European Journal of Plant Pathology **104:** 725–736, 1998.

Histology of root rot of flax seedlings (*Linum usitatissimum)* infected by *Fusarium oxysporum* f.sp. *lini*

G.M.L.W. Kroes[1], R.P. Baayen[2,*] and W. Lange[1]
[1] *Centre for Plant Breeding and Reproduction Research (CPRO-DLO), PO Box 16, NL-6700 AA Wageningen, The Netherlands;* [2] *Research Institute for Plant Protection (IPO-DLO), PO Box 9060, NL-6700 GW Wageningen, The Netherlands;* * *Author for correspondence*

Accepted 21 July 1998

Key words: appositions, cell wall degradation, colonisation, infection, resistance, root tip

Abstract

The histology of root rot of flax seedlings infected by *Fusarium oxysporum* f.sp. *lini* was studied using semi-thin sections of plastic-embedded roots. Within two days, the fungus colonised the root cap cell layers by intercellular and intracellular growth. Attempted intercellular penetration of root cap cells via the middle lamella induced the formation of appositions next to penetrating hyphae. Other cells next to invading hyphae collapsed, which was accompanied by swelling of the cells neighbouring the collapsing cells. Invasion of the root cap and growth towards the protodermis seemed retarded to some extent by the natural sloughing off of root cap cell layers. The protodermis and cortex were reached and penetrated in four days, which was followed by rapid and massive colonisation of the entire root tip. The protoxylem was reached in eight days. From eight to sixteen days after inoculation, the lower parts of the roots were colonised throughout and the cortical region was degraded. Colonised tissues were severely plasmolyzed. Heavily colonised roots were hollowed out, the only remaining tissues being the epidermis and exodermis outside, and remainders of the colonised xylem inside. Upward spread of root rot was restricted in the period studied to the first 10 mm from the root tip, the upper parts of the root and the hypocotyl being unaffected except for invasion through lateral roots infected at their respective tips. Mature roots with a well-developed epidermis and exodermis were not invaded from outside. Disease development was similar in partially resistant 'Hermes' and susceptible 'Regina', except for rot development that was consistently slightly more extensive in the susceptible cultivar. Distinct extravascular resistance factors were not detected in 'Hermes', suggesting that extravascular resistance in flax to *F. oxysporum* f.sp. *lini* is of a quantitative nature.

Introduction

Fusarium oxysporum f.sp. *lini* (Bolley) Snyder and Hansen, causal agent of wilt disease in flax (*Linum usitatissimum* L.), is one of the most important diseases in this crop all over the world. The fungus is soil-borne and infection takes place mainly through the roots, although the hypocotyl may also be infected (Nair, 1956). Several early studies on this disease concern the mode of infection and colonisation of flax by *F. oxysporum* f.sp. *lini* (Tisdale, 1917; Boyle, 1934; Millikan, 1951; Nair, 1956; Nair and Kommedahl, 1957). Although *F. oxysporum* f.sp. *lini* induces wilt symptoms in flax and colonizes the vascular tissues of roots and stems, typical root rot symptoms also are a well-known part of the disease syndrome. Rotted roots are observed commonly and consistently in field trials; sudden death of flax crops often coincides with massive root rot (G.M.L.W. Kroes, personal observations from various countries over several years). Boyle (1934) considered root rot and wilt of flax to represent two distinct types of the disease, each with independently inherited resistance factors. If indeed the same fungus is able to incite both rot and wilt symptoms, root rot may facilitate or initiate vascular colonization, or be a secondary stage of disease, or a combination of these. Alternatively, a complex of pathogenic strains may be involved differing in their

mode of action. No conclusive evidence is available, however, for the existence of races among aggressive isolates of *F. oxysporum* f.sp. *lini* (Kroes, 1997).

Several flax cultivars including 'Hermes' exhibit high levels of partial (field) resistance to *F. oxysporum* f.sp. *lini* even when susceptible cultivars such as 'Regina' succumb totally to the disease (Beaudoin 1991). In the laboratory, foliar symptom development during the first three weeks after root infection does not reflect field resistance and susceptibility levels; in contrast, differences in sprout length reduction (an indicator of root damage) correlate well to field resistance (Kroes et al., 1998). Differential root invasion and root rot development in the initial phase of disease may therefore contribute to resistance to *F. oxysporum* f.sp. *lini*, additional to vascular resistance factors in the stem that are expressed in later phases. However, the contribution of root-bound resistance factors would seem to be relatively small, since sprout length in susceptible cultivars is reduced only slightly (about 30%) more than in resistant ones (Kroes et al., 1998). The present study was undertaken to investigate the early stages of root pathogenesis and rot development in flax, as well as to check whether or not appreciable qualitative differences in root rot development exist between cultivars with contrasting levels of field resistance. We used a bioassay developed for this purpose (Kroes et al., 1998) and a highly aggressive isolate used as a standard in French greenhouse tests (INRA, Versailles) to determine *Fusarium* resistance in new flax cultivars. Some general aspects of root infection and early stages of vascular colonization have been described by Turlier et al. (1994) using hydroponically grown flax seedlings and a GUS-transformed strain of the pathogen. The present study, however, is the first to provide an accurate description of root rot development in flax infected with *F. oxysporum* f.sp. *lini.*

Materials and methods

Host and pathogen

Flax seeds from partially resistant cv. Hermes and susceptible cv. Regina were obtained from Landbouwbureau Wiersum, Dronten, The Netherlands, and CPRO-DLO stock collection, respectively. Seeds were sterilised prior to use for 15 seconds in 70% ethanol, followed by 15 min in 1% hypochlorite.

Single-spore cultures of *F. oxysporum* f.sp. *lini* (Fof), isolate Fof-F60 (a highly aggressive isolate used as a standard in greenhouse tests at INRA, Versailles), were provided by Dr. G. Fouilloux, Versailles, France. Stock cultures were stored at −80 °C on PROTECT bacterial preservers (Technical Service Consultants Ltd, UK).

Experimental design

Seedlings were grown on moist filter paper in closed preserving jars as described and illustrated by Kroes et al. (1998). In short, the seeds were placed on the upper edge of a 5 cm high strip of moist filter paper located against the inner wall of two-litre glass preserving jars in such a way that the young roots could develop in between glass and paper. The lowermost 5 mm of the filter paper was suspended into 100 ml of a 10% MS-solution (Murashige and Skoog 1962) at the bottom of the jar. Prior to placing the seeds, the glass jars with the filter paper and nutrient solution were autoclaved for 20 min. Sixteen preserving jars were used, each of which contained three sterilised seeds of each cultivar that were randomly placed in the jar. The jars were placed in a climate chamber with 16 h light (Philips 84 HF, 1100 lux) per day, at a temperature of 23 °C. The outside of the jars was covered with aluminium foil to protect the developing roots from direct light. The seeds germinated rapidly and seedling roots reached the nutrient solution within three days.

Inoculation of seedlings

Plants were inoculated six days after sowing, when the young roots extended for several cm into the nutrient solution. Inoculum was prepared from 14-day-old potato dextrose agar cultures of the fungus by flooding these with sterile water, scraping the surface with a transferring needle and adjusting the resulting spore suspension with a hemacytometer to 10^5 spores per ml. The seedlings from 12 jars were inoculated by pouring 1 ml spore suspension onto each root, while four jars were treated with sterile water as a control. For seedling root tips suspending in the nutrient solution, this resulted in an inoculum dosage of 6×10^3 spores per ml nutrient solution. After two, four, eight, and sixteen days, three seedlings per cultivar were harvested for further processing from each of the three inoculated jars, and three seedlings per cultivar were harvested from the control jars.

Light microscopy

Immediately after harvesting, segments of 3–5 mm length of root tips, branched root segments, root segments from the part of the root 1 cm below the junction of stem and root, and hypocotyl segments, were fixed in 3% glutaraldehyde in 0.025 m phosphate buffer (pH 6.8). Fixed segments were dehydrated in a graded ethanol series and embedded in Technovit 7100 (Heraeus Kulzer GmbH, Friedrichsdorf, Germany). From all harvests, four to ten samples of root tips of both cultivars were examined, and two to six samples per cultivar of root branching sites, of parts 1 cm below the junction of stem and root, and of the hypocotyl. Using a Jung 2040 rotary microtome and Ralph glass knives, sections of 1.5 – 2.0 μm thickness were made at various levels of the root tips and up to 0.5 cm above the root tips, and from the other plant parts described. The sections were stained with toluidine blue O (Jensen, 1962), a metachromatic stain allowing for discrimination between acid polysaccharides such as pectic acid (purple-red metachromasy) and phenolics-containing materials such as lignin and suberin (blue-green orthochromasy) (Clark et al., 1981; O'Brien and McCully 1981; Krishnamurthy 1988). Sections were viewed with a Zeiss Axioplan microscope (Zeiss-Nederland B.V., Weesp, The Netherlands) using bright-field light microscopy and differential interference contrast, and photographs were recorded using Zeiss MC-100 photographic equipment on Ilford Pan F50, Kodak Technical Pan and Kodak Ektachrome 320 T film.

Results

Control seedlings

Roots and shoots of water-treated controls remained healthy throughout the experiment. Root anatomy followed the usual pattern for dicots, except for the root cap that extended upwards along the root for several mm (for an illustration, see Turlier et al., 1994). Root cap cells were generated from protodermal cells with similar cytoplasmic density as the cortical cells, but distinguishable from these by their longitudinal rather than isodiametric shape and by tangential cross walls reflecting the meristematic activity of the protodermal cell layer. This condition is illustrated in Figure 1, from an inoculated but still virtually unaffected root. Higher up, roots were covered by a single epidermal layer, separated from the cortex by a single layer of suberized exodermal cells.

Macroscopic symptoms

The fungus showed a distinct preference for the root tip throughout the experiment. Two days after inoculation, the fungus was ubiquitously present around the root tips but much less so in the zone of elongation or at lateral root branches. Three to five days after inoculation, most root tips turned purple over the first 1.5 mm, assumedly due to the production of fungal pigments. After about eight days the root tips decayed and turned brown. After twelve days, the brown zone extended to 3–5 mm from the tip. Decay spread right across the root, eventually resulting in hollow roots consisting of merely the epidermis and exodermis as outer coat and, internally, remainders of the stele. After sixteen days, decay had spread to maximally 10 mm from the tip, and both cultivars had developed disease symptoms such as leaf yellowing and necrosis of the shoot apex. Disease development was similar in both cultivars, although discolouration and rot consistently were slightly more extensive in 'Regina' than in 'Hermes'. No appreciable differences in histopathology were encountered between both cultivars that could explain these minor differences in rot development. In the following sections, root infection, colonization and rot are therefore described without further reference to 'Regina' or 'Hermes'.

Infection and colonisation of the root tip

Two days after inoculation, fungal hyphae proliferated in between the cell layers of the root cap as well as inside the cells of the outer, senescent and already detached layers of the cap (Figure 1A to C). From the colonised outer layers, the fungus attempted to invade the inner, healthy layers of the root cap that still were closely pressed to the root. Fungal hyphae grew through the middle lamella, and in the mucilage that covered the root cap cells (Figure 1C). Fungal growth in new root cap layers initially was intercellular only, although rapidly followed by intracellular growth in detaching cell layers.

Penetration of hyphae into the middle lamellae between the anticlinal walls of root cap and protodermis cells induced the production of disc-shaped to globose appositions in these cells (Figure 1C), as well as the collapse of cells (Figure 1A to C). Collapsing cells were often bordered by swollen cells, and had darkly

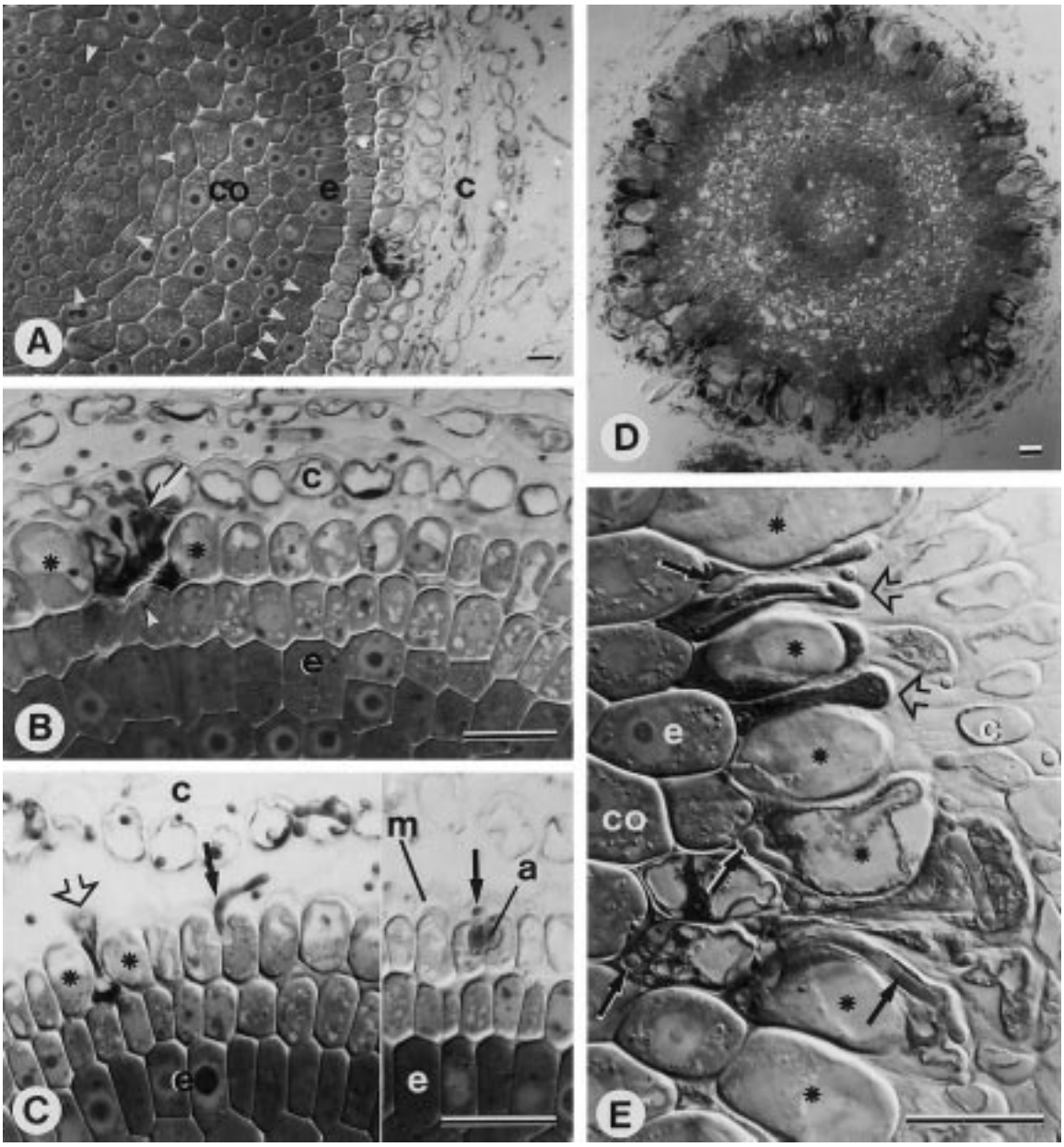

Figure 1. Micrographs of flax seedling roots, two days (A–C) and four days (D–E) after inoculation with *Fusarium oxysporum* f.sp. *lini*. Sections of the meristematic zone close to the root tip. Bar = 20 μm.

A. General view. Fungal hyphae are present abundantly in between the cell layers of the root cap (c). The protodermis (e) is covered by several cell layers that together form the root cap. The cortex (co) consists of isodiametric cells. Note meristematic activity in the protodermis and the pericycle (arrowheads). Cv. Regina.

B. Detail of A. Cell collapse at the site of invasion (arrow) of a fungal hypha into the root cap. Cell contents are intensely stained with toluidine blue O. Neighbouring root cap cells are swollen (∗), and root cap cells underneath have formed an apposition-like structure (arrowhead).

C. Swollen cells (∗) next to a collapsed cell (open arrow), and appositions (a) produced in other root cap cells next to penetration hyphae (black arrows) coming from the mucilage (m) covering these cells. Cv. Hermes.

D. General view of an infected root showing darkly stained, collapsed root cap cells and swollen neighbouring cap cells. The cortex has not yet been invaded. Cv. Hermes.

E. Detail of E. Many cells have collapsed (open arrows), and their contents are intensely stained with toluidine blue O. Neighbouring cells are heavily swollen (*). The penetration hyphae (black arrows) have just reached the protodermis and the cortex (co).

staining contents. Appositions stained blue-green (orthochromatic) with toluidine blue O, indicative of the deposition of phenolics. Appositions were also observed in senescent, plasmolysed root cap cell layers in the process of detachment and at later stages of pathogenesis often also in cells of fully detached root cap layers that had been sloughed off (not shown). Invasion of the root cap and growth towards the protodermis seemed retarded to some extent by the natural sloughing off of root cap cell layers.

Four days after inoculation (Figure 1D through 3B), the root cap had been massively invaded in both cultivars up to 1.5 mm from the root tip. Most root cap cells were heavily plasmolysed and seemed dead. Collapsing, darkly staining cells neighboured by inflated and swollen ones were at this stage commonly observed in the inner root cap layers next to the protodermis (Figure 1D and 1E). The fungus had passed the root cap and reached the protodermis at several places (Figure 1E), which was followed by rapid and massive colonisation of the entire root tip, as shown in Figure 2 for successive sections of the same root tip. Close to the tip of this root (about 0.2 mm), the fungus had colonised the root throughout, including the cortical and stelar tissues (Figure 2A). Cellular disorganisation and incipient plasmolysis were observed throughout the root. Slightly further off from the tip of the same root (Figure 2B), the same condition was observed except for one side of the root where a single row of living, more or less unaffected protodermal cells was present. Still farther back (Figure 2C, overview and Figure 3A and B, detail), about two-thirds of the root was diseased whereas one third part was unaffected. A sharp boundary existed between affected and unaffected cells. No hyphae were present in the unaffected region, that apparently connected with the single row of unaffected protodermal cells closer to the tip. At 0.3 mm from the tip (Figure 2D), the entire root including the stele seemed unaffected except for the root cap. Typical appositions were not produced in cortex cells. The cortex was colonised intercellularly, although the fungus was also observed inside splitted cell walls and eventually also inside the cells (Figure 3A and B). Colonised parts of the cortex underwent plasmolysis and cell contents were disorganised (Figure 1E; Figure 3A and B).

Eight days after inoculation (Figs. 4 and 5), root tips were invariably colonised throughout and the contents of colonised cells stained little or not compared with four days earlier. Cell disorganisation was more severe than four days after inoculation (Figure 4A). Intercellular growth of fungal hyphae induced severe plasmolysis of neighbouring cortical cells (Figure 4B and 4C) and, eventually, dissolution of cell walls and decay of the colonised tissues (Figure 5). Behind the zone of elongation ($>$ 2 mm from the root tip), infection via the epidermis was not observed, even though fungal hyphae did now and then occur on the surface of mature parts of the root (not shown). Mature parts of roots were generally colonised from within, apparently from lower situated colonised parts (Figure 5A). Fungal hyphae grew into the differentiating protoxylem vessels (Figure 5B, overview and Figure 5C, detail). In general, fungal hyphae in and around the stelar tissues stained intensely while in the outer parts of the root the hyphae were poorly stained (Figs. 4A, 4B and 5B), suggesting a withdrawal of fungal cytoplasm from previously colonised areas to the colonisation front.

Sixteen days after inoculation, tissue death was complete up to 10 mm from the root tip and, as judged by their staining ability, fungal hyphae had withdrawn most of their cytoplasm from this region. Dissolution of cortical tissues was extensive (Figure 6A). Typically, the cortex of mature roots was hollowed out, the diseased roots being covered by only the exodermis and the epidermis (Figure 6B and 6C). While this was invariably the case in susceptible ‘Regina’, degradation was less extensive in ‘Hermes’, which at this stage frequently had unknown occluding materials in the intercellular spaces materials that stained orthochromatically with toluidine blue, indicating the presence of phenolics, and had similarly greenish stained remainders of cortical cell walls (Figure 6D).

Upper root parts and the hypocotyl region

Throughout the experiment, fungal hyphae remained confined to the lower parts of the roots although the entire root surface had been inoculated. In the upper parts of the root (10 mm from the hypocotyl) and in the hypocotyl region itself no fungal material or host reactions to colonisation were observed at all. However, in a number of cases fungal hyphae were encountered inside the cortex and xylem of roots at branching sites (not shown); such observations were only made at 16 days after inoculation. In such cases infection seemed to have taken place at the tip of the lateral root.

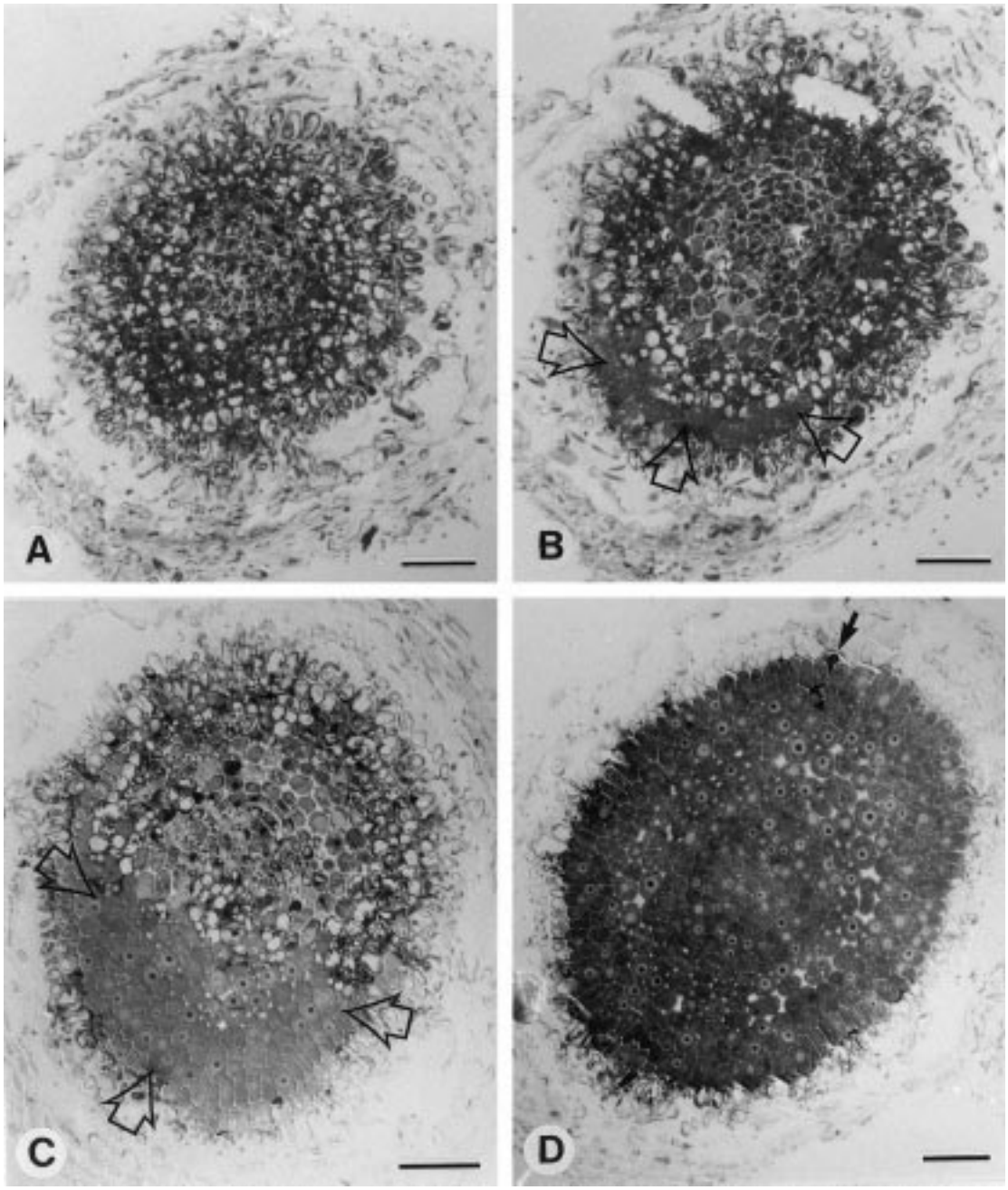

Figure 2. Micrographs of successive sections of a seedling root, four days after inoculation with *Fusarium oxysporum* f.sp. *lini*. Sections of the meristematic zone, 0.2 – 0.3 mm from the tip. Bar = 50 μm. Cv. Hermes.
A. Section taken at about 0.2 mm from the root tip. Hyphae are present abundantly in the root cap layers surrounding the root tip. All parts of the root have been invaded at this level.
B. Section taken slightly farther from the tip. Same condition as in A, except that locally the protodermal cells are unaffected (open arrows).
C. Section taken still farther from the root tip. Two-third of the root is infected as in A and B, while a third part (connecting to the healthy protodermal cells shown in B) is unaffected (open arrows). A sharp boundary exists between invaded and unaffected areas.
D. Section taken at about 0.3 mm from the root tip. The root is unaffected throughout, except for the presence of fungal hyphae in the root cap and a single invasion site (arrow).

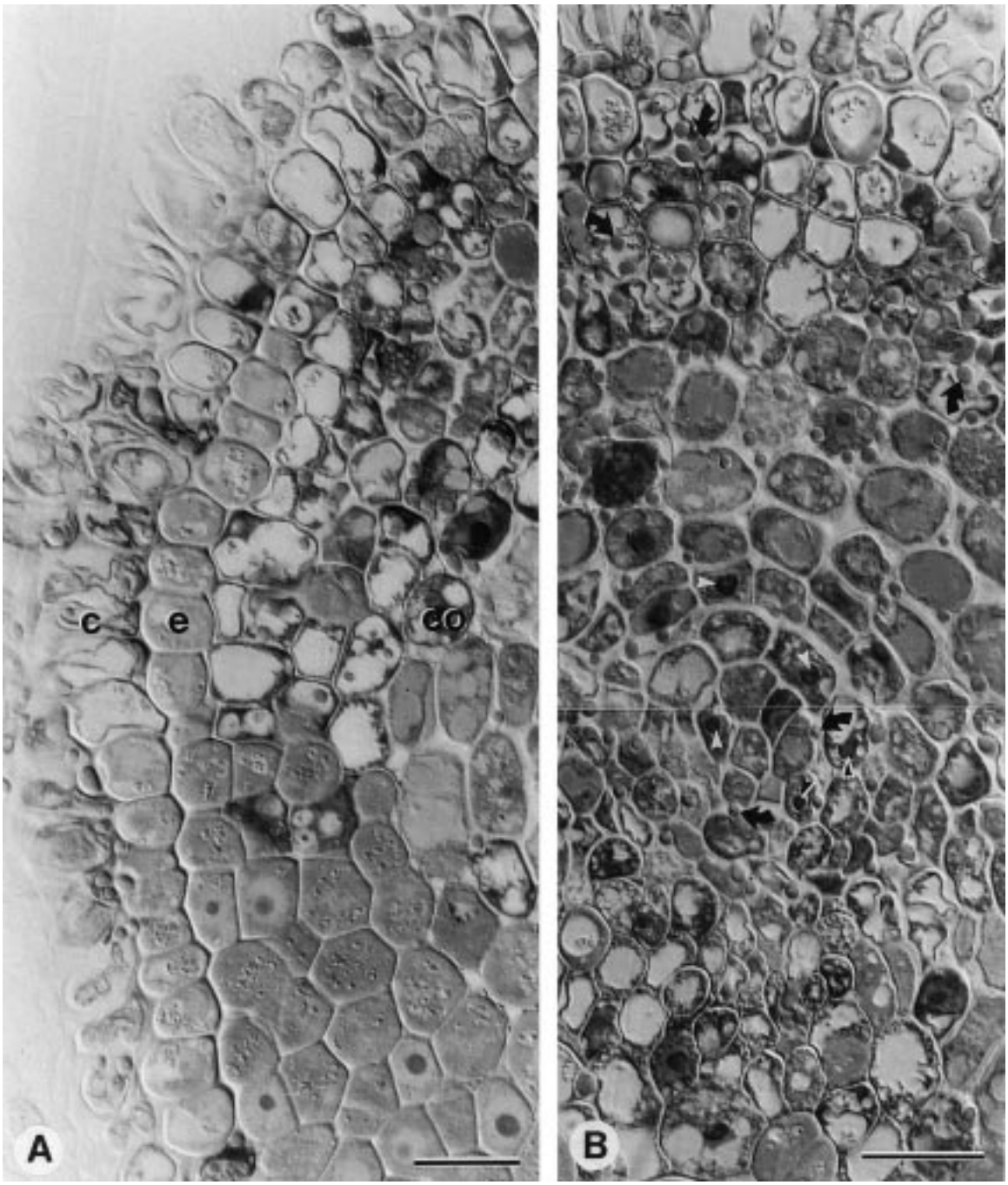

Figure 3. Details of Figure 2 C. Bar = 20 μm.
A. Transition zone from unaffected root tissues (lower part) to invaded ones (upper part of micrograph). Fungal hyphae are present abundantly in between cortical cells and often also inside these. Affected cells have darkly stained contents and show incipient plasmolysis; several of them appear to be largely empty. Abbreviations: c, root cap; e, protodermis; co, cortex.
B. Invaded cortical and stelar tissues. Hyphae (arrows) are present abundantly in between the cortical and stelar cells, and also inside several cortex cells. Do not confuse with cell nuclei (arrowheads). Incipient plasmolysis is seen in many cortical cells of which the cytoplasm otherwise seems relatively unaffected (compare with lower part of A).

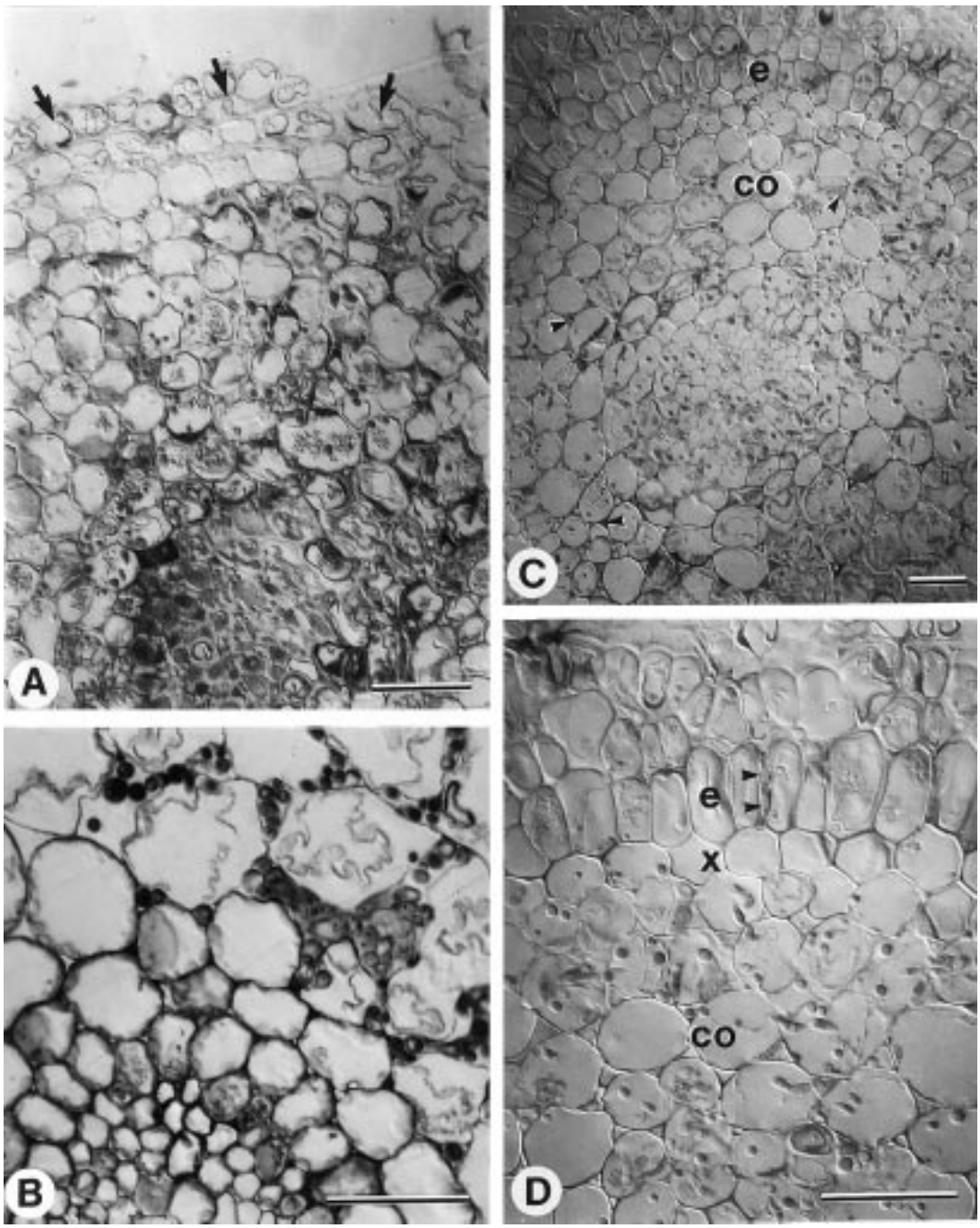

Figure 4. Micrographs of flax seedling roots, eight days after inoculation with *Fusarium oxysporum* f.sp. *lini*. A, C and D, sections from the meristematic zone, 0.25 - 0.30 mm from the tip: B, from an area further away (1.4 mm from the tip). Bar = 30 μm.

A. Root tip in similar condition as in Figure 3 (four days after inoculation) except that cell disorganisation and plasmolysis are more severe. Few or no cortical cells have normal appearing cytoplasm; rather, cell contents have condensed against the wall. Massive tissue destruction is not yet apparent. Fungal hyphae in the root cap (arrows) are poorly stained compared with those in the cortex. Cv. Regina.

B. Intercellular growth of hyphae at the colonisation front. Note severe plasmolysis of cells in the invaded area. Cv. Regina.

C. The cortex and stele have been colonised intensely. Note severe plasmolysis in otherwise empty-looking cortical cells. Tissue destruction is not yet apparent. Some of the intercellular spaces are occluded (arrowheads). Abbreviations: e, protodermis; co, cortex. Cv. Hermes.

D. Detail of C. Cortical cells have been heavily colonised and are probably dead. Fungal hyphae in the root cap are poorly stained compared with those in the cortex. Note the hypha in between two protodermal cells (arrowheads) touching upon towards the exodermis (x).

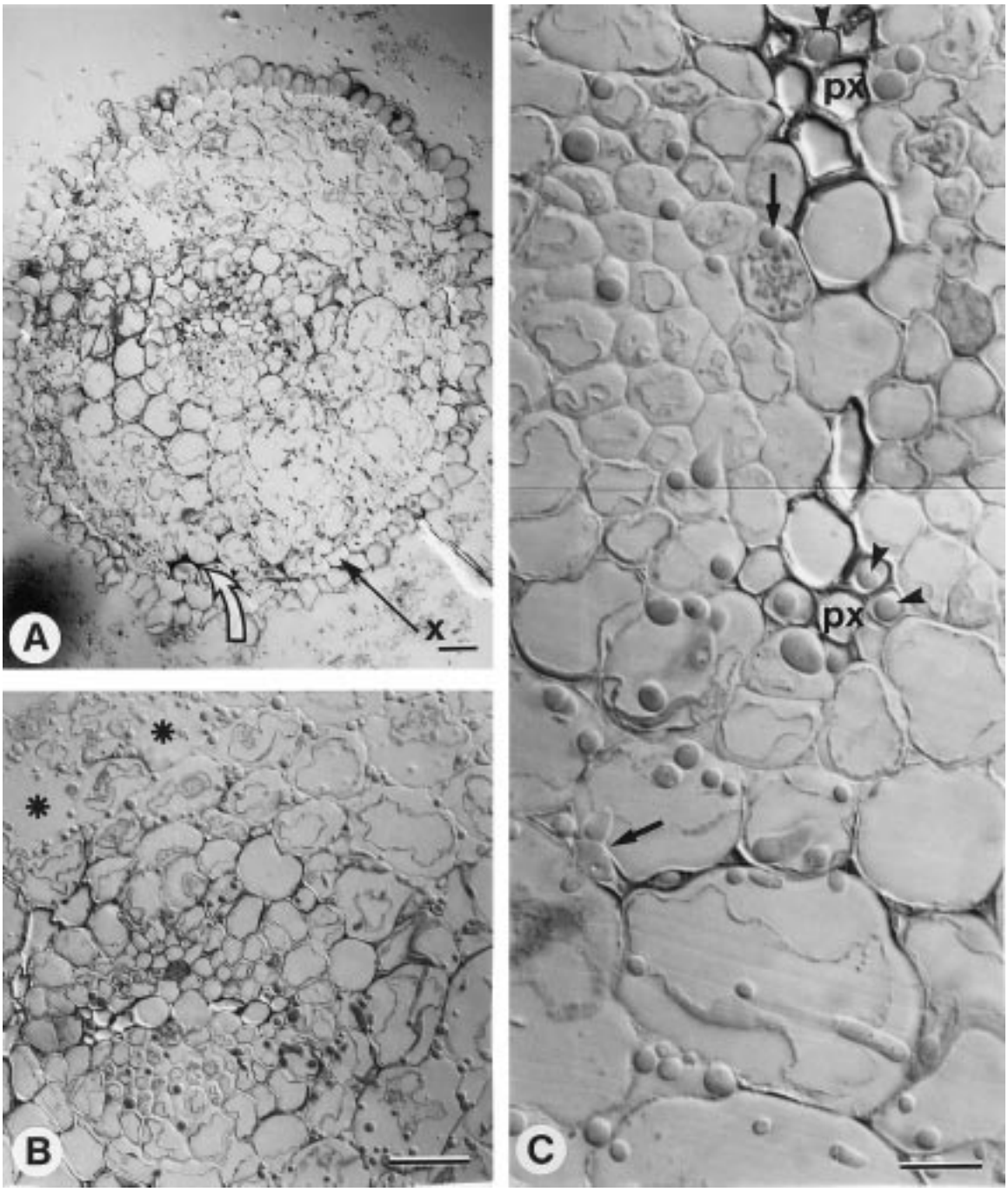

Figure 5. Micrographs of flax seedling roots, eight days after inoculation with *Fusarium oxysporum* f.sp. *lini*. Sections of a young part of a root at 1.4 mm from the tip. Cv. Hermes.
A. Heavily colonised and partially hollow root. Plasmolysis is visible throughout the cortex. Whereas many cell walls are in the process of degradation, others are darkly stained with toluidine blue O and are relatively unaffected (left hand part of root). Note occluding materials in intercellular spaces at bottom part of root (curved arrow). A well-developed exodermis (x) is present. Bar = 30 μm.
B. Detail of A, showing colonisation of the stele and degradation of the cortex ($*$). Bar = 30 μm. C. Detail of B. Fungal hyphae have colonised the cortical and stelar parenchyma, and have reached the protoxylem vessels (px; arrowheads). From the intercellular spaces, hyphae initially colonise parenchyma cell walls and middle lamellae and, subsequently, invade the cells (arrows). Bar = 10 μm.

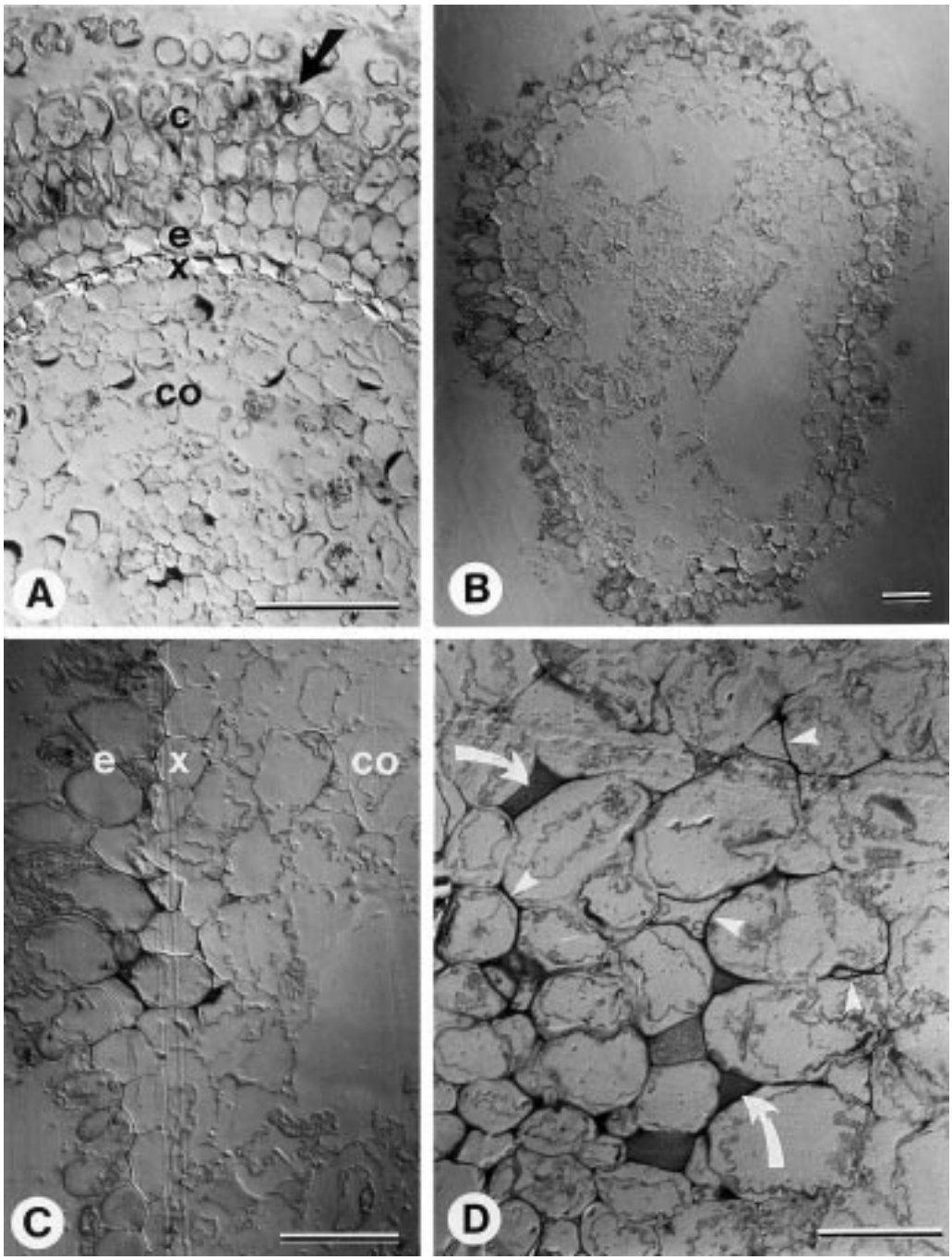

Figure 6. Micrographs of flax seedling roots, sixteen days after inoculation with *Fusarium oxysporum* f.sp. *lini*. A, section from the meristematic zone, 0.25 – 0.3 mm from the root tip; B – D, sections from root parts at 3.0 mm from the tip. Bar = 50 μm.
A. Cell wall degradation of the colonised cortex has resulted in incipient hollowing out of the root. Fungal hyphae are largely unstained. Note remaining apposition in root cap (arrow). Cv. Regina. Abbreviations: c, root cap; e, protodermis; x, exodermis; co, cortex.
B. General view of a hollow root. The epidermis and exodermis have remained. Cv. Regina.
C. Detail of B showing epidermis (e) and exodermis (x). Except for a few darkly stained walls, little remains of the cortex (co).
D. Thickened, darkly stained and apparently degradation-resistant walls (arrowheads) and occluding compounds in the intercellular spaces (arrows) of a largely hollow root. Cv. Hermes.

Discussion

Flax seedling roots were infected at the root tip, by invasion of the root cap and through the protodermal layer into the cortex. Mature parts of roots, with a fully differentiated epidermis and a suberised exodermis, were not subject to infection. Mature roots were colonised either from the invaded root tip, or from diseased lateral roots that had been invaded at their own tips. These observations support those of Turlier et al. (1994), who reported that hyphae of a GUS-marked transgenic strain of *Fusarium oxysporum* f.sp. *lini* were particularly active at the root tip and the tips of lateral roots, while mycelium was not active on the mature non-exudating root surface. Resistance to infection of the mature root surface, comprising both the epidermis and a suberized exodermis, has also been observed in lilies infected by *F. oxysporum* f.sp. *lilii* (Baayen and Rijkenberg, unpublished results).

Invasion of the root cap and growth towards the protodermis seemed retarded to some extent by the natural sloughing off of root cap cell layers. Some similarity exists with the sloughing off of occluded xylem tissue from roots of resistant carnations infected with *F. oxysporum* f.sp. *dianthi* as part of their defense response (Baayen et al., 1989, 1996). In flax, it is difficult to judge whether or not the sloughing off process is used by the host to actively defend itself against the pathogen. The host certainly attempts to defend itself, as evidenced by the appositions formed next to penetration hyphae in the root cap cells and the protodermal cells.

Appressoria were not observed in this study. Turlier et al. (1994) interpreted short hyphal branches as representing appressorium-like structures, although the mycelium never seemed to penetrate under these structures. In the present study fungal hyphae rather invaded the middle lamella region of the anticlinal cell walls of root cap and protodermis cells in the same manner as described for lily, pea and tomato infected by *F. oxysporum* f.sp. *lilii*, f.sp. *pisi* and f.sp. *lycopersici*, respectively (Baayen and Rijkenberg, unpublished results; Bishop and Cooper, 1983). Infection of the inner cell layers of the root cap and the protodermis induced the formation of appositions in some cells, while other cells collapsed. The reason for these different responses is not clear. Swelling of cells adjacent to the collapsing ones may be a matter of osmotic pressure.

Once fungal hyphae had reached the protodermis, colonisation of the cortex and stele was rapid and massive. The fungus rapidly degraded the cortex, hollowing out the root, and eventually also the xylem. Colonisation and disease in flax seedling roots thus resemble root rot such as has been described for lily root tips infected by *F. oxysporum* f.sp. *lilii*, including severe plasmolysis of affected cells and degradation of their walls (Baayen, 1992, 1996; Baayen and Rijkenberg, unpublished results). Although *F. oxysporum* f.sp. *lini* also induces wilt symptoms in flax, typical root rot symptoms, as presently observed, are a well-known part of the disease syndrome. Boyle (1934) considered root rot and wilt of flax to represent two distinct types of the disease, each with independently inherited resistance factors. However, in the present study root rot was induced by an aggressive wilt isolate applied to the root tips in low effective dosage. Also, rot developed at the root tips growing freely in the nutrient solution and not over the root surface adhering to the filter paper. Altogether, it is therefore unlikely that the present observations reflect incidental saprophytic behaviour of the fungus. Rather, root rot of flax may precede and facilitate vascular invasion. This is supported by the eventual colonization of protoxylem vessels from out of the rotting root tip, a condition observed only at the end of the study period but presumably being the starting point for vascular colonization. Root rot and wilt thus may represent two successive phases of the same disease.

The observations of this study lead to a model of root infection and colonisation that differs from that proposed by Turlier et al. (1994). The latter authors concluded that the fungus penetrates into undifferentiated protodermis cells and root cap cells, and then reaches the subapical meristem where it remains endophytically as a permanent internal site of infection for the differentiating xylem. Endophytic fungal growth would follow cell division, the hyphae being later eliminated from the differentiated cortex and phloem cells while remaining alive in the epidermis, and in and between the vessels and the stelar parenchyma. In the present study, however, colonised protodermal and cortical cells underwent severe plasmolysis and disorganisation of cell contents and eventually suffered degradation. Colonised cells appeared moribund or dead rather than supportive of endophytic hyphae. Fungal growth inside cortical cells without apparent damage, such as in present Figure 3 (easily mistaken for endophytic growth), was at best a short transient phase in disease development. Also, the protoxylem was reached by a massive front of hyphae out of the cortex, rather than by differentiation of endophytically

colonised undifferentiated stelar cells into protoxylem vessels. No indications at all were obtained that the fungus spreads endophytically into the epidermis and cortex, later on to be eliminated from the cortex but not from the epidermis. The presence of hyphae in stele and epidermis but not in the cortex mentioned by Turlier et al. (1994) may have been due to progressive colonisation of the stele after onset of the vascular phase of the disease, coinciding with restricted invasion of the upper outskirts of the root cap. In contrast to the model proposed by Turlier et al. (1994), the observations presented here fit well with earlier models of root infection by *F. oxysporum* (Bishop and Cooper, 1983; Benhamou et al., 1994), in which the root tip xylem is reached by centripetal growth of hyphae through the cortex and paratracheal parenchyma.

In line with previous observations (Kroes et al., 1998), pathogenesis in field resistant 'Hermes' did not differ appreciably from that in susceptible 'Regina' with the method used in this study. Differences in pathogenesis between susceptible and resistant flax cultivars have been reported previously, the fungus remaining restricted to the cortex in 20-day-old seedlings of resistant 'Redwood', whereas the phloem and xylem of susceptible 'Punjab' had been invaded by that time (Nair, 1956; Nair and Kommedahl, 1957). Although no substantial evidence for extravascular resistance in 'Hermes' was found in the present study, the existence of extravascular resistance factors in other cultivars cannot be excluded. Future studies should consider possible vascular resistance factors in resistant flax cultivars.

Acknowledgements

This work was financially supported by the Dutch Commission for Flax, the Dutch breeding companies CEBECO Seeds, Lelystad, Van de Bilt Zaden, Sluiskil, Landbouwbureau Wiersum, Dronten, and Procotex Breeding, St. Jansteen. We thank M.G. Förch (IPO-DLO) for technical assistance and J. Hoogendoorn (CPRO-DLO) and J.E. Parlevliet (Wageningen, Agricultural University) for discussions and critical reading of the manuscript.

References

Baayen RP (1992) Resistance mechanisms of plants to rot and wilt diseases caused by *Fusarium oxysporum*. Acta Horticulturae 325: 675–682

Baayen RP (1996) Afweermechanismen bij lelie tegen aantasting door *Fusarium oxysporum*. Report 96-03, DLO Research Institute for Plant Protection, Wageningen, The Netherlands, pp. 33

Baayen RP, Ouellette GB and Rioux D (1996) Compartmentalization of decay in carnations resistant to *Fusarium oxysporum* f.sp. *dianthi*. Phytopathology 86: 1018–1029

Baayen RP, Van Eijk C and Elgersma DM (1989) Histology of roots of resistant and susceptible carnation cultivars from soil infested with *Fusarium oxysporum* f.sp. *dianthi*. Netherlands Journal of Plant Pathology 95: 3–13

Benhamou N, Fortin JA, Hamel C, St-Arnaud M and Shatilla A (1994) Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f.sp. *chrysanthemi*. Phytopathology 84: 958–968

Bishop CD and Cooper RM (1983) An ultrastructural study of root invasion in three vascular wilt diseases. Physiological Plant Pathology 22: 15–27

Boyle LW (1934) Histological characters of flax roots in relation to resistance to wilt and root rot. Technical Bulletin, United States Department of Agriculture, Washington, D.C., 458: 1–18

Clark G (1981) Staining procedures. Fourth edition. Williams and Wilkins, Baltimore, USA, pp. 512

Jensen WA (1962) Botanical histochemistry. W. H. Freeman and Co., San Francisco, USA, pp. 408

Krishnamurthy KV (1988) Methods in plant histochemistry. S. Viswanathan Printers and Publishers, Chetput, Madras, India, pp. 104

Kroes GMLW (1997) Aspects of resistance in flax and linseed (*Linum usitatissimum*) to *Fusarium oxysporum* f.sp. *lini*. PhD thesis, Wageningen Agricultural University, The Netherlands, pp. 159

Kroes GMLW, Sommers E and Lange W (1998) Two *in vitro* assays to evaluate resistance in *Linum usitatissimum* to *Fusarium* wilt disease. European Journal of Plant Pathology 104 (in press)

Millikan CR (1951) Diseases of flax and linseed. Technical Bulletin 9, Department of Agriculture Victoria, Australia, pp. 140

Murashige T and Skoog F (1962) A revised medium for rapid assays with tobacco tissue cultures. Physiologia Plantarum 15: 473–497

Nair PN (1956) Factors affecting resistance of flax to *Fusarium lini*, Bolley. PhD thesis, University of Minnesota, USA, pp. 90

Nair PN and Kommedahl T (1957) The establishment and growth of *Fusarium lini* in flax tissues. Phytopathology 47: 25 (abstract)

O'Brien TP and McCully ME (1981) The study of plant structure; Principles and selected methods. Thermacarphi Pty. Ltd., Melbourne, Australia, pp. 280

Tisdale WH (1917) Flaxwilt: a study of the nature and inheritance of wilt resistance. Journal of Agricultural Research 11: 573–606

Turlier MF, Eparvier A and Alabouvette C (1994) Early dynamic interactions between *Fusarium oxysporum* f.sp. *lini* and the roots of *Linum usitatissimum* as revealed by transgenic GUS-marked hyphae. Canadian Journal of Botany 72: 1605–1612