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Early stages of infection of maize (*Zea mays*) and *Pennisetum setosum* roots by the parasitic plant *Striga hermonthica*

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Abstract

Infection of young roots of maize (*Zea mays* L.) by the parasitic plant *Striga hermonthica* (Del.) Benth. was examined. Attachment to and penetration of roots occurred within 1–2 days after inoculation. Subsequent growth through the cortex to the host stele and proliferation of parasite xylem tissue was commonly completed by 3–4 days after inoculation. Histochemical staining showed that young maize roots do not contain major wall-thickening components. However, an increase in cell wall fluorescence and endodermal cell wall thickness was often seen at the site of infection and in the surrounding maize root tissue at 3 days after inoculation. This host response was variable and did not prevent rapid and successful penetration by the parasite. In contrast, uninfected roots of *Pennisetum setosum* (Sw.) L. Rich., a species resistant to *S. hermonthica*, had substantial thickening of the inner endodermal cell walls and exhibited further cell wall thickening at the stele upon infection. Examination of infections on both hosts demonstrated the presence of autofluorescent material at the host-parasite interface. This material was thicker and more extensive at the *P. setosum*-*S. hermonthica* interface than at the maize-*S. hermonthica* interface, and contained polyphenols and lignin. Examination of the host-parasite xylem connections in maize revealed substantial invasion of the host stele by both parenchyma and tracheary elements. In a few cases of *P. setosum* infection, parasite cells entered the stele; however, this did not lead to successful establishment of the parasite.

Introduction

Striga spp. (Scrophulariaceae) are obligate root parasites which infect a range of crop plants and cause serious constraints to yield (Parker, 1991). *S. hermonthica* infects several important crops, notably maize, millet, rice and sorghum. Seeds of *Striga* spp. germinate in response to chemicals secreted by host roots. When in contact with a host root, the radicle tip swells and penetrates the root, forming an haustorium which grows through the host tissue (Musselman, 1980; Stewart and Press, 1990). Following vascular contact, the parasite produces an extensive xylem system to facilitate transport of water and nutrients from the host (Visser and Dorr, 1987; Mallaburn and Stewart, 1987). The parasite seedling then develops leaves and eventually emerges from the soil; it may also develop adventitious roots which form further connections with the host root system (Musselman, 1980). Although the parasite is capable of photosynthesis, some carbon is still obtained from the host, experimental evidence indicating that approximately 38% and 85% of the parasite carbon is host-derived in sorghum and millet respectively (Graves et al., 1989; Graves et al., 1990).

Although detailed studies have been made of the interaction of *Striga* spp. with sorghum (Maiti et al., 1984; Elhiweris, 1987; Olivier et al., 1991), cowpea (Okonkwo and Nwoke, 1978; Reiss, 1995) and millet (Ba, 1988), little information is available on the infection of maize. Varieties of maize which tolerate infection by *S. hermonthica* with little yield loss have been identified (Kim, 1991), but none are so far known which are fully resistant to the establishment and growth of the parasite (Lane and Bailey, 1992).

However, resistant and partially resistant varieties do exist in several other crops, including sorghum (resistance to *S. hermonthica* and *S. asiatica*), rice (resistance to *S. hermonthica*) and cowpea (resistance to *S. gesnerioides*) (Lane and Bailey, 1992; Harahap et al., 1993). Examination of the host response to infection in susceptible and resistant reactions may indicate future directions for breeding or targets for the manipulation of increased resistance to parasite establishment. The present study describes a preliminary examination of the early stages of infection of the roots of two hosts by the same *S. hermonthica* strain; a maize variety which is highly susceptible to infection and allows full development of the parasite, and the tropical weed *P. setosum* which, although susceptible to penetration by *S. hermonthica*, does not support successful infections (J.A. Lane. unpubl.). The aim was to identify any consistent changes in cell wall thickness and composition in the roots of both hosts during penetration, to assess whether these changes had an effect on the progress of infection, and to attempt to identify factors which might be important in the susceptibility or resistance of the host to parasite establishment.

Materials and methods

Growth and infection of host plants

All plants were maintained in a Fisons F600H controlled growth chamber (conditions: 30 °C light, 25 °C dark, 16 h photoperiod, 75% relative humidity, 180 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density over the waveband 400–700 nm). Maize and *P. setosum* seedlings were grown and inoculated with *S. hermonthica* using the seedling culture system described by Lane et al. (1991). Maize seeds (cv. 'LG21-90', Force Limagrain) were surface-sterilised for 3 min in a 12.5% (v/v) sodium hypochlorite solution, soaked in water for 3 h then grown for 4-5 days in perlag (Silvapearl, UK). *P. setosum* seeds (obtained from Thailand) were grown for 25 days to produce a root system which is similar to that of 4-5 days-old maize seedlings. After transfer to the seedling culture system, maize plants were allowed to grow for 2 days before inoculation. *P. setosum* plants were grown in the seedling culture system for 12 days in order to produce young roots suitable for inoculation. Because of the longer growth period, *P. setosum* seedlings were watered with nutrient solution rather than the distilled water used for maize seedlings (Lane et al., 1991).

S. hermonthica seeds from Kenya (accession number 91-01) were surface-sterilised for 5 min, rinsed several times in distilled water then preconditioned on water-saturated GF/A discs (Whatman) in the dark at 30 °C for 10-12 days (Lane et al., 1991). The discs were then placed upon young host root tips. After 24 h, seedlings with short radicles were transferred to young lateral roots with a paint brush. Uninfected control tissue was grown in the seedling culture system to the same age as the infected plants. Samples of infected and uninfected roots were taken daily from 1-5 days after inoculation (dai).

Clearing and staining of infected roots

Infected roots were removed and cleared for 30 min in ethanol-chloroform (3:1), stained for 5 min in Aniline Blue in lactophenol (0.01% w/v), mounted in lactophenol and viewed under bright field illumination (adapted from O'Connell et al., 1993).

Fixation of tissue

Fixation, dehydration, wax infiltration and embedding were based on the protocol of Sylvester and Ruzin (1993). Material was fixed in formaldehyde (50% ethanol/10% acetic acid/4% formaldehyde (v/v) in phosphate buffered saline) for 4 h with 5 min vacuum infiltration at the beginning of each hour, followed by a change of fixative, after the first, second and third hour. Fixed tissue was dehydrated in an ethanol series then saturated gradually with 2-methylpropan-2-ol tert-butanol (TBA, Merck Ltd.). Infiltration with paraffin wax was done over 3 days with 3–4 changes of 100% wax. Sections (7–10μm thickness) were cut using a microtome fitted with glass knives.

Material for embedding in LR White resin (London Resin Co. Ltd., Hampshire) was fixed for 1 h in 4% formaldehyde/5% glutaraldehyde (v/v) in 0.1M cacodylate buffer (pH 7), rinsed in water for three 1 h periods, then dehydrated through an ethanol series. Resin was infiltrated by 45 min incubations in 25%, 40%, 50%, 75%, 85%, 90% (v/v) resin, a 1 h incubation in 100% resin, then three 24 h incubations in 100% resin, the first at 4 °C and the following two at room temperature (R. Pring, pers. comm.). Sections (1–2μm thickness) were cut for examination.

Staining for light microscopy

Sections from infected (3 dai) and uninfected roots were examined for autofluorescence and with several stains to study cell wall thickness and composition, and the connections between host and parasite xylem. All stains and solvents were purchased from Merck Ltd. Wax sections were stained with: a) Haematoxylin (0.5% w/v) and Aniline Blue (0.1% w/v) to detect fluorescent components, including callose (blue/white under fluorescence) (Jefferies, 1979); b) Aniline Blue (0.005% w/v) in conjunction with Toluidine Blue O (0.05% w/v), to detect fluorescent components, including callose, and polyphenolics (blue to blue-green under bright field) (Fernandez and Heath, 1986); c) aqueous Safranin O (1% w/v) and 0.2% Fast Green (0.2% w/v), to highlight lignified, cutinised or suberised cell walls (red) and cytoplasm and cellulosic cell walls (green) (Conn, 1953), using xylene to clear before mounting; d) a saturated solution of Phloroglucinol in 20% (v/v) HCl, to stain lignin (pink/red) (Fernandez and Heath, 1986); e) Methylene Blue (1% w/v) as a general cytoplasm stain (Suzuki, 1963). Sections in methacrylate resin (1-2μm) were stained with: f) 0.1% (w/v) Aniline Blue in 0.15M Na_2HPO_4, pH 9, to examine the wall thickness of uninfected roots.

Sections were viewed with a Zeiss Axiophot microscope under epi-fluorescent illumination using filter set II (G 363, FT 395, CP 420) (for stains a, b and f) and under bright field with (stain b) or without (stains c, d and e) differential interference contrast. Results were recorded on Kodak EPT160, Kodak Technical Pan or Kodak Gold400 film. Kodak Technical Pan was developed in Diafine developer (Acufine, USA).

Results

Timing and progress of infection of maize roots

Soon after inoculation, radicle tips of the parasite became swollen and produced short hairs. Attachment to maize roots and penetration of the epidermis occurred within a short time once the germinated seedlings were on or near the root, and was usually achieved by 1 dai (Figure 1a). However, infection was not synchronous and in some cases penetration did not occur until 2 dai. At the point of penetration some compression of host cells was commonly observed. Growth of columnar intrusive cells into the host cortex was usually accomplished by 2-3 dai (Figure 1b), although in some cases the stele was reached by 1 dai. Differentiation of tracheary elements in the haustorium and external parasite tissue was observed at 3 dai, with the parasite forming a complex xylem system (Figure 1c). This was succeeded by expansion of the cotyledons at 4-6 dai (Figure 1d), causing the seed coat to rupture. The first true leaves developed by 5-6 dai (Figure 1e). Infection in the seedling culture system was efficient; the majority (c. 70-80%) of germinated *S. hermonthica* seedlings placed on the host root formed successful infections.

Anatomy of uninfected roots of maize and P. setosum

There was a clear difference in the extent of cell wall thickness and fluorescence between uninfected maize and *P. setosum* roots. Young maize lateral roots had thin cortical cell walls, slightly thicker epidermal cell walls and little noticeable cell wall thickening of the stele, apart from the mechanical strengthening of the xylem vessels and the Casparian strip (Figure 2a). Some fluorescence was observed in the maize epidermis, xylem vessels and Casparian strip after staining with Aniline Blue. However, examination of unstained sections indicated that the majority of this fluorescence was due to autofluorescence rather than specific detection of callose. Young *P. setosum* lateral roots also had little thickening or fluorescence in the cortical and thinner epidermal cell walls (Figure 2b). However, they exhibited conspicuous thickening of the inner tangential walls of the endodermis and of the cells and elements in the stele (Figure 2b). These thickened walls were highly fluorescent in both stained and unstained sections. The anatomy of both host roots varied slightly along the root and between different roots, with respect to the number of cell layers in the stele, the extent of thickening in the endodermis and the size and number of xylem vessels.

Effects of infection on cell wall fluorescence, thickness and composition

Sections from infected and uninfected maize and *P. setosum* roots were stained with Haematoxylin and Aniline Blue and viewed under UV epi-fluorescence to examine the effect of infection on the distribution and accumulation of fluorescent compounds and callose (Figure 3). Infection of maize led to a general increase in fluorescence both at the infection site (Figure 3a) and in the infected root c. 5mm from the infection site (Figure 3b) at 3 dai. The fluorescence followed

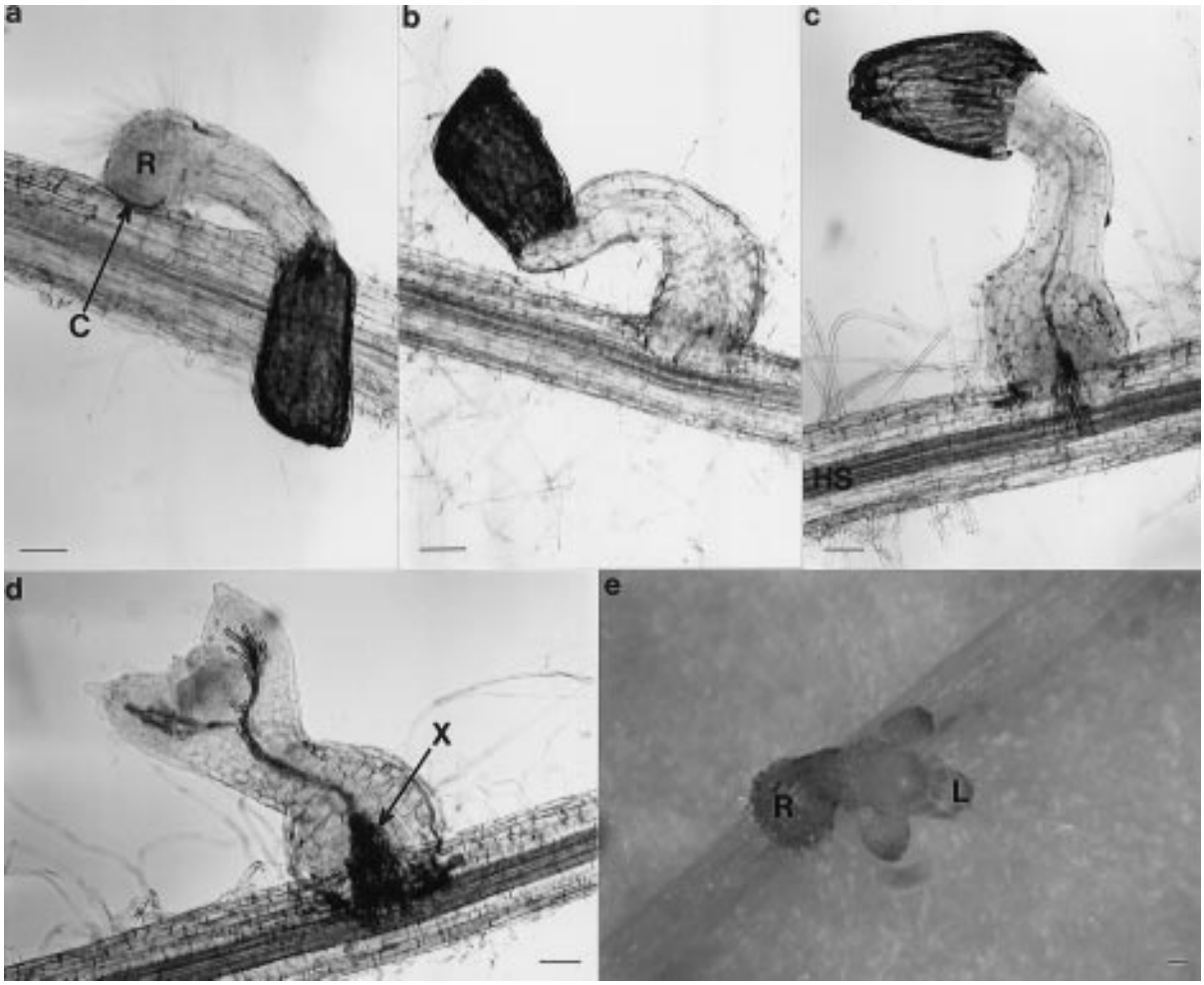

Figure 1. Early infection stages of maize roots by *Striga hermonthica*. Development of the interaction is shown in cleared roots stained with Aniline Blue in lactophenol showing typical stages at 1, 2, 3 and 5 days after inoculation (dai, a-d). a) Attachment to and early penetration of the host at 1 dai; b) growth into the host root cortex at 2 dai; c) penetration of the stele and development of the parasite xylem at 3 dai; d) expansion of the cotyledons and further xylem development at 5 dai; e) the development of leaves, shown here in unstained tissue growing in the seedling culture system at 7 dai. R – swollen *S. hermonthica* radicle tip; C – compression of host cells at point of attachment; HS – host stele; X– proliferation of parasite xylem; L – parasite leaf. Bar = 100μm.

the same pattern of distribution as that observed in the uninfected root of an equivalent age (Figure 3c). Some infected maize roots had noticeable thickening of the inner endodermal cell walls (Figure 3a, b) compared with the endodermal cell walls in uninfected roots; however, the extent of this thickening was variable and it was absent in some infected roots. Apart from general cell wall fluorescence, small points of fluorescence were also observed in the stele and, occasionally, in the cortex of both uninfected (Figure 3c) and infected maize (Figure 3a, b); these may represent deposits of callose or a similar substance. There was no clear difference in the density and distribution of these deposits between infected and uninfected roots.

P. setosum roots also showed a general increase in cell wall fluorescence on infection with *S. hermonthica* (Figure 3d, e) compared to the uninfected roots (Figure 3f). There was little change in the fluorescence of the inner endodermal wall, which remained high, but the cortical cell walls fluoresced more intensely both at the infection point (Figure 3d) and in the root tissue surrounding the point of penetration (Figure 3e) than in uninfected tissue (Figure 3f). Again, small points of fluorescence were observed and were present in similar amounts in the infected and uninfected roots.

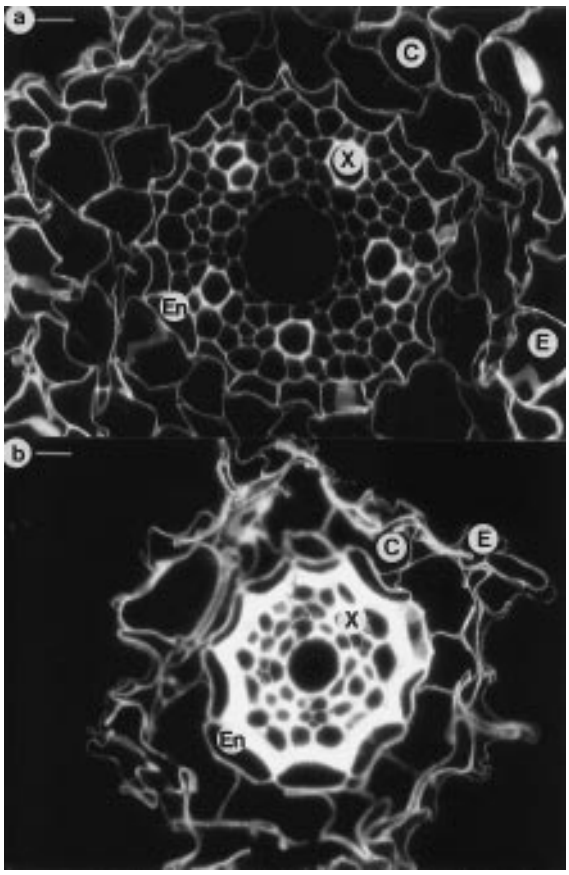

Figure 2. Anatomy of uninfected maize and *Pennisetum setosum* roots. Transverse resin sections (1–2μm) from uninfected host roots were stained with Aniline Blue and viewed under UV-fluorescence to demonstrate differences in cell wall thickening between the two hosts. a) Uninfected maize roots; b) uninfected *P. setosum* roots. C – cortex; E – epidermis; En – endodermis; X – xylem vessel. Bar = 10μm.

In both hosts there was an additional layer of fluorescence at the host-parasite interface (Figure 3a, d). This was commonly more intense in *P. setosum* than in maize. Crushed and compacted host cells were often observed at the flanks of the host-parasite interfaces, leading to a concentration of cell wall material which may cause or contribute to this fluorescent layer.

To further examine the accumulation and distribution of fluorescent material at the infection point, sections of infected roots were stained with Aniline Blue in conjunction with Toluidine Blue, to reduce or quench the natural autofluorescence (Figure 4). When viewed under UV epi-fluorescence, the *P. setosum-S. hermonthica* infections showed patches of very bright fluorescence at the flanks and the invasive front of the haustorium and along the host stele (Figure 4c). In contrast, the fluorescence in infected maize roots was variable, less intense and confined to the sides of the haustorium, usually close to the point of penetration (Figure 4a). When the same sections were viewed under bright field, the *P. setosum-S. hermonthica* infection exhibited strong blue-green staining with Aniline Blue and Toluidine Blue, both at the interface and along the host stele close to the infection, indicating the presence of polyphenolic material (Figure 4d). No significant accumulation of polyphenols was detected in maize with this stain (Figure 4b).

To investigate the accumulation of wall-thickening components, sections of infected and uninfected roots were stained with Safranin and Fast Green to identify cellulosic walls (blue-green) and the presence of lignin, cutin or suberin (red) (Figure 5). The patterns of staining and cell wall thickening observed in infected maize roots were very variable. In young uninfected maize roots (Figure 5c), the inner tangential cell walls showed variable levels of thickening, but always stained blue-green. Red staining was only observed in the radial walls of the endodermal cells (possibly the Casparian strip) and in the xylem vessel walls. In some infected maize roots, the inner endodermal cell walls were substantially thickened and stained red, both at the infection site (Figure 5a) and in the root tissue c. 5mm away from the site of penetration. However, in other infections the inner endodermal cell walls stained blue-green and showed either slight or no thickening (Figure 5b). The extent and composition of the thickening did not appear to be related to the extent of parasite penetration.

Both infected and uninfected *P. setosum* roots usually exhibited a strong red staining of the thickened inner endodermal cell walls after staining with Safranin and Fast Green, and this did not appear to be significantly increased by infection. However, the *P. setosum* xylem vessels, which also stained red, were observed to have increased layers of thickening near to the invading parasite (Figure 5d).

Deposition of red-staining compounds was commonly observed at the host-parasite interface. In maize, this deposition was only apparent in patches at the flanks of the interface, and the amount of Safranin red-staining components varied between infections (Figure 5a, b). In *P. setosum* roots a substantial layer of red-staining material was apparent, usually surrounding the entire interface (Figure 5d). It was not clear whether this layer at the interface was derived from the host or from the parasite.

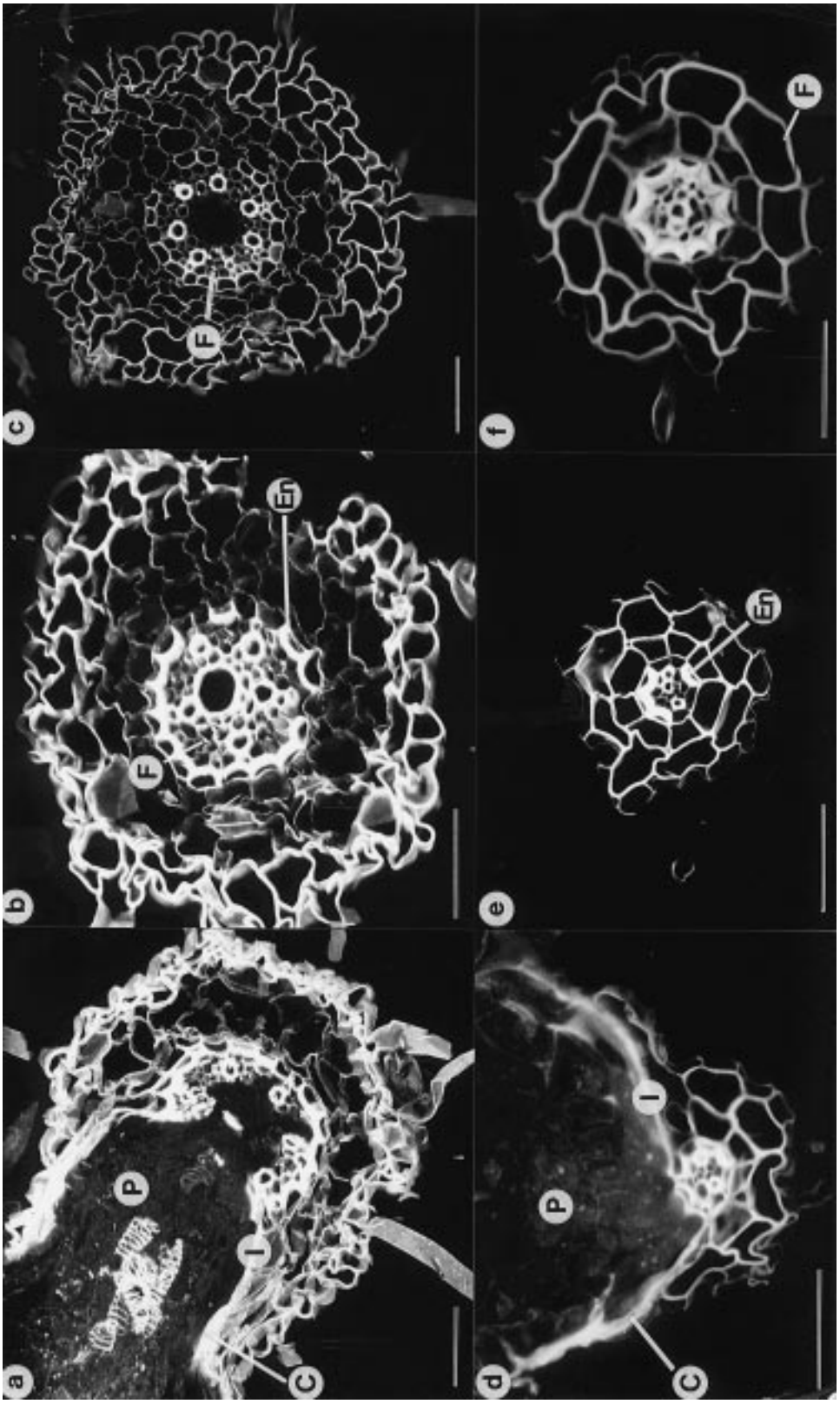

Figure 3. Effect of *Striga hermonthica* infection on host root fluorescence. Semi-thin (7-10μm) transverse wax sections from maize (a-c) and *P. setosum* (d-f) were stained with Haematoxylin and Aniline Blue and viewed under UV epi-fluorescence. a) The maize-*S. hermonthica* infection site; b) infected maize root c. 5mm from the infection site; c) uninfected maize root of an equivalent age; d) *P. setosum*-*S. hermonthica* infection site; b) infected *P. setosum* root c. 5mm from the infection site; c) uninfected *P. setosum* root of an equivalent age. I – host-parasite interface; En – endodermis; P – parasite haustorium; F – fluorescent deposit; C – crushed host cells. Bar = 50μm.

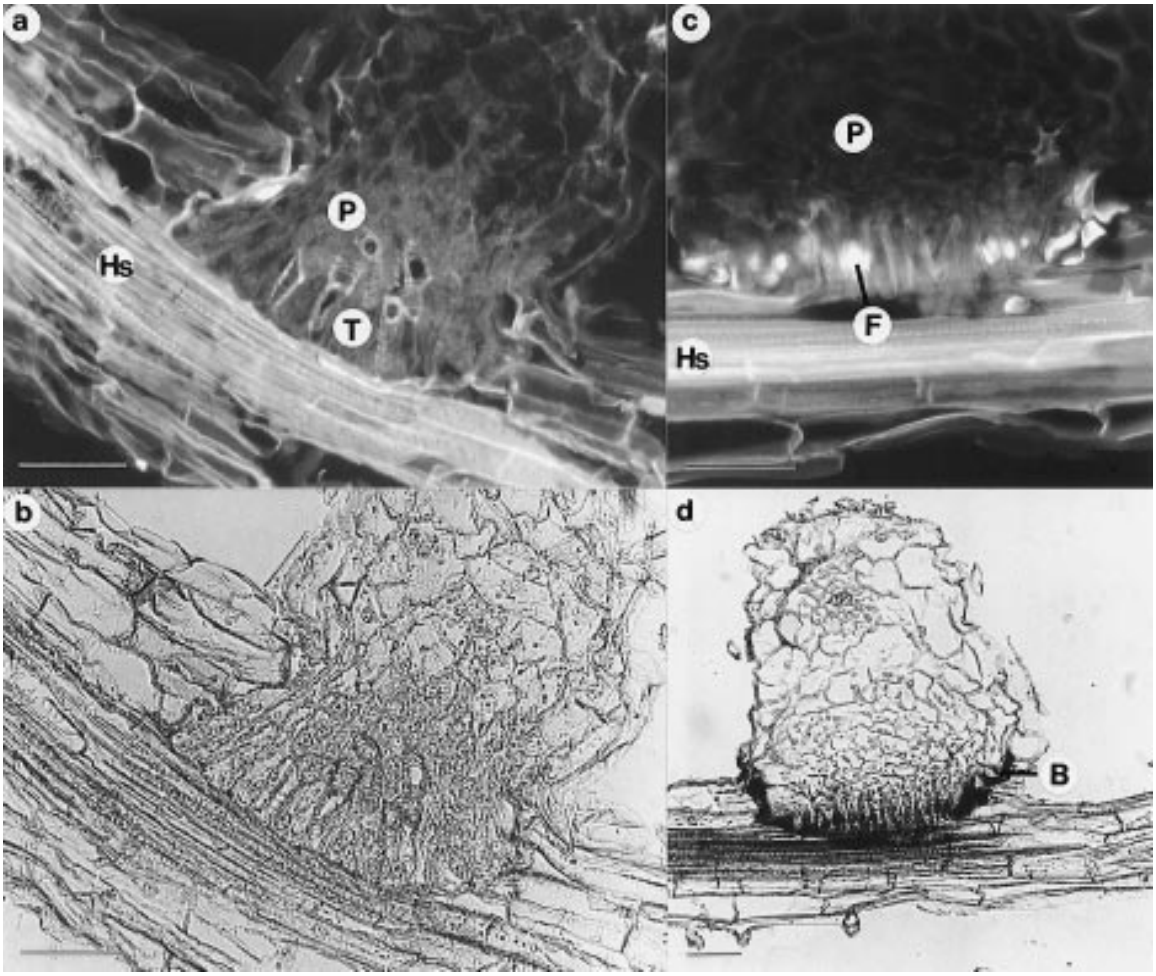

Figure 4. Aniline Blue/Toluidine Blue fluorescence and staining at the host-parasite interface. Longitudinal wax sections (7–10μm) of the infection site were stained with Aniline Blue in conjunction with Toluidine Blue to detect the presence of callose, other strongly fluorescent components and polyphenolics. a) The maize-*S. hermonthica* infection site viewed under UV epi-fluorescence, showing small areas of fluorescence at the side of the interface and in parts of the host stele; b) section (a) viewed under bright field with differential interference contrast, showing no significant accumulation of blue-green staining polyphenolics; c) the *P. setosum*-*S. hermonthica* infection site viewed under UV epi-fluorescence, showing strong areas of fluorescence at the base and sides of the interface; d) section (c) viewed under bright field with differential interference contrast, showing strong blue-green staining at the interface and along the host stele close to the infection site. Hs – host stele; P – parasite haustorium; F – fluorescence; B – blue-green staining; T – parasite tracheary element. Bar = 50μm.

Staining with Phloroglucinol-HCl confirmed the presence of lignin at the *P. setosum*-*S. hermonthica* interface and in the host root endodermis, with the strongest staining in regions where the parasite met the host stele. Some lignin was detected in infected maize roots in the xylem vessels and at the flanks of the parasite tissue, but the staining was much less extensive and less intense than in *P. setosum* infections (data not shown).

Parasite connections to the host stele

Examination of the host-parasite connections at the maize-*S. hermonthica* infection site revealed that the maize stele was invaded by both parenchymatous cells and tracheary elements of the parasite, with the cells and elements appearing to grow into and along the host vessels (Figure 6a). However, although tracheary elements were present in every infection studied, the majority of connections were formed by parenchymatous cells. The haustorial cells above the interface consisted of parenchyma cells containing extremely dense

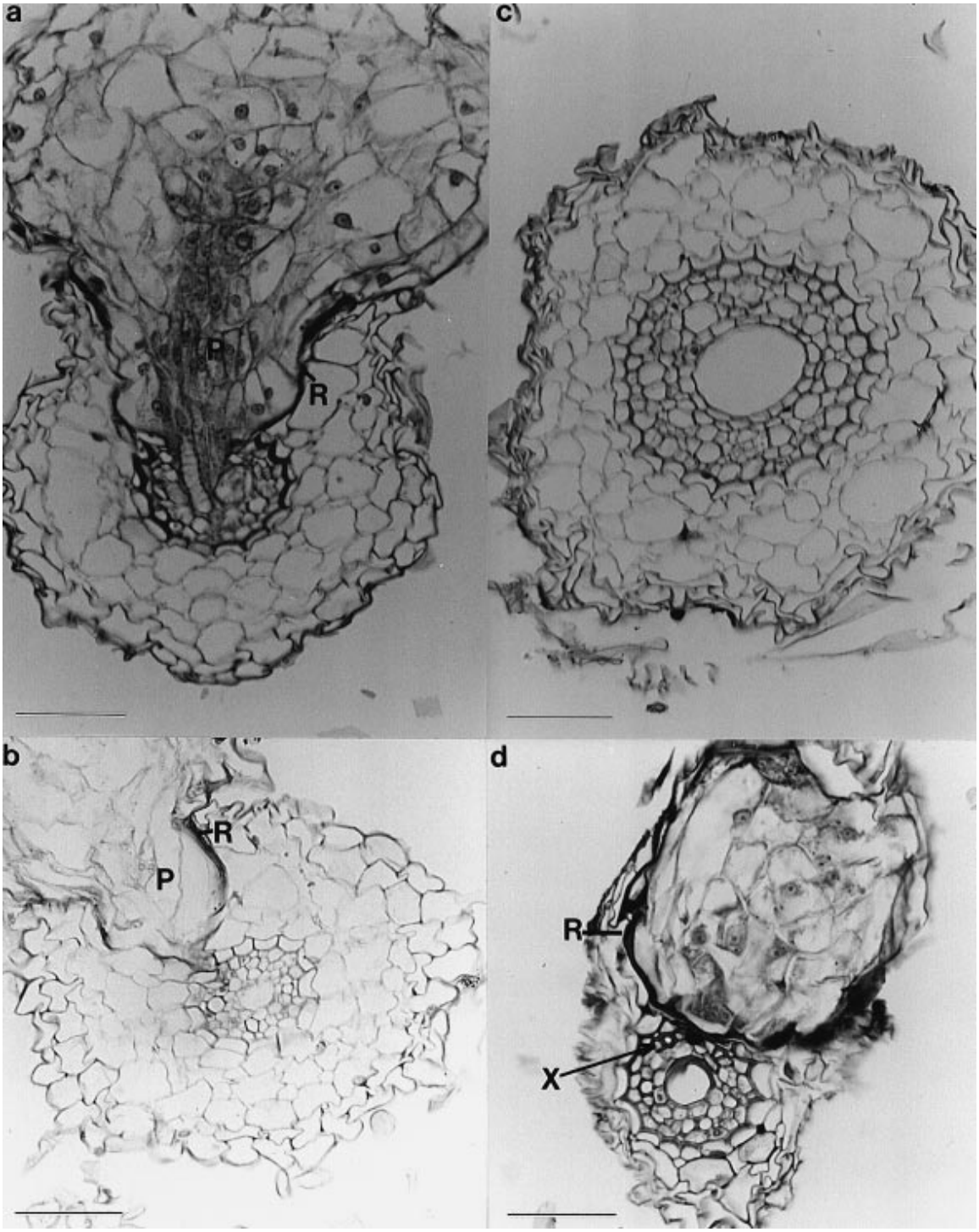

Figure 5. Cell wall thickening and composition in infected and uninfected roots. Transverse wax sections (7-10μm) were stained with Safranin and Fast Green and viewed under bright field to identify lignified, cutinised or suberised cell walls (red) and cellulosic cell walls (blue-green). a), b) Maize-*S. hermonthica* infection sites demonstrating the variation in endodermal thickening and staining between different infected roots; c) uninfected maize root showing no significant accumulation of Safranin red-staining compounds; d) *P. setosum*-*S. hermonthica* infection site showing a strong accumulation of Safranin red-staining components surrounding the host-parasite interface and increased deposition in the host xylem vessels near to the parasite. P – parasite haustorium; R – red staining; X – thickened xylem vessel. Bar = 50μm

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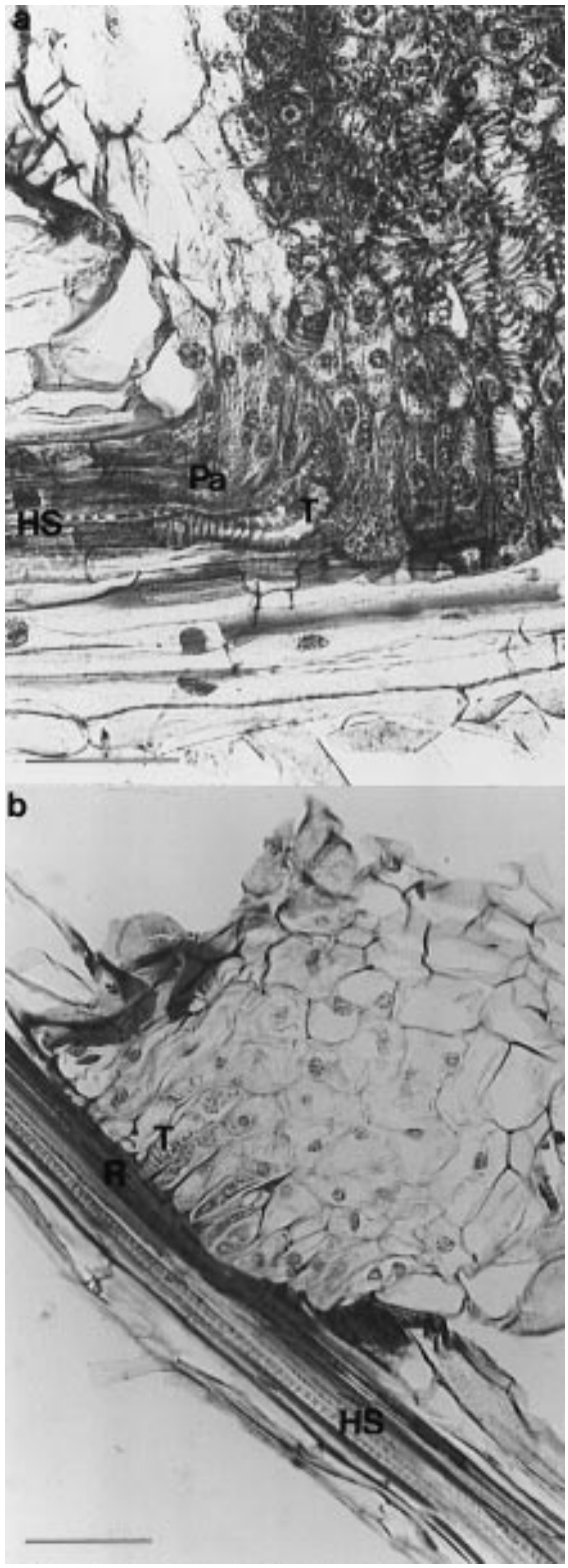

Figure 6. Parasite connections to the host stele. Longitudinal wax sections (7-10μm) were stained with Methylene Blue (a) or Safranin and Fast Green (b) and viewed under bright field. a) The maize-*S. hermonthica* infection site, showing the invasion of the maize stele by both parenchymatous cells and tracheary elements of the parasite; b) the *P. setosum*-*S. hermonthica* infection site, showing a rarely produced tracheary element. HS – host stele; Pa – parasite parenchyma growing into the host stele; T – parasite tracheary element; R – red staining. Bar = 50μm.

cytoplasm and a network of tracheary elements with spiral thickenings.

The vast majority of infections of *P. setosum* terminated outside the endodermis. Tracheary elements were produced in a few interactions (Figure 6b) and the host stele was occasionally breached. But despite penetration, these infections did not develop any further than the cotyledon expansion phase and no network of parasite tracheary elements was observed.

Discussion

Timing and progress of infection of maize roots

Infection of maize by *S. hermonthica* was rapid, an established parasitic interaction being produced within 3-4 dai. *S. hermonthica* penetrated *P. setosum* roots at the same rate as maize but the infection commonly halted at the endodermis at 3-4 dai, with eventual necrosis at the infection site (J.A. Lane, unpubl.). The timing of early infection by *S. hermonthica* was similar to that reported previously for *S. asiatica* on sorghum (Ramaiah et al., 1991) and *S. gesnerioides* on cowpea (Reiss, 1995), but shoot development of these parasites was slower. In all cases, the timing of individual infections could vary by around 24 h, but Reiss (1995) found that this variation was due mainly to the timing of attachment and penetration, with the subsequent steps occurring in a fixed order.

Effects of infection on cell wall fluorescence, thickness and composition

Investigations of stained and unstained root sections demonstrated that the cell walls of both maize and *P. setosum* have a high level of fluorescence which increases on infection. We attempted to determine whether this increase was caused by an accumulation of naturally fluorescent compounds, such as lignins,

small molecular weight phenolic compounds, cutin or suberin, which contribute to the plant's autofluorescence (O'Brien and McCully, 1981), or to a specific deposition of callose, which is known to occur in response to injury or infection (Clark, 1981). Callose is frequently observed as small 'points' of fluorescence in sections stained with Aniline Blue and viewed under UV epi-fluorescence (O'Brien and McCully, 1981). Small deposits of callose were observed in both infected and uninfected roots, but their distribution and occurrence did not change noticeably on infection. When the autofluorescence and non-specific binding of the Aniline Blue fluorochrome were reduced with toluidine blue (O'Brien and McCully, 1981), strong patches of fluorescence were still visible at the host-parasite interfaces which may be due to large concentrations of callose; however, by their appearance they are more likely to have been caused primarily by a large accumulation of fluorescent phenolic material which was too concentrated to be fully quenched by the toluidine blue. This assumption is supported by the strong staining of polyphenolic material at the *P. setosum*-*S. hermonthica* interface and along the infected host's stele. Further work at the level of electron microscopy may give a clearer indication of whether callose is deposited in significant amounts near the infection site and/or at the interface.

Staining with Safranin and Fast Green confirmed the presence of thickening and strengthening substances in the stele of infected and uninfected *P. setosum* roots, in the steles of some infected maize roots and at the host-parasite interface in both hosts. The low specificity of Safranin makes it unclear whether this thickening derives from lignification, cutinisation or suberisation, or a combination of polymers. A more specific, but less sensitive, test with Phloroglucinol-HCl confirmed that lignin was present at the interface and in the steles of the infected hosts.

The presence and accumulation of fluorescent and phenolic compounds and thickened cell walls was significantly different between the susceptible maize and the resistant *P. setosum*. *P. setosum* roots have what would appear to be a natural barrier to penetration of the stele - an endodermis and stele with thickened walls which are lignified and may also contain other phenolics and wall polymers. In addition to this preformed barrier, infection leads to an increased wall thickness in parts of the stele, possibly to increase the mechanical resistance and to decrease permeability. It has been established that host cell wall changes on infection are important in the resistance of plants to fungal infection (Rioux and Briggs, 1994; Kolattukudy et al., 1994). Lignin, cutin, suberin and callose are well-known as preformed or induced barriers to invasion by strengthening cell walls, thus increasing their protection against mechanical pressure and cell wall degrading enzymes and decreasing their permeability, which may prevent the pathogen from taking up plant nutrients (Rioux and Briggs, 1994). The presence of mechanical barriers has also been suggested as a mechanism for host resistance in plant-parasite infections, by preventing efficient penetration of the host cortex or stele (Saunders, 1933). Some correlation has been shown between the presence of endodermal and pericycle thickening in uninfected plants and field resistance in sorghum, but it may not be the only source of resistance in these plants (Maiti et al., 1984; Elhiweris, 1987). While it is probable that the resistance of *P. setosum* is due in part to the endodermal barrier and stelar thickenings, those parasites which penetrate the stele successfully still do not establish an effective interaction. This indicates that there may be a further mechanism of resistance, such as the production of toxic substances by the host, which eventually leads to the observed necrosis and death of the parasite and the surrounding host root tissue (Lane, unpublished). The observed build-up of phenolics may contribute to this (Rioux and Briggs, 1994). Elhiweris (1987) discovered increases in the phenolic content of sorghum plants infected with *S. hermonthica*, and indicated that the concentration of phenolic compounds was closely related to the resistance or susceptibility of the plants. The expression of resistance after successful penetration of the host stele also occurs in the resistant cowpea (B301)-*S. gesnerioides* interaction, where a necrosis of parasite and host similar to a hypersensitive response was observed (Lane and Bailey, 1992; Reiss, 1995). An examination of enzymes, chemicals and free phenolics at the *P. setosum*-*S. hermonthica* infection site may reveal factors important in the expression of resistance.

A very obvious layer of polyphenolic and fluorescent material was observed at the *P. setosum*-*S. hermonthica* interface, surrounding the invading haustorium. However, it was impossible to determine by light microscopy whether this accumulation was derived from the host, as a defense response to seal off the parasite, or from the parasite to strengthen the invading tissue and aid penetration. This requires further study by electron microscopy. Heide-Jorgensen and Kuijt (1995) showed both thickening and lignification of the parasite parenchyma cells at the *Triphysaria*-host interface. Reiss (1995) observed thickening of the

S. gesnerioides haustorial cells of the interface with cowpea, but also showed additional autofluorescence of host cell walls adjacent to the interface. Part of the fluorescence seen at both the *P. setosum*- and the maize-*S hermonthica* interfaces may also be derived from compressed cell walls pushed aside by the invading parasite. Localised crushing and distortion of host cells has been observed at the flanks of other invading plant parasites (Heide-Jorgensen and Kuijt, 1995; Pate et al., 1990; Reiss, 1995).

In maize the response to infection was inconsistent. A slight increase in fluorescence was always visible in the infected root; this may represent an increased accumulation of fluorescent phenolic compounds and wall polymers, in an attempt to strengthen the maize cell walls and hinder infection. The thickening of the inner endodermal cell walls observed in a number of infections was not always present; examination of a large number of sections indicated that cellulosic thickening of the endodermis was a factor of the stage of root development rather than a response to infection, as thickened endodermal cell walls were observed in uninfected roots as the distance from the root tip increased. However, the accumulation of Safranin red-staining components at the maize endodermis in some roots may be a specific response to infection, as it was not observed in uninfected roots of the same age. The difference in response may be due to natural variation in maize seedlings, or to some seedlings being more competent to respond to invasion, perhaps because of prior exposure to wounding or stress. The presence of fluorescent and Safranin red-staining material in the maize endodermis had no apparent effect on the speed or ability of the parasite to establish itself. This is in agreement with the results of Olivier et al., (1991), who found that although the accumulation of cellulosic and phenolic deposits was seen in both susceptible and resistant sorghum cultivars infected with *S. hermonthica*, accumulation in the susceptible cultivar was later and failed to halt penetration. Similarly, the changes in maize on infection may be either insufficient or too late to affect the progress of the parasite. An interesting point is that even in those roots which exhibit Safranin red staining of the endodermis, no accumulation of red-staining materials or lignin was observed at the invasive front of the parasite, only in patches at the sides. This indicates that the maize root does not seal off the haustorium as a defense response, and further that the parasite does not require significant thickening of its invasive cells to penetrate the maize root. The deposits of Safranin red staining compounds at the side of the interface may derive from the host, as a response to wounding, or from the parasite, possibly to reduce diffusion and direct the flow of water and nutrients from the host stele to the parasite xylem.

Parasite connections to the host stele

Invasion of the host stele was shown to involve both parenchymatous cells and tracheary elements, with the former appearing more frequent. This observation agrees with previous examinations of parasite-host xylem connections (Ba, 1988; Mallaburn and Stewart, 1987; Pate et al., 1990; Visser and Dorr, 1987). Work on *Striga* spp. and *Olax phyllanthi* has demonstrated apoplastic flow through both parenchyma and tracheary elements, indicating that both cell types are involved in uptake from the host (Kuo et al., 1989; Stewart and Press, 1990). Visser and Dorr (1987) observed that haustorial cells actually grow within host xylem vessels, as seems to be the case in the maize-*S. hermonthica* interaction (Figure 6a). In the *P. setosum*-*S. hermonthica* interaction, penetration of the endodermis and formation of tracheary elements was rare and growth into and along the host xylem vessels was not observed.

Conclusions

This preliminary study confirms that *S. hermonthica* is an efficient parasite of maize, with an infection process similar to that described in other hosts of *Striga* spp. While some young maize roots do exhibit an increased accumulation of fluorescent wall components on infection with *S. hermonthica*, the response varies widely between roots and does not successfully impede the establishment of the parasite. The resistance of *P. setosum* to *S. hermonthica* could be due at least in part to the preformed, highly thickened endodermal and stelar cell walls, rich in lignin and possibly other wall polymers, which are absent in maize. Investigation of the enzymes and soluble phenolics present at the *P. setosum*-*S. hermonthica* interface may reveal components which prevent further parasite development and could be important in the expression of resistance. Examination at the level of electron microscopy is required to determine whether the layer of fluorescent material and polyphenolics observed at the host-parasite interface is associated with a specific host response or part of the parasite invasion mechanism. A fuller understanding and comparison of the parasite infection process and host

response in susceptible and resistant hosts may identify factors which are important to the successful establishment of the parasite, or to the successful expression of a host resistance response. This may in turn aid the selection or production of varieties with a higher resistance to parasitism.

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