Neth. J. Pl. Path. 91 (1985) 119-135

Colonization and histopathology of susceptible and resistant carnation cultivars infected with Fusarium oxysporum f. sp. dianthi

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Accepted 20 February 1985

Abstract

Stems of the susceptible 'Early Sam' and resistant 'Novada' carnations were inoculated with a conidial suspension of *Fusarium oxysporum* f. sp. *dianthi.* Stem segments of either cultivar were sampled regularly and used for determination of fungal growth and for microscopical investigation.

'Early Sam' showed typical *Fusarium* wilt symptoms and its stems were colonized intensively. The observed vascular browning appeared to be caused by discolouration of primary walls of infected vessels and surrounding cells. Vessels were rarely occluded with gel. Cell wall degradation led to the formation of stem cavities. Hyperplasia of xylem parenchyma was not seen.

In 'Novada', fungal colonization remained low throughout the experiment. Macroscopic symptoms were absent except for longitudinal bursts in the stem, which appeared to be caused by hyperplasia of xylem parenchyma bordering infection. Vascular gelation occurred in the infected tissues, causing some vascular browning also. Xylem vessel regeneration was observed in the hyperplastic layer. Cavities were not formed, and wall discolouration was rare. Vascular gelation is considered part of the *Fusarium* wilt resistance mechanism. It is followed by xylem vessel regeneration, which expresses a general plant response to vascular dysfunction rather than being part of the resistance mechanism.

Although of different origin, vascular browning as such occurs in both susceptible and resistant interactions. In breeding for resistance, care should hence be taken with the current use of browning as an indication of disease.

Additional keywords: Dianthus caryophyllus, resistance mechanism, vascular browning, gel formation, vessel regeneration.

Introduction

Wilt diseases have long inflicted serious damage to commercial carnation crops. Bacterial wilt and *Phialophora* wilt have been the subject of many investigations (Hellmers, 1958; Nelson and Dickey, 1966; Péresse, 1975). Up to 15 years ago *Phialophora cinerescens* (Wollenw.) v. Beyma was considered the most important wilt-inducing pathogen of carnation, and research as well as plant breeding programmes were focused on this pathogen. During the 1970's, however, *Phialophora* wilt was almost completely replaced by *Fusarium* wilt (Garibaldi, 1978). Research has since centered on *Fusarium* wilt; histopathological work has been carried out by Pen-

nypacker and Nelson (1972) with a susceptible cultivar (White Sim), and recently by Harling et al. (1984) and Harling and Taylor (1985) with a susceptible (Red Baron) and a resistant cultivar (Carrier 929).

The present study is part of a research project that aims at characterizing carnation resistance to *Fusarium oxysporum* f. sp. *dianthi* in order to aid resistance breeding. In the present study, special attention has hence been paid to aspects of possible practical importance for resistance breeding, and carnation cultivars have been used that are currently grown in the Netherlands. Of the cultivars chosen, the spray carnation 'Early Sam' is susceptible. 'Novada' is one of the most *Fusarium* wilt-resistant selections bred at the Institute for Horticultural Plant Breeding, Wageningen, where a long-term carnation resistance breeding programme has been carried out. The programme was initially directed at *Phialophora* wilt but later at *Fusarium* wilt (Sparnaaij and Demmink, 1977). The genetic origin of 'Novada' differs from that of the resistant selection used by Harling et al. (1984) and Harling and Taylor (1985) (L.D. Sparnaaij and J.F. Demmink, personal communication), rendering this cultivar especially interesting for further investigation. Both susceptible and resistant interactions are characterized, and possible anatomical bases for resistance are discussed.

Materials and methods

Plant and fungal material. Rooted cuttings of the susceptible carnation cultivar Early Sam, obtained from M. Lek & zonen B.V., Nieuwveen, and the resistant cultivar Novada, obtained from the Institute for Horticultural Plant Breeding, Wageningen, were planted in steamed soil (8 cm diameter pots) and grown for 3 weeks prior to inoculation in the glasshouse at 20 tot 24 °C.

A virulent isolate (WCS 816) of *F. oxysporum* Schlecht. f. sp. *dianthi* (Prill. & Delacr.) Snyder & Hansen, obtained from the Research Station for Floriculture, Aalsmeer, was grown on potato dextrose agar slants.

Preparation of inoculum and inoculation of plants. After culturing the fungus in Tchernoff's medium (Tchernoff, 1965) amended with 0.1% yeast extract on a reciprocal shaker for 5 days at 23 °C, mycelial fragments were removed by filtering the culture through sterile glasswool. The conidial suspension was washed twice in sterile tapwater and adjusted to a concentration of 10^7 conidia ml^{-1}.

Of both cultivars, 60 plants which had been kept dry for two days were stem-inoculated between the third and fourth node above the soil, where an artificial (Parafilm) 'pair of leaves' had been constructed. After sterilization of the stem surface with 70% ethanol, 20 μl of the conidial suspension was deposited on the Parafilm and against the stems on either side. The stems were incised horizontally through the droplets just into the xylem cylinder. Stem segments of 10 plants of either cultivar were sampled at 1, 3, 7, 11, 18 and 35 days after inoculation, and used for microscopical investigation as well as to determine fungal growth.

Determination of fungal growth. Stem pieces between 10 and 65 mm above the site of inoculation were freed of leaves and after surface sterilization, stem parts 15 to 25 and 50 to 60 mm above the site of inoculation were cut out, chopped into small pieces and homogenized for 30 sec in 10 ml of sterile tap-water by means of an Ultra-Turrax

homogenizer. The homogenates were filtered through cheese-cloth. The filtrate was plated out in serial dilutions on Tchernoff's medium to which 80 IU sodium benzylpenicillin and 160 IU streptomycin sulphate ml^{-1} had been added. After 3 days incubation at 22 °C, fungal growth was determined by means of colony counting and calculation of mean propagule numbers over stem segments of 10 plants.

Microscopical preparations. When sampling carnation stems, the plants used in the above described paragraph were cut off below the site of inoculation and immersed into water immediately. Stem parts 6 to 10 mm above the inoculation site were cut off with a razor blade under water level and immediately fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 6.8-7.7, 22 °C).

To improve fixation, air was removed from the material by placing it under vacuum. After three washings in sodium cacodylate buffer at 3 °C for minimally 4h, specimens were dehydrated following the method described by Feder and O'Brien (1968) and stored several weeks in *n*-butanol at −20 °C. Specimens were embedded in polyethylene glycol methacrylate, obtained from Polysciences (Warrington, PA, USA) as JB-4 kit, following the provided instructions except for prolonging infiltration (one to three weeks). During this procedure the specimens were cut longitudinally and any remaining air was removed by incubation under vacuum. The cured blocks were further hardened during storage for several weeks in a desiccator and trimmed for better orientation of the specimens. Sections 3 to 4 μm thick were made using a Leitz rotary microtome equipped with glass knives, placed separately on drops of water on a microscope slide and dried down on a slide warmer at 55 °C. Sections were stained in the plastic, dried again on a slide warmer and mounted in Euparal. Generally the sections were stained in toluidine blue (Feder and O'Brien, 1968; O'Brien and McCully, 1981); other stains proven to be useful were aqueous ruthenium red (Jensen, 1962) and safranin 0-fast green FCF (Clark, 1981) used separately or in combination. Photographs of sections were made with a Leitz Orthoplan microscope with a Leitz Orthomat camera attachment and recorded on Agfapan 25 film (15 DIN).

Results

Macroscopic symptoms. Inoculated 'Early Sam' carnations exhibited typical *Fusarium* wilt symptoms. Chlorotic leaves, crook-neck shoots and wilt were seen from 18 days after inoculation on. At the 35th day, most plants were completely wilted. In this cultivar vascular browning was first observed after 7 days, 1 cm above the site of inoculation, becoming ever more prominent up to the 35th day. At about 7 cm from the inoculation site a little browning was seen after 11 days, becoming prominent also at the 18th to 35th day.

In 'Novada' carnations, only longitudinal bursts in the stem cortex were observed from the 11th day on, and locally a little vascular browning occurred shortly above the site of inoculation from the 7th day on. All inoculated 'Novada' carnations were still healthy at the end of the experiment.

Host colonization. Conidia appear to have been taken up into the stems of either cultivar in comparable quantities (Fig. 1). In 'Early Sam', after a short decrease an enormous increase in propagule quantity was seen in the stem segments at 15 tot 25

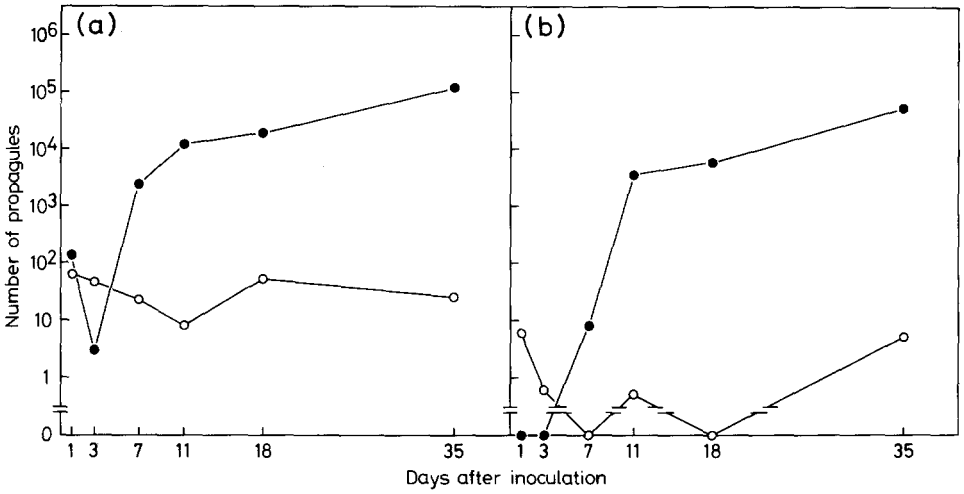
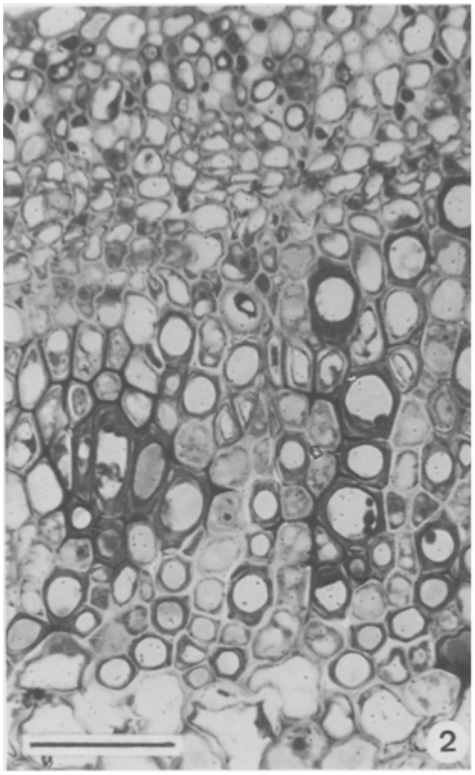


Fig. 1. Average number of propagules of *F. oxysporum* f. sp. *dianthi* in stem pieces of 10 mm length at 15-25 mm (a) and 50-60 mm (b) above the site of inoculation from 10 susceptible ('Early Sam', •) and 10 resistant ('Novada', ○) carnation plants at various times after inoculation with a conidial suspension of 10^7 conidia ml^{-1}.

mm height. A similar increase was seen some days later at 50 to 60 mm height. Propagules were found in all plants checked at 15 to 25 mm from 7 days on and at 50 to 60 mm height from 11 days on. In 'Novada', fungal growth remained very low throughout the experiment. Generally, small propagule quantities appeared to be present in stem parts 15 to 25 mm high, but almost none in parts 50 to 60 mm high. At either height, propagules were never found in all plants checked throughout the experiment. Contrary to the enormous spread of the fungus in 'Early Sam' plants, the fungal propagule quantity seemed to remain at inoculation level in 'Novada', i.e. a few propagules at 15 to 25 mm and almost none at 50 to 60 mm height.

In microscopical sections, the fungus was detected in vessel elements from the 1st day after inoculation on. In 'Early Sam', the fungus was present only in isolated vessels in small areas during the 1st week, spreading continuously, however, during the experiment (Figs 2-7). At the beginning of the 2nd week hyphae were present in larger areas, generally still in isolated vessel elements, but sometimes small xylem areas were completely colonized. The major part of the xylem, with exception of the fibers, and often the vascular cambium and phloem were colonized during the 2nd and 3rd week (Figs

Figs 2-4. Parts of stem sections from 'Early Sam' carnations infected with *F. oxysporum* f. sp. *dianthi*. All preparations stained with toluidine blue. Magnification bars represent 50 μm.
Fig. 2. Transverse section showing discoloured primary walls around xylem vessel elements containing hyphae in otherwise healthy vascular tissue (3 days after inoculation).
Fig. 3. Longitudinal section showing xylem vessels containing hyphae and gels (7 days after inoculation).
Fig. 4. Transverse section showing intensively colonized vascular tissue in which wall degradation is occurring (middle to right) and unaffected vascular tissue (left) (cx-cortex, f-fiber cylinder, p-phloem, x-xylem, m-medulla; 11 days after inoculation).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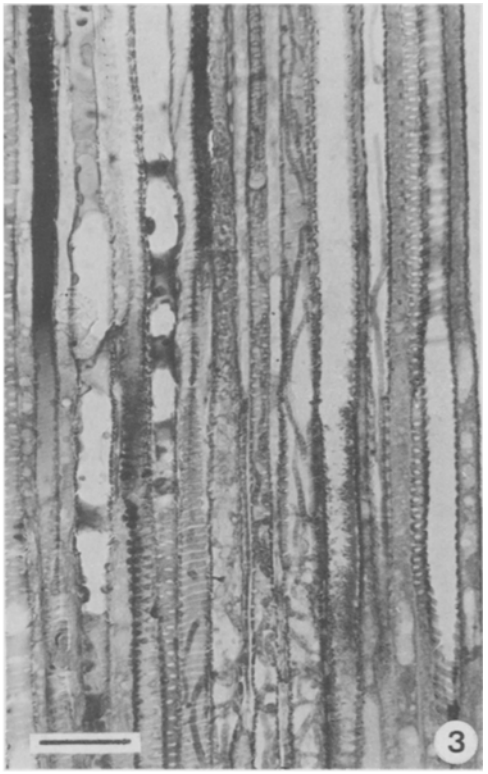
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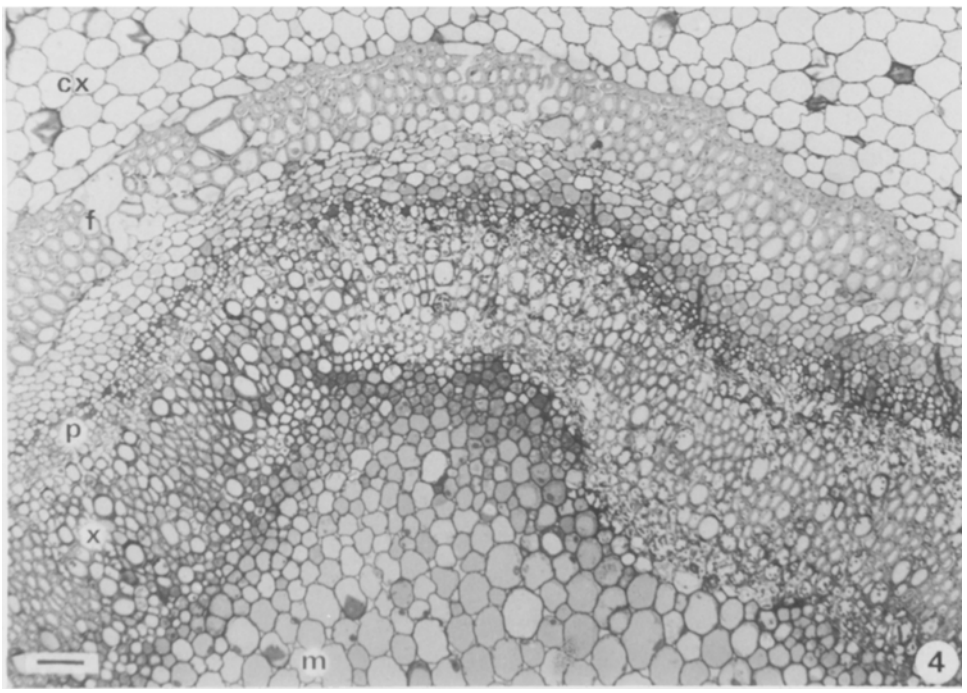
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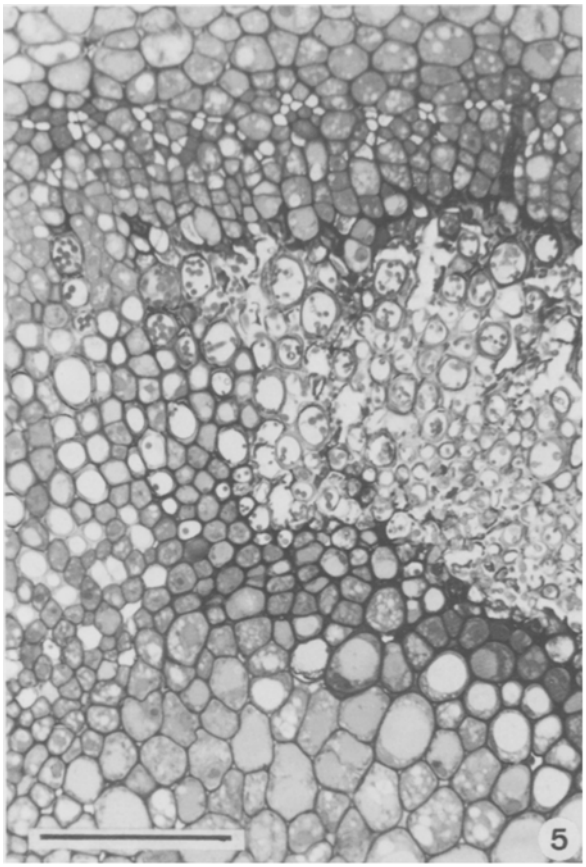
4-6). After 5 weeks the vascular cylinder generally was completely colonized; the fungus often spread further through medulla and cortex, and sometimes even formed sporodochia at the surface of the stem (Fig. 7). In 'Novada', fungal spread was very limited. The fungus could be detected only in a few isolated vessel elements in relatively small areas during the entire experiment (Figs 8-18), and seemed to be isolated effectively, though not eradicated, by the host. Sometimes fungal material could not at all be detected. In some plants, however, hyphae were present in larger, though still very restricted areas. In these cases, first seen 7 days after inoculation, only the xylem bordering the medulla was colonized, the major part of the vascular cylinder remaining unaffected (Fig. 14). In either cultivar, fungal spread was mainly vertical. Very little horizontal spread of the fungus occurred in 'Novada', whereas this was more common in 'Early Sam'.

Histopathology of the susceptible interaction. In 'Early Sam', discolouration of primary walls of infected vessels and surrounding xylem parenchyma cells was the first microscopically detectable response to the fungus (Fig. 2). Discoloured primary walls often were thicker than primary walls in unaffected tissue. Discoloured walls were first seen three days (rarely one day) after inoculation and were one of the most prominent host responses during the development of the disease. Corresponding intercellular spaces often were filled with discoloured material too. Cells with discoloured walls always formed the border zone of infected areas and occurred in the xylem or, when the entire xylem had been colonized, in phloem and medulla (Figs 5, 6). Finally formed cavities were also bordered by heavily discoloured walls and intercellular spaces containing discoloured material, representing the dark brown strands which can be seen macroscopically in diseased 'Early Sam' carnations (Figs 6, 7). Primary wall discolourations appeared to be associated with changes in cell metabolism. With onset of colonization, normally unstained parenchyma cells became densely chromophilic and filled with vesicles. Discolouration of primary walls sometimes was already seen at this stage (Fig. 5), but more often in connection with condensation of this chromophilic material against the cell wall and the formation of a large, unstained vacuole (Fig. 6).

Figs 5-7. Parts of stem sections from 'Early Sam' carnations infected with *F. oxysporum* f. sp. *dianthi*. All preparations stained with toluidine blue. Magnification bars represent 100 μm.
Fig. 5. Transverse section through vascular tissue showing the edge of a colonized area. Primary walls have disappeared within this area; at its edges primary walls and intercellular spaces are discoloured and neighbouring parenchyma cells contain many vesicles and stain darkly (11 days after inoculation).
Fig. 6. Transverse section through diseased vascular tissue. The xylem shows the beginning of cavity formation and is bordered by cells with heavily discoloured walls. In the medulla, a sharp border occurs between darkly stained parenchyma cells rich in vesicles (a) at some distance and unstained cells with discoloured walls (b) nearby the colonized tissues (18 days after inoculation).
Fig. 7. Transverse section showing the final stage of disease. The fungus has colonized the entire stem, caused large cavities in the xylem and formed sporodochia at the stem surface (35 days after inoculation).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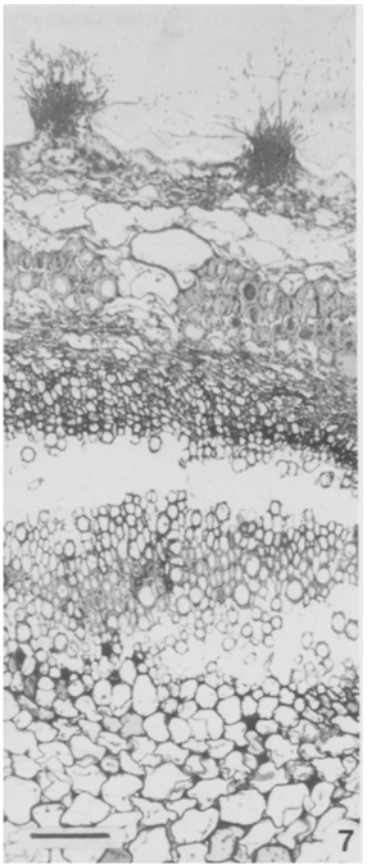
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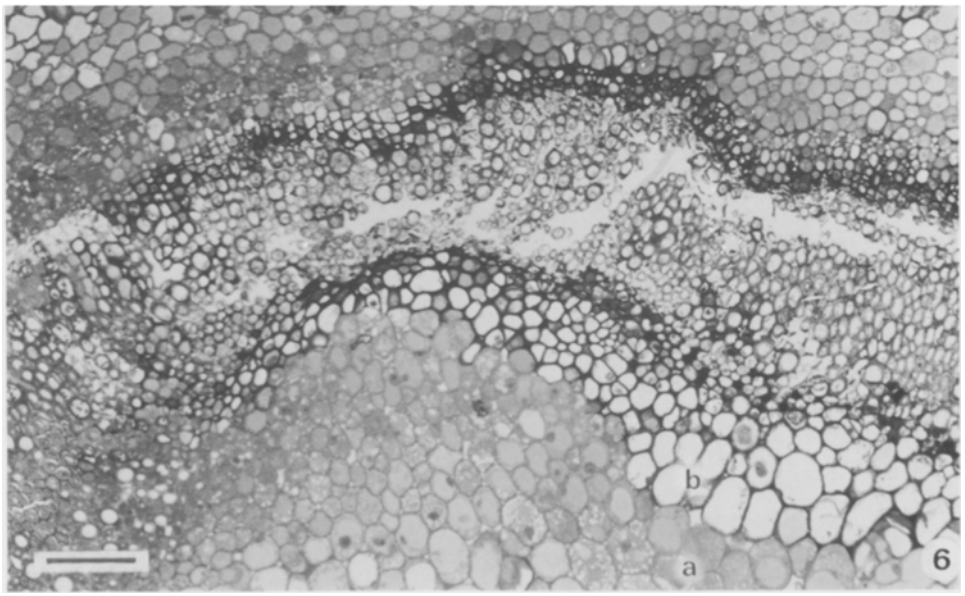
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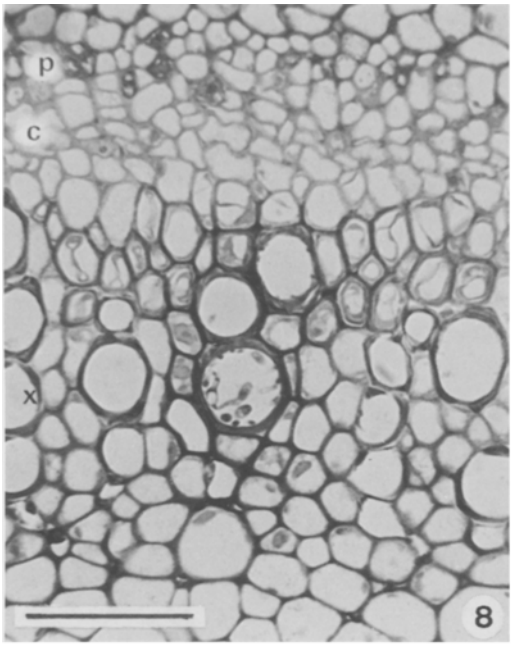
Gels were observed only seldom in vessels bordering the medulla. Hyperplasia or hypertrophy of xylem parenchyma was not observed.

Cell wall degradation was observed from the 3rd day on and resulted in cavity formation. Primary cell walls were broken down in xylem tissues colonized for some time (Fig. 5). Secondary walls were broken down more slowly so that apparently free-floating vessels occurred in the so-formed cavities in which no fungus was deteced any longer (Fig. 7).

Histopathology of the resistant interaction. In inoculated 'Novada' carnations, discolouration of primary walls was rare. Vessel occlusion by orange to dark brown gels appeared to be the predominant response to fungal invasion. Gels were often seen in vessels immediately surrounding those in which hyphae were growing; infected vessels often did not contain gels (Fig. 10). The gels showed considerable variation in structure and location. Often vessel pits as well as lumina were entirely filled with a dark, uniformly stained gel (Figs 9, 12); frequently, however, vessel lumina were only partly filled with gels which consisted of several differently stained zones (Figs 10, 11). Vessels containing gels or hyphae usually were restricted to the xylem part bordering the medulla, the younger metaxylem, cambium and phloem remaining unaffected (Fig. 14). Xylem parenchyma cells adjacent to infected vessels or vessels filled with gel regularly were densely stained and full of vesicles. Gel droplets were seen protruding into vessel lumina out of pits shared with such cells (Fig. 11). In other cases the contents of vessel-bordering cells became discoloured and granular, and wall discolourations were observed also (Fig. 10). Both vessels containing gels and adjacent xylem parenchyma cells with discoloured, granular contents are responsible for the relatively small brown strands in 'Novada' carnations. Stem cavities were not formed.

Hyperplasia of xylem parenchyma was detected in plants from seven days on. The shape of xylem parenchyma cells surrounding infected areas became irregular and cell contents stained densely (Fig. 10). Next, cell divisions occurred, generally only in the xylem parenchyma at the side of the medulla (Fig. 14), but rarely also at the cambial side (Fig. 13). Vessel parts sometimes were pushed away from the vascular cylinder by dividing cells (Fig. 12). Cell division resulted in rays of small, elongate cells forming a layer up to twice as broad as unaffected xylem of the same age from 11 days on (Figs 14-18). Tension created by the formation of this hyperplastic tissue led to the formation of bursts in the cortex bordered by wound tissue with discoloured cell walls already

Figs 8-11. Parts of stem sections from 'Novada' carnations infected with *F. oxysporum* f. sp. *dianthi*. All preparations stained with toluidine blue. Magnification bars represent 50 μm.
Fig. 8. Transverse section showing the fungus in one xylem vessel element in otherwise healthy vascular tissue (p-phloem, c-cambium, x-xylem, m-medulla; 1 day after inoculation).
Fig. 9. Transverse section showing gels completely filling lumina and pits of xylem vessel elements (7 days after inoculation).
Fig. 10. Transverse section through the vascular region showing xylem vessel elements only partly filled with gel and granular, discoloured xylem parenchyma cells (arrow) surrounding infected vessels (7 days after inoculation).
Fig. 11. Longitudinal section showing gel droplets (arrow) protruding into a xylem vessel element from pits shared with a neighbouring darkly stained parenchyma cell rich in vesicles (7 days after inoculation).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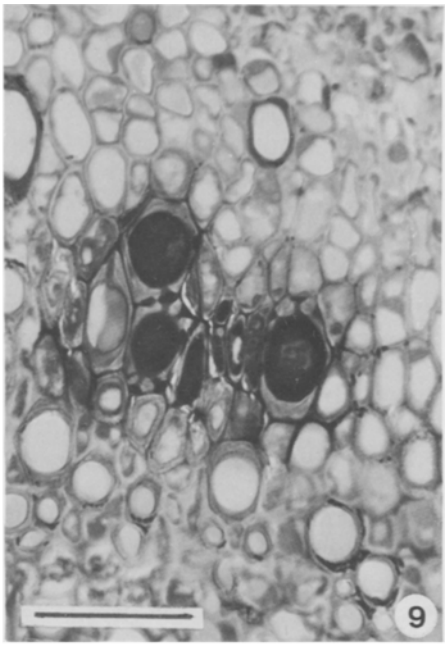
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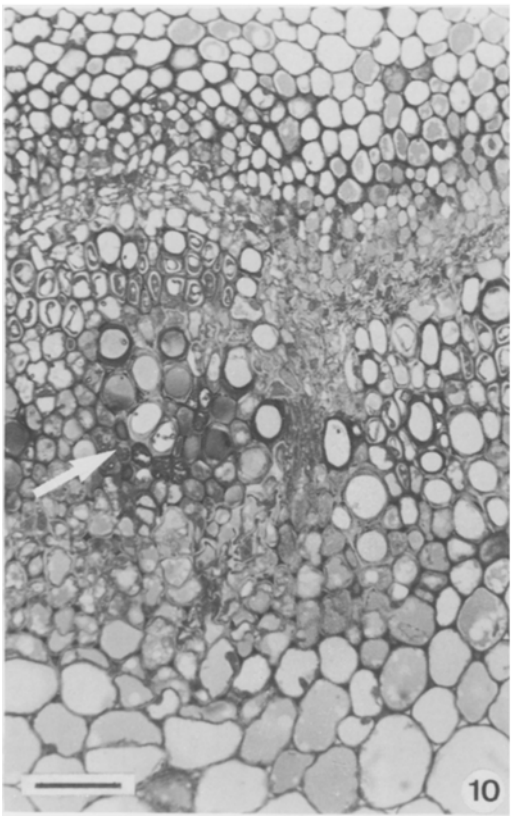
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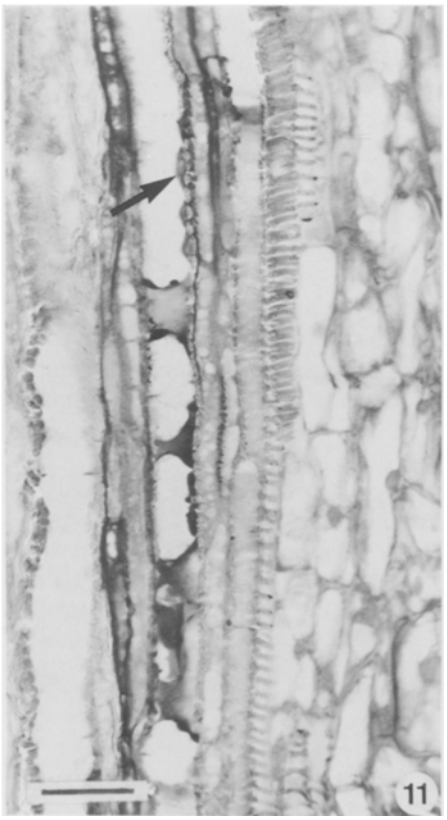
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in early stages (Figs 14-18). This discolouration, however, probably is due to a superficial, secondary infection.

Hypertrophied xylem parenchyma cells with virtually unstained walls were observed adjacent to the area of vessels containing gels after 35 days (Figs 16, 17).

In the hyperplastic layer xylem vessel regeneration was observed already after 11 days. The formation of secondary wall thickenings in individual cells layered in a vertical line was followed by the disappearance of the intermediate cell walls (Fig. 15). This resulted in the formation of scalariform metaxylem vessels like those produced by the cambium. After five weeks the hyperplastic zone was still changing into regenerated xylem tissue, on cross-section apparently replacing the diseased parts of the xylem and bridging the unaffected parts (Fig. 16).

Histochemistry of gels and discoloured walls. In healthy tissues, primary cell walls were stained deep blue-purple with toluidine blue, indicating the presence of polycarboxylic acids (e.g. pectic acids), polyphosphates or polysulphates. Secondary walls, however, were stained a greenish blue because of lignin and some polyphenols (O'Brien and McCully, 1981). In sections treated with ruthenium red for pectic acids (Clowes and Juniper, 1968), the primary walls were stained red. With fast green FCF, a test for cellulose (Clark, 1981), no staining was observed with the staining periods used in this study.

In infected carnations, gels were stained dark blue with toluidine blue, dull reddish with ruthenium red, and bright green with fast green, indicating the presence of various amounts of polysaccharides, among which pectin and probably hemicellulose. Discoloured walls stained like, but more intense than primary walls in unaffected tissue, i.e. dark blue with toluidine blue, red with ruthenium red, and more (burst edges) or less (around infected tissues) greenish with fast green, which also is indicative of polysaccharides.

Discussion

'Early Sam' carnations became heavily colonized and severely diseased after inoculation with *F. oxysporum* f. sp. *dianthi.* Inoculated 'Novada' carnations remained healthy and showed a permanently small amount of *Fusarium* propagules: the pathogen appeared to be localized effectively by the host without being eliminated or

Figs 12-15. Parts of stem sections from 'Novada' carnations infected with *F. oxysporum* f. sp. *dianthi.* All preparations stained with toluidine blue. Magnification bars represent 200 μm.
Fig. 12. Longitudinal section through the border of xylem (x) and medulla (m). Xylem vessels close to the medulla contain gels and are separated from the medulla by a thin zone of hyperplastic tissue (h) by which a vessel has partly been pushed aside (7 days after inoculation).
Fig. 13. Transverse section through the vascular region showing hyperplasia of xylem parenchyma on both cambial and medullar side of infection (18 days after inoculation).
Fig. 14. Transverse section through the vascular region showing gels in xylem vessels closest to the medulla (m) only, a hyperplastic zone consisting of orderly rays of small cells (h), and a burst in the stem cortex lined by cells with darkly stained walls (18 days after inoculation).
Fig. 15. Longitudinal section showing xylem vessel regeneration in the hyperplastic tissue (18 days after inoculation).


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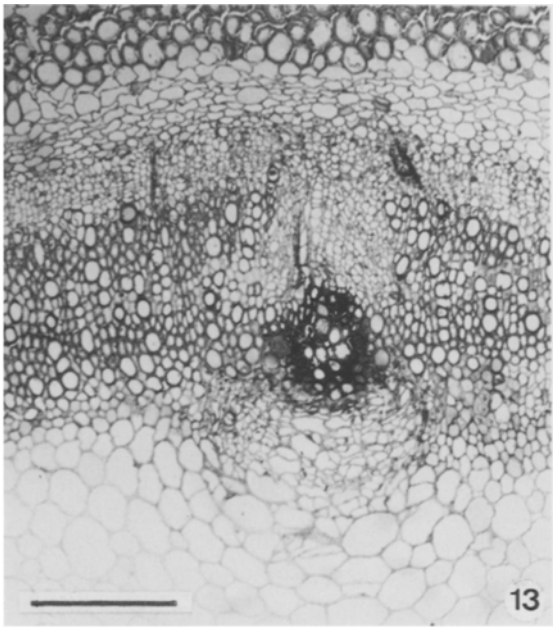
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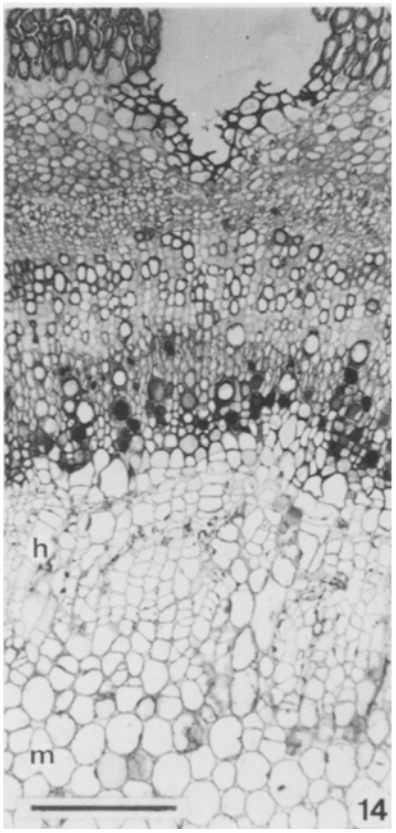
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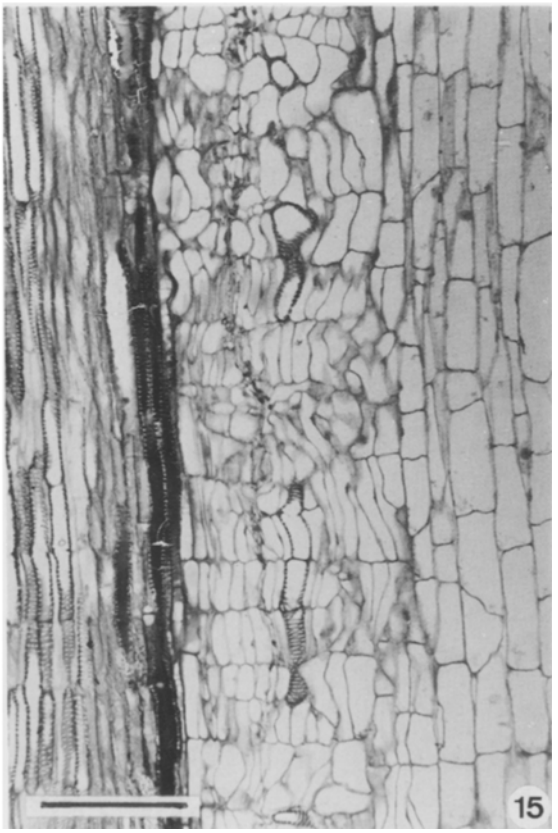
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being able to induce disease symptoms. Since conidia were introduced directly into the stem, the resistance observed in 'Novada' must be based on defence mechanisms within the vascular system itself. Anatomical differences found in this study that possibly allow for resistance are alterations of primary cell walls, gel formation in vessels, and hyperplasia of xylem parenchyma followed by xylem regeneration.

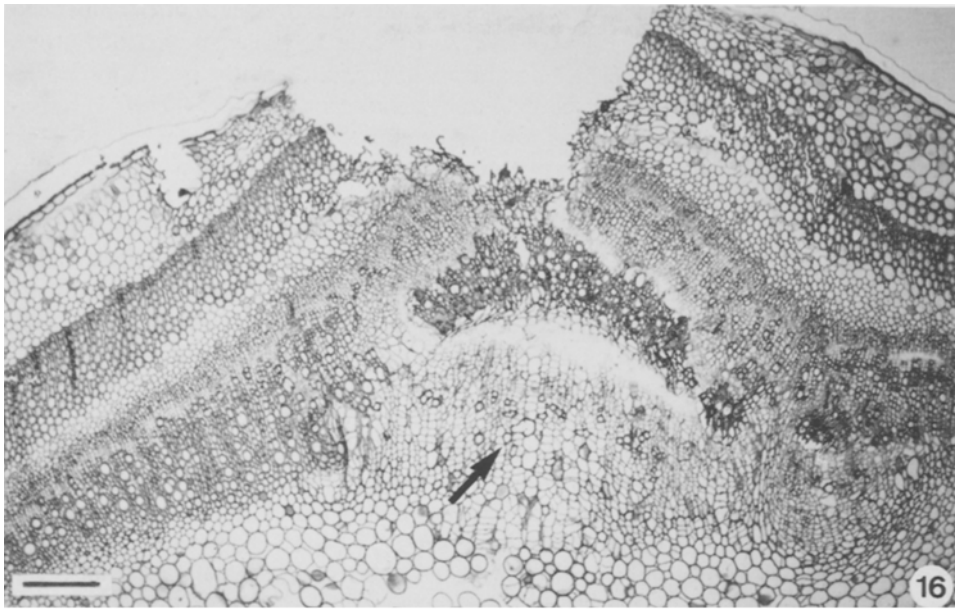
Discoloured and thickened primary walls have been reported to occur after inoculation of carnation (Pennypacker and Nelson, 1972; Péresse, 1975), chrysanthemum (Emberger and Nelson, 1981; Stuehling and Nelson, 1981), and tomato (Chambers and Corden, 1963) and are interpreted as deposition of material formed by infected cells or by accumulation of products of nearby disintegrating cells. Other possible explanations for the thickening of walls are cell wall swelling or lysis induced by the pathogen. Distension of primary wall and middle lamella constituents, possibly the cause of gel formation (VanderMolen et al., 1977), may be responsible for wall thickening too, as may wall lysis preceded by swelling. We suggest, however, that cell wall-impregnating components originate from metabolically active cells as indicated by their intense staining reaction with toluidine blue (Harling and Taylor, 1985), and that phenolics are released by cell decompartmentalization (MacHardy and Beckman, 1981). Beckman and Talboys (1981) discussed the deposition of additional wall layers and phenolics as a means of prevention of colonization. However, discoloured walls probably do not contribute to resistance as they occurred mainly in the susceptible cultivar. Discoloured walls as reported for tomato (Chambers and Corden, 1963) have even been considered to enhance susceptibility by 'waterproofing' the vessels (Corden and Chambers, 1966).

Vascular gelation has often been reported for carnations infected with various pathogens (Catesson et al., 1976; Harling and Taylor, 1985; Pennypacker and Nelson, 1972; Péresse, 1975). The histochemical tests used indicated that these gels are composed of various amounts of polysaccharides, among which are pectin and probably hemicellulose, together with oxidized polyphenols, all of which are considered to be normal gel components (Beckman and Talboys, 1981). Opinions on gel origin differ in the literature. Swelling or lysis of the constituents of primary walls and middle lamella of vessels followed by infusion of phenolics (Beckman and Talboys, 1981; MacHardy and Beckman, 1981; Pennypacker, 1981; VanderMolen et al., 1977) and secretion of gel by paravascular parenchyma cells (Catesson et al., 1976) have been held responsible. Our observations on protrusion of gel droplets into vessel lumina through pits shared with paravascular parenchyma cells are in accordance with reports by Catesson et al. (1976) and Harling and Taylor (1985); this observation itself, however, fits both other hypotheses also. Gel formation is a general phenomenon and represents a factor in the response of plants that provides for resistance to vascular infections

Figs 16-18. Parts of a transverse stem section from a 'Novada' carnation 35 days after inoculation with *F. oxysporum* f. sp. *dianthi*. Magnification bars represent 200 μm.

Fig. 16. General view showing hyperplastic tissue with regenerated xylem vessels (arrow) bridging the unaffected parts of the xylem (left, utmost right). A large stem burst has formed (preparation stained with toluidine blue).

Fig. 17, 18. Details of 16 showing vascular gelation, abundant xylem vessel regeneration (arrow), and wall discolouration of cells bordering the stem burst (preparation stained with safranin 0 and fast green FCF, respectively).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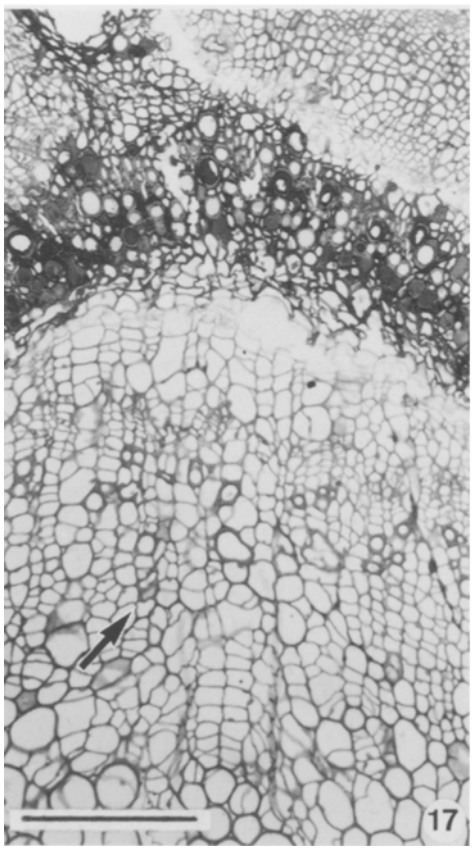
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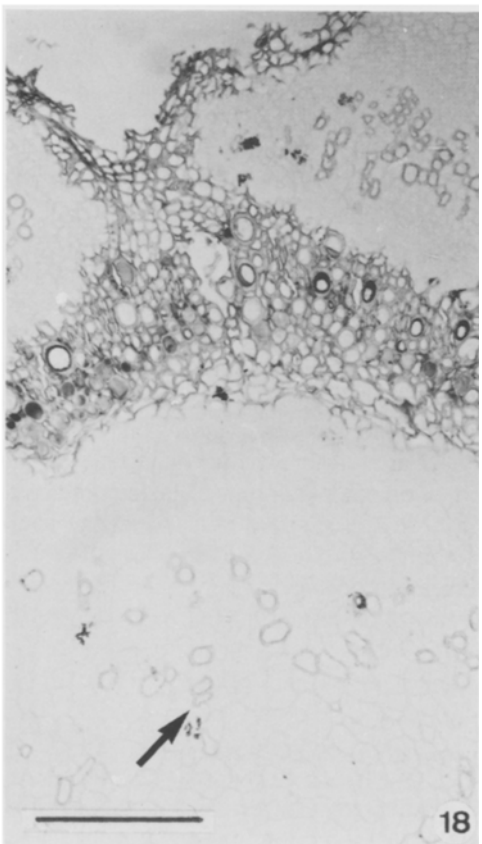
18

(VanderMolen et al., 1977; MacHardy and Beckman, 1981). Gels would limit fungal spread by occluding the vessels. The present report confirms that, in carnation, abundant gelation is characteristic of *Fusarium* wilt-resistant interactions, as reported previously (Harling and Taylor, 1985; Pennypacker and Nelson, 1972). However, vascular occlusion by gels has been shown to be one of the main causes of wilt symptoms in several other plant-pathogen interactions, e.g. *Phialophora* wilt of carnation (Péresse, 1975), *Verticillium* wilt of cotton (Misaghi et al., 1978) and *Fusarium* wilt of banana (Beckman and Halmos, 1962). Preliminary dye transport experiments have shown that – where present – gelation blocks the water transport in *Fusarium* wilt resistant carnations also (R.P. Baayen, unpublished). Lateral water transport from healthy parts of the vascular system and xylem regeneration in the hyperplastic tissue enable the plant to overcome local vascular dysfunction. Generally, a localized disruption of vascular function in the region of the initial trapping site – as seen in e.g. *Fusarium* wilt resistance – is considered insignificant in general plant health (Beckman and Halmos, 1962). In *Fusarium* wilt of banana but *Phialophora* wilt of carnation as well, the sequence of vascular occlusion seems to be disrupted, but not prevented, occlusion thus becoming systemic and probably the primary cause of wilting (Beckman and Halmos, 1962, Moreau et al., 1978; Péresse, 1974, 1975). A different disease mechanism, however, seems to be operating in *Fusarium* wilt of carnation, where destruction instead of occlusion of the vascular tissues causes wilting.

In carnation, hyperplasia and hypertrophy of xylem parenchyma have been reported after inoculation with *P. cinerescens* and *F. oxysporum* f.sp. *dianthi,* the resulting tension forces sometimes causing stem bursts (Harling et al., 1984; Harling and Taylor, 1985; Pennypacker and Nelson, 1972; Péresse, 1975). Hyperplasia and hypertrophy were not observed in susceptible 'Early Sam' contrary to previous reports for susceptible carnations (Pennypacker and Nelson, 1972; Harling and Taylor, 1985).

Pennypacker and Nelson (1972) observed that hyperplastic xylem parenchyma cells were arranged in orderly columns with a less virulent *Fusarium* isolate only, closely resembling the situation in 'Novada' and 'Carrier 929' (Harling and Taylor, 1985). In both latter cultivars xylem regeneration was observed in the hyperplastic tissue (Harling et al., 1984). In 'Novada', dysfunctional parts of the xylem appeared to be completely replaced by a broad hyperplastic zone changing gradually into new metaxylem tissue and bridging the unaffected parts of the vascular cylinder. Xylem regeneration in parenchymatic tissues, however, is a normal physiological phenomenon when vascular function has been disrupted (Sinnott and Bloch, 1945; Péresse, 1971). An auxin gradient established by a basipetal flux of auxin is responsible for rate and orientation of vessel differentiation (Aloni and Zimmermann, 1983; Jacobs, 1952; Sachs, 1969, 1981). Since xylem hyperplasia and regeneration only occurred in 'Novada' where little fungus was present, the responsible auxin would likely be of plant origin. Probably, susceptible carnations also are capable of xylem regeneration under favourable circumstances. Xylem vessel regeneration should hence be considered to be a general physiological response, only occurring when the invading pathogen is under control.

In conclusion, in susceptible carnation-*F. oxysporum* f. sp. *dianthi* interactions destruction of the vascular tissue very likely induces wilting; only in resistant interactions the infection is quickly localized, especially by defence systems within the

vascular cylinder, and fungal growth remains low. Of the observed histopathological differences between both cultivars studied, only vascular gelation may have played a role in determining resistance. Vascular gelation was responsible for a little vascular browning in resistant carnations, whereas wall discolouration was responsible for abundant vascular browning in susceptible carnations, corresponding with reports for cotton (Misaghi et al., 1978) and tomato (Chambers and Corden, 1963), respectively. Although considered the most prominent symptom of *Fusarium* wilt, vascular browning as such occurs in both susceptible and resistant interactions and probably only indicates injury to phenolic-storing cells (MacHardy and Beckman, 1981). In carnation wilt resistance breeding, vascular browning is commonly used as a proof of disease. The present report explains why Carrier (1977) reported that "light discolouration may have been 'normal' in resistant 'Carrier 929' ", although browning was otherwise used for disease determination. Misinterpretation of healthy, resistant carnation showing vascular browning may have affected estimated carnation wilt resistance levels, which never exceed 80-90% (L.D. Sparnaaij and J.F. Demmink, personal communication). Consequently, care should be taken when using vascular browning as an indication of disease.

Acknowledgements

These investigations were supported in part by the Netherlands Foundation for Technical Research (STW), future Technical Science Branch of the Netherlands Organization for the Advancement of Pure Research (ZWO). This research was carried out in collaboration with the Institute for Horticultural Plant Breeding (IVT), Wageningen and the Research Station for Floriculture, Aalsmeer.

Thanks are expressed to Miss J. Janssen and Mrs A. Slootman-Koelman for assistance with inoculations and determination of *Fusarium* growth curves.

Samenvatting

Kolonisatie en histopathologie van een vatbare en een resistente anjercultivar na inoculatie met Fusarium oxysporum f. sp. dianthi

Anjers van de vatbare cultivar Early Sam en de resistente cultivar Novada werden geïnoculeerd met een conidiënsuspensie van *Fusarium oxysporum* f. sp. *dianthi.* Van beide cultivars werden regelmatig stengeldelen geoogst om deze microscopisch te onderzoeken en om de schimmelgroei te bepalen.

'Early Sam' vertoonde de voor deze verwelkingsziekte kenmerkende symptomen en werd intensief gekoloniseerd. Aan het vaatweefsel waargenomen bruinkleuring bleek veroorzaakt te worden door verkleuring van de primaire wanden van geïnfecteerde vaten en de hen omringende cellen. Zelden trad er in de vaten gomvorming op. Celwandafbraak veroorzaakte de vorming van holten in de stengel. Hyperplasie van het houtparenchym werd niet waargenomen.

In 'Novada' bleef de schimmelgroei gedurende het hele experiment beperkt. Macroscopisch waren er enkel lengtescheuren in de stengel te zien, die veroorzaakt bleken te worden door hyperplasie van aan de infectie grenzend houtparenchym. In het geïnfecteerde vaatweefsel optredende gomvorming veroorzaakte ook enige

bruinkleuring. In het hyperplastische weefsel werd regeneratie van houtvaten waargenomen. In de stengel werden geen holten gevormd, en verkleuring van de celwanden kwam weinig voor. De vorming van gommen in de houtvaten maakt waarschijnlijk deel uit van het resistentiemechanisme. De daarop volgende houtvatregeneratie is eerder een algemene reactie van de plant op vaatverstopping dan een deel van het resistentiemechanisme.

Vaatverbruining, zij het van verschillende oorsprong, komt voor in zowel vatbare als resistente interacties. Om die reden moet men in de resistentieveredeling bij de anjer voorzichtig zijn met het gebruik van bruinkleuring als ziekteïndicatie.

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