Neth. J. Pl. Path. 95 (1989) 3-13

Histology of roots of resistant and susceptible carnation cultivars from soil infested with Fusarium oxysporum f.sp. dianthi

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Accepted 11 February 1988

Abstract

Fusarium wilt-resistant 'Novada' and wilt-susceptible 'Early Sam' carnations were planted in soil infested with *Fusarium oxysporum* f.sp. *dianthi*, and their roots studied after five and ten weeks. Both in 'Novada' and 'Early Sam', the extravascular tissues of undamaged young root parts were scarsely colonized. In roots of 'Novada', infected xylem vessels were usually occluded with gums and surrounded by phellem tissue. In mature parts of roots, the phellem surrounding occluded vessels often merged with the external phellem surrounding the vascular cylinder, after which the occluded vessels were shed from the roots. The phellem at the root surface appeared to be a strong barrier to fungal invasion. In roots of 'Early Sam' carnations, as well as in a few roots of 'Novada' carnations, the defence responses did not result in compartmentation of the fungus, and colonization and degradation of the vascular tissues followed. Diseased roots finally became hollow. The shoots of most of the 'Novada' carnations were not colonized and remained healthy. Shoots of 'Early Sam' carnations, and eventually a few shoots of 'Novada' carnations, were colonized and developed wilt symptoms.

Additional keywords: Dianthus caryophyllus, phellem, gums.

Introduction

Histological studies on resistance and susceptibility of carnation to fusarium wilt, caused by *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f.sp. *dianthi* (Prill. & Del.) Snyder & Hansen, have hitherto been confined to the stem (Pennypacker and Nelson, 1972; Baayen and Elgersma, 1985; Harling and Taylor, 1985; Baayen, 1986, 1988). In the stem, resistance to *F. oxysporum* f.sp. *dianthi* is associated with localization of the pathogen by gum formation in and phellem formation around infected xylem vessels. However, the determinative phase of the interaction with a soil-borne pathogen is more likely to be found in the roots. Resistance of carnation roots to *F. oxysporum* f.sp. *dianthi* may be expressed in the epidermis and cortex (lignituber formation) the endodermis (suberization, presence of phenolics), the vascular system (occlusion, possibly phytoalexin production), or in a combination of these (Beckman and Halmos, 1962; Mueller and Beckman, 1976; Beckman and Talboys, 1981; Flood, 1985). If different and independent resistance mechanisms operate in various parts of the carnation plant, these probably could be combined in resistance breeding programmes.

The present study was undertaken to investigate the expression of resistance to *F. oxysporum* f.sp. *dianthi* in roots of carnations growing in soil infested with the pathogen, i.e., under semi-natural conditions. To this end, rooted cuttings of the resistant cultivar Novada and, as a reference, the susceptible cultivar Early Sam were grown in infested soil for several months. Both cultivars were previously used in the stem inoculation experiments (Baayen and Elgersma, 1985; Baayen, 1986, 1988). Root samples for microscopical study were taken several weeks after planting so as to ensure that well-developed interaction stages would be present. As precise timing and location of the infection process were not well predictable with this method, a time-sequence study on the early stages of the infection process was not attempted.

Materials and methods

Plant material. Rooted cuttings of the fusarium wilt-resistant carnation cultivar Novada and the wilt-susceptible cultivar Early Sam were obtained from the Institute for Horticultural Plant Breeding (IVT), Wageningen.

Preparation of inoculum and inoculation of plants. A virulent isolate (WCS 816) of *F. oxysporum* f.sp. *dianthi* race 2 was cultured in Czapek Dox liquid medium (Oxoid) on a reciprocal shaker for one week, and 5 × 100 ml shake culture mixed into 10 l of autoclaved soil amended with 0.5 l malt extract (Oxoid, 3%). After two weeks incubation at 20 °C, the soil contained c. 2×10^4 *F. oxysporum* propagules g^{-1} soil as determined by plating soil suspensions in serial dilutions on malt extract agar (Oxoid) to which 80 IU sodium benzylpenicillin and 160 IU streptomycin sulphate ml^{-1} had been added. After three weeks incubation, the soil was mixed with steamed soil (1 : 3) and used. Sixty rooted cuttings of 'Novada' and 40 of 'Early Sam' were inoculated by planting them in the final soil mixture (8 cm diameter pots). Fourty 'Novada' and 20 'Early Sam' control cuttings were planted in uninoculated steamed soil. The planted cuttings were kept in a glasshouse and watered by hand.

Light microscopy. Five and ten weeks after inoculation, ten inoculated and three control 'Novada' carnations and three inoculated and one control 'Early Sam' carnations were lifted, carefully washed to remove most of the soil, and freed of leaves. For each plant, 8-mm-long root segments of three roots (four per root, two adjacent segments taken close to the base and two adjacent ones taken close to the apex of the root), and stem segments taken at 0-1 cm, 4-5 cm and 9-10 cm height were fixed in FAPA (formaldehyde (40%)-ethanol-water-propionic acid-acetic acid, 5 : 45 : 45 : 2.5 : 2.5). Two procedures were used for sectioning and staining the fixed segments. Part of the segments were embedded in polyethylene glycol methacrylate (JB-4 plastic), sectioned at 3-4 μm and stained with toluidine blue (Baayen and Elgersma, 1985). Other segments were sectioned by hand with a razor blade and studied in one of the following ways: 1) unstained, 2) after staining with toluidine blue, 3) after staining with ethanolic sudan III or ethanolic sudan IV for detecting suberin (Jensen, 1962), or 4) after treatment with phloroglucinol-HCl for detecting lignin (Clark, 1981). Photographs of sections were made with a Leitz Orthoplan photo-microscope and recorded on Agfapan 25 film.

Results

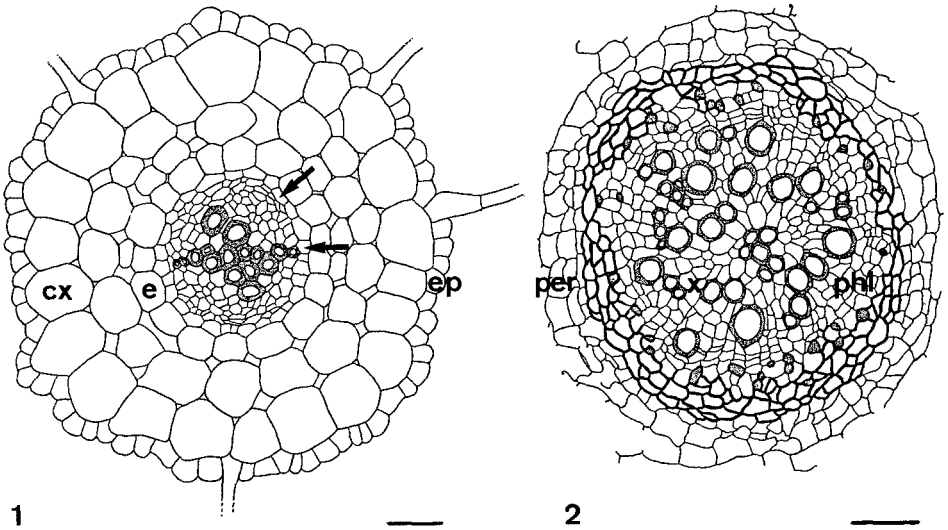
Disease progress in 'Novada' and 'Early Sam'. Five weeks after planting in soil infested with *F. oxysporum* f.sp. *dianthi*, wilt symptoms had not developed in 'Novada' carnations. Some roots and particularly young root parts of the carnations were healthy white and in microscopical preparations appeared to be free of the pathogen. Other roots (particularly root bases) had been colonized by the pathogen and sometimes were browned. The stems appeared to be free of the pathogen in most plants, although in one plant a small area with defence responses was observed at the base of the stem. Unilateral fusarium wilt symptoms developed in only a few 'Novada' carnations from 8 weeks on after planting. Ten weeks after planting, the stems of the remaining symptomless plants still appeared to be free of the pathogen (at most, some defence responses to colonization had occurred at the very base of the stem), while the stems of diseased plants were colonized on the affected side high up into the stem. The colonized vascular tissue of stems was brown-edged.

Fusarium wilt symptoms developed in 'Early Sam' carnations already 3.5 weeks after planting in soil infested with *F. oxysporum* f.sp. *dianthi*. Five weeks after planting, the majority of plants showed yellowing and wilting of leaves and, sometimes, also stunting of shoots. The majority of the roots of affected plants was colonized and macroscopically browned. Stems of affected plants were colonized high up into the stem. The colonized vascular tissue of the stem was degraded, and on cross-section appeared brown-edged. In most plants, the vascular tissues were colonized and degraded all around the stem, rendering the stems hollow and dried out. Ten weeks after planting, all plants had wilted completely.

Root anatomy in 'Novada' and 'Early Sam' (Figs 1 and 2). The epidermis of young parts of roots of 'Novada' and 'Early Sam' (Fig. 1) consisted of a single layer of narrow cells, elongated in axial direction, some giving rise to root hairs. The cortex consisted of c. 2-4 layers of wide, less elongate parenchymatous cells. The innermost cortical layer, the endodermis, consisted of similarly shaped but smaller cells of which the radial and transverse walls, and often also the tangential walls, appeared to contain suberin (the walls gave a positive staining reaction with sudan III and IV). The vascular cylinder of young root parts consisted of xylem, cambium and phloem arranged in a diarch pattern, surrounded by a pericycle consisting of one layer of small, often flattened parenchyma cells.

In maturing root parts, the pericycle differentiated into a phellogen which produced 1-3 layers of enlarged, suberized phellem cells even while the epidermis and cortex remained unimpaired. The endodermal cells gradually collapsed at this stage. Phellem differentiation was often restricted to the vicinity of single collapsed endodermal cells, or to those sides of roots where the cortex had been damaged. The vascular cylinder was covered in all cases studied by a continuous layer of endodermal cells, phellem cells, or cells of both types.

In mature parts of roots (Fig. 2), the extravascular tissues (including the endodermis) were sloughed off. Well-developed phellem tissue surrounded the vascular tissues of these root parts. The vascular tissues at this stage consisted of a thick, solid core of xylem surrounded by vascular cambium and phloem; the diarch arrangement of the xylem had disappeared. Healthy mature root parts were white to yellowish (due to the



Figs 1 and 2. Camera lucida drawings of transverse sections of healthy carnation roots. Bars = 50 μm.

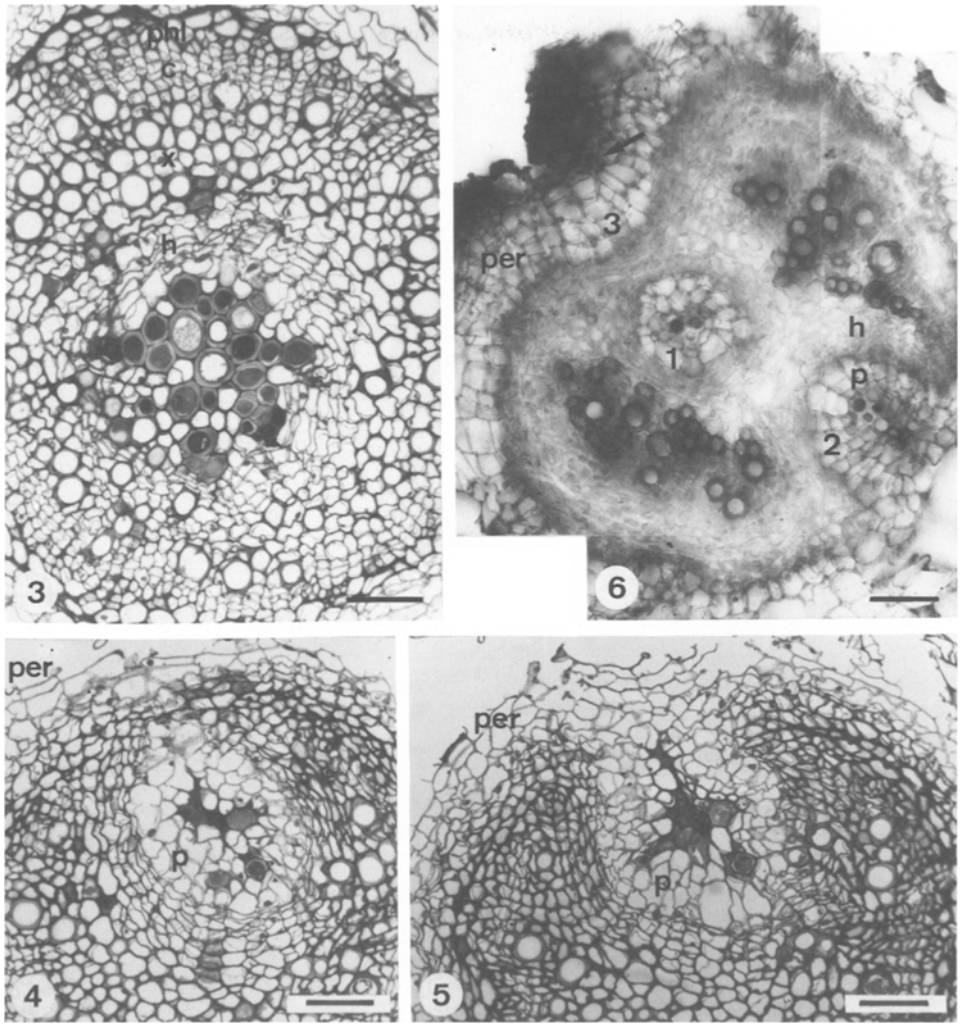
Fig. 1. Young root of 'Early Sam' showing epidermis (ep), cortex (cx), endodermis (e), pericycle (arrows) and diarch xylem.

Fig. 2. Older root of 'Novada' showing root periderm (per), phloem (phl), cambium and xylem (x).

external phellem); healthy young root parts were white.

Histopathology of roots of resistant 'Novada' (Figs 3-12). Colonization of the extravascular tissues was scarce in young parts of roots of 'Novada' carnations, but sometimes occurred when these tissues were damaged or, in due course, were shed. Lignituber-like structures were not encountered. Colonization of the phellem tissue surrounding mature roots parts was never observed.

Both in young and in mature root parts, affected xylem vessels were occluded with gums (Figs 3-6) which were yellow to dark brown in unstained hand-sectioned material. The affected vessels often had yellowish secondary walls. The primary walls and adjacent intercellular spaces of xylem parenchyma cells next to occluded or colonized vessels were impregnated with lignin-like material (Figs 4 and 5; see also Fig. 15) as previously described for the resistant response in carnation stems (Baayen, 1988). The brown gums and lignified primary walls stained greenish with toluidine blue and a deep red with phloroglucinol-HCl. Around occluded vessels a broad zone of hyperplastic xylem parenchyma tissue was formed (Figs 3-6). The inner 1-4 cell layers of the hyperplastic tissue differentiated into enlarged, thin-walled phellem cells with suberized walls (staining orange to red with sudan III and IV, and staining pinkish with phloroglucinol-HCl) (Figs 4-9). These phellem cells were identical in shape and staining properties to those of the periderm surrounding mature parts of roots. Vascular regeneration was not observed in the non-suberized, outer part of the hyperplastic tissue. Outside of the hyperplastic tissue, the vascular tissue usually appeared entirely unaffected.



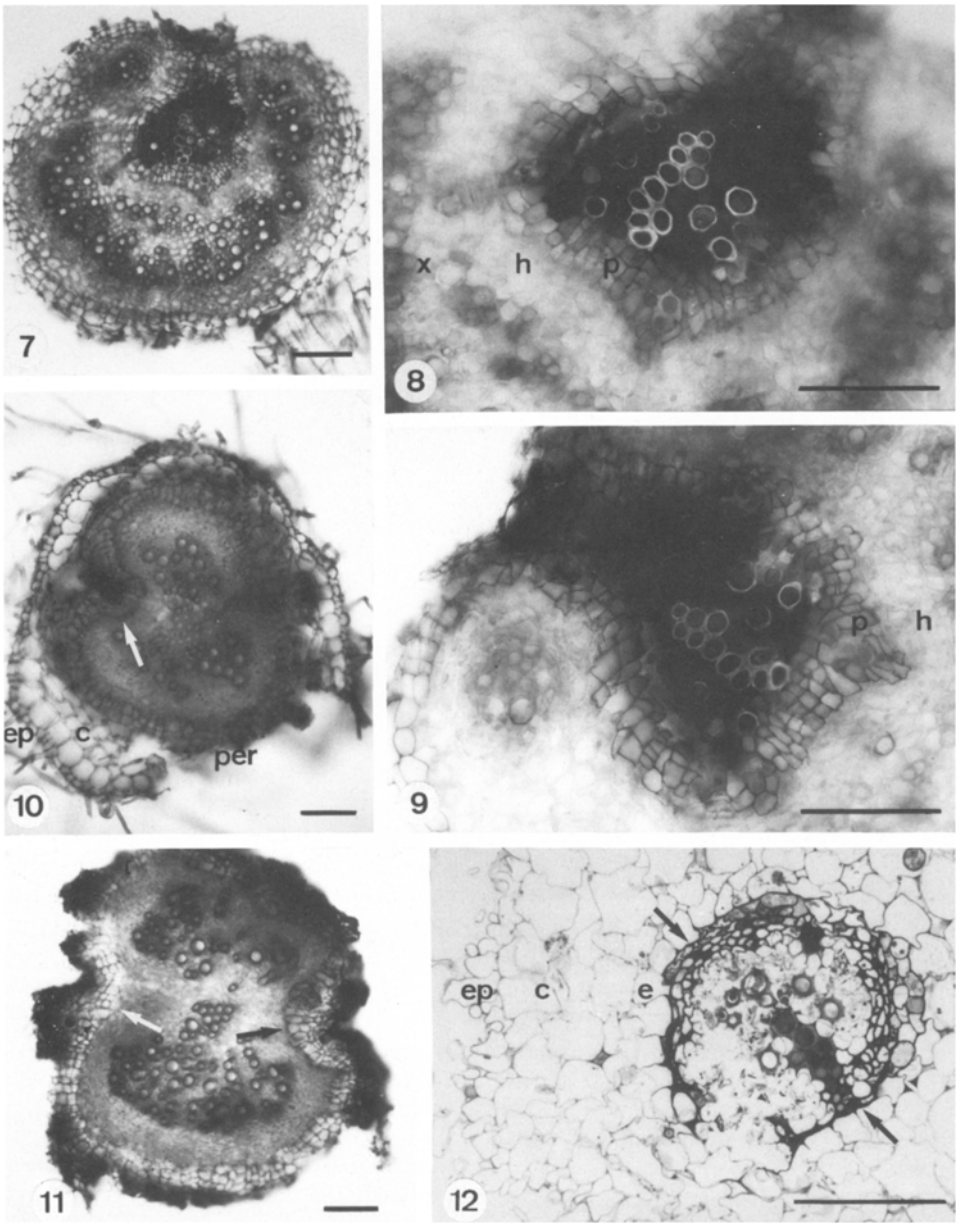
Figs 3-6. Defence responses in relatively mature parts of roots of 'Novada' carnations, five weeks after planting in soil infested with *Fusarium oxysporum* f.sp. *dianthi*. Figs. 3-5. Transverse microtome sections of plastic-embedded material stained with toluidine blue; Fig. 6. Transverse hand section stained with toluidine blue. Bars = 50 μm.

Fig. 3. Affected vessels occluded with gums and surrounded by hyperplastic xylem parenchyma tissue (h). Phellem cells have not yet been formed. The root is otherwise unaffected. Phloem – phl, cambium – c, xylem – x.

Fig. 4. A layer of phellem cells (p) has formed in the inner part of the hyperplastic tissue surrounding a few occluded vessels. The responding tissues are still situated completely within the vascular tissues. Periderm – per.

Fig. 5. Section of the same root as in Fig. 4 but made c. 2 mm further up. The phellem (p) surrounding the occluded vessels has merged with the phellem of the root periderm (per). The root otherwise appears to be unaffected. Note the darkly stained, lignified walls of xylem parenchyma cells neighbouring the occluded vessels.

Fig. 6. Occluded vessels (arrow) surrounded by phellem tissue (p) and hyperplastic tissue (h) 1. situated entirely within the vascular tissues, 2. connecting with the root periderm, and 3. situated at the outer side of the periderm (per). The remaining vascular tissues appear to be unaffected.



Figs 7-11. Defence responses in relatively mature parts of roots of 'Novada' carnations, ten weeks after planting in soil infested with *F. oxysporum* f.sp. *dianthi*. Transverse hand sections. Bars = 100 μm.

Fig. 7. General view of a root in which the intravascular phellem surrounding affected vessels has made connection to the external root periderm. The remaining vascular tissues appear to be unaffected. Section stained with sudan IV and toluidine blue.

Figs 8 and 9. Details of the section shown in Fig. 7. Fig. 8. Unstained section; Fig. 9. Section stained with sudan IV and showing staining of the phellem cells (p) surrounding the affected tissue and of those of the root periderm. Xylem – x, hyperplastic tissue – h.

The occluded vessels surrounded by phellem tissue were situated in the roots as long, axial brown strands which were macroscopically visible. When only a few vessels were occluded with gums, phellem tissue did usually not form, and little or no browning of the roots occurred. In such cases, the occluded vessels in axial direction disappeared gradually within the unaffected vascular tissue. When many vessels were occluded with gums, and the surrounding phellem tissue was well-developed, the affected tissue was often eliminated by a sloughing-off process. In these cases the occluded vessels in axial direction appeared to move from the center to the margin of the vascular cylinder of the root; the phellem surrounding the occluded vessels merged with that of the periderm surrounding the vascular cylinder, and the affected tissue was shed from the roots (Figs 4-11). This even occurred in maturing roots before the extravascular tissues had been sloughed off (Fig. 10). As a consequence of the sloughing-off process, dark brown axial strands repeatedly occurred on the surface of, instead of within affected roots. In microscopical preparations, remainders of occluded vascular tissue were commonly seen outside the root periderm, which was curved inwards at such places (Fig. 11).

Compartmentation of the pathogen in the vascular tissues was not always achieved. In plants which exhibited defence responses in some roots, the xylem, cambium and phloem of others were colonized and degraded just as described below for roots of 'Early Sam' (compare Figs 12 and 13). As with 'Early Sam', the colonized and sometimes hollow vascular cylinder was often surrounded by cells of which the primary walls were impregnated with brown, lignin-like material, which gave the diseased roots a brown appearance. Locally formed vascular gums and phellem cells were frequently present within the colonized roots and did not appear to be degraded (Fig 12).

Histopathology of roots of susceptible 'Early Sam' (Figs 13-17). Colonization of the extravascular tissues of roots of 'Early Sam' carnations was scarce, as described for roots of 'Novada'. The vascular tissues of most roots were colonized, however. Colonization sometimes was limited to the xylem vessels (Fig. 15), but usually affected all vascular tissues including the cambium and phloem (Figs 13, 14, 16, 17). Heavy degradation of the vascular tissues by the fungus resulted in hollow roots (Figs 14, 17). Adjacent to colonized tissue and sometimes surrounding the entire vascular cylinder, the primary walls of parenchyma cells were impregnated with brown, lignin-like material (Figs. 13, 15; compare also Fig. 12) with similar staining properties as the gums. The

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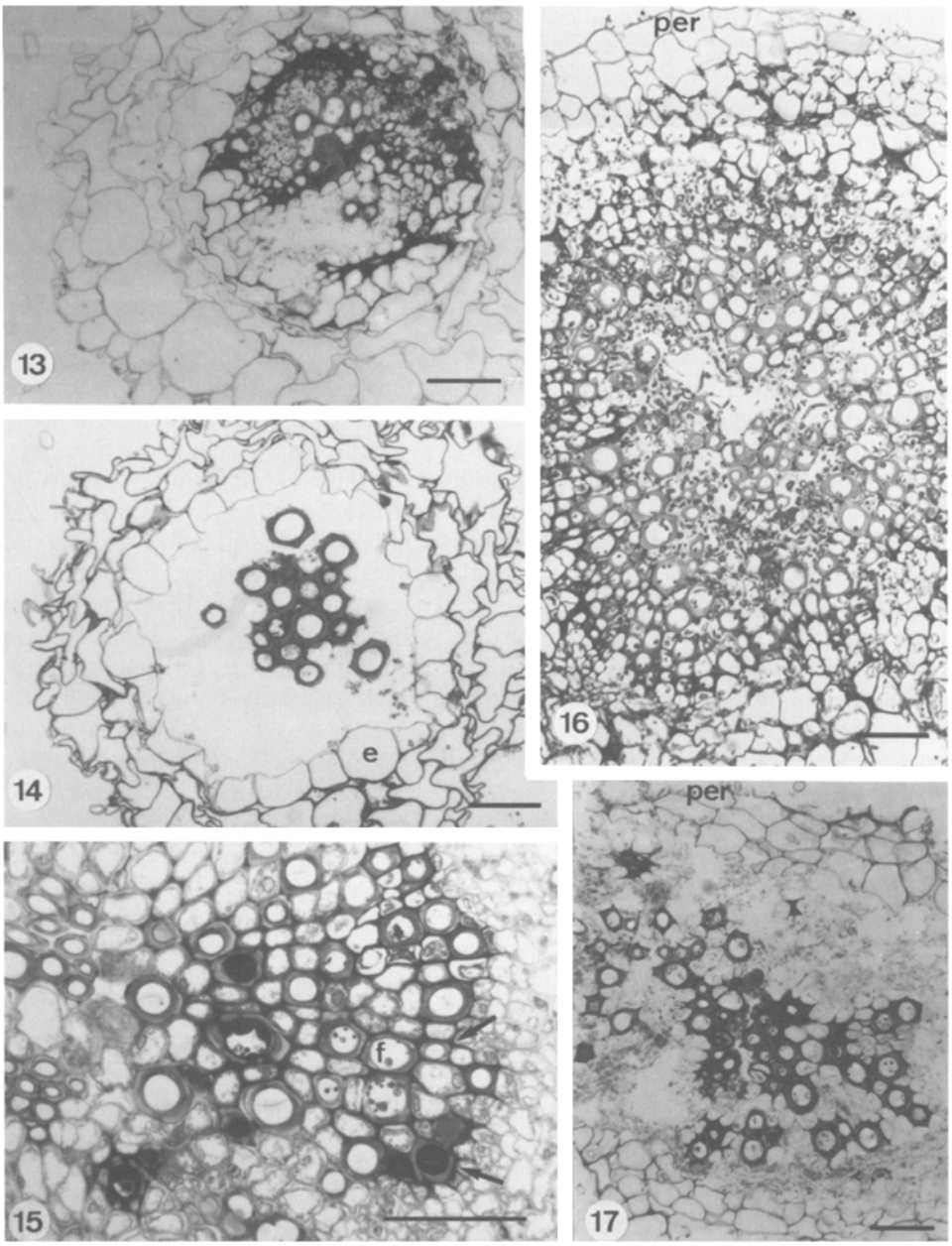
Fig. 10. Elimination of occluded vessels out of the vascular cylinder by phellem connections (arrow) occurring while the extravascular tissues are still present. Section stained with toluidine blue. Epidermis – ep, cortex – c, periderm – per.

Fig. 11. Functional root from an inoculated plant showing remainders of eliminated tissue (occluded vessels surrounded by phellem) at the outer side of the root periderm, which is locally curved inwards (arrows). Section stained with sudan IV and toluidine blue.

Fig. 12. Diseased young root of a 'Novada' carnation, five weeks after planting in soil infested with *F. oxysporum* f.sp. *dianthi*. Transverse section of plastic-embedded material stained with toluidine blue. Bar = 100 μm. The vascular tissues have partially been degraded, except for the lignified xylem vessel walls, vessels containing gums and adjacent primary walls impregnated with (darkly stained) lignin-like material. The primary cell walls at the periphery of the vascular cylinder have also been impregnated with such material (arrows). Epidermis – ep, cortex – c, endodermis – e.



Figs 13-17. Diseased roots of 'Early Sam' carnations, five weeks after planting in soil infested with *F. oxysporum* f.sp. *dianthi*. Transverse sections of plastic-embedded material stained with toluidine blue. Bars = 50 μm.

Fig. 13. Young root of 'Early Sam' in similar condition as shown for 'Novada' in Fig. 12.

Fig. 14. Young root in which the vascular tissues have been degraded up to the endodermis (e), except for the lignified xylem vessel walls. The extravascular tissues have not been degraded.

browned walls appeared to be responsible for the macroscopic browning of diseased roots. The xylem vessels were rarely occluded with gums (Figs 13, 15). Hyperplasia of xylem parenchyma cells sometimes accompanied the gummosis; a few hyperplastic cells sometimes differentiated into suberized phellem cells. Primary walls impregnated with the lignin-like material, locally formed gums and phellem cells, and the regularly lignified secondary walls of xylem vessels did not appear to be degraded and remained present in otherwise hollow roots (Figs 13, 14, 16, 17). The phellem tissue surrounding more mature root parts was never colonized, even when colonization and degradation of the vascular tissues immediately next to the phellem were extensive (Figs 16, 17).

Discussion

Resistance of 'Novada' carnations to *F. oxysporum* f.sp. *dianthi* was expresssed in the vascular system of roots in the same way as reported for the stems (Baayen and Elgersma, 1985; Baayen, 1988). Vertical restriction of the pathogen was accomplished by the occlusion of colonized xylem vessels with phenol-infused gums, and horizontal localization by lignification responses in the xylem parenchyma cells next to affected vessels and by the differentiation of phellem tissue around the affected tissue. The occluded vessels were eventually shed from the roots. This response is unique; such a process does not or only rarely take place in carnation stems (Baayen, 1988). In stems, the localization response is followed by vascular regeneration in the non-suberized, outer parts of the hyperplastic tissue surrounding the occluded vessels (Baayen, 1986, 1988); this was not observed in carnation roots. Vascular regeneration would probably be superfluous in roots, since the sloughing-off of occluded parts of the xylem allows the root to resume its normal development.

The present results emphasize the importance of suberized cells as a barrier to fungal invasion. It is generally assumed that the endodermis of roots constitutes such a barrier (Beckman and Talboys, 1981), even though the transverse walls of the endodermal cells are not always suberized (in carnation roots they mostly are, though). In the present study, phellem cells proved to be an efficient barrier to the fungus. Phellem tissue, formed around the vascular cylinder well before the extravascular tissues were shed, kept exposed mature root parts entirely free of the fungus. Colonization or degradation of phellem cells was not observed, not even when extensive degradation of the vascular tissues had occurred. The endodermis and the phellem tissue surrounding the vascular cylinder apparently formed a more important barrier to fungal invasion than the epidermis and cortex, because occluded vessels were shed from the vascular cylinder even when the extravascular tissues had not yet been sloughed off.

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Fig. 15. More mature root in which some xylem vessels have been colonized (f), and others occluded with gums. Impregnation of primary walls with lignin-like (darkly stained) material has occurred in xylem parenchyma cells next to affected vessels (arrows).

Fig. 16. Mature root showing heavy colonization of the vascular cylinder, the onset of cavity formation, and limitation of colonization by the root periderm (per).

Fig. 17. Decaying mature root in which the vascular tissues have been degraded, except for the lignified xylem vessel walls, a few vessels containing gums and primary walls impregnated with lignin-like material. The periderm (per) surrounding the root has remained intact.

The vascular system of roots of susceptible 'Early Sam' carnations was colonized and degraded, just as reported for the stems (Baayen and Elgersma, 1985; Baayen et al., 1988). Virtually all roots and stems studied were colonized, and all plants wilted completely. However, wilting usually is unilateral initially, as presently observed with 'Novada'. Apparently, the infection pressure in the present experiment was very high; the situation encountered in 'Novada' (which normally does not develop disease symptoms at all) perhaps better represents the responses of 'Early Sam' under lower infection pressures. Resistance and susceptibility of carnation to *F. oxysporum* f.sp. *dianthi* is partial after all; the difference is quantitative (Sparnaaij and Demmink, 1977). Some defence responses were observed in roots of susceptible 'Early Sam', and some roots and shoots of resistant 'Novada' eventually became diseased just as in 'Early Sam'. Cultivar resistance to fusarium wilt is probably determined by the capacity of the vascular tissues of the roots to localize an invading pathogen. A high localization capacity leads to an increased time lag before a root, and eventually the shoot will be successfully colonized and wilting will follow. Fusarium wilt therefore develops more slowly or not at all in more resistant cultivars.

Colonization of extravascular root tissues was scarcely observed in the present study. This may to some extent be due to resistance of the extravascular tissues to fungal invasion. However, penetration of epidermal cells and subsequent colonization of cortical cells was observed (although infrequently) in preliminary studies on carnation roots surface-inoculated with a conidial suspension (R.P. Baayen, unpublished results). Moreover, the inoculation procedure presently followed is likely to have favoured a direct entrance of the pathogen into the vascular system (the planting of recently uprooted cuttings always results in wounding of roots), and once there, the vascular system is the preferred habitat for vascular pathogens. This may be a more important reason for the scarcity of extravascular colonization in the present study.

Acknowledgements

These investigations were supported by the Netherlands Technology Foundation (STW). Thanks are expressed to Ms L.P. Waterreus for technical assistance.

Samenvatting

Histologie van wortels van resistente en vatbare anjercultivars uit grond besmet met Fusarium oxysporum f.sp. dianthi

Resistente 'Novada' en vatbare 'Early Sam' anjers werden geplant in grond besmet met *Fusarium oxysporum* f.sp. *dianthi*. Na vijf en tien weken werden de wortels van de planten bestudeerd. De extravasculaire delen van onbeschadigde jonge wortelgedeelten waren in beide cultivars nauwelijks gekoloniseerd. In de wortels van 'Novada' waren geïnfecteerde houtvaten meestal verstopt door gommen en omgeven door kurkweefsel. In oudere wortelgedeelten sloot het kurkweefsel rond verstopte vaten vaak aan op het kurkweefsel aan de buitenkant van het vaatweefsel, waarna de aangetaste vaten werden uitgestoten uit de wortel. Het kurkweefsel aan het worteloppervlak leek een belangrijke barrière te vormen tegen het binnendringen van de schimmel. In de wortels van 'Early Sam' en in enkele wortels van 'Novada' mislukte het afweerproces, en werd het vaatweefsel

door de schimmel gekoloniseerd en vervolgens afgebroken. Aangetaste wortels werden na afloop van tijd hol. De bovengrondse delen van de meeste 'Novada' anjers werden niet gekoloniseerd en bleven gezond. De bovengrondse delen van de 'Early Sam' anjers, en op den duur die van enkele 'Novada' anjers, werden gekoloniseerd en verwelkten.

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