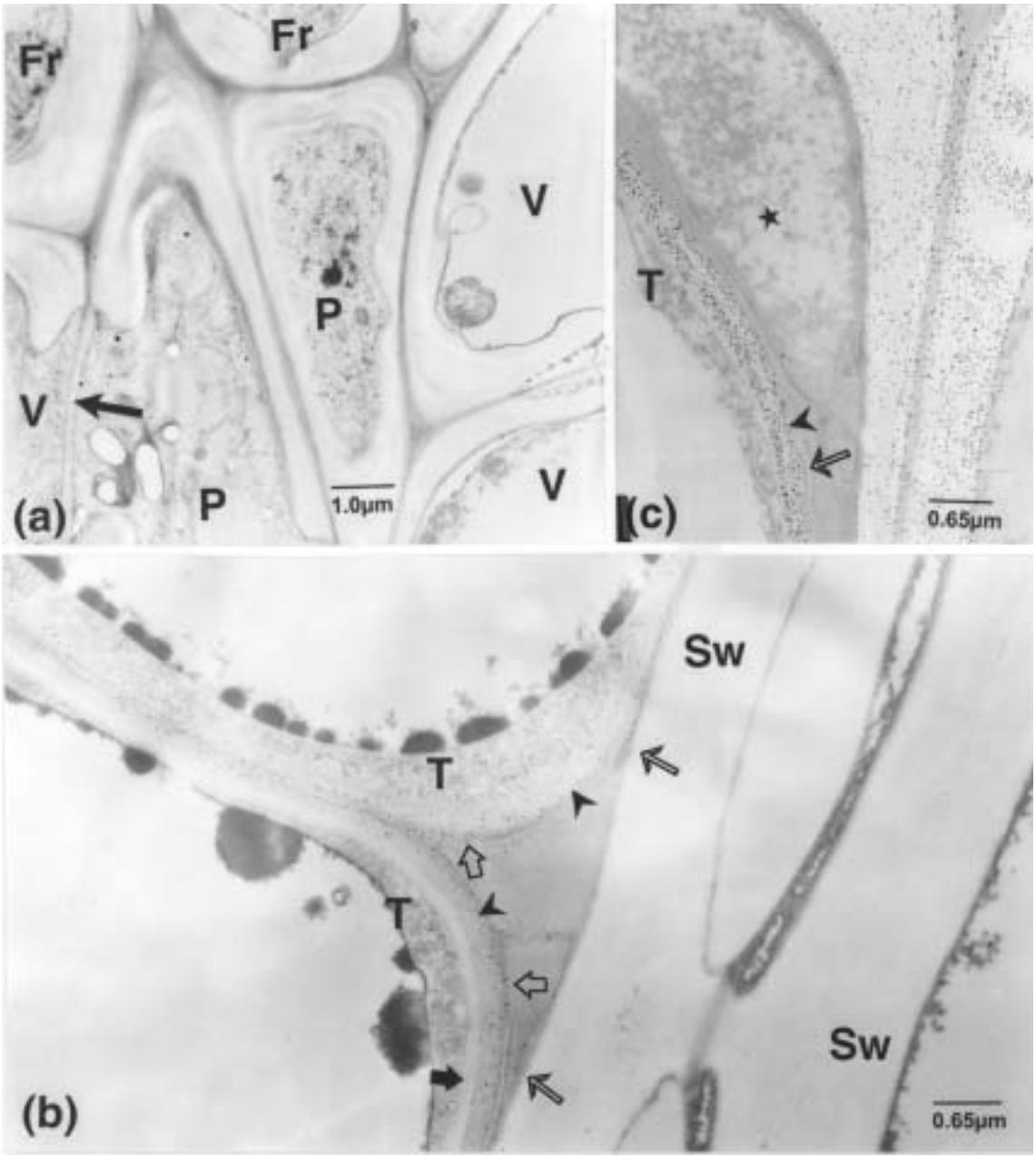

Figure 1. Samples from staghorn sumac inoculated with FOC, shortly after inoculation. **(a):** newly differentiated tissue in a non-wounded sample. Arrow = a long and thin half-bordered pit membrane. **(b):** labelling for pectin. Outer wall layers (thick, light arrows) and the inner portion of the adjoining layers (arrowheads) in the two confluent tyloses are labelled, as is some material surrounding them (thin, light arrows). An additional inner layer occurs in these tyloses, thicker in one (dark arrow) than in the other. **(c):** labelling for cellulose. Strong labelling for cellulose of the inner tylosis wall layer (arrowhead) and of the V-secondary wall. The thin, outer layer (arrow) is unlabelled, as is the other matter present between it and the V-wall (star). FOC, *Fusarium oxysporum* f. sp. *callistephi*; Fr, fibre; P, parenchyma cell; Sw, vessel secondary wall; T, tylosis; V, vessel.

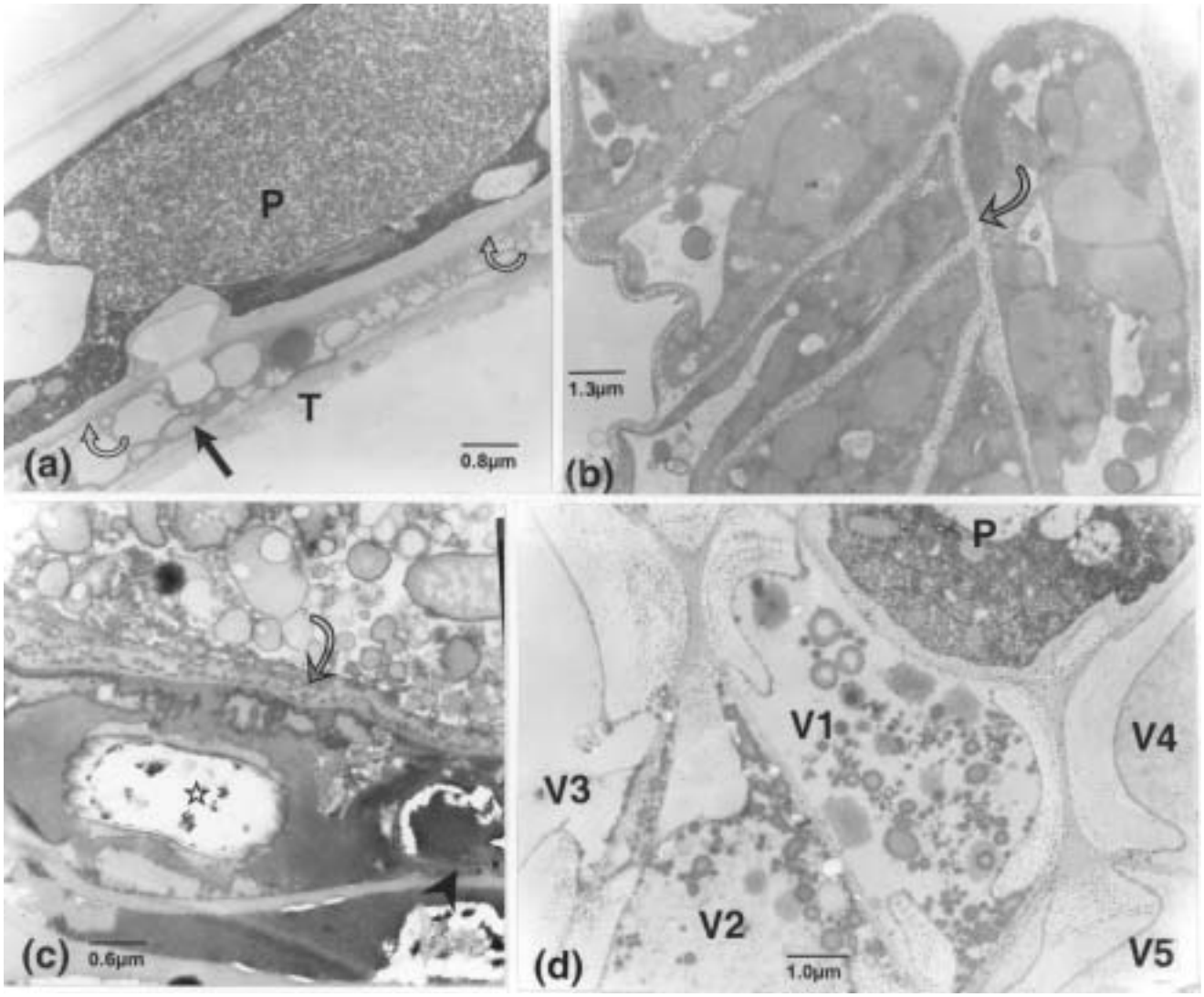

Figure 2. (a): from a sample injected with H_2O. Latex-like material (straight arrow) included between a tylosis and the V-wall. Note: the long pit membranes (curved arrows); small portions of secondary wall in the P with dense content, including that of the large vacuole. **(b):** FOC-inoculated. Labelling for cellulose. Rows of P, with labelled walls (curved arrow), forming the margin of a normal laticifer. **(c):** natural infection. Abundant latex material (upper part) occurs next to a cell wall, highly impregnated with similar material (curved arrow). A thin wall divides the cell (arrowhead), and a void area (star) is clearly delimited. **(d):** FOC-inoculated. Labelling for cellulose. In newly differentiated tissue, many latex bodies occur in vessels V1 and V2, next to a P with dense content; vessels V3, V4, and V5 are nearly free of similar content. FOC, *Fusarium oxysporum* f. sp. *callistephi*; P, parenchyma cell; T, tylosis; V, vessel.

(suberin-like) was frequently noticeable in these as well as in the adjoining or intercalary parenchyma cells (Fig. 1b).

Increase in latex production

In healthy plants, laticifers were noted in the pith and in the outside phloem. In wounded controls as well as in inoculated plants, close to and at a short distance from the inoculation points, large amounts of material, some of latex appearance, abounded in some vessel elements (Fig. 2a). The latex likely flowed into these vessels following wounding of the bark and the pith upon inoculation, from laticifers that were typically organized according to classical models, that is with rows of parenchyma cells surrounding the opening of the canal (Fig. 2b). As a possible reaction to infection, inoculated plants produced numerous pockets of latex in the phloem region (Fig. 2c). A characteristic of these pockets was that walls of disintegrating or adjoining cells related to latex production were heavily impregnated by the compounds (Fig. 2c). The presence of host wall residues mixed with the latex was indicated by a positive reaction for pectin (not illustrated). The abundance of latex observed in the newly differentiated vessel elements (Fig. 2d) or intercellularly between these and paratracheal cells, both of which showed wall alterations, as in Figure 2c, likely originated from these pockets. The

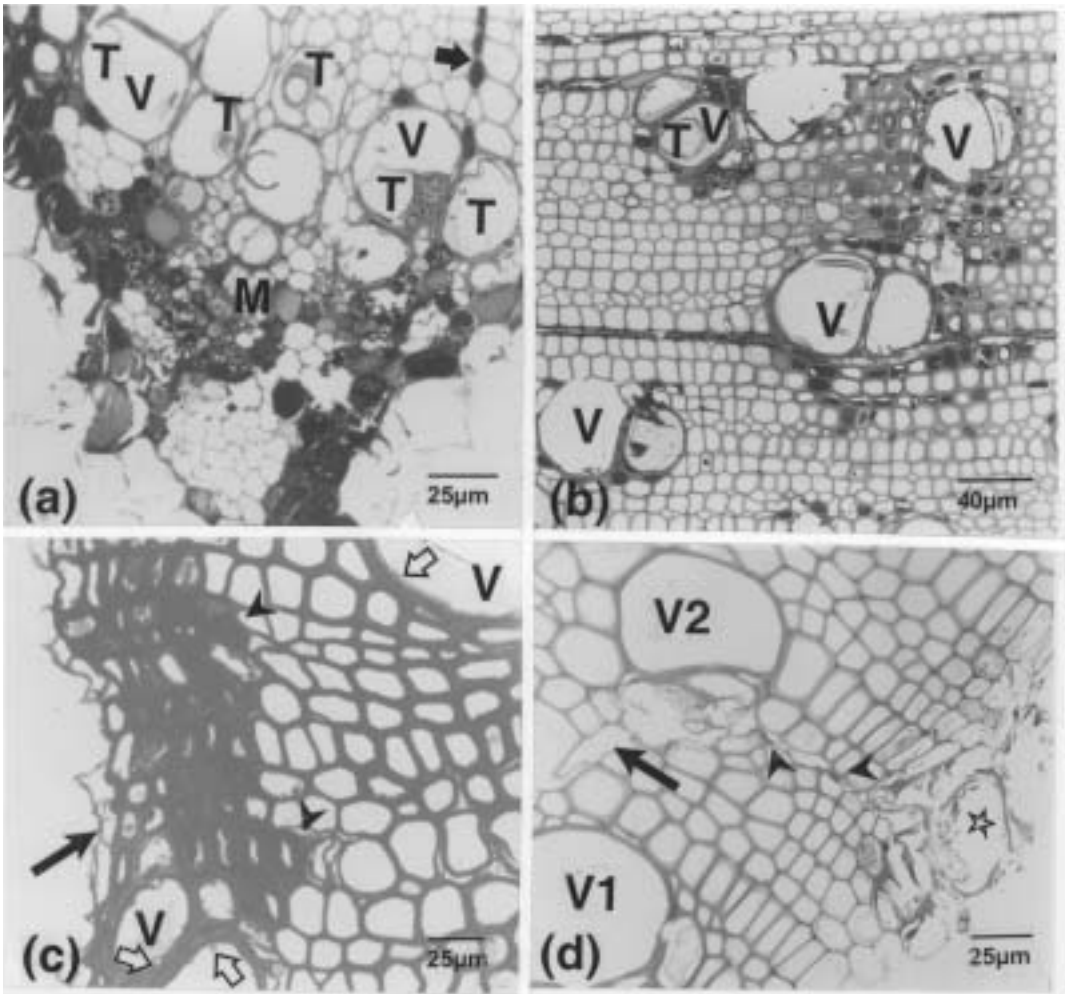

Figure 3. From FOC-inoculated samples. LM observations. **(a):** cells of medullary sheath, in the periphery of large pith cells, and some ray cells (arrow) are densely stained, except the paler vacuoles. **(b):** numerous cells with strongly stained content are included around and between groups of V-elements in the mid-growth ring. **(c):** groups of recently deposited cells (dark arrow) have thick opaque walls. Arrowheads = some opaque matter in intercellular areas, and light arrows = V-walls covered with extraneous material. **(d):** highly altered and hypertrophied cells, at the cambium interface (star), are in line with a row of distorted cells from deeper in the xylem (arrow). Traces of opaque matter (arrowheads) occur in this path of cell alterations. FOC, *Fusarium oxysporum* f. sp. *callistephi*; M, medullary sheath; T, tylosis; V, vessel.

latex was ordinarily white in healthy plants; the one emerging close to the infected areas was often pink, purple or yellow, according to disease severity. No thorough investigations were made on the nature of these variations, but the yellow latex was noted to predominantly occur in xylem regions bordering the invaded xylem in plants showing slight or no external symptoms.

Reactions in parenchyma and fibre cells: overview, LM observations

Pronounced reactions frequently occurred in cells of the medullary sheath, which were densely stained as were neighbouring ray cells (Fig. 3a). Groups of cells with similar, strongly staining content also surrounded groups of vessels in the invaded xylem (Fig. 3b). The majority of these cells had large vacuoles filled with fibrillo-granular matter (as in Fig. 2a).

In some parts of the recently deposited cells, many appeared to have thickened walls (Fig. 3c), and distal to these, many other cells, including vessels, were distorted and showed only thin walls (Fig. 3d). All these disorders occurred in zones where vessel walls were covered with layers of opaque matter and small particles or with small particles alone (Fig. 3c) and in line with altered ray cells or with paths of cell ruptures deeper in the xylem (Fig. 3d).

Host cell wall appositions and modifications, TEM observations

As in many other systems, appositions and new wall layers were deposited in many cells. Other types of reactions were also observed. The material identifiable as appositions was often very thick and heterogeneous. In tests for cellulose, labelling was associated with some apposition components but not with

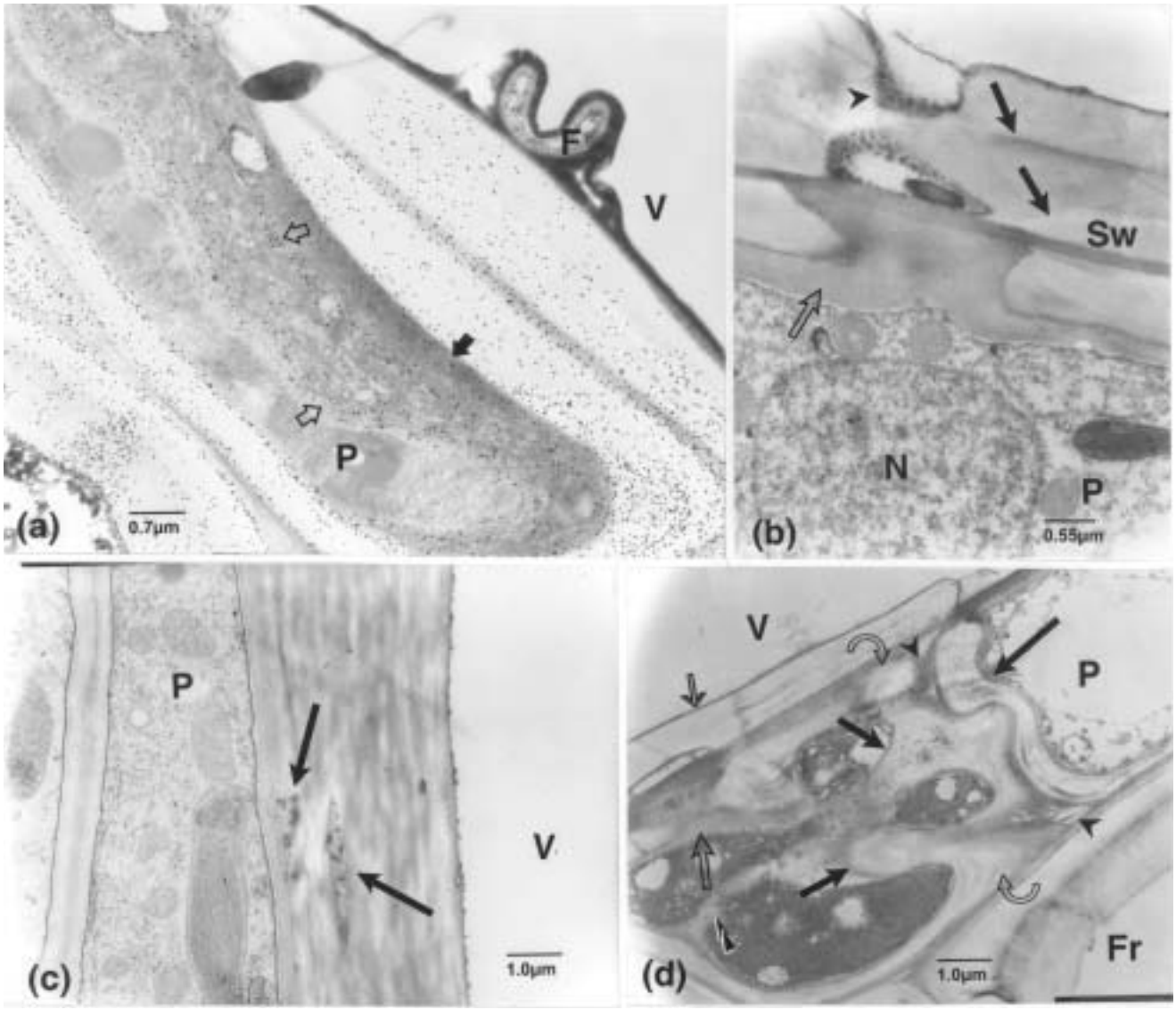

Figure 4. From FOC-inoculated samples. **(a):** labelling for cellulose. From the vessel side inward, the P contains an unlabelled, mostly homogeneous layer (opaque arrow), a labelled layer (light arrows) circumscribing an unlabelled mass, and unlabelled cell content remnants next to the unequally labelled secondary wall. **(b):** three demarcated layers (dark arrows) are distinguishable in the V-wall. A thick protective layer seals off the pit in the P (light arrow). The arrowhead = opaque bodies bordering the pit chamber, confluent with an opaque layer lining the V-wall. **(c):** shows a thickened marbled vessel wall containing opaque matter (arrows) in a region likely corresponding to an occluded pit chamber. **(d):** atypical wall depositions in P characterized by: a cross-wise secondary wall covered by a protective-like layer (long, dark arrow) abutting on the pit membrane region; secondary wall extensions (short, dark arrows) from the cell secondary wall (curved arrows) resulting in intercellular-like areas (arrowheads); a protective layer portion sealing off a pit (thick, light arrow) or bordering (superimposed arrowheads) locules with cell content. The V-wall is covered with a thin opaque layer (thin, light arrow). F, fungal cell; FOC, *Fusarium oxysporum* f. sp. *callistephi*; Fr, fibre; N, nucleus; P, parenchyma cell; Sw, vessel secondary wall; V, vessel.

others (Fig. 4a); the mostly unlabelled layer, contacting the native cell wall in these cases, differed from the often very thick protective layer (Fig. 4b), which generally labelled for both cellulose and pectin (not illustrated). Vessel walls also appeared to be thicker and more opaque than normal, appearing at times as if this thickening had occurred by the addition of new wall layers, as shown by a distinct demarcation between each layer (Fig. 4b, c). These modifications, which were occasionally associated with the presence of opaque matter in the middle lamella (not illustrated), were apparently concomitant with partial or complete obstruction of pit chambers (Fig. 4b, c). Vessel secondary walls frequently labelled unequally for cellulose (Figs. 2d, 4a), excluding the situation (Fig. 4a) where foreign bodies, apparently overlaying the wall, seemed to have interfered with labelling. Some paratracheal parenchyma cells also contained some intricate layers of wall (Fig. 4d), separated from one another by intercellular-like areas, or by

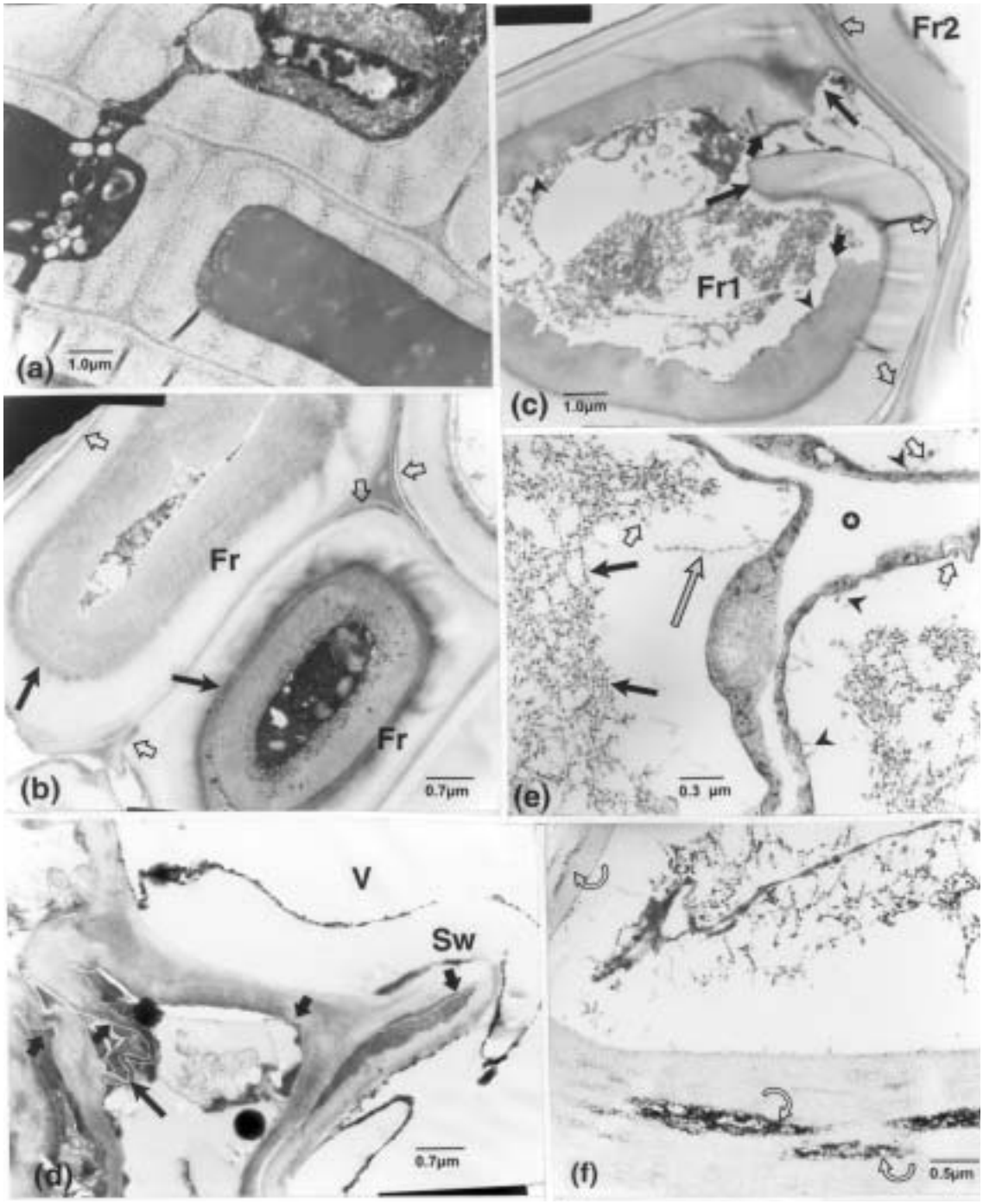

Figure 5. From samples that were FOC-inoculated, **a-c,** or naturally infected, **d-f. (a-c):** from tissues in the vicinity of invaded vessel elements. **(a):** labelling for cellulose. Rows of cells having thick walls and opaque content, much of it filling vacuoles. **(b):** new, inner gelatinous-like layers present over a thin, more compact layer (dark arrows) in fibres. Wall alterations (light arrows) are associated with traces of opaque matter between or in the walls of these cells. **(c):** thin bands (light arrows) line regions of wall alterations in and between fibres. In Fr1, margins of alterations and ruptures visible in both wall layers do not match (corresponding ends indicated by identical arrows: dark, long and short, respectively). Traces of a thin, inner lucent layer also occur in the cell (arrowheads). **(d):** a thin lucent layer (short, dark arrows) is apposed to the native or adventitious-like (long, dark arrow) walls in distorted or collapsed cells adjoining a "capsized" vessel element lined with opaque matter, close to the cambial region. **(e):** a much retracted fibre cell content (star) is surrounded at a distance by networks of thin structures (dark arrows) beaded with small opaque bodies. Larger, vesicular-like bodies (short, light arrows), some also beaded (long, light arrow), occur close to the cytoplasm (arrowheads) or at the margin of the network. **(f):** a thin band of much altered cell content is here closely surrounded by structures similar to those in **e**. Pockets of somewhat similar matter occur in the thick gelatinous layer (curved arrows). FOC, *Fusarium oxysporum* f. sp. *callistephi*; Fr, fibre; Sw, vessel secondary wall; V, vessel.

bifurcations from the protective layer of the cell, or as extensions of the secondary wall layer. A thin, suberin-like layer occurred in some of these cells (not illustrated).

As shown in LM observations, a reaction in many cells, forming a barrier zone, was a thickening of cell walls and an increased density of the cell content. TEM observations of corresponding regions indicated that many rows of cells showed these features, where the intense staining of cell content was mainly due to opaque matter filling vacuoles (Fig. 5a). Wall alterations in the proximity of this type of cell often occurred, associated with the presence of opaque bands and material, present in or between the walls of other cells (Fig. 5b, c). Occasionally, the new wall layers were also altered or ruptured and appeared to be locally separated into two layers. The additional wall layers in fibres were identified as gelatinous layers, which occasionally were also covered by a thin suberin-like layer (Fig. 4c), as in parenchyma cells (Fig. 5d), and in the majority of cells with dense content in the medullary sheath (not illustrated).

Noteworthy features in affected cells

Some host cells contained large amounts of small particles, often occurring as beaded chains, surrounding much retracted but still well delimited content (Fig. 5e) or just a tiny band, almost membrane-like, of altered cytoplasm (Fig. 5f). Some of these cells had laid down a gelatinous-like layer, in which were included patches of opaque material (Fig. 5f). Numerous tiny fibrils were also observed in other cells that only contained some remnants of cytoplasm surrounding pathogen cells, an association that was similar to that in vessel elements (not illustrated).

Unusual tissue hypertrophy

As already mentioned, tissue disorders occurred in the outer xylem as apparent reactions to cambium cell alterations. Thus, new layers of cells with opaque walls were deposited through which occurred many disruptions (Fig. 6a, b). The peculiarities to be noted in these cases were that groups of much disordered cells overlaid bands of altered ones that likely corresponded to the former cambial interface and that, perpendicularly to these cells, other groups of seemingly non-differentiated cells occurred (Fig. 6a). Pronounced tissue proliferation was also traceable to the medullary sheath and pith regions, which seemingly intruded across existing xylem tissue (Fig. 6c). This likely corresponded to the initiation point of numerous warty outgrowths present on the stems of inoculated plants, in the vicinity of invaded xylem (Fig. 6d). Indeed, sections through these mounds displayed a continuity of cells from the pith inside out, laid down at right angles with inner xylem cell layers and reaching the native bark tissue (Fig. 6e). The juncture of the hyperplastic tissue and the existing xylem was also marked by chaotic tissue including some differentiated elements, where opaque matter occurred intercellularly (Fig. 6f). These hyperplastic, seemingly empty cells displayed muroid divisions whose origins were not easily explainable. However, when these were viewed in TEM, some type of wall ingrowths were observed to be associated with traces of content (Fig. 6g).

The cortical and bark-like tissue layers, in the largest mounds, appeared to have displaced the native cortex and periderm and to have overlaid them (Fig. 6h). The types of cells in these cases ranged from large, normal pith cells, followed by others with several cross divisions, leading to a region containing some apparently better differentiated cells with some as periderm (Fig. 6h). Laticifers and some larger, vessel-like cells also occurred therein. The frequency of these mounds was relatively high. Thus, for example, in branches 5 to 6 mm in diam, 11 of these mounds occurred over a length of 8 cm, in one instance, and 26 mounds, over a length of 15 cm, in another.

DISCUSSION

Observations regarding the general infection process of staghorn sumac by *Fusarium oxysporum* were reported in Ouellette *et al.* (2005a, b). The present observations concern some of the main reactions occurring in these plants and their similarities with those observed in other wilt diseases (Baayen *et al.* 1996; Ouellette 1981a; Ouellette and Rioux 1992, 1993; Ouellette *et al.* 2004a; Rioux and Ouellette 1991). These similarities pertain to the thickening and deposition of new wall layers, including gelatinous ones in fibres, and to the deposition of suberin-like layers in tyloses, paratracheal parenchyma cells, and in cells of the medullary sheath and of the barrier zones formed. Labelling of tylosis walls for pectin and cellulose was equivalent to that observed in elms (Rioux *et al.* 1998). The areas containing cells with very opaque content and thickened walls, surrounding groups of vessel elements, also recalled those occurring in elms (American, Siberian, and different clones) and in non-hosts inoculated with the Dutch elm disease (DED) pathogen (Et-Touil *et al.* 2005; Ouellette and Rioux 1992; Rioux and Biggs 1994). Vessel secondary walls also appeared to be modified as in elms (Ouellette 1981b). As these types of reactions were noted in plants known to be resistant, particularly carnation (Baayen *et al.* 1996), and in plants showing milder external symptoms in the other systems studied, the reactions in staghorn sumac may be considered to be of the same nature.

The observed reacting host cells were often located next to wall alterations in adjacent cells, as in other systems (Ouellette *et al.* 2004a), and likewise associated with opaque matter (Ouellette *et al.* 2005b). An indication that wall ruptures were not attributable to an effect of sample manipulation was that paths of wall alterations, reaching in some cases from one cell to another across their walls, had been sealed off by the deposition of a continuous new wall layer (Ouellette, unpublished observations). This layer in some paratracheal cells did not always label for cellulose as the protective layer generally does; in this situation, the cellulose unlabelled wall was somewhat similar to appositions labelling for callose (Mueller and Morgham 1996). However, adjacent layers in the cells were neatly labelled for cellulose, which indicates that these cells were probably subjected to more than one attack, as in other situations (see Fig. 5b, c).

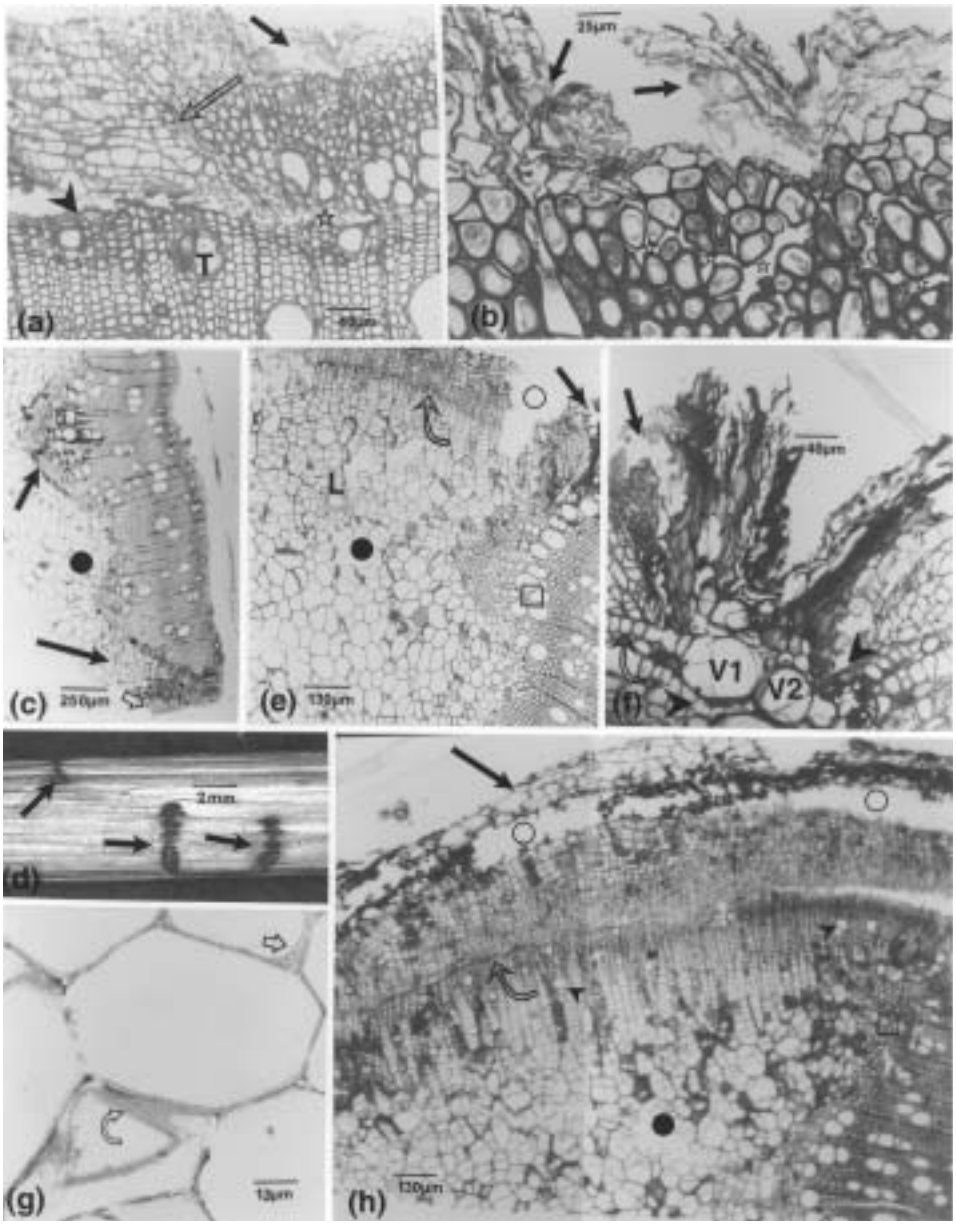

Figure 6. From FOC-inoculated samples. LM observations. **(a):** hyperplastic and distorted cells (dark arrow), overlaying a zone of small or thin-walled cells (star), preceded by layers of cells (arrowhead) with thick, opaque walls (see Fig. 4c). Seemingly undifferentiated cells have been laid down at a right angle (light arrow) with the other more normally aligned cells. **(b):** enlarged portion from **a** (in the area next to and below the dark arrow). Many highly distorted cells with thin walls occur in the outer xylem (arrows), over rows of irregular cells having opaque and seemingly thickened walls; gaps occur between some of these (stars). **(c):** in a twig, the first invaded area is indicated by cells having opaque content next to V-elements or in the medullary sheath (short, dark arrow). A zone of tissue (long, dark arrow), confluent with the pith (dark circle), and also bordered by darkly stained cells, extends through apparently displaced xylem tissue (light arrow). **(d):** opaque bands (arrows) present cross-wise a twig of a freshly inoculated plant. **(e):** a section through one similar band, showing a mound of tissue having proliferated from the pith (dark circle) and medullary sheath, at a right angle with the xylem (square). This tissue contains cells with many cross walls, decreasing in size towards a band of smaller cells (curved arrow), over which occur more diversified cells. Next to a gap in these tissues (empty circle), some cells are again oriented at an angle with the xylem, bordering a zone of distorted cells (dark arrow). **(f):** deformed tissue (dark arrow) in an area confluent with that indicated by the arrow in e, shown at a higher magnification. Adjoining tissues are also disordered, some stemming obliquely (curved arrow) to vessel elements (V1 and V2); these are also bordered by altered cells (arrowheads). **(g):** pith cells, one showing a type of cross wall (curved arrow), display traces of content (thick arrow). **(h):** mounds of tissue having proliferated from cells bordering the pith (opaque circle), as in e, with the following differences: occurrence of small V-like elements (arrowheads) below an opaque band (curved arrow), above which some of the cells are analogous to bark tissue, itself separated by collapsed cells (empty circles) from a layer of larger cells (dark arrow), with both regions likely corresponding to the original periderm. Hyperplastic tissue, of greater opacity than the other tissues, also swirls from the inner xylem (square). FOC, *Fusarium oxysporum* f. sp. *callistephi*; L, laticifer; T, tylosis; V, vessel.

An apparently novel feature, at least in terms of anatomy, is the intricate formation of axial and crosswise layers in some paratracheal cells, sealing off pit membranes and thus communications with adjoining vessel elements. These types of reactions, when taken singly, may not be that meaningful in compartmentalizing infection, but taken in conjunction with the other types of wall modifications, they may be important. In any event, all these reactions resulted from alterations to host cell walls.

Another feature of interest in infected sumac plants was the abundant occurrence of latex in vessel elements in the vicinity of inoculated wounds or of recently differentiated ones in the vicinity of the cambium. The first case may reflect an artificial condition, but the latex effect on fungal cells and their reactions to its contact (Ouellette *et al.* 2005a) are nevertheless informative, concerning, in particular, the production of microfilaments by these cells. The presence of latex in newly differentiated vessels may be more meaningful in terms of infection, as these were free of fungal cells, even though they often adjoined other colonized vessels (not illustrated). However, the extensive alterations of cell walls related to latex production, and not from typical laticifers, may not be that beneficial to the plant.

The striking cell reactions observed in relation to the presence of numerous opaque particles thus appeared to be in response to these possibly foreign agents. Indeed, these structures may be analogous to those observed in other hosts (Ouellette and Baayen 2000; Ouellette *et al.* 2004b; and unpublished observations), and associated with pathogen cells. This possibility once again points out the need to properly differentiate between components of pathogen and host origin, or a mixture thereof, and to better understand the host-pathogen relationships of these diseases. Moreover, a question may be raised as to when exactly the content of affected host cells can be qualified as becoming completely inactive.

Finally, the outstanding proliferation of pith-like cells terminating in mimically differentiated or non-differentiated tissue in the outer xylem may also be considered a curiosity, except that this condition (also apparently related to host wall alterations) may reflect an increased production of growth-promoting substances, including ethylene, which have been thought to play an important role in fungal wilt diseases (Beckman 1987; Cooper *et al.* 1998; Pegg 1981, 1984). Theoretically, as these masses of tissue interrupted the xylem, they might, if in any great quantity, hinder normal sap movement and contribute to the development of external symptoms. Similar proliferation and improper differentiation of tissues have also been observed in elms infected with DED (Ouellette *et al.* 2004a; and unpublished observations). In infected elms (Ouellette 1960), the pathogen was found to pass from one side of the shoots and twigs to the opposite side via the pith, in which reacting cells were also observed (Ouellette, unpublished observations). These observations once more indicate that the development of fungal wilt diseases, at least those under discussion, is not solely a question of rapid invasion of vessel elements, and that all tissues should be taken into consideration to adequately understand all the types of actions and reactions occurring in these diseases.

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