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Differences in pathogenesis observed among susceptible interactions of carnation with four races of Fusarium oxysporum f.sp. dianthi

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Abstract

Susceptible interactions of 'Early Sam' carnations with races 1, 2, 4 and 8 of *Fusarium oxysporum* f. sp. *dianthi* differed in pathogenesis, both after stem and after root inoculation. Race 1 induced pallescence and withering of leaves. Affected vascular tissue had a uniform pallid to pale brown colour; though heavily colonized, it was not or virtually not degraded. Defence reactions developed only slowly. Race 2 induced yellowing, of the midribs in particular, and withering of leaves. Affected vascular tissue was white with dark brown margins. Colonized tissue was degraded to leave vascular cavities. At lower heights of colonization, many defence reactions developed, which sometimes resulted in localization of the pathogen. Race 4 induced a similar pathogenesis as race 2, except for less intensive defence reactions. Race 8 induced midrib lesions on, and pallescence, withering and necrosis of leaves. Affected vascular tissue had a uniform light brown colour. Degradation of colonized vascular tissues was rare; instead, many defence reactions were observed, even at high heights in the plants.

Races 1, 2 and 4 of *F. oxysporum* f. sp. *dianthi* did not induce disease symptoms in 'Novada' carnations, known to be highly resistant to race 2. Stem-inoculated plants localized the infection close to the inoculation site; stems of root-inoculated plants remained unaffected. The localization response also occurred in 'Early Sam' and 'Novada' carnations stem-inoculated with *F. oxysporum* f. sp. *lycopersici*.

Additional keywords: Dianthus caryophyllus L., pathotypes, *Fusarium oxysporum* f. sp. *lycopersici*.

Introduction

The existence of physiological races of formae speciales of *Fusarium oxysporum* has long been known. Well-known examples are the races of *F. oxysporum* f. sp. *lycopersici* (Gabe, 1975), *F. oxysporum* f. sp. *pisi* (Kraft and Haglund, 1978), *F. oxysporum* f. sp. *conglutinans* (Armstrong and Armstrong, 1966) and *F. oxysporum* f. sp. *melonis* (Armstrong and Armstrong, 1978). Eight arbitrarily numbered races ('pathotypes') were reported for *F. oxysporum* f. sp. *dianthi* by Garibaldi (1975, 1977, 1983). The most common and first-known one is race 2, against which only partial resistance is known and which is the only race occurring world-wide. The other races are known mainly from

the Italian and French Rivièra; of these, race 1 in particular is remarkable in only attacking Mediterranean and spray carnations, but not at all the American 'Sim' varieties which are highly susceptible to race 2 (Garibaldi, 1983). The virulence pattern of races 1 and 2 was confirmed by Matthews and Arthur (1978). According to Matthews (1978), all English isolates probably belong to race 2; the situation in the Netherlands is believed to be similar. The virulence pattern of the three important races 1, 2 and 4 has recently been investigated under Dutch conditions in comparison with Dutch isolates of the pathogen (Baayen et al., 1987; Demmink and Baayen, 1987). The present report does not concern virulence patterns, but the pathogenesis of susceptible and resistant interactions of carnation with four races (1, 2, 4 and 8) of *Fusarium oxysporum* f. sp. *dianthi* as observed in (race 2-highly susceptible) 'Early Sam' and (race 2-highly resistant) 'Novada' carnations.

Materials and methods

Plant and fungal material. Rooted cuttings of the carnation (*Dianthus caryophyllus* L.) cultivars Early Sam and Novada, obtained from M. Lek & Zonen B.V., Nieuwveen were planted in steamed soil (8 cm diameter pots) and grown for several weeks in the glasshouse prior to inoculation.

Isolates of *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. & Delacr.) Snyder & Hansen race 1 (Garibaldi 1 and G F101), race 2 (G 75, G F107, Willie Commelin Scholten 816 and WCS 843), race 4 (G 310 and G F79) and race 8 (G s.n.), and of *F. oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyder & Hansen (WCS 801) were grown on potato dextrose agar slants. Isolates obtained from Dr A. Garibaldi, Turin, Italy were used with permission of the Dutch Plant Protection Service, Wageningen after phytosanitary precaution measures had been taken.

Inoculation and sampling of plants. Single spore isolates of *F. oxysporum* f. sp. *dianthi* race 1 (G 1), race 2 (G 75, WCS 816 and WCS 843) and race 4 (G 310), and of *F. oxysporum* f. sp. *lycopersici* (WCS 801) were cultured in carboxymethylcellulose medium (Cappellini and Peterson, 1965) on a reciprocal shaker at 20 °C. After 4 days, mycelial fragments were removed by filtering the cultures through sterile glasswool. The conidial suspensions were washed twice in sterile tapwater by centrifugation, adjusted to a concentration of 10^7 conidia ml^{-1}, and used for stem inoculation (30 μl at either side of the stem) as described by Baayen and Elgersma (1985) of 15 plants of all 12 host-pathogen combinations. Ten control plants of each cultivar were treated similarly with sterile water. Stem segments of three stem-inoculated plants of all host-pathogen combinations and of two controls were sampled at 3 days and 1, 2, 4 and 8 weeks after inoculation. Stem parts 10 to 15 mm above the inoculation site were embedded in plastic and sectioned with a rotary microtome as described below; remaining stem parts were partially sectioned by hand as well.

In a second experiment, isolates of *F. oxysporum* f. sp. *dianthi* race 1 (G 1 and G F101 single spore culture), race 2 (G 75 and G F107), race 4 (G 310 and G F79 single spore culture) and race 8 (G s.n.) were cultured in Czapek Dox liquid medium (Oxoid) on a reciprocal shaker at 20 °C. After 10 days, conidial suspensions (10^7 conidia ml^{-1}) were prepared as described above and used for stem inoculation (20 μl at one side of the stem only) and root inoculation (3 ml $plant^{-1}$ poured on the soil) of 16 and 24

plants, respectively, of each host-pathogen combination. Stem-inoculated plants had been kept dry for several days before inoculation to ensure the inoculum would be taken up, whereas root-inoculated plants had been kept wet to minimize passive translocation of conidia into the stem following inoculation (Baayen and De Maat, 1987; Sparnaaij and Demmink, 1987). All seven interactions with 'Early Sam' were studied, both after root and stem inoculation, but with 'Novada' only that with race 1 (G 1). A same number of control plants of each cultivar was treated similarly with sterile water. Development of external disease symptoms was followed in detail during three months. Development of internal symptoms and histopathology were studied on hand-made sections from all regions of the stems of minimally five plants per host-pathogen combination and inoculation method used.

Light microscopy. Stem parts to be embedded in plastic were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer (pH 6.8), and placed under vacuum to remove any remaining air. After three rinses in phosphate buffer, specimens were dehydrated following Feder and O'Brien (1968), embedded in polyethylene glycol methacrylate (JB-4, Polysciences), sectioned at 3-4 μm, and stained with aqueous toluidine blue 0 or ethanolic sudan III (Jensen, 1962; O'Brien and McCully, 1981). Hand-made sections were studied either unstained, after staining with sudan III or phloroglucinol-HCl (Clark, 1981), or after applying Mäule's test (Gerlach, 1969). Photographs of sections were made with a Leitz Orthoplan photo-microscope and recorded on Agfapan 25 film. All illustrations in this paper show microtome sections of plastic-embedded material stained with toluidine blue.

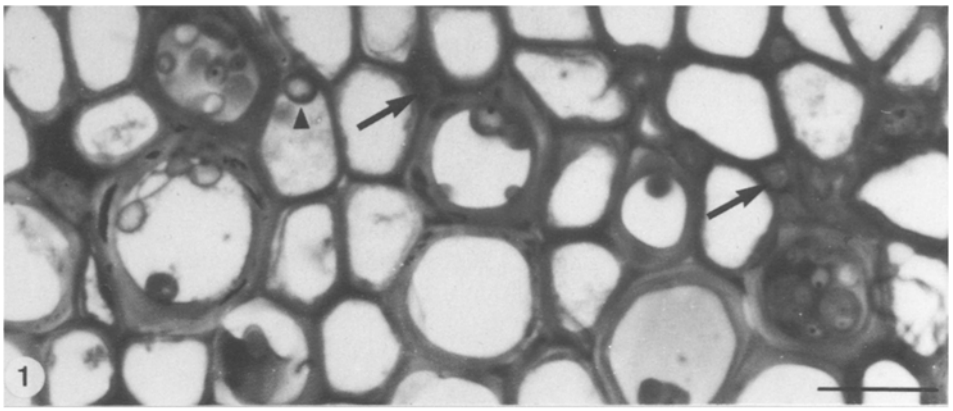
Results

'Early Sam' – Fusarium oxysporum f. sp. dianthi, race 1. Both race 1 isolates induced unusual, often unilateral wilt symptoms in 'Early Sam' carnations. Affected leaves turned a pale (rarely yellowish) green, and gradually withered and wilted while the midrib remained dark green or turned brownish. Burnt leaf tips sometimes occurred. The stem surface was not visibly affected, but for rare cracks. The small plants used in the first experiment regularly developed crook-necks, the larger ones of the second experiment did not. Wilt developed quicker in stem-inoculated plants (after 2-4 weeks) than in root-inoculated ones (after c. 6 weeks).

Affected vascular tissue showed an unusual pallid to pale brownish colour throughout, instead of the healthy greenish white. The brownish tinge was stronger near the infection source (especially in stem-inoculated plants) and diminished at higher colonization levels, where it sometimes disappeared altogether.

Infected plants were heavily colonized (Figs 1, 2). The fungus not only colonized xylem vessels, but also moved out into the intercellular spaces (Fig. 1) and into the cambium and phloem. Fungal hyphae in xylem vessels were sometimes coloured a bright red in unstained hand sections. Degradation of vascular tissues did virtually not occur (Figs 1-5). Even wilted, dead plants were left undegraded, their stems being dried out and hard instead.

A limited amount of defence reactions (Baayen and Elgersma, 1985; Baayen, 1986, 1987, 1988) was observed at a late stage, mainly near the infection source, and developed only slowly (Figs 3-5). Pale yellow vascular gums, xylem parenchyma cells with granular,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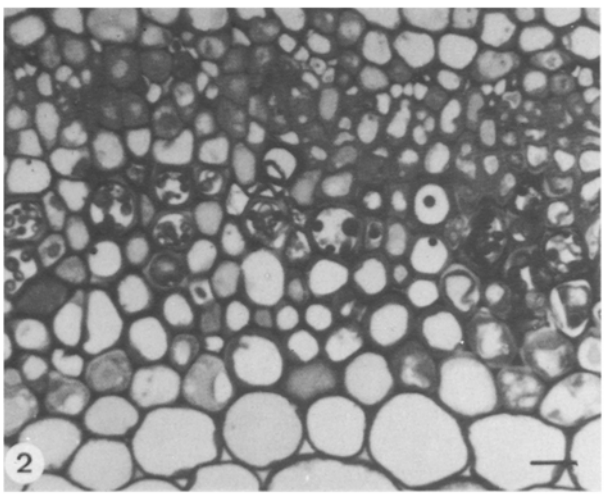
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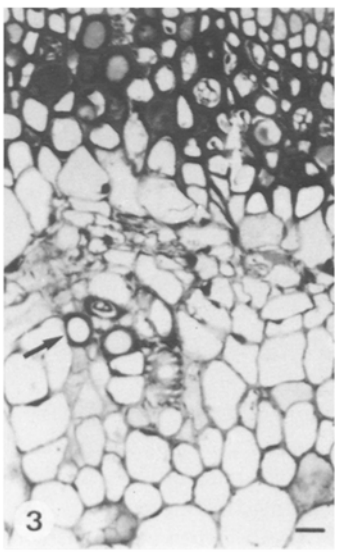
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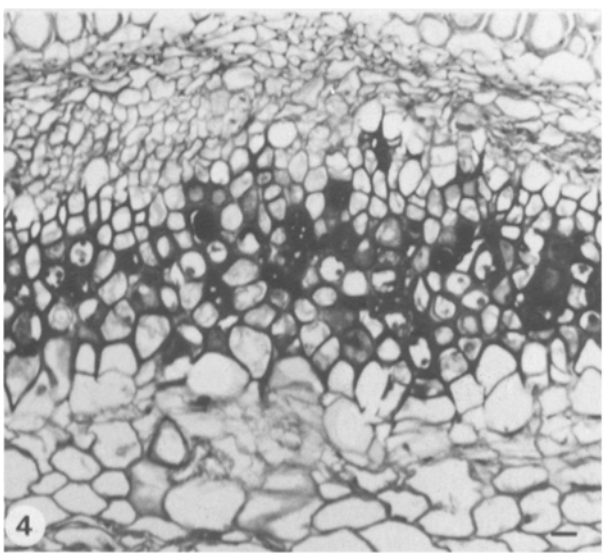
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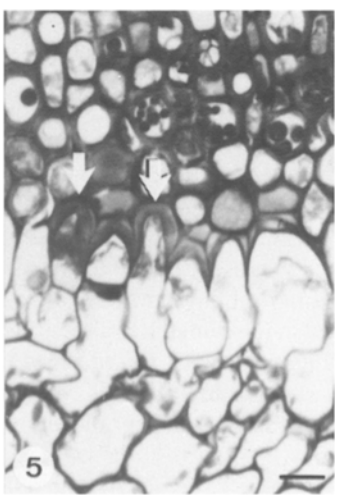
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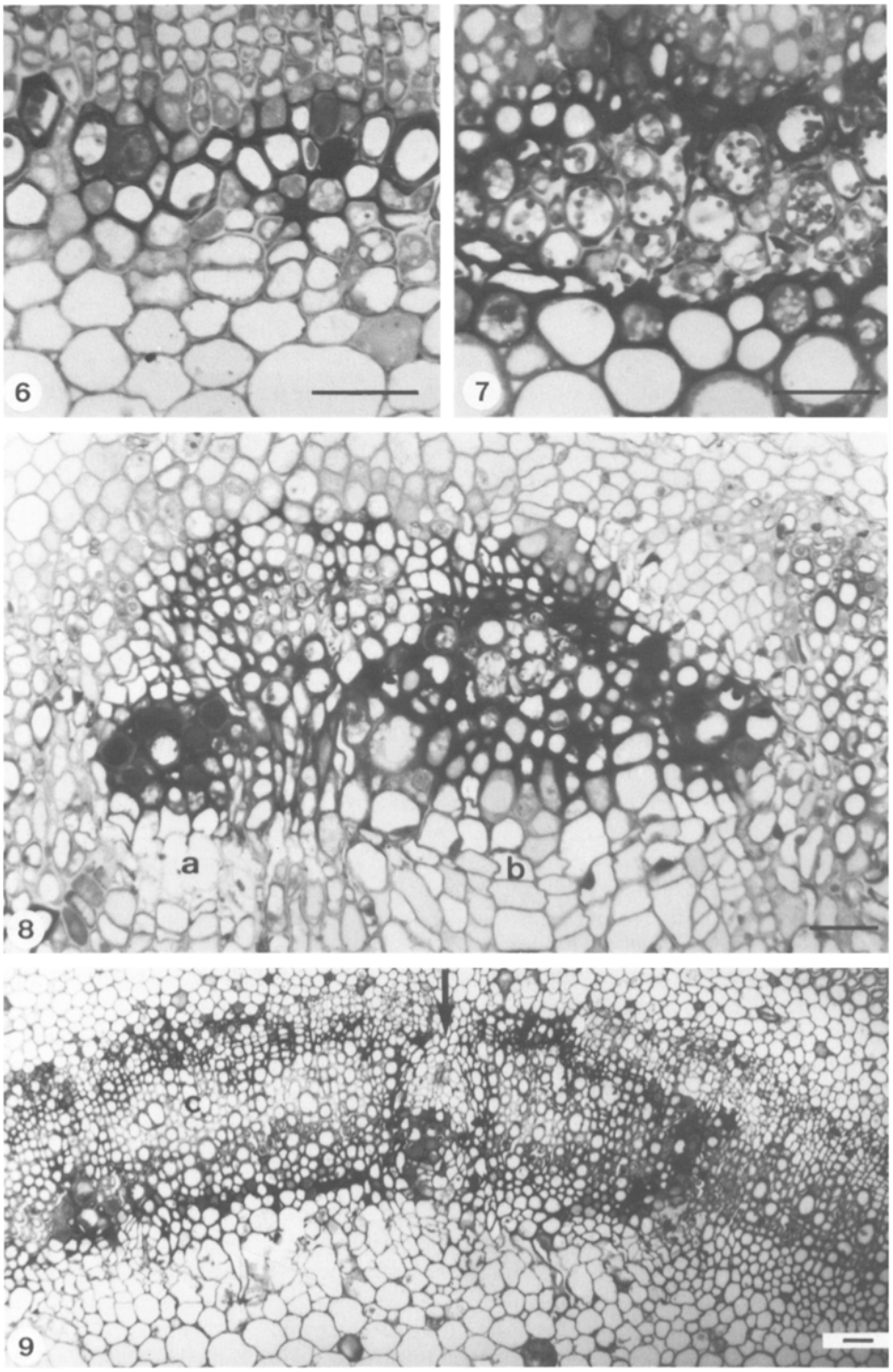
Figs 1-5. Parts of transverse sections of 'Early Sam' stem-inoculated with *F. oxysporum* f. sp. *dianthi* race 1 (G 1), showing vascular colonization virtually not followed by degradation. The medulla is at the bottom of the photomicrographs. Magnification bars represent 13 μm.
Fig. 1. Hyphae growing in xylem vessels, xylem parenchyma cells (arrowhead) and intercellular spaces (arrows) (two weeks after inoculation).
Fig. 2. General view of colonized xylem (one week after inoculation).
Fig. 3. Hyperplastic xylem parenchyma tissue with regenerating xylem vessels (arrow) next to colonized xylem with gums (four weeks after inoculation).
Fig. 4. General view of deformed colonized xylem with gums and impregnated, darkly stained primary cell walls (eight weeks after inoculation).
Fig. 5. U-shaped wall thickenings (arrows) bordering colonized xylem (eight weeks after inoculation).

brown contents, and at the medullary margin of colonized tissues in particular, various wall thickenings (Fig. 5) and wall appositions were formed. In due course, some xylem parenchyma cells adjacent to affected vessels showed renewed meristematic activity, which sometimes led to the formation of a layer of hyperplastic tissue (Fig. 3). Several hyperplastic cells adjacent to colonized xylem tissue eventually acquired suberized walls; locally, distinct phellem tissue thus was sometimes formed at the medullary side of the xylem. Few attempts of vascular regeneration occurred in non-suberized parts of hyperplastic tissues. Moderate yellowish brown discolouration of the primary walls of xylem parenchyma cells occurred throughout the colonized tissues, and was mainly responsible for the pallid vascular discolouration. The brown material was of lignin-like nature, staining red with phloroglucinol-HCl but not with Mäule's test. At medium heights, wall discolouration was the only visible response to colonization. Still higher up, this lignification-like response sometimes no longer accompanied colonization. Due to cell divisions and cell wall rigidification caused by the lignification-like response, affected xylem tissue often was quite distorted (Fig. 4).

'Early Sam' – Fusarium oxysporum f. sp. dianthi, race 2. The various race 2 isolates induced the usual unilateral wilt symptoms in 'Early Sam' carnations. The midribs or, more rarely, halves of affected leaves yellowed strongly, while the lamina otherwise remained green. Affected leaves eventually withered and wilted. Alternatively, withering of leaf bases without any yellowing (Baayen and De Maat, 1987) also induced wilting. The surface of the affected side of the stem regularly turned grey and withered, or turned yellow as well. Stem cracks also occurred. Repeatedly, unilaterally wilting plants were encountered of which the stem was grey, dried out and hollow on the wilted side, while the other side was healthy and even produced flowers. The small plants used in the first experiment regularly developed crook-necks; the larger ones of the second experiment did not. Wilt developed quicker in stem-inoculated plants (after 2-4 weeks) than in root-inoculated ones (after c. 6 weeks).

Affected vascular tissue was white and bordered on both sides by a striking dark brown layer. Although the brown discolouration sometimes extended upwards entirely along the colonization, it usually diminished at upper colonized parts, leaving the affected vascular tissue a bright white only. In lower parts of root-inoculated plants, affected vascular tissue sometimes was brownish throughout.

Infected plants were heavily colonized on the affected side. Fungal hyphae in xylem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Figs 6-9. Parts of transverse sections of 'Early Sam' stem-inoculated with *F. oxysporum* f. sp. *dianthi* race 2 showing vascular colonization followed by degradation. The medulla is at the bottom of the photomicrographs. Magnification bars represent 33 μm.

Fig. 6. Initial colonization stage with few vessels occluded with gums and many xylem parenchyma cells with impregnated, darkly stained primary walls (three days after inoculation with isolate WCS 816).

Fig. 7. Impregnated, darkly stained primary walls and intercellular spaces at the margins of colonized xylem undergoing degradation. In the colonized xylem, merely the lignified secondary walls of the vessels and the fungal hyphae have not been degraded (one week after inoculation with isolate G 75).

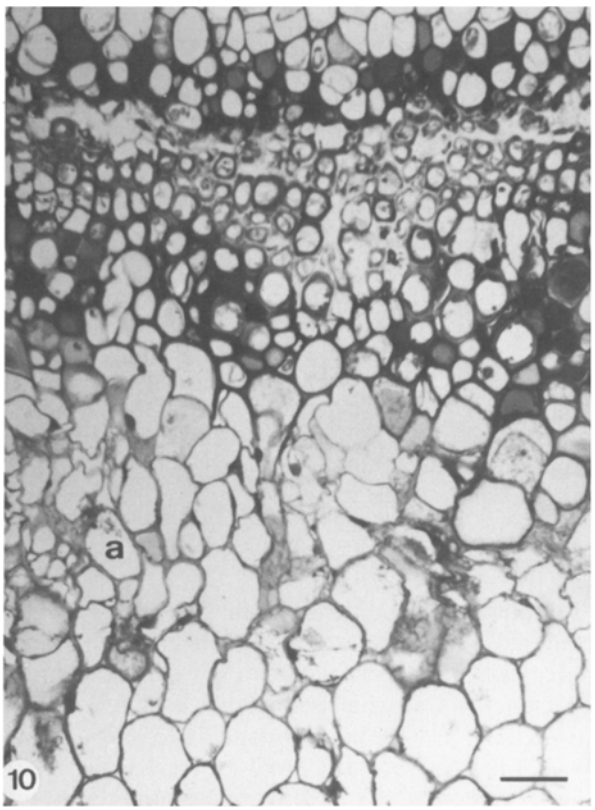
Fig. 8. A 'well-developed' localization area (a) with vessels occluded with gums and surrounded by hyperplastic parenchyma tissue, next to a colonized area (b) which lacks the gums but is also bordered by hyperplastic parenchyma tissue. In the upper middle, another colonized area bordered by parenchyma cells with only slightly impregnated walls (two weeks after inoculation with isolate WCS 816).

Fig. 9. Colonized xylem (c) bordered by parenchyma cells with impregnated walls, and with some gums and hyperplastic parenchyma cells on the medullar side, interrupted by a small 'localization area' (arrow). Unaffected xylem at the right side (8 weeks after inoculation with isolate WCS 816).

vessels were sometimes coloured a bright red. Colonization of the xylem was accompanied by degradation of primary cell walls and lysis of xylem parenchyma cells (Baayen and Elgersma, 1985). Colonized xylem was thus bleached and often transformed into cavities containing air and cell wall remnants. Cambium and phloem usually were colonized and degraded along with the xylem, in some cases eventually followed by the cortex, epidermis and medulla as well. Lignified secondary walls of vessels and fibers, however, and the epidermal cuticle were comparatively resistant to degradation. At the stem base surface, the fungus eventually formed sporodochia.

Already three days after vessel colonization, the primary walls and adjacent intercellular spaces of xylem parenchyma cells around affected vessels became impregnated with additional material. The impregnated structures gradually turned dark brown, due to accumulation of lignin-like material (staining red with phloroglucinol-HCl but not with Mäule's test). Initially, the response affected the primary walls shared with colonized vessels (contact walls) and the adjacent lateral walls of xylem parenchyma cells (Fig. 6). Due to the degradation process, the response developed only fully and therefore caused macroscopic browning in the parenchyma cells at the cortical and medullary margins of colonized vascular tissue (Figs 7, 9). The brown, lignification-like phenomenon at colonization boundaries was the prevailing response to colonization. At the upper colonized parts the lignification-like response sometimes was absent, leaving the colonized xylem a mere white. Heavily impregnated, brown walls and suberized ones as mentioned below were resistant to degradation and appeared to form a barrier to lateral spread of the fungus.

Vascular colonization was followed by many defence reactions beside the lignification-like response (Figs 6-11). Host responses in stem-inoculated plants initially (Fig. 6) were similar to those of resistant 'Novada' carnations (Baayen and Elgersma, 1985; Baayen, 1988) except for the presence of fewer vascular gums (in the first experiment slightly less already after three days, and clearly less one week after inoculation). Localization of the pathogen was sometimes succesful. Usually, however, the pathogen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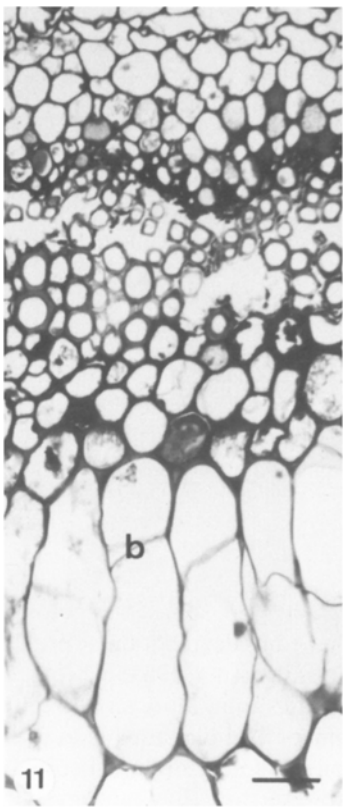
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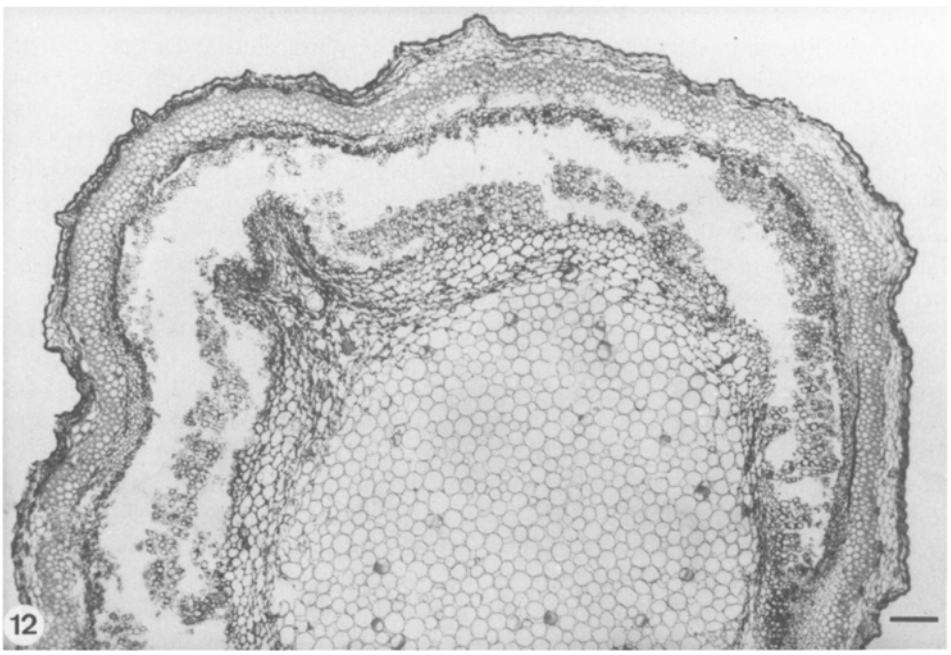
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Figs 10, 11. Parts of transverse sections of 'Early Sam' stem-inoculated with *F. oxysporum* f. sp. *dianthi* race 2, showing irregularly hyperplastic (a) and hypertrophied (b) xylem parenchyma cells at the medullary side of vascular tissue undergoing degradation. Other defence responses such as gums and impregnated, darkly stained walls at colonization margins are visible as well. Magnification bars represent 33 μm.

Fig. 10. Four weeks after inoculation with isolate WCS 843.

Fig. 11. Eight weeks after inoculation with isolate WCS 816.

Fig. 12. Part of transverse section of 'Early Sam' four weeks after stem-inoculation with *F. oxysporum* f. sp. *dianthi* race 4 (G 310) showing cavities at the site of the vascular tissues, with remainders of the secondary walls of vessels and fibers. Magnification bar represents 130 μm.

escaped (Fig. 8) within a week after stem inoculation, initially to induce no further defence or only slight browning of primary walls at colonization margins. Nevertheless, defence reactions such as gum formation, hyperplasia of xylem parenchyma followed by suberization (i.e., differentiation of phellem tissue), the lignification-like response and the formation of various wall thickenings and appositions eventually occurred in many cases at lower colonized parts. This was observed particularly on the medullary side of the xylem, while the cortical side was colonized, degraded and brown-edged (Figs 8-11). These defence reactions generally were ill-developed and irregular (such as irregular hyperplasia and hypertrophy; Figs 10, 11), but sometimes were sufficiently developed to allow complete vascular regeneration next to colonized xylem (Baayen, 1986). At lower colonization levels, small localization areas often alternated with brown-edged colonized areas with or without defence reactions on the medullary side (Fig. 9).

Due to effective localization responses, a few stem- and root-inoculated plants remained healthy throughout the experiments. In other plants, infection was effectively localized on one side of the stem but not on the other, resulting in unilateral colonization and wilting. A resistant response frequently occurred below the inoculation site in stem-inoculated plants, thus restricting disease to the part of the shoot above the inoculation site. Exceptionally, brown-margined colonized xylem could be traced into a leaf, leaving the plant as a whole unaffected.

'Early Sam' – Fusarium oxysporum f. sp. dianthi, race 4. Both race 4 isolates induced comparable symptoms and histopathology in 'Early Sam' carnations as did race 2. However, affected xylem had a bright white colour and usually only at lower colonization levels was slightly brown-edged or brownish throughout. Defence reactions, including the brown lignification-like response at colonization boundaries were virtually absent in the first experiment, and never abundant in the second one. Correspondingly, degradation of vascular tissues (Fig. 12) was heavy; in the first experiment, it occurred already within a week after stem inoculation. Xylem vessel regeneration was sometimes initiated in medulla (rarely cortex) parenchyma cells next to vascular cavities.

Wilt developed quicker in stem-inoculated plants (within 2 weeks in the first experiment, after c. 4 weeks in the second one) than in root-inoculated ones (after c. 8 weeks). Virtually all stem-inoculated plants of the second experiment finally were severely diseased or wilted; however, for unknown reasons most root-inoculated ones remained healthy, and their stems internally unaffected and free of colonization.

'Early Sam' – Fusarium oxysporum f. sp. dianthi, race 8. The single race 8 isolate

induced unusual, unilateral wilt symptoms in 'Early Sam' similar to those provoked by race 1. Unilateral necrosis of the basal margins of otherwise healthy leaves, necrosis of leaf tips, and the formation of lesions around leaf midribs occurred as well. The midribs themselves often turned brown, bordered by a narrow yellow zone; yellowing otherwise was uncommon. Wilt developed very slowly, though quicker in stem-inoculated plants (after 4 weeks) than in root-inoculated ones (after 6 weeks). Internally, affected vascular tissue had a deep light brown colour throughout at lower levels, diminishing to a pallid discolouration higher up. Stems of infected plants were colonized to moderate heights. Degradation of vascular tissues occurred only rarely. Instead, many defence reactions like the formation of pale coloured gums, xylem parenchyma cells with brown and granular contents, browned primary walls and a broad zone of hyperplastic xylem parenchyma tissue with yellowish wall thickenings (compare Fig. 5) at its border accompanied colonization up to high levels. The hyperplastic zone repeatedly developed an inner zone of suberized phellem cells, and an outer zone in which some vascular regeneration took place.

'Novada' – Fusarium oxysporum f. sp. dianthi, races 1, 2 and 4. Races 1, 2 and 4 did not induce visible disease symptoms in 'Novada' carnations throughout both experiments. Microscopic examination of infected material showed that all stem-inoculated plants had localized the infection within a few cm from the inoculation site as previously reported (Baayen and Elgersma, 1985; Baayen, 1986, 1987), whereas stems of root-inoculated plants were entirely unaffected. The localization response invariably consisted of the occlusion of xylem vessels with brown gums, and 'hypersensitive' browning and death of some xylem parenchyma cells, as well as the occurrence of various wall modifications in other xylem parenchyma cells. The wall modifications comprised the formation of wall thickenings and appositions, and impregnation with phenolic-containing material. The brown gums and impregnated walls eventually contained phenolic polymers related to lignin. The occluded zone was surrounded by hyperplastic xylem parenchyma tissue, of which the inner cell layers differentiated into suberized phellem tissue, whereas vascular regeneration occurred in the outer layers. These phenomena are described in detail elsewhere (Baayen and Elgersma, 1985; Baayen, 1986, 1987, 1988).

'Early Sam' and 'Novada' – Fusarium oxysporum f. sp. lycopersici. Stem inoculation both of 'Early Sam' and 'Novada' with *F. oxysporum* f. sp. *lycopersici* did not induce visible disease symptoms. Microscopic examination of the infected material showed that all plants had localized the infection within a cm from the inoculation site. The localization response was similar to the response described for 'Novada' inoculated with races 1, 2 and 4 of *F. oxysporum* f. sp. *dianthi*, although it took place very closely above the incision.

'Early Sam' and 'Novada' controls treated with sterile water. Treatment of stems or roots of 'Early Sam' and 'Novada' carnations with sterile water did not induce disease. Microscopic examination only revealed normal wound healing responses at the stem incisions as described by Baayen (1986). In both experiments, a few plants exceptionally became diseased after several months. As judged by internal symptoms, this appeared to be due in all cases to infection by *F. oxysporum* f. sp. *dianthi* race 2.

Discussion

'Early Sam' carnations proved to be susceptible, and 'Novada' carnations resistant to all races studied of *F. oxysporum* f. sp. *dianthi*. Differences in susceptibility of 'Early Sam' to the four races were not determined, nor would this be easy as the external symptoms provoked by the races were not the same. The presently described differences in pathogenesis among susceptible interactions of carnation with races of *F. oxysporum* f. sp. *dianthi* have consistently been observed both with several carnation cultivars (Demmink, Baayen and Sparnaaij, unpublished), with several isolates of each race, and with root as well as stem inoculation (present data).

'Early Sam' carnations inoculated with the most common race 2 developed the usual fusarium wilt symptoms (Bickerton, 1942; Hantschke, 1961). At low heights, colonization by race 2 was accompanied by defence reactions such as gum formation and hyperplasia of xylem parenchyma as noticed by Pennypacker and Nelson (1972) and Harling and Taylor (1985). Presumably due to deficient formation or quick degradation of the gums, these reactions often were ineffective for localization of the pathogen. At higher heights colonized tissue was typically brown-edged, due to the deposition of brown, lignin-like material (lignification *sensu lato*) as also deposited to a smaller extent in resistant interactions (Baayen, 1988). 'Lignified' as well as suberized tissues formed at colonization edges were resistant to degradation by the pathogen, and presumably formed a lateral barrier to fungal spread. Degradation of the vascular tissues of carnation by *F. oxysporum* f. sp. *dianthi* has long been noticed (Bickerton, 1942; Pennypacker and Nelson, 1972; Baayen and Elgersma, 1985; Harling and Taylor, 1985). Other formae speciales degrade their host as well (Ho et al., 1985). *F. oxysporum* readily produces many cell wall-degrading enzymes (Pegg, 1985); four endopolygalacturonase isozymes are presently known for f. sp. *dianthi* (Scala et al., 1981). Of the symptoms induced by race 2, wilting is probably due mainly to vascular destruction (Baayen and De Maat, 1987). 'Lignified' and suberized lateral barriers may additionally hamper water dislocation from colonized xylem into the leaves. The yellowing response resembled premature senescence. Crook-neck formation seems to occur only after unilateral colonization of young, growing shoot tips (Snyder, 1941; Bickerton, 1942). The phenomenon is probably due to inhibition of the elongation of the colonized side of the shoot, as crook-necks always curve towards the affected side. Inhibition of elongation may result from death of the elongating tissues because of degradation, from rigidification of the affected tissues because of the deposition of lignin-like material, or from both.

Disease development in 'Early Sam' carnations infected with race 4 was comparable to that provoked by race 2 but for less intensive defence reactions such as the brown lignification-like response, and more severe degradation. The differences appear to be largely quantitative.

Disease development in 'Early Sam' carnations infected with race 1 was entirely different. Foliar symptoms resembled a slow necrosis, and vascular degradation did virtually not occur. Deposition of lignin-like material and other defence reactions developed only well after colonization and were not effective in localizing the pathogen. Symptoms induced by race 1 may be due to accumulation to toxic levels of fungal metabolites produced in the xylem. A severe necrosis sometimes follows (Demmink et al., 1987). Although not always present, the formation of 'lignified' and suberized lateral barriers may have additionally hampered lateral water transport.

The wilt symptoms and pathogenesis induced by race 8 resembled those induced by race 1, rather than those induced by races 2 and 4, and may be caused in a similar way.

With several of the races, fungal hyphae in xylem vessels were repeatedly coloured a bright red in unstained hand-made sections, which is remarkable. The red colour may well be a pigment such as the fungus produces in vitro.

Defence was elicited in susceptible as well as resistant interactions with race 2. The differences initially were merely quantitative; the localization response was always successful in 'Novada', but only rarely in 'Early Sam'. Resistance of carnation cultivars to race 2 shows a large, quantitative variation (Sparnaaij and Demmink, 1977). It is polygenic, and to some extent correlated with resistance to phialophora wilt which also is partial and polygenic (Sparnaaij and Demmink, 1976, 1977, and unpublished results). Partial resistance of carnation to race 2 apparently is largely a property of the cultivar, independant of interactions with the pathogen; i.e., it is horizontal sensu Vanderplank (1984). We propose that quantitative variation between cultivars in resistance components which contribute to the localization response determines their localization capacity and thereby the resistance level. The same holds for resistance to race 4, which also is partial and seems to be largely correlated to resistance to race 2 as well (Garibaldi, 1983; Demmink, Baayen and Sparnaaij, unpublished results). Both races 2 and 4 could belong to one variable complex. Minor variant interactions within such a (hypothetical) race 2-race 4 complex occur even within races 2 and 4 themselves (Sparnaaij and Demmink, 1987; Demmink, unpublished results), and not necessarily imply gene-for-gene interactions. Physiological variation among pathogenic strains may also interact with physiological differences in resistance expression between the cultivars. Variation in production of different fungal cell wall-degrading enzymes may interact with variation in composition of host cell walls and gums, as may variation in fungal sensitivity to different phytoalexins with variation in production by the host of the different phytoalexins, for example.

Race 1 clearly differs from races 2 and 4. Its virulence pattern is entirely different (Garibaldi, 1983; Demmink et al., 1987), and it does virtually not degrade its hosts. Race specificity in *F. oxysporum* f. sp. *dianthi* apparently is linked to genes coding for distinctive physiological characters. This is no exception in *F. oxysporum*: cultural distinctions occur between races of f. sp. *apii* (Puhalla, 1984) and between races of f. sp. *cubense* (Su et al., 1986), and pathogenetical ones between races of f. sp. *melonis* (Armstrong and Armstrong, 1978) and between f. sp. *lycopersici* and f. sp. *radicis-lycopersici* (Jarvis and Shoemaker, 1978). More information on the biological relationships between the races of f. sp. *dianthi* is required.

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Samenvatting

Verschillen in pathogenese waargenomen tussen vatbare interacties van anjer met vier fysio's van Fusarium oxysporum f. sp. dianthi

Tussen interacties van 'Early Sam'-anjers met fysio's 1, 2, 4 en 8 van *F. oxysporum* f. sp. *dianthi* werden verschillen in ziekteontwikkeling gevonden na wortel- zowel als stengelinoculatie. Fysio 1 gaf verbleking en verdroging van de bladeren. Aangetast vaatweefsel was gelijkmatig vaal of lichtbruin van kleur, en werd hevig gekoloniseerd, maar vrijwel niet afgebroken. Afweerreacties kwamen slechts traag op gang. Fysio 2 gaf vergeling, in het bijzonder van de hoofdnerven, en verdroging van de bladeren. Aangetast vaatweefsel was wit met donkerbruine randen. Gekoloniseerd weefsel werd afgebroken, hetgeen leidde tot de vorming van holten in het vaatweefsel. In de lagere gekoloniseerde delen traden veel afweerreacties op, hetgeen soms lokalisatie van het pathogeen tot gevolg had. Fysio 4 gaf eenzelfde ziekteontwikkeling als fysio 2, maar minder afweerreacties. Fysio 8 gaf lesies bij de hoofdnerven, en verbleking, verdroging en necrose van bladeren. Aangetast vaatweefsel was gelijkmatig lichtbruin van kleur. Afbraak van gekoloniseerd vaatweefsel werd zelden waargenomen; veel afweerreacties vergezelden de kolonisatie tot hoog in de stengel.

Inoculatie van 'Novada' anjers met fysio's 1, 2 en 4 van *F. oxysporum* f. sp. *dianthi* had geen ziektesymptomen tot gevolg. Via de stengel geïnoculeerde planten lokaliseerden de infectie ter hoogte van het inoculatiepunt; de stengels van via de wortels geïnoculeerde planten waren onaangetast. De lokalisatiereactie trad ook op in 'Early Sam' en 'Novada' anjers na inoculatie via de stengel met *F. oxysporum* f. sp. *lycopersici*.

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