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Invasion of naturally senescing root cortices of cereal and grass seedlings by *Microdochium bolleyi*

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Summary In glasshouse experiments, invasion of wheat and grass (*Dactylis glomerata*) seedling roots by *Microdochium bolleyi* was strongly correlated with the pattern and rate of natural senescence of the root cortex. The fungus did not enhance cortical senescence and did not damage roots except in a few instances when it invaded and killed their tips. *M. bolleyi* behaved as a weak parasite, largely restricted to invasion of naturally senescing cortices of cereal and grass roots.

Introduction

The fungus *Microdochium bolleyi* (Sprague) de Hoog and Hermanides-Nijhof is commonly isolated from the rhizosphere and haulm base of cereals and grasses[3,13,15,16,18,19]. It is classed as a 'minor pathogen'[17], which implies that it causes yield losses, either alone or in association with other pathogens. However, *M. bolleyi* seldom causes serious damage to cereals in artificial inoculations, unless it is present on seedling roots at an overwhelming inoculum level[7]. It can even be beneficial, because it can control infection by more aggressive pathogens[12,14,15,17]. The uncertainty surrounding the role of this fungus in disease needs to be resolved. With this in mind, we studied the pattern of colonization of wheat and grass roots by *M. bolleyi* in glasshouse conditions, with special reference to the 'natural' pattern of senescence of the root cortex, which occurs in the normal course of root development[5,9,10].

Experimental

Wheat (cv. Cappelle-Desprez) and cocksfoot grass (*Dactylis glomerata* L. cv. S26) were grown from seeds in either horticultural vermiculite or John Innes Number 1 compost in 7 cm diameter plastic drinking cups. The rooting media were brought to, and maintained at, 60% saturation with Hoagland's solution, and the containers were incubated in a glasshouse at 20° or 25°C. In most experiments the seeds were sown over 11 mm diameter inoculum disks of *M. bolleyi*, cut from the margins of colonies on potato-dextrose agar; controls received no inoculum. In one experiment a 5 mm band of 3% maizemeal-sand inoculum of *M. bolleyi* (28 days old) was placed either 2 cm or 4 cm deep in the rooting medium, and seeds were sown on the surface so that the roots grew into

the inoculum. In all, six isolates of *M. bolleyi* were used, most being freshly obtained from wheat or barley, but two isolates (from barley) had been in culture for more than 4 years.

On sampling of the experiments, roots were preserved in 70% methanol, then hydrolysed briefly in dilute HCl, treated with acridine orange, mounted in buffer and examined under a fluorescence microscope for presence and distribution of nuclei in the cortical cells[9]. Cells were considered dead if they did not contain a stainable nucleus. Growth by *M. bolleyi* could not be assessed by direct means because its hyaline hyphae were indistinguishable from those of other fungi. Instead, the characteristic darkly pigmented groups of cells of *M. bolleyi*[6,16] were recorded under a compound microscope at × 100 magnification. Then the roots were stained with phloroglucinol-HCl[2], mounted in concentrated HCl and assessed for distribution of lignified papillae formed by roots in response to attempted invasion by *M. bolleyi*.

Results

Comparison of invasion of wheat and grass roots

This experiment compared growth by three isolates of *M. bolleyi* on wheat and grass roots in vermiculite, from agar disks immediately below the seeds. Three replicate cups of each treatment and of controls were sown with five seeds and sampled after 25 days. Assessments were made on the first-formed seminal roots of two seedlings randomly selected from each cup.

Roots of uninoculated plants did not contain dark fungal cells or lignified papillae, and they showed a normal pattern of cortical senescence as described elsewhere[9]. In the inoculation treatments a few roots did not emerge from the inoculum disks because their tips were killed by *M. bolleyi*; these roots are excluded from the presented results. However, most roots emerged and grew normally, and their final lengths and extent of cortical senescence were unaffected by *M. bolleyi* (Table 1). This was confirmed in a second experiment with wheat and barley.

Assessments of the distributions of dark fungal cells or lignified papillae showed that the isolates of *M. bolleyi* differed from one another in extent of invasion down roots (Table 1). The most invasive isolate (MB3) had been freshly obtained from field plants whereas the other two isolates (MB1 and MB2) had been in culture for more than 4 years. A similar difference between these isolates was found in a subsequent experiment, but in this some fresh isolates grew as poorly as did MB1 and MB2, so invasiveness is not simply related to length of storage in culture. The term invasiveness rather than growth is used in this respect, because *M. bolleyi* might have grown further than we could detect. But if it did so, it did not attempt invasion of the living root cells.

Each isolate of *M. bolleyi* invaded less far down grass than wheat roots, but it invaded a similar proportion of the total root length of grass and wheat. In other words, *M. bolleyi* did not overwhelm the slower-growing grass roots, as it would have done if it had colonized them as

Table 1. Extents of invasion of seminal roots of wheat and grass (*Dactylis glomerata*) by isolates of *Microdochium bolleyi* from inoculum disks positioned immediately below seeds; means ± SE for 6 replicate roots grown for 25 days in vermiculite at 20°C

Mean extent (cm) of:	Isolate			Control[a]
	MB1	MB2	MB3	
Wheat				
Roots	18.1 ± 0.70	13.3 ± 0.65	18.1 ± 2.17	15.2 ± 0.89
Dead root epidermis	6.3 ± 1.70	5.5 ± 1.12	9.4 ± 1.71	6.1 ± 1.02
Dark cells of *M. bolleyi*	4.3 ± 0.71	4.3 ± 1.72	9.3 ± 1.84	—
Lignified papillae	5.3 ± 0.77	5.4 ± 1.23	7.3 ± 1.94	—
Per cent root length invaded	29	41	51	—
Dactylis glomerata				
Roots	6.6 ± 0.76	7.1 ± 0.58	6.3 ± 1.11	8.7 ± 1.03
Dead root epidermis	2.4 ± 0.42	3.4 ± 0.97	3.1 ± 0.80	4.3 ± 0.96
Dark cells of *M. bolleyi*	1.9 ± 0.41	2.0 ± 0.20	2.3 ± 0.20	—
Lignified papillae	1.8 ± 0.47	2.4 ± 0.23	2.7 ± 0.65	—
Per cent root length invaded	29	34	43	—

[a] Uninoculated roots.

rapidly as on wheat (Table 1). Moreover, in each instance the extent of invasion down roots was just less than the extent of death of the root epidermis.

Pattern of invasion of roots by M. bolleyi

M. bolleyi had a characteristic pattern of invasion of roots in the experiment described above and in all other experiments of this type (Fig. 1). In the oldest part of the root axes, groups of dark fungal cells and lignified papillae were seen in the root epidermis and occasionally in the underlying (second) cortical cell layer, but not deeper in the cortex. Papillae were usually seen in host cells immediately beneath those containing dark fungal cells. Further down the roots, groups of dark fungal cells were seen in the outer one or two cortical cell layers and in the innermost cell layers (layers 5 and 6 in wheat, and 4 in *D. glomerata*) but seldom in the middle cortex; papillae were seen in most cell layers in this region. Still further down the roots, dark fungal cells and lignified papillae occurred mainly in the inner cortex but also were seen locally in the outer cortex of root axes around the bases of laterals (Figures 1 and 2). This changing pattern down roots is shown quantitatively for wheat in Figure 1, wheat being chosen because its six cortical cell layers can be assigned equally to 'outer', 'middle' and 'inner' cortex. The data were pooled for five isolates growing on wheat in vermiculite. Chi-squared analysis at each distance down the roots shows a highly significant ($P = 0.01$ or $P = 0.001$) departure from randomness in these data.

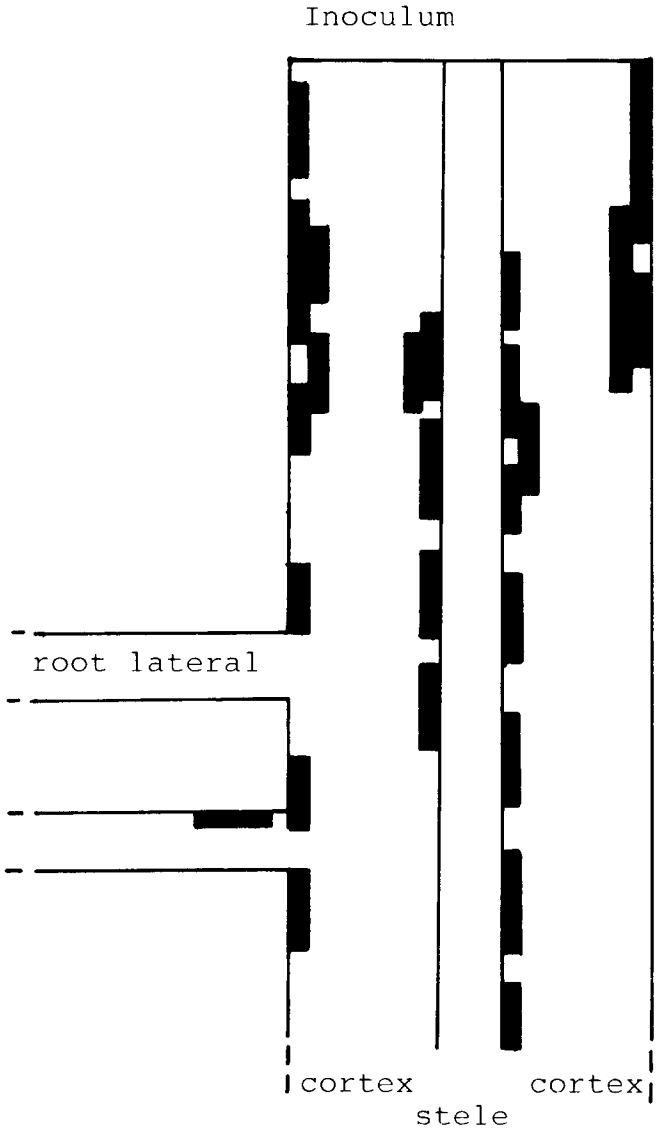

Fig. 1. Diagrammatic representation of the distribution of groups of dark cells of *M. bolleyi* (solid blocks) in a wheat or a grass root cortex as the fungus grows down roots from inoculum immediately below the seed. Figures shown below are numbers of wheat roots (maximum 30) containing groups of dark cells in the outer cortex (cell layers 1, 2), middle cortex (cell layers 3, 4) and inner cortex (cell layers 5, 6) at different distances down the roots.

	Cortex		
	Inner	Middle	Outer
0–5 mm	6	2	27
10–15 mm	17	5	25
20–25 mm	21	4	11
30–35 mm	20	1	4

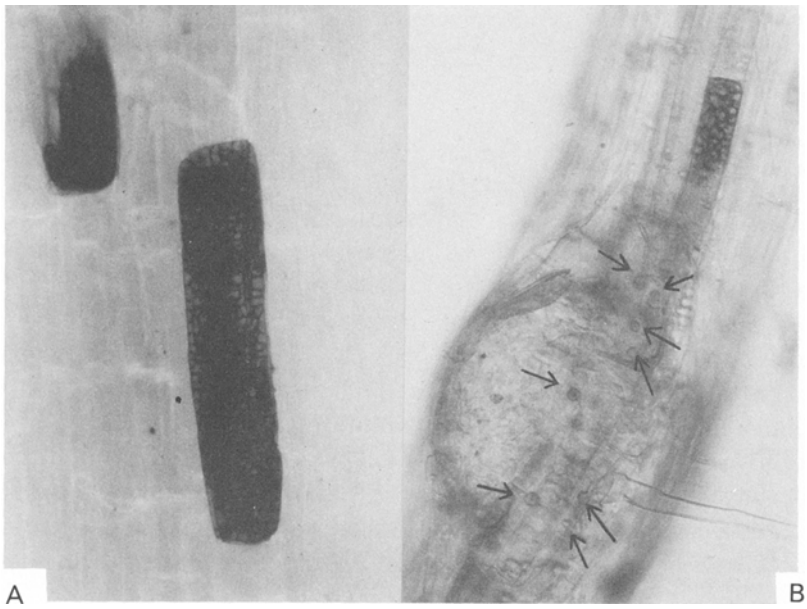

Fig. 2. **A**. Groups of dark cells of *Microdochium bolleyi* in cortical cells of a wheat root axis. **B**. Lignified papillae (*arrows*) and a group of dark cells of *M. bolleyi* in outer cortical cells of a wheat root axis near an emerging root lateral; root stained with phloroglucinol-HCl.

Relationship between invasion by M. bolleyi *and cortical senescence*

Wheat plants were grown for 12 days in vermiculite with a layer of inoculum of *M. bolleyi* 2 cm or 4 cm below the seeds. For each depth of inoculation, six replicate roots (from three replicate cups) were hand-sectioned at 5 mm intervals and the sections were assessed for (1) number of nucleate cortical cell layers (maximum 6) and (2) innermost cortical cell layer containing groups of dark cells of *M. bolleyi*. All roots showed a normal pattern of cortical senescence, as would occur in the absence of the fungus. Nuclei disappeared first from the epidermis behind the zone of nucleate root hairs, and then from successively deeper cortical cell layers in older regions of the roots. The 'end point' of this sequence had just been reached near the tops of the roots of the 12 day old plants, where five of the six cell layers were anucleate; the innermost (sixth) cortical cell layer is known to remain nucleate for most or all of the life of a root[9]. The depth of invasion by *M. bolleyi* paralleled the amount of cortical senescence, because dark fungal cells usually were seen immediately outside the layers of host cells that contained nuclei. Combining the results for the 2 cm and 4 cm inoculum depths, because they were similar, this relationship is represented by the equation

$$y = 0.62x + 0.91 \qquad (r = 0.663;\ P = 0.01),$$

where y is the innermost cell layer containing fungal cells, and x is the number of dead cortical cell layers in sections cut at different distances along the roots.

Discussion

All the evidence in this paper suggests that *M. bolleyi* causes damage only to root tips or very young root regions. Other workers have reported substantial damage of inoculated wheat and grass seedlings[7,18], but plants often recover fully from this damage when they produce more roots to replace those killed by *M. bolleyi*. The fungus does not even enhance cortical senescence of roots that escape early death, and yet the cortex has a short natural life in the conditions used here and its resistance to invasion must decline rapidly with age. The evidence, therefore, strongly suggests that *M. bolleyi* is a weak parasite (in addition to being a weak pathogen). Holden[11] reached a similar conclusion for *Phialophora graminicola* (Deacon) Walker, based on similar evidence, and this was subsequently confirmed[3,5].

The pattern of invasion of roots by *M. bolleyi* shown in Figure 1 can now be explained. At 20–25°C wheat root axes can extend at more than 2 cm $24\,h^{-1}$ whereas *M. bolleyi* grows at 1.5–4.0 mm $24\,h^{-1}$ on potato-dextrose agar (unpublished observations). So, as the fungus grows down a root from an inoculum disk it must encounter root regions that have matured ahead of it and which show progressively more cortical senescence ahead of colonization. Immediately below the inoculum the fungus colonizes root regions with a living cortex. Here even the outer cortical cells respond to invasion by forming lignified papillae, the fungus penetrates poorly and evidently responds by producing groups of darkly pigmented cells. Slightly further down the root, the cortex is at first alive, restricting invasion, but then senesces. This would explain why *M. bolleyi* forms groups of dark cells in both the outer and inner cortex. Still further down the root, the fungus encounters cortical cells that have already senesced. It may colonize these, depending on the degree of microbial competition for them, or grow beneath them, next to the outermost living root cells. In either case it forms groups of darkly pigmented cells only in the vicinity of living host cells.

In support of these arguments, lignified papillae and groups of dark fungal cells are found locally in the outer cortex further down the root where laterals emerge. The cortex of the root axis remains nucleate — at least temporarily — around the bases of laterals[5,9]. Furthermore, in conditions of severe root impedance (as in this study) the successive cortical cell layers die rapidly once the epidermis becomes anucleate[9]. This explains why relatively few groups of dark fungal cells occurred in

the middle cell layers of the cortex (Fig. 1). The patterns of occurrence of these dark fungal cells are similar to those of *P. graminicola* and related fungi[4]. They have been termed 'growth cessation structures' and may be temporary survival structures from which the fungus can resume invasion of the cortex as host resistance declines[4].

We studied only invasive growth by *M. bolleyi*. If the fungus grew further along roots than we detected then it would have done so as a saprophyte, and this was not of primary concern in this study. However, *M. bolleyi* is reported from the rhizosphere of a range of non-graminaceous plants[6] in addition to its many graminaceous hosts[18], and it differs from *P. graminicola* and related fungi in this respect[1]. Whether *M. bolleyi* grows as a parasite or saprophyte on non-graminaceous plants merits further study.

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