Cytology and Histology

Compartmentalization of Decay in Carnations Resistant to *Fusarium oxysporum* f. sp. *dianthi*

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ABSTRACT

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The anatomy of well-developed defense responses in carnation to *Fusarium oxysporum* f. sp. *dianthi* was studied in light of the compartmentalization of decay in trees (CODIT) model. Compartmentalization of the fungus was achieved by occlusion of vessels with gums (wall 1) and circumscription of the affected area with reaction zones (walls 2 to 4). Reaction zones were characterized by thickening, lignification, and suberization of parenchyma cell walls; suberization of fiber walls; and proliferation of xylem parenchyma cells bordering the reaction zone. A cork layer was formed from the proliferating cells. Wall thickenings stained for methyl ester groups (indicative of pectins or xylans) and lignin aldehydes, but not for cellulose, callose, or lipids. Induced lignin stained like cork rather than vessel or fiber lignin. Gums stained for pectin and lignin. Pressure exerted by proliferating parenchyma caused the stem to burst. Cells at crack margins stained for lignin and suberin. No essential differences existed between vascular and extravascular reaction zones, which sometimes merged fluently. Compartmentalized tissues were replaced by vascular regeneration within the proliferating xylem parenchyma on the side of the medulla. Defense responses focused for protection of regenerated cambium and xylem (wall 2) rather than the original cambium (wall 4), as in trees.

Additional keywords: *Dianthus caryophyllus*, Fusarium wilt, histochemistry.

Reaction zones appear essential to the restriction of pathogen-induced decay in trees. According to the compartmentalization of decay in trees (CODIT) concept proposed by Shigo and Marx (67), fungal colonization of infected wood can be inhibited both vertically and laterally by four distinct defense responses termed "walls." Colonization is inhibited vertically by occlusion of conducting elements of the xylem (wall 1). Radial growth is restricted by tangential barriers formed in existent xylem at the inner margin of the affected area (wall 2). Existing ray parenchyma cells may function to restrict lateral spread of the pathogen (wall 3). Radial growth towards the periphery of the xylem is restricted by a barrier zone (wall 4) formed by the cambium after the onset of infection. Wall 4 is considered the most important of the four walls of the CODIT model (19,21, 45,62,63,64,66). In the same manner, infected bark is protected by ligno-suberized boundary tissue developing from extant cells and necrophylactic periderm derived from newly differentiated phellogen (17,18,60).

Histochemical studies of reaction zones in trees have shown that the barriers mostly arise from parenchyma cells, although fibers were also described as principal components of some barrier zones (63,64). Accumulation of phenolics in cell lumina and lignification and suberization of walls of parenchyma cells have been described (14,16,17,19,58,59,63,64).

Defense responses in carnations resistant to Fusarium wilt, a disease caused by the soilborne fungus *Fusarium oxysporum* Schlecht.:Fr. f. sp. *dianthi* (Prillieux & Delacroix) Snyder & Hansen, resemble the formation of reaction zones in trees. In susceptible cultivars, xylem vessels of roots and stems are colonized by means of mycelial growth (4,6,10,11,32); passive transport of microconidia is not involved, although it can be induced artificially (5). Colonization of stems is unilateral because of lateral inhibitory reactions of the host, while vertical growth of the fungus is not inhibited (6,7). Degradation of colonized xylem by fungal cell-wall-degrading enzymes such as polygalacturonase and pectin methyl esterase (8) results in dry rot and hollowing-out of stems and withering of leaves (6,7, 11,46). Strands of laterally compartmentalized, colonized xylem may disappear into leaf traces, resulting in yellowing and withering of one or two affected leaves, while the remainder of the plant is not colonized any further and remains healthy (10). These observations have been taken as an indication of the occurrence of compartmentalization responses in carnation similar to those described for trees (10).

Several carnation cultivars are available with high levels of partial resistance to Fusarium wilt. These cultivars virtually never develop wilt symptoms and are not, or only incidentally, colonized upon inoculation (6,8,10,32). The most resistant cultivar known is the one used in this study, 'Novada'. Resistance to Fusarium wilt in such cultivars is characterized by compartmentalization of infection. This is achieved by the occlusion of invaded xylem vessels with various types of material including gums (gels), the formation of wall appositions in xylem parenchyma cells next to colonized vessels, the formation of a cork layer lining the inner side of the infected part of the xylem, and regeneration of functional vascular tissue (2,3,6,11,32,33). High concentrations of phytoalexins are localized within the occluded xylem (48). Occasional failure of the compartmentalization response in partially resistant cultivars results in disease development. Inversely, plants of susceptible cultivars may occasionally be successful in compartmentalizing the invading fungus and remain free of disease

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(10,11). When a plant is inoculated by incision through a droplet of inoculum placed in the leaf axil on two opposite sides of the stem, successful isolation of the fungus sometimes occurs on one side of the stem, while the fungus escapes from compartmentalization on the other side and induces unilateral wilt (R. P. Baayen, *unpublished data*). Cultivars with higher levels of partial resistance to the fungus are better able than susceptible ones to compartmentalize infection through such responses and to retard the colonization process when compartmentalization fails (10). Similar defense responses occur in carnation roots, in which the compartmentalized area may eventually be excluded from the root (11).

Previous papers have presented a detailed description of the chronology of defense responses in carnation at the light microscopical level in relation to the qualitative and quantitative development of the fungus in space and time and in comparison with the events observed in susceptible cultivars (2,3,4,6,11,32,33). The role of phytoalexins has also been investigated, and phytoalexins have been localized in the vascular tissues of susceptible and resistant cultivars by pyrolysis-gas chromatography-mass spectrometry microanalyses (48,49). The current paper builds on our previous studies of defense responses in resistant cultivars of carnation. The aim of this paper is to demonstrate that the cellular modifications that culminate in the defense of resistant carnation plants against *F. oxysporum* f. sp. *dianthi* can be interpreted in a novel manner in light of the CODIT model as developed for trees. This paper, therefore, focuses on the responses that are characteristic of mature compartmentalized xylem and involves the use of histochemical tests and stains that are commonly used in forest pathology. We hope that the paper will assist in bridging the gap between forest pathology and the histopathology of herbaceous plants.

MATERIALS AND METHODS

Inoculation and sampling of plants. Rooted cuttings of the highly resistant carnation cultivar Novada, obtained from M. Lek & Sons B.V., Nieuwveen, the Netherlands, were planted in steam-sterilized soil (85% peat, 10% clay, and 5% sand) and grown in the greenhouse at 22°C for 4 weeks.

Inoculum was prepared by flooding 1-week-old potato-dextrose agar (PDA) (Oxoid; Unipath Ltd., Basingstoke, England) slant cultures of isolate WCS816 of race 2 of *F. oxysporum* f. sp. *dianthi* (from R. P. Baayen's culture collection) with 1 ml of water, scraping the colony with a transfer needle, and adjusting the resulting spore suspension to 1×10^7 conidia/ml. A droplet of 20 µl of conidial suspension was placed against the stem in the axil of one of the lower leaves, after which the stem was incised across the droplet as described previously (9). Eighteen plants were inoculated, 12 others were treated with sterile water instead of the conidial suspension, and six remained untreated.

Stem segments were collected at 3 to 10 mm above the incision at 1, 2, 4, 8, 16, and 32 days after inoculation from three inoculated plants, two water-treated plants, and one untreated plant. Samples were fixed in 2.5% glutaraldehyde in 0.025 M phosphate buffer (pH 6.9) and stored in this solution at 3°C before sectioning. Additional inoculated plants and untreated controls were sampled and studied without any fixation to exclude artifacts such as fixation-induced autofluorescence (24,36). No differences were seen in brightfield and fluorescence properties between fresh and fixed sections, except for the natural red fluorescence of chlorophyll seen in fresh sections, which was extinguished by fixation in glutaraldehyde.

The experiment was repeated with the same cultivar and isolate, but slightly different sampling dates. Observations made on this material were similar to those described here (data not shown).

Microscopical study of sections. Sections made by hand with razor blades were stained and viewed with a Zeiss Axioplan microscope (Zeiss-Nederland B.V., Weesp, the Netherlands) using brightfield light microscopy (B) and epifluorescence microscopy (F) (UV light: excitation filter G 365, beam splitter FT 395, barrier filter LP 420; Zeiss-Nederland B.V.) separately or in combination. Birefringence was studied with polarization contrast, as well as with differential interference contrast, with the prism positioned to give birefringence against a dark background. Sections were photographed using Zeiss MC-100 photographic equipment (Zeiss-Nederland B.V.).

Delignification treatments. Before some tests, sections were treated with 2 N NaOH for 30 min to remove alkali-soluble lignin including phenolic acids esterified to the cell wall (29,41), as well as phenolic aldehydes bound to the wall by alkali-labile ether bonds (34). Lignified tissues that would not stain using conventional procedures commonly became accessible to stains after treatment with NaOH. Prolonged treatments with NaOH will remove most of the xylans and other hemicelluloses from the wall (29,42,51).

Alternatively, sections were immersed in 1% aqueous KMnO4 for 5 min. Permanganate oxidizes lignin and quenches its autofluorescence (26,42,70). However, specific walls remained autofluorescent unless treated beforehand with NaOH. This is presumably because of improved accessibility of walls to permanganate by alkali-induced swelling or the presence of permanganate-insensitive wall-bound aromatic acids and aldehydes. Esterified phenolic acids are abundant in carnation cell walls (50) and are strongly autofluorescent (70). Suberin retains its autofluorescence even after successive treatments with alkali and permanganate.

Stains for lignin, suberin, and lipids. Mäule's test (31) was used to distinguish syringyl (dimethoxyphenol) from guaiacyl (monomethoxyphenol) lignin, which will not stain with Mäule's test. Sections were also stained with auramine O (30), a pseudo-Schiff's fluorochrome (22) that will stain lignin (53).

Suberin and lignin were distinguished with a number of different tests and stains. Acid phloroglucinol stains the aldehyde groups (22,23,34) of lignin and suberin, but quenches lignin autofluorescence and retains suberin autofluorescence (15,53). Phenolic acids are not stained by acid phloroglucinol (1). Sections were, in some cases, subjected to alkaline hydrolysis or oxidation with permanganate before staining to remove the stainable compounds (26). Sudan black B (40) results in staining and loss of suberin autofluorescence, whereas lignin is not stained and retains its autofluorescence (15). Ammoniacal gentian violet (28) was applied to stain suberin deep violet, whereas regular lignin stains pink (58).

Lipids were stained with Sudan black B and also with rhodamine B, which results in white-pink fluorescence of lipids (30). Lignin is also stained with rhodamine B (31), but its fluorescence is orange-red rather than white-pink. The use of auramine O as a stain for acidic waxes (30,40) is questionable (25).

Stains for phenolics. Various stains were used for phenols including Gibb's reagent (40) and toluidine blue O (53), which will stain phenols (Gibb's reagent), as well as tannins, lignin, and suberin (blue-green reactions with toluidine blue O). The nitroso test (40) was used for staining phenols and catechol tannins. The ferric ferricyanide reduction test (22) was also applied, leading to a blue staining of reducing phenols and other reducing substances such as proteins containing sulphydryl groups (30,40).

Stains for aldehydes. Lignin aldehydes and other natural aldehydes were stained red by treating sections with Schiff's reagent (40) and stained yellow by staining sections with 2,4-dinitrophenylhydrazine (DNPH) (34,40).

General test for polysaccharides. The periodic acid-Schiff's reaction (PAS) (40) was applied to sections for obtaining a red staining of polysaccharides with free vic-glycol groups. Before the

test, naturally occurring free aldehydes (lignin) were blocked with DNPH in part of the sections (40,65).

Stains for cellulose, chitin, and callose. Chlor-zinc-iodide (30) and chlorazol black E (22,40) were used to stain cellulose. In both cases, lignified cellulose stained when sections were treated beforehand with NaOH. Sections were also treated with the optical brightener Uvitex 2B (Ciba-Geigy Corp., Basel, Switzerland) (0.1% in water), which has affinity for crystalline β-linked glucans such as cellulose (22,40,53,68), chitin, and callose (35). Lignified cellulose proved to fluoresce only when sections were treated beforehand with NaOH.

For callose, sections were stained with resorcinol blue (1% in water) or with aniline blue (0.1% in 0.1 M K_2HPO_4, pH 9.5) (22,40). With aniline blue, lignin autofluorescence was quenchable by oxidation with permanganate before staining.

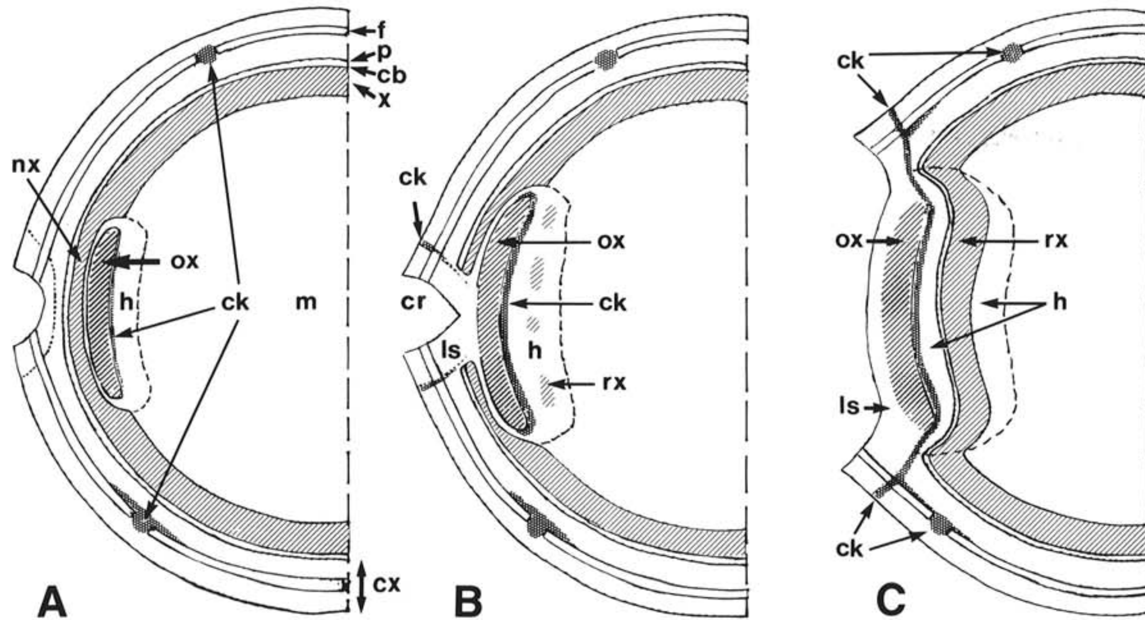

Fig. 1. Schematic representation of well-developed defense responses in stems of resistant carnation cultivar Novada inoculated with *Fusarium oxysporum* f. sp. *dianthi* **A,** at 10 mm distance from the incision; **B,** at intermediate levels; and **C,** at 3 mm from the incision at 32 days postinoculation. Nonincised and mock-inoculated controls lacked the illustrated phenomena, except for having gaps in the cortical fiber sheath filled with cork cells. **A,** Occluded xylem (ox), within which the fungus is compartmentalized, is covered by an equally thick layer of normal xylem (nx) formed by the cambium (cb) after inoculation that remains free of colonization. Particularly on its inner side, the occluded xylem is surrounded by hyperplastic parenchyma (h). In some cases, the stem has burst longitudinally to form a shallow cortical crack (cr) that is lined by ligno-suberized tissue (ls). Gaps in the fiber sheath (f) are naturally filled with cork cells (ck), a thin layer of which also lines the occluded xylem. **B,** Occlusion of a larger xylem area coincides with stronger hyperplasia and a deeper crack in the stem. Regenerating xylem (rx) is observed within the hyperplastic area. The ligno-suberized tissue next to the crack merges with that of the occluded xylem. Cork layers internally lining the ligno-suberized cortical tissues are not in contact with the cork layer internal to the occluded xylem. **C,** Occluded xylem is part of the ligno-suberized boundary zone next to the crack. Cork layers internal to the ligno-suberized cortical tissues, as well as the occluded xylem, connect fluently with one another and sometimes also with the cork filling gaps in the cortical fiber sheath. Regenerated xylem, phloem, and cambium formed in the hyperplastic area have taken over the role of the occluded tissue and connect laterally to the unaffected vascular tissues. Further abbreviations: cx = cortex; m = medulla; p = phloem; and x = xylem.

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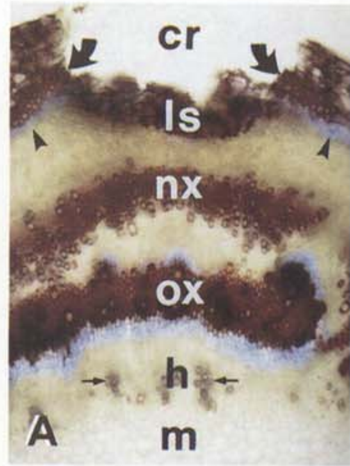
Fig. 2. Reaction zones in the **A to K,** xylem and **L and M,** cortex of resistant carnation cultivar Novada inoculated with *Fusarium oxysporum* f. sp. *dianthi* (32 days postinoculation [dpi] unless mentioned otherwise). **A,** Occluded xylem (ox) is circumscribed by cork cells (blue fluorescence) and hyperplastic xylem parenchyma (h), separating it from the medulla (m) as well as the new xylem produced by the original cambium (nx). Note the regenerating xylem (small arrows) in the hyperplastic area on the side of the medulla. Ligno-suberized boundary tissue (ls) along the cortical crack (cr) connects to the cork cells (arrowheads) internal to the fiber sheath (curved arrows). Lignin stains red with acid phloroglucinol (×49). **B,** Syringyl lignin of fibers in the xylem and in the fiber sheath (f) within the cortex (cx) stains red with Mäule's test. The compartmentalized xylem (*) is more or less in continuity with newly formed regular xylem, while regenerating xylem (arrows) is protected by a well-defined cork layer (blue fluorescence; arrowheads). Vessels stain weakly compared with fibers and retain some autofluorescence. The yellow-brown material in the defense area is not stained (×49). **C,** Suberized cells at the inner margin of the defense area (black arrowheads) are stained black with Sudan black B, as are the ligno-suberized cells (white arrowheads) lining the crack. Fluorescence of suberin is quenched, but not that of lignin in the compound middle lamella and the material filling the intercellular spaces of cells next to the crack (×99). **D,** Version of **C** without autofluorescence. **E,** Detail of **B** (×99). Note residual fluorescence of fiber (black arrowheads) and vessel walls (white arrowheads) at the external margin of the defense area. **F,** Cork cell walls (black arrows), vessel-occluding materials (arrowheads), and xylem parenchyma cell walls throughout the compartmentalized area stain for lignin with acid phloroglucinol. A small area (open arrow) is being colonized and degraded and does not stain any longer (×99). **G,** Secondary walls of vessel elements (v), fiber cells (f), apposition walls of xylem parenchyma cells (arrows), and cork cells (ck) stain red with hydroxylamine-ferric chloride for esterified uronides (×238). **H,** Ammoniacal gentian violet stains lignin of cork cells, lignified xylem parenchyma, and gums deep violet, while vessel and fiber lignin stained pale pink (×198; 16 dpi). **I,** Lignified walls and gums in the defense area fluoresce greenish yellow with auramine O (×198; 16 dpi). **J to L,** Reaction zone lignin in **J,** xylem (×99) and **L,** cortex (×99) fluoresces red with rhodamine B. **K,** Overview (×49). Cork cells lining the inner side of the compartmentalized xylem (black arrowheads) and necrophylactic periderm lining the inner side of the ligno-suberized boundary zone (white arrowheads) retain their blue autofluorescence, as do cork cells filling the gap in the fiber sheath (curved arrow) and those partially lining the inner side of the sheath next to the gap. Secondary walls of fibers fluoresce salmon red, although the inner wall layers of older fibers retain their autofluorescence, as do the vessel walls. Staining of lignified parenchyma walls (white arrows) decreases with distance from the cork cells. **M,** Walls of cortical fibers stain red for syringyl lignin with Mäule's test preceded by treatment with NaOH, but those of the ligno-suberized boundary zone do not. Suberin and cutin retain their blue autofluorescence. Fibers partaking in the ligno-suberized boundary zone have suberin linings inside their walls. Suberization in the boundary zone increases towards the necrophylactic periderm (np) (×148).



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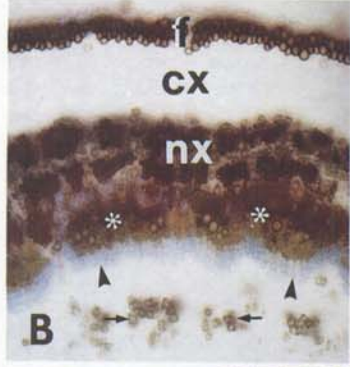

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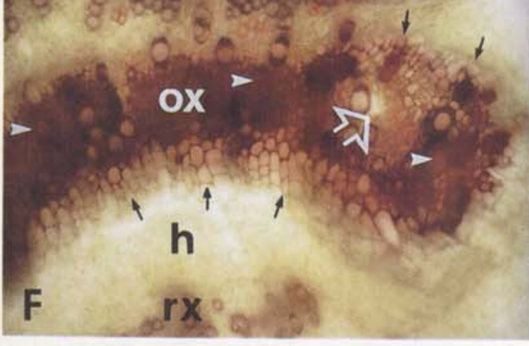

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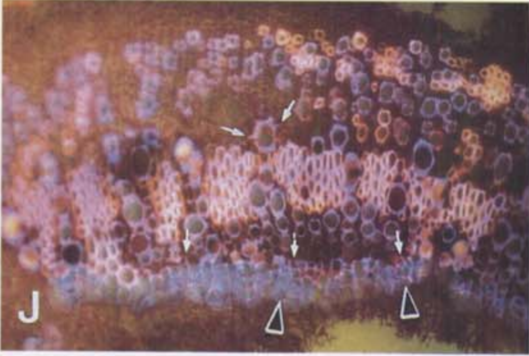

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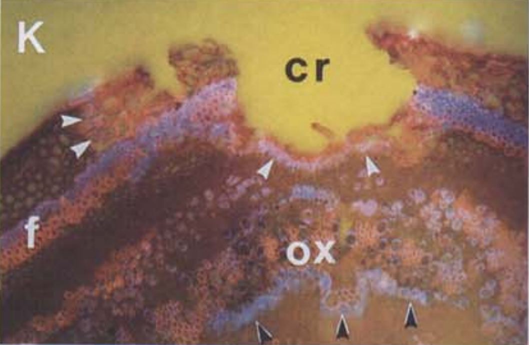

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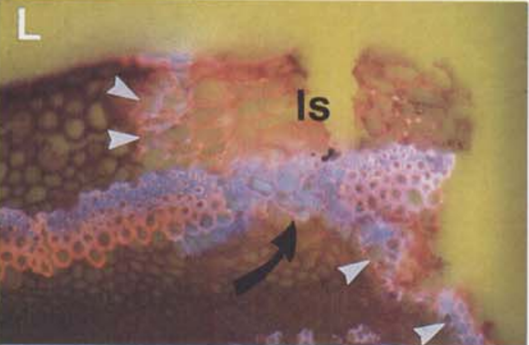

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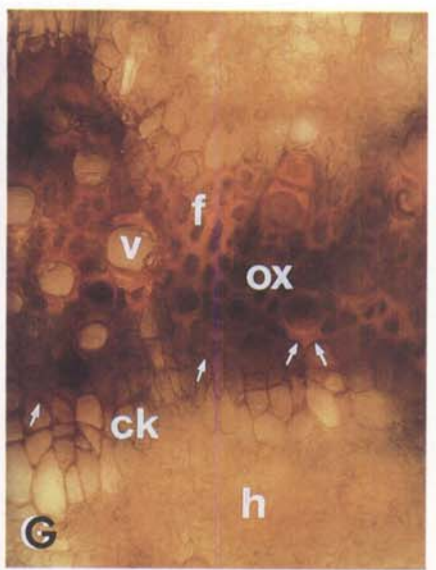

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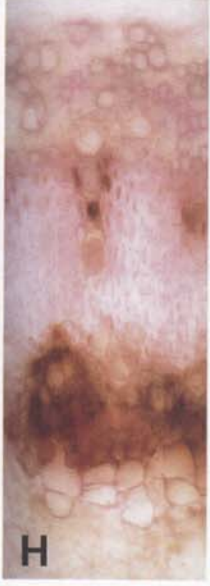

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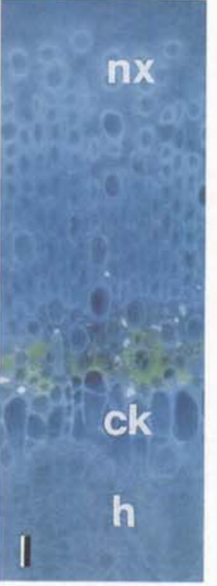

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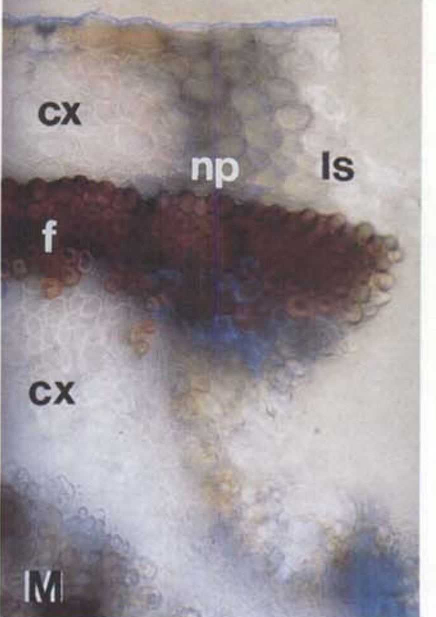

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Stains for nonesterified and methyl-esterified uronic acids. Pectic acids were stained with ruthenium red (30,37), toluidine blue O (53) (purplish red staining reactions only), and alcian blue 8GX (22). Nonesterified pectin was stained blue-black by treating the sections with tannic acid-ferric chloride (40). Hydroxylamine-ferric chloride (29,30) was applied on sections to stain methyl-esterified carboxyl groups of acid polysaccharides. Control tests involved the saponification of the ester groups with NaOH before staining (no color produced any longer) and increased methylation by placing the section in a hot solution of methanol containing 0.5 N HCl (intensified staining) (29,37,40).

Birefringence tests. Birefringence was used to locate walls with oriented crystalline cellulose. Cutin and suberin also impart birefringence to cell walls (15,25).

TABLE 1. Responses of the xylem of resistant carnations inoculated with *Fusarium oxysporum* f. sp. *dianthi* to histochemical tests for polysaccharides

		Vessels[a]		Fibers[a]		Parenchyma[a]		
Histochemical test	Specificity	M	S	M	S	M	A	Gums
Periodic acid-Schiff's	Noncrystalline polysaccharides	+	±[b]	+	+[b]	+	+	+
Birefringence with polarized light	Crystalline polysaccharides	–	+	–	+	–	–	–
Chlor-zinc-iodide	Cellulose	+	+[c]	+	+[c]	+	–	–
Chlorazol black E	Cellulose	+	+[c]	+	+[c]	+	–	–
Uvitex 2B fluorescence	Cellulose, chitin, callose	+	+[c]	+	+[c]	+	–	–
Resorcinol blue	Callose	–	–	–	–	–	–	–
Aniline blue fluorescence	Callose	–	–	–	–	–	–	–
Ruthenium red	Pectic acid	+	–	+	–[d]	+	–[d]	+
Toluidine blue O	Pectic acid[e]	+	–	+	–[d]	+	–[d]	+
Alcian blue 8GX	Pectic acid	±	–	±	–	+	–	+
Tannic acid-ferric chloride	Pectic acid	+	–	+	–	+	–	+
Hydroxylamine-ferric chloride	Esterified uronides	+	+	+	+	+	+	+

[a] M = compound middle lamella; S = secondary wall; and A = apposition wall.
[b] Distinctly lamellar, with multiple alternating narrow red (+) and yellow (–) layers.
[c] Only after treatment with NaOH.
[d] Sometimes faintly stained after treatment with NaOH.
[e] Positive when red or purple-red; negative when blue, blue-green, or unstained.

RESULTS

Development of defense responses. The chronology of defense responses from 1 to 32 days postinoculation (dpi) was similar to previous reports (2,3,6,32,33). The following paragraphs pertain to the structural and chemical modifications observed at 32 dpi unless mentioned otherwise. At the levels sampled in this study (3 to 10 mm above the incision), no differences were encountered up to 32 dpi in the vascular and cortical regions between untreated and water-treated controls, confirming previous reports (2). Illustrations of the anatomy of untreated plants and the wound-healing responses at the site of incision in water-treated controls are available in previous studies (2,32).

Previous studies on similar material have shown that 1 month after inoculation of a resistant cultivar the fungus is mainly in the first 5 mm above the incision (2,3,4,6). In this region immediately above the inoculated sector, fungal hyphae are abundant and incidental degradation of the colonized xylem may be observed. The abundance of fungal hyphae decreases drastically from 3 to 10 mm above the incised sector, with an upper limit reached by the hyphae of 30 mm to, rarely, 60 mm (4,6,10). The region from 3 to 10 mm, thus, represents an area with abundant defense responses, but relatively few fungal hyphae, mainly in the center of the compartmentalized xylem in still-open xylem vessels that were occluded higher up in the stem. Defense responses decreased considerably in intensity and affected volume from 3 to 10 mm in height, sometimes disappearing entirely within the 7-mm-long sample itself. A schematic representation of the defense responses at 32 dpi is shown in Figure 1.

As a result of the incision of stems at inoculation, the fungus was introduced simultaneously into existing protoxylem and metaxylem vessels close to the medulla, as well as into differentiating metaxylem vessels next to the cambium. As was shown previously (3,6), infection was mostly compartmentalized in those parts of the xylem that had existed at the time of inoculation. Because of continuing cambial activity and differentiation of new xylem, the thickness of the xylem had doubled at the end of the experiment. The colonized area was restricted to a pocket of altered tissue located within the inner half of the total layer of xylem tissue at the end of the experiment, with the outer half (next to the cambium) being mostly unaffected (Figs. 1A and B and 2A to D). Many of the illustrations at 32 dpi, thus, show both the

TABLE 2. Responses of the xylem of resistant carnations inoculated with *Fusarium oxysporum* f. sp. *dianthi* to histochemical tests for lignin, suberin, phenolics, and lipids

Histochemical test	Specificity	Color[a]	Vessel walls	Fiber walls	Apposition walls	Cork cell walls	Gums
Phloroglucinol-HCl	Lignin, suberin	B - red	+	+[b]	+	+	+
	Suberin	F^{au} - blue-white	–	– (+[c])[d]	– (+[c])	+[c], –[b]	–
Sudan black B	Suberin	B - black	–	– (+[c])	– (+[c])	+[c], –[b]	–
	Lignin	F^{au} - blue-white	+	+ (–[c])	+ (–[c])	–[c], +[b]	+
NaOH-$KMnO_4$	Suberin	F^{au} - blue-white	–	– (+[c])	– (+[c])	+[c], –[b]	–
$KMnO_4$ only	Suberin, esterified phenolic acids	F^{au} - blue-white	+	+[b] (+[c])	– (+[c])	+[c], –[b]	–
Mäule's test	Syringyl lignin	B - red	±	+	–	–	–
Gentian violet	Regular lignin	B - pale pink	±	+	–	–	–
	Wound lignin	B - dark violet	–	–	+	+	+
Toluidine blue O	Lignin, phenolics	B - blue-green	+	+	+	+	+
Ferric ferricyanide	Phenolics	B - blue	±	±[b]	+[c]	+	+
Nitroso test	Phenolics	B - orange to red	±	±	+[c]	+	+
Gibb's reagent	Phenolics	B - blue	–	–	+[c]	+	±
Auramine O	Regular lignin	F^{au} - blue-white	+	+[b]	–	+	–
	Wound lignin	F - green	–	–	+	–	+
Rhodamine B	Lignin	F^{au} - blue-white	+	+[b]	–	+[b]	–
	Lignin	F - salmon to red	+[e]	+[e]	±	–	–
	Lipids	F - white-pink	–	–	–	+[c]	–

[a] B = brightfield; F = fluorescence; and F^{au} = autofluorescence.
[b] Particularly the compound middle lamella.
[c] Particularly the narrow wall layer next to the cell lumen.
[d] Results within brackets hold for some cells only.
[e] Partially or entirely overshadowed by blue autofluorescence unless treated beforehand with NaOH.

colonized and the newly formed tissues. As illustrations from all histochemical tests cannot be presented, those of tests considered to be most significant or representative have been selected. A summary of the results from all tests is shown in Tables 1 and 2.

Location of reaction zones in the xylem. Compartmentalization of the fungus within the invaded xylem was achieved vertically by occlusion of vessels with gums (wall 1) and horizontally by circumscription of the affected area with reactions zones (walls 2 to 4). Xylem vessels and intercellular spaces in the defense area were occluded with yellow to brown material (Fig. 2D and E). The reaction zones were characterized by thickening (appositions), lignification, and suberization of walls of xylem parenchyma cells and, at times, suberization of walls of xylem fibers, as well as by proliferation (hyperplasia) of xylem parenchyma cells immediately outside of the reaction zone (Figs. 2 to 4).

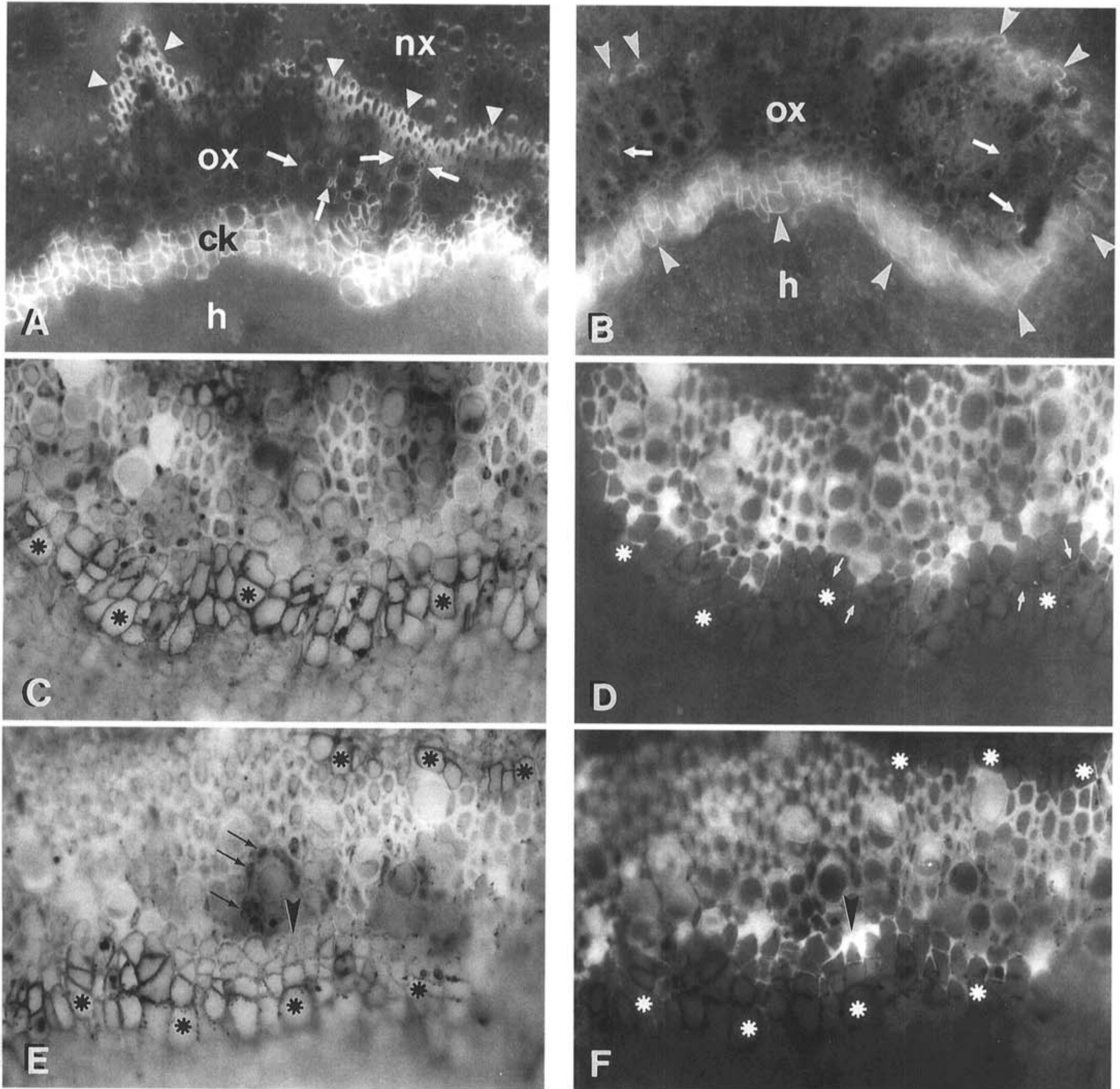

Fig. 3. Reaction zones in the xylem of resistant carnation cultivar Novada inoculated with Fusarium oxysporum f. sp. dianthi (32 days postinoculation). **A,** Residual fluorescence counterpart of Figure 2E (Mäule's test without NaOH; ×125). Several layers of cork cells (ck) separate the occluded xylem (ox) from the hyperplastic tissue (h) (wall 2). Suberized cells are within the compartmentalized xylem (white arrows). The occluded xylem is separated from the new xylem produced by the original cambium (nx) by a layer of xylem fibers (wall 4) of which the secondary walls contain little or no syringyl lignin (Fig. 2E) and, thus, have enhanced residual autofluorescence (white arrowheads). Note residual fluorescence of vessel walls. **B,** Residual fluorescence counterpart of Figure 2F (acid phloroglucinol; ×125). Cork cells circumscribe the compartmentalized xylem (white arrowheads), but defense responses are weaker laterally (wall 3) and on the side of the newly produced xylem (wall 4) than on the side of the medulla (wall 2). Suberized cells within the compartmentalized xylem (white arrows) can be detected, but with some difficulty. **C to F,** Details (×250) of the section stained with Sudan black B shown in Figure 2D. **C and E,** Suberin of cork cells (a few examples marked by asterisks) is stained; but **D and F,** not the lignified primary wall region (white arrows) that partially retains its fluorescence. Suberized xylem parenchyma cells (black arrows) can be detected only with difficulty. Note fluorescence of lignified U-shaped appositions (black arrowheads) of wall 2 reaction zone cells immediately next to the cork cell layer.

The first layers of proliferating cells differentiated into thin-walled cork cells with thick suberin layers in a gradient to the existing thick-walled apposition cells that had lignified walls, but only some of which had thin suberin-containing layers (Figs. 2F to K and 3A to F). Suberin was recognized by its autofluorescence that was quenched by Sudan black (Fig. 3C to F), but not by acid phloroglucinol (Fig. 3B) or alkali-permanganate treatments. Lignification and suberization decreased from the reaction zone towards the colonized inner part of the compartmentalized area (Fig. 3A to F).

Compartmentalized tissues were replaced by regeneration of phloem, cambium, and xylem within the remaining proliferating xylem parenchyma next to the medulla (Figs. 1B and C and 2A and B). Proliferation of medulla parenchyma cells next to the proliferating xylem parenchyma cells occurred incidentally as described previously (2,3,6) but, in general, the perimedullary zone

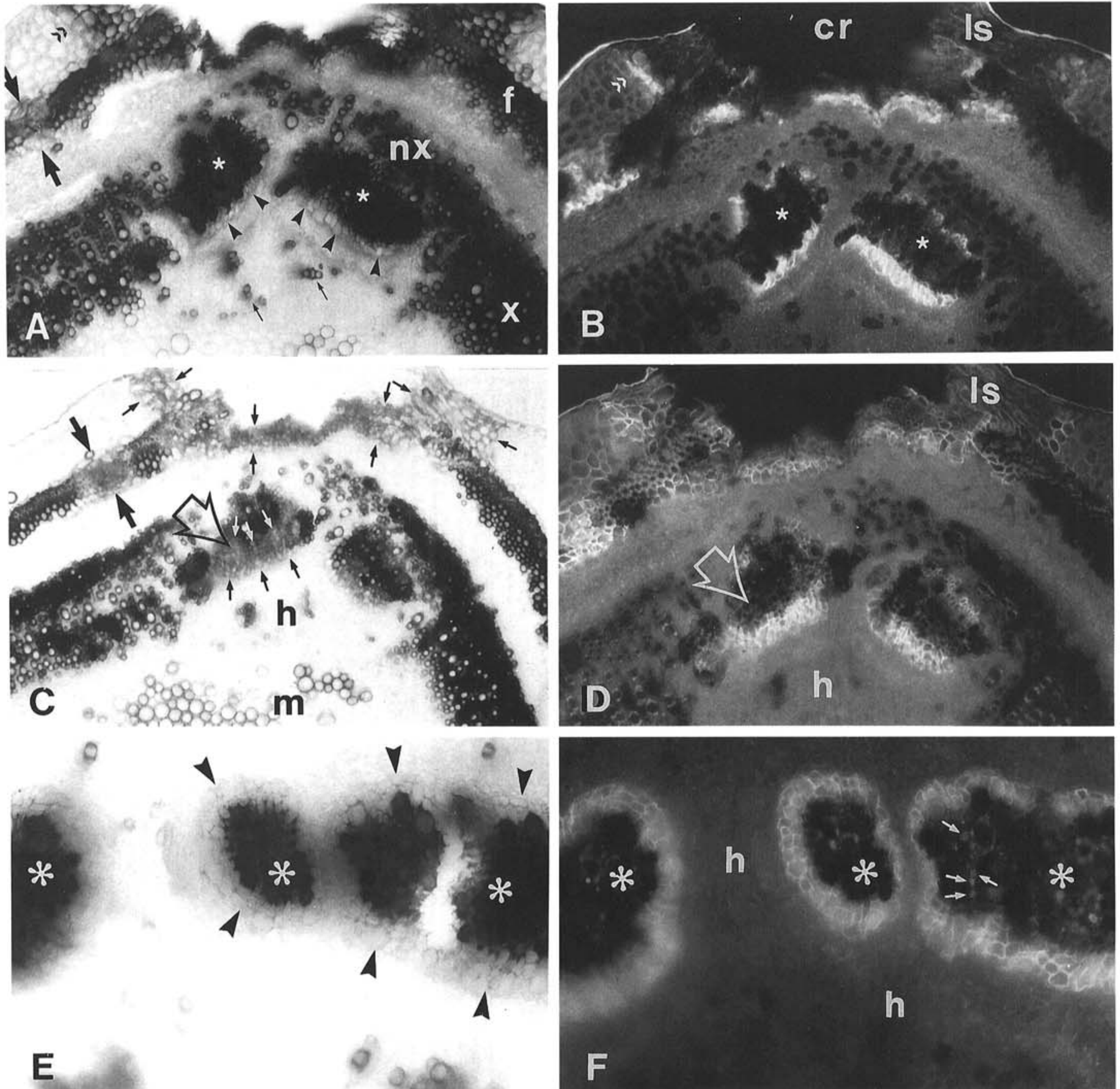

Fig. 4. Reaction zones in the xylem and cortex of resistant carnation cultivar Novada inoculated with *Fusarium oxysporum* f. sp. *dianthi* (32 days postinoculation). **A and B,** Compartmentalized xylem (*) is separated from the regenerating xylem (small arrows) by a well-developed cork layer (arrowheads) (wall 2), while a less well-developed cork layer exists on the side of the new xylem (nx) produced by the original cambium (wall 4). The margin of the crack (cr) in the cortex discloses several ligno-suberized cells (ls) and, next to these, cork cells as part of a necrophylactic periderm (»). A gap in the fiber sheath (f) is filled with cork cells (large arrows). **A,** Both lignin and suberin stain red with acid phloroglucinol (brightfield), but **B,** only suberin retains its fluorescence (×63). **C and D,** Same area as in **A and B,** but stained with Mäule's test without NaOH. Walls of fiber cells in cortex and xylem are stained dark red. **C,** Unstained, slightly brownish walls of cork cells, ligno-suberized boundary zone cells, and lignified parenchyma cells in the center of the compartmentalized xylem (open arrow) have been marked by small arrows (brightfield; ×50). **D,** Suberized walls remain fluorescent, as do vessel walls (×63). **E and F,** Compartmentalized xylem (*) circumscribed completely by cork (arrowheads) along walls 2, 3, and 4. **E,** Both lignin and suberin stain red with acid phloroglucinol (brightfield), but **F,** only suberin retains its fluorescence. Some xylem parenchyma cells in the compartmentalized area have suberin (arrows) as judged by their residual fluorescence (×125). Further abbreviations: h = hyperplastic xylem parenchyma; m = medulla; and x = xylem.

sensu Esau (27) did not contribute significantly to the process of hyperplasia and vascular regeneration. The regenerating vascular tissues, in some cases, connected laterally with the unaffected vascular tissues (Fig. 1C) (2).

Well-developed reaction zones were mainly formed in the region next to the medulla (wall 2), while defense responses decreased in intensity along the lateral reaction zones (wall 3) towards the outer tangential zone (wall 4) (Figs. 1A and B, 2A to K, 3A and B, and 4A to D), although incidentally the compartmentalized area was completely circumscribed by well-developed reaction zones (Fig. 4E and F). Reaction zone formation, thus, seemed focused at protection of the regenerated cambium and xylem (wall 2) rather than the original cambium (wall 4).

Location of reaction zones in the cortex. Pressure exerted by extensive proliferation of the xylem parenchyma next to the medulla caused the stems to burst. Longitudinal cracks were, thus, formed that were deeper close to the incision (3 mm height), but often extended several centimeters upwards. The cracks crossed the cortical fiber sheath and were lined with a boundary zone of cells that were positive for lignin and suberin (Figs. 1; 2A, C, D, and K to M; and 4A to D). Suberin deposition was thick and abundant on the inner side of these reaction zones where a necrophylactic periderm could be recognized, but was less noticeable towards the edge of the crack (Fig. 4A and B). Suberin was also observed in cortical fibers situated in the reaction zone in continuity with the band of suberized cortex parenchyma (Figs. 2M and 5A). In both healthy-looking and infected parts of plants, as well as in untreated controls (data not shown), a thin layer of corklike cells often lined the inner side of the fiber sheath, frequently in association with gaps in the sheath that were usually filled with such corklike cells (Figs. 4A and B and 5B and C). The corklike cells lining cracks in the stems of inoculated plants often connected fluently to those lining the inner side of the fiber sheath and, in some cases, also to those lining the borders of the compartmentalized xylem (Fig. 1). Close to the point of incision, merging of vascular and extravascular reaction zones allowed the compartmentalized vascular tissues to be sloughed off through the cracks. In such cases, the necrophylactic periderm along the crack connected fluently with the periderm cell layer on the inner side of the affected xylem (Fig. 1C) (2). Cracks were not observed in untreated or mock-inoculated controls, nor were any other changes observed in the cortical tissues of control plants.

Histochemistry of additional wall layers of xylem parenchyma cells (polysaccharides). As expected, the compound middle lamella of xylem parenchyma cells stained for cellulose and pectin (Table 1). The pectin was partially esterified. When sec-

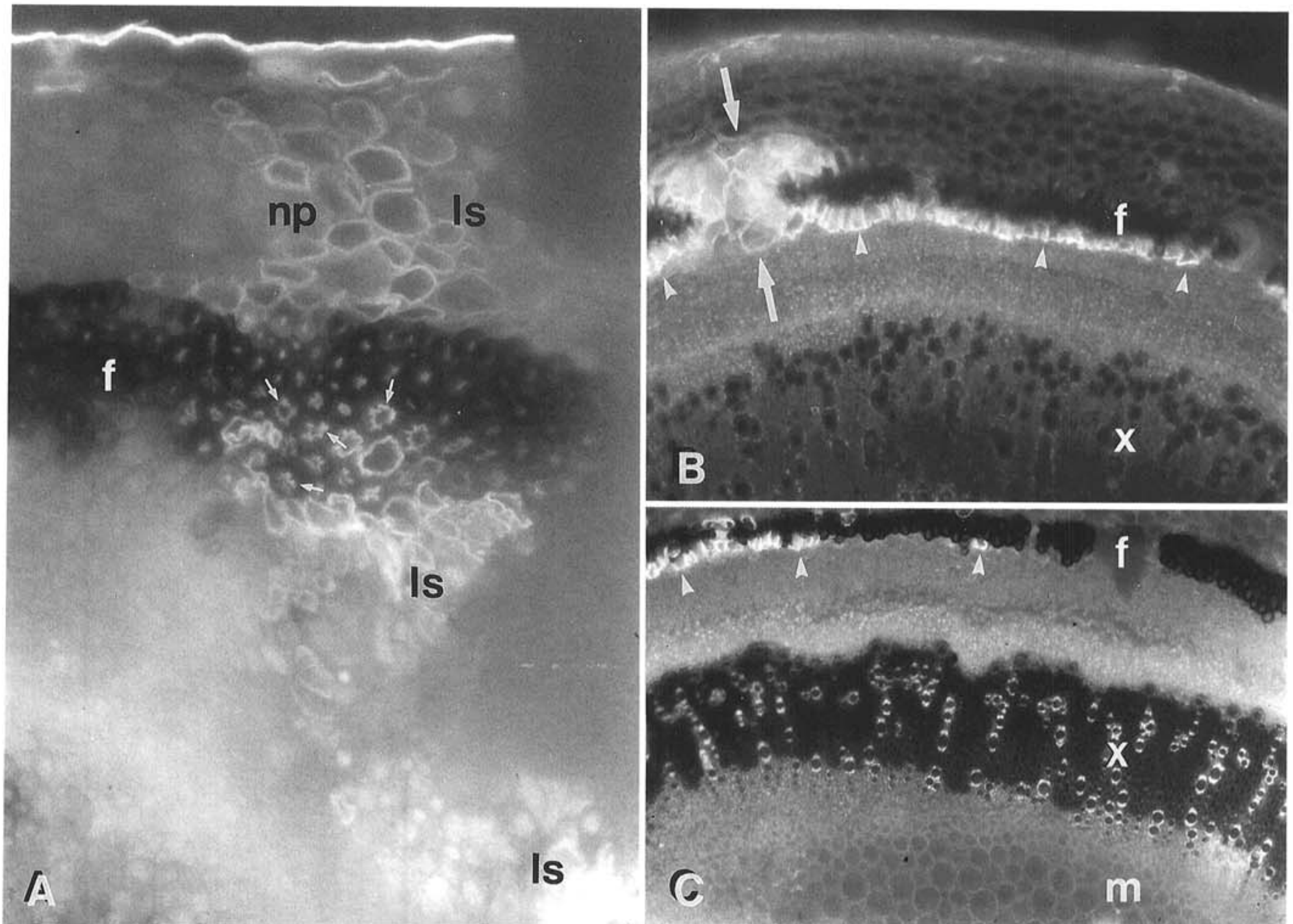

Fig. 5. Defensive structures and defense responses in the cortex of resistant carnation cultivar Novada inoculated with *Fusarium oxysporum* f. sp. *dianthi* (32 days postinoculation [dpi] unless mentioned otherwise). **A,** Residual fluorescence counterpart of Figure 2M (Mäule's test; ×235) showing the ligno-suberized boundary zone (ls) along a cortical crack. Suberin and cutin fluoresce; autofluorescence of lignin has been removed by treatment of sections with NaOH. Fibers partaking in the ligno-suberized boundary zone have suberized walls (arrows). Suberization in the boundary zone increases towards the necrophylactic periderm (np), even in the fiber sheath (f). **B,** The fiber sheath is lined internally by cork cells (arrowheads) that also fill a gap in the sheath (arrows). Residual autofluorescence of suberin after staining with acid phloroglucinol (×63; 16 dpi). **C,** Fiber sheath lined by cork cells (arrowheads) as in **B**. Residual autofluorescence of suberin and nonsyringyl lignin (secondary walls of vessels) after treatment with Mäule's test without NaOH (×63). The xylem area shown is unaffected by the fungus (m = medulla and x = xylem).

tions were treated with NaOH to remove ester groups, ruthenium red-positive middle lamellae stained considerably stronger (data not shown). Middle lamellae stained with hydroxylamine-ferric chloride for methyl-esterified uronic acid groups (esterified pectin), with staining being intensified by methylation of free carboxyl groups in hot acid methanol. Sections treated with NaOH did not react with hydroxylamine-ferric chloride.

The structure of wall appositions has been described previously (3) in detail from light micrographs of semithin sections of plastic-embedded material stained with toluidine blue. Apposition material reacted positively for noncrystalline polysaccharides with free vic-glycol groups (periodic acid-Schiff's test), particularly at the margin of the compartmentalized xylem. In sections that had been treated with the aldehyde blocker DNPH before the PAS test, lignified secondary walls of vessels and fibers were characterized by alternating red (PAS-positive) and yellow (DNPH-positive and PAS-negative) layers (Table 1), presumably indicating the presence of alternating layers of crystalline and noncrystalline polysaccharides. This was not the case for the appositions (data not shown).

The appositions did not convincingly stain for unesterified pectin with ruthenium red or other tests for pectic acids (Table 1). However, all secondary walls in cortex, xylem, and medulla, as well as the thick appositions at the margin of the compartmentalized xylem, were stained with hydroxylamine-ferric chloride for methyl-esterified uronic acid groups (Fig. 2G).

The apposition material did not react generally for cellulose, chitin, or callose (Fig. 6; Table 1). While lignified cellulose in secondary walls of vessels and fibers in the compartmentalized area was stained by chlor-zinc-iodide, chlorazol black E, and Uvitex 2B if sections had been treated with NaOH; apposition layers were not stained even after such treatment (Fig. 6A and B). Lack of birefringence of apposition walls also indicated the lack of oriented crystalline polysaccharides such as cellulose or chitin; secondary walls of vessels and fibers in the defense area were strongly birefringent (Fig. 7). Enhanced birefringence of walls, but not the compound middle lamella, of ligno-suberized cells (Fig. 7B and E) was presumably because of the presence of suberin rather than additional or newly oriented layers of cellulose.

Minute spots of callose were in regular and regenerated phloem as judged with alkaline aniline blue fluorescence (Fig. 6C). Callose was not detected in appositions or in other parts of the compartmentalized xylem.

Histochemistry of additional wall layers of xylem parenchyma cells (lignin, suberin, phenolics, and lipids). Appositions of parenchyma cells in the compartmentalized xylem stained for lignin aldehydes with acid phloroglucinol (Table 2). Staining was intense close to the marginal reaction zones, but unequal in the center of the compartmentalized area (Fig. 2F). Treatment of sections with NaOH slightly reduced the staining response of the appositions and the vessel walls, but more considerably that of the secondary walls (but not the compound middle lamella) of fibers, indicating the prevalence of esterified lignin aldehydes in fiber walls but not in appositions. Oxidation of lignin aldehyde groups by treatment of sections with permanganate for 5 to 60 min com-

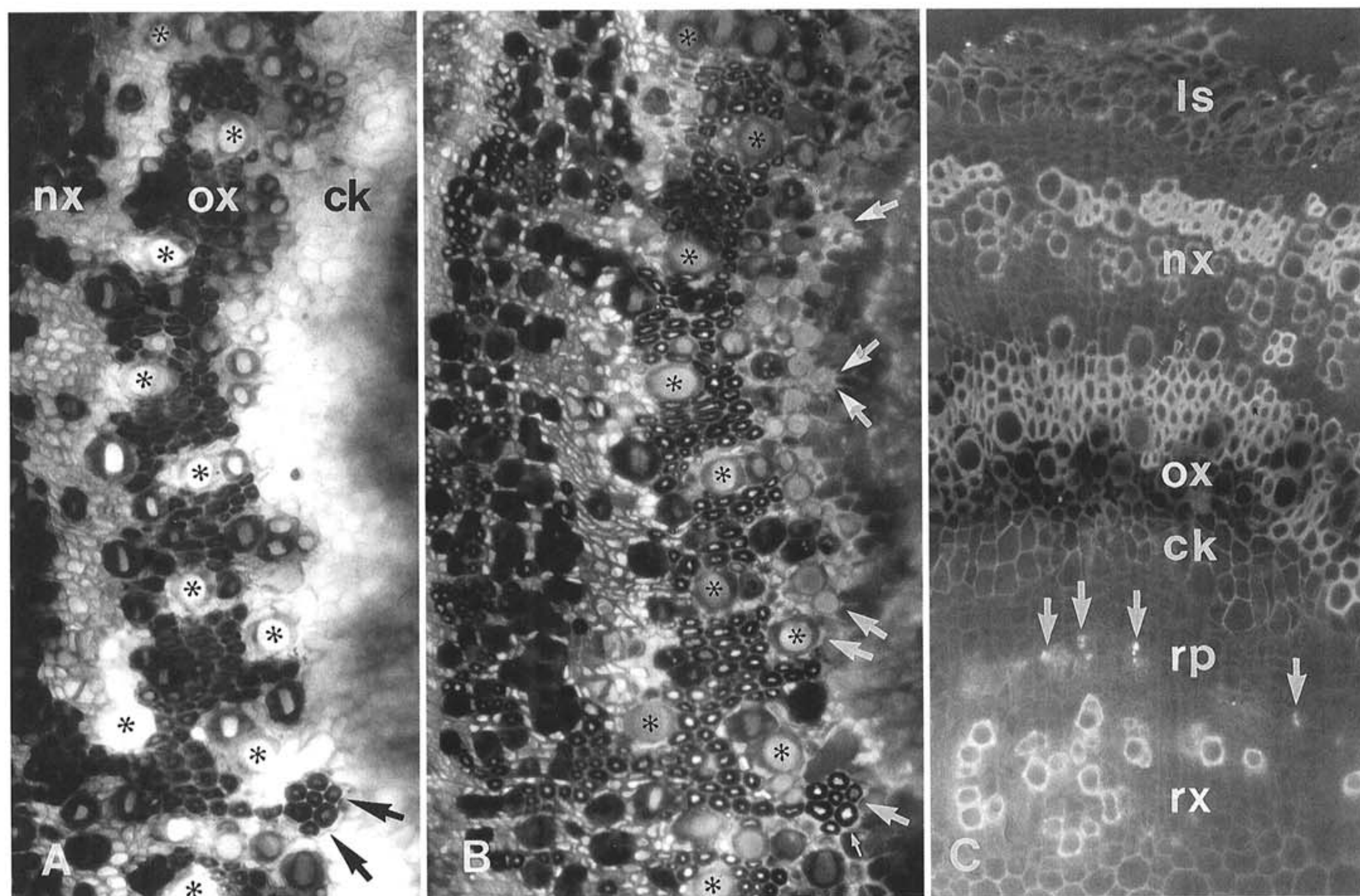

Fig. 6. Tests for cellulose and callose performed on sections from stems of resistant carnation cultivar Novada inoculated with *Fusarium oxysporum* f. sp. *dianthi* (32 days postinoculation). **A and B,** Chlor-zinc-iodide test preceded by NaOH (×167). Cellulosic primary walls and secondary walls of vessels and fibers **A,** stain blue-black in brightfield and **B,** are black under ultraviolet light. **A,** Apposition walls (arrows) all along the border of the compartmentalized xylem and the cork layer stain yellow and are not black under ultraviolet light. Cork cell walls also stain yellow, as do the contents of occluded vessels (*). **C,** Aniline blue fluorescence (×167). Callose (arrows) is in regenerated phloem in the hyperplastic area, but not in the compartmentalized xylem. The remaining fluorescence visible results from background autofluorescence of lignified and suberized cell walls. Abbreviations: ck = cork cell layer; ls = ligno-suberized boundary zone; nx = new xylem produced by the original cambium; ox = occluded xylem; rp = regenerated phloem; and rx = regenerated xylem.

pletely removed the staining response. Compared with the lignified walls of unaffected vessels and fibers, walls lignified as part of the defense response, as well as vascular gums, were more resistant to permanganate treatment and, thus, appeared more rich in lignin aldehydes. Indeed, these walls and gumlike materials were intensely stained with Schiff's reagent for aldehydes. Staining of these sites with Schiff's could not be entirely blocked by prior treatment with DNPH even after several days, whereas regular lignin aldehydes in walls of vessels and fibers had been blocked completely. The same sites stained intensely greenish yellow with auramine O (Fig. 2I), again indicating the presence of lignin.

Appositions were mostly unstained with Mäule's test for syringyl lignin (Fig. 2E; Table 2), indicating the prevalence of guaiacyl lignin, phenyl lignin, or both over syringyl lignin. Only exceptionally, walls close to the cork cell layer stained slightly (data not shown). Results were unaltered when sections had been treated with NaOH before the test, indicating that negative staining responses were not because of the inaccessibility of lignified walls to the stain.

Autofluorescence of lignin aldehydes in appositions and other lignified cell walls was quenched by acid phloroglucinol (Figs. 3B and 4B and F; Table 2). Lignin autofluorescence was also

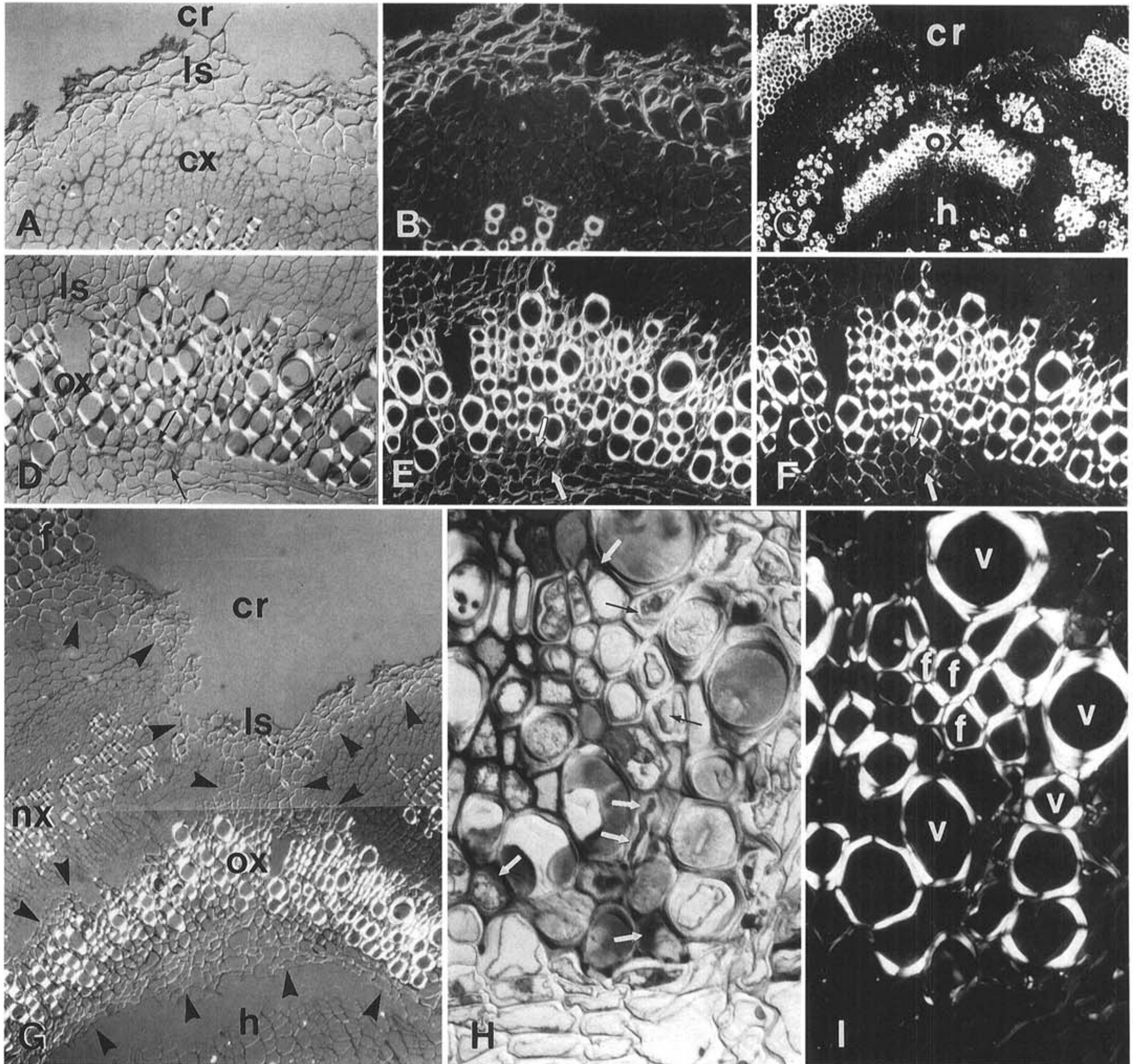

Fig. 7. Tests for birefringence performed on semithin sections from glycol methacrylate-embedded samples from a previous experiment (6) representing similar defense responses as in Figures 1 to 6 (42 days postinoculation). **A, B, D, E, and G,** Differential interference contrast and **C and F,** polarization contrast micrographs of an unstained section. **B and E,** The prism was positioned to give birefringence against a dark background, resulting in a better resolution than with polarization contrast. **A and B,** Details of the ligno-suberized boundary zone (ls) along the crack (cr) in the cortex (cx) showing birefringence of suberin (×167; ×210). **C and G,** General view (×42; ×104). A fluent connection exists (arrowheads in **G**) between the ligno-suberized boundary zone along the crack, the reaction zone bordering the occluded xylem (ox) (wall 4), and wall 2. **D to F,** Details of the compartmentalized xylem showing birefringence of cellulosic secondary walls of vessels and fibers. Suberized reaction zone cells also display birefringence, while walls of regular parenchyma cells are, at best, weakly birefringent (×167). **H,** Brightfield and **I,** polarization contrast micrographs of a section stained with toluidine blue O (×625). Secondary walls of vessels (v) and fibers (f) display birefringence, but appositions (arrows) do not. Further abbreviations: h = hyperplastic parenchyma and nx = new xylem produced by the original cambium.

quenched by treatment with permanganate, except for the compound middle lamella of fibers and the secondary walls of vessels, the autofluorescence of which was only removed when sections had been treated successively with NaOH and permanganate (Table 2). Residual autofluorescence of tissues in sections treated with Mäule's test, which includes a permanganate step, was identical to that after treatment with permanganate only. Lignin autofluorescence was not quenched by Sudan black B (Fig. 3D and F).

Autofluorescence of suberized walls and suberin-containing linings of appositions was quenched by staining with Sudan black B (Fig. 3D and F), but not by acid phloroglucinol or alkali-permanganate treatments (Figs. 3A and B and 4).

Lignified secondary walls of vessels, fibers, and other medullary and cortical cells with secondary walls fluoresced orange-red with rhodamine B. However, the compound middle lamella of fibers and the secondary walls of vessels retained their blue autofluorescence (Fig. 2J to L). Residual autofluorescence of these walls was removed with NaOH treatment, rendering such walls orange-red throughout (Table 2). Appositions were stained slightly with rhodamine B close to the suberized margins of the defense area, but generally not in the central part (Fig. 2J). These results indicated that defense-induced lignin differs chemically from the lignin in vessels and fibers.

Walls of corklike cells, lignified apposition walls, and gumlike material stained deep violet with gentian violet, while regular lignin of fibers and vessels stained pink (Fig. 2H). Defense-induced lignin in appositions and gums, thus, resembles the lignin fraction of suberin rather than vessel or fiber wall lignin.

Histochemistry of corklike cells. Well-developed cork cells (Fig. 3) had thin walls coated with suberin. There was a gradual transition from this type of cell to thick-walled xylem parenchyma cells, the appositions of which were ultimately lined with suberin. Staining responses with the histochemical tests used were essentially the same in both types of cells. With rhodamine B, the well-developed suberin layer of corklike cells fluoresced blue, lined with white-pink on the side of the cell lumen (Table 2), indicative of the presence of lipids. The cuticle was the only other structure to fluoresce white-pink rather than orange-red with rhodamine B.

Histochemistry of cortical parenchyma cells along cracks. Cortical parenchyma cells lining cracks in the stem had slightly thickened walls and stained for lignin and suberin, giving a reaction similar to that observed in appositions produced by xylem parenchyma cells. Thickened walls of cortical parenchyma stained for methyl-esterified uronic acid groups rather than for cellulose or callose. Walls stained for lignin aldehydes (Fig. 4A), but virtually not for syringyl lignin (Fig. 2M), and they fluoresced greenish-yellow with auramine O, thus, resembling defense-induced lignin in the xylem in all aspects. At the edge of the crack, cells had a very thin suberin layer (Fig. 5A) and were not, or very slightly, stained with rhodamine B (Fig. 2L). Further off, the suberin layer increased in thickness and cell walls fluoresced orange-red with rhodamine B. The final cell layers consisted of typical cork cells in an area of meristematic activity (necrophylactic periderm) in which blue autofluorescence was superimposed on the orange-red fluorescence with rhodamine B as it was for the periderm-like layer bordering the compartmentalized area (Fig. 2J to L). Although the basis for staining of lignin with rhodamine B is not known, the staining responses in cortical defense areas with rhodamine B clearly resembled those in the vascular region.

Histochemistry of gumlike materials. The yellow, orange, or brown material in vessel elements stained for polysaccharides with free (pectic acid) or methyl-esterified uronic acid groups, but not for cellulose or callose (Table 1). This material did not stain for lipids; it stained for lignin aldehydes and phenolics, but the lignin did not stain with Mäule's test for syringyl lignin, nor did it fluoresce with rhodamine B (Fig. 2E and J; Table 2). Gumlike materials fluoresced greenish yellow with auramine O (Fig. 2I). Yellow to brown amorphous material with similar staining properties also permeated the intercellular spaces at the margins of the compartmentalized xylem and slightly permeated the ligno-suberized boundary zone along stem cracks (Fig. 2D).

DISCUSSION

Reaction zones formed in carnation xylem in response to infection by *F. oxysporum* f. sp. *dianthi* were characterized by thickening (appositions), lignification, and suberization of walls of xylem parenchyma cells and, at times, also the suberization of walls of xylem fibers. Thick appositions at the margin of the compartmentalized xylem stained similarly to secondary walls of vessel elements, fiber cells, and sclerified parenchyma cells with hydroxylamine-ferric chloride. This test for methyl-esterified uronic acids (29) is employed for the detection of pectin (30,37,65). However, the presence of unesterified pectin could not be convincingly demonstrated in the appositions. Secondary walls are also known to contain very little pectin (12,51). It is, therefore, possible that hydroxylamine reacts with methyl-esterified carboxyl groups of cell wall polysaccharides other than pectin. In secondary walls, the reacting polysaccharides are probably xylans (29,51). Xylans, the major hemicelluloses of secondary walls of angiosperms, have one glucuronic acid side group for every 10 xylose groups, and most of these are esterified (38,51,52). Whether the structural polysaccharides in appositions are xylans, esterified pectins, or still other polysaccharides will be elucidated by the use of gold-labeled antibodies. Secretion of xylans or pectins and their deposition in walls, followed by infusion with phenolics and lignification, would at any rate provide a functional and rapid response to attack by pathogens or wounding. Lignin is markedly resistant to most microbial wall-degrading enzymes and is linked covalently to pectin (primary walls) and glucuronoxylans (secondary walls), but not to the cellulosic frame of the wall (38,39).

Callose was not detected in apposition walls. Although callose has been found in other plants (43,44,69), its production is by no means a common feature of defense responses in the xylem (14,58,63).

The absence of cellulose and unesterified pectin from wall appositions, except for the innermost layer, has also been observed by others (13,72). Although the tests for cellulose and unesterified pectin were not positive, the presence of these compounds in the appositions cannot be completely excluded. Indeed, transmission electron microscopy (TEM) observations of appositions have indicated that they may be heterogeneous and that traces of pectin and cellulose can sometimes be observed, but perhaps not in sufficient amounts to be detectable with the tests used here at the light microscope (LM) level (55). Our TEM studies with labeled probes have helped to confirm many of the current observations and will be presented in more detail in forthcoming publications. It is clear that appositions eventually become covered by other types of wall layers as observed in other plants (54), and the boundary between such layers and the appositions may not always be clear-cut enough to completely delineate them at the LM level. Furthermore, the normal deposition of cellulose and other compounds in wall appositions may be quite disturbed; for example, orientation of crystalline cellulose, if present at all in the appositions, may be such as not to give any birefringence.

Wall apposition lignin differed from that of vessels and fibers and rather resembled the lignin of cork cells in its lack of syringyl groups. Vessels are themselves poor in syringyl lignin compared with fibers (47) and stained less intensely with Mäule's test. Syringyl lignin is degraded more rapidly than guaiacyl, followed by p-hydroxyphenyl lignin, which is the most resistant (20), and in-

deed is selectively removed from infected carnation xylem (48,49) as judged by pyrolysis-mass spectrometry. Cork cell walls and appositions were equally stained dark violet with ammoniacal gentian violet, a stain used to locate suberin (28), but more probably specific for the nonsyringyl lignin fraction of suberized walls.

In the neoplastic tissues (cortical and vascular defense responses), a gradual transition occurred from thin-walled cork cells with a well-developed suberin layer to parenchyma cells with thick appositions lined by a thin suberin layer. Although part of the paratracheal parenchyma cells laid down at the onset of infection had apposition walls not lined by suberin, it seems apparent that cork cells and apposition-bearing xylem parenchyma cells share some homology. The resemblance extends to the cells of the ligno-suberized boundary zone, the necrophylactic periderm along cracks in the surface of the stem, and probably also to the exophylactic periderm of roots. Both in stems (2) and in roots (11), intravascular periderm may connect with the necrophylactic or exophylactic periderm during sloughing-off of compartmentalized xylem. Similar connections have been described for trees (17).

Sloughing-off of compartmentalized areas is well-known for trees. Localized bark infections are eventually shed as bark scales (18,71,72). However, infected wood cannot be removed, but is rather walled-off and sometimes overgrown by new wood layers produced by the cambium (18,19). Preservation or regeneration of the cambium is extremely important for the survival of infected trees (21). This explains why the strongest defense reactions of trees (suberization of parenchyma cells and fibers) usually are those in wall 4 of the CODIT model (45,57,63). Walls 1 to 3 may also involve suberization, however (16,61). Compared with trees, carnations are highly flexible in growth. This flexibility allows them to regenerate vascular tissues internal to the infected ones, a phenomenon apparently not possible in trees. Typically, the regenerated vascular tissues (including a new cambium) rather than the original cambium are protected from the pathogen. In terms of the CODIT model, wall 2 is more important for carnations than wall 4. The underlying mechanism (particularly suberization of parenchyma cells and fibers) and its purpose (protection of the functional cambium) are clearly the same. Newly differentiated vascular tissues may even function in the vicinity of colonized ones suffering degradation thanks to internal periderm (2).

As proposed by Shigo (66), wall 2 in trees restricts the inward spread of pathogens and is composed of preexisting cells such as fibers in the latewood that possess thick walls. Wall 3, restricting lateral colonization, is formed out of existing ray cells. Although similar in location, the phellem cells produced by the hyperplastic xylem parenchyma internal and lateral to the infected xylem in carnation clearly differ in ontogeny. It may be questioned, however, whether in trees a limited amount of meristematic activity may also accompany suberization in walls 2 and 3. On the other hand, the observed continuity of necrophylactic periderm along cortical cracks with suberized preexisting sclerenchyma cells of the fiber sheath suggests that suberization itself, not the associated development of secondary meristems, is the essential factor in defense.

Tyloses, particularly suberized ones, form a vertical barrier to invasion (wall 1) in many plant species, but are extremely rare in carnation as judged by light (3) and electron (G. B. Ouellette, *unpublished data*) microscopy. Rather, vessels are occluded with lignified gums with similar composition as wall appositions, except for the additional presence of pectin. Like cork lignin, that of gums was phloroglucinol-positive, but Mäule-negative. Substances with these characteristics have been described in a general term as wound gum (22). Gumlike material was not only found in xylem vessels, but also within the intercellular spaces demarcating ligno-suberized cells of xylem and cortex. They probably reflect active secretion of apposition materials through walls into the apoplast.

Defense responses in carnation are strikingly similar to compartmentalization processes in bark and wood of trees. For bark, similar conclusions were drawn by Biggs (14) who stated that boundary zone formation in herbaceous and woody plants is fundamentally similar. Little attention has been given thus far to compartmentalization responses in xylem of herbaceous plants, which may shed a new light on similar responses in trees. One example is balsam poplar, in which wall 2 closely resembles that of carnation in structure and ontogeny; it also comprises a band of corklike cells that are formed out of dedifferentiating xylem and perimedullary parenchyma cells (56,63; D. Rioux, *unpublished data*). Inversely, compartmentalization processes may explain part of the symptomatology of wilt diseases in herbaceous plants. A clear example is the strong vascular browning in stems of unilaterally wilting carnations, which has been considered typical for the disease. A close examination of this phenomenon reveals that the browning is restricted to the outer and inner margins of the colonized xylem and can be identified as lignified and suberized reaction zones similar to those described in this study (7). This is supported by the observation that the limited quantities of phytoalexins found in diseased susceptible carnations are largely present within these brown reaction zones (48). Unilateral wilt of carnation plants is apparently due to failing vertical defense responses rather than failure of lateral compartmentalization. A more general application of the CODIT model to fungal diseases in herbaceous plants, thus, seems promising for achieving a better understanding of various features of defense in time and space.

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