Chapter (non-refereed)

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Cellulolysis of cotton by fungi in 3 upland soils

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1 *Summary*
This laboratory study aimed to improve our limited knowledge of the fungal colonization of cotton strips inserted into soil for decomposer studies. The results indicated the following points.

i. Fungi cellulolytic on cotton cloth were isolated from cotton strips retrieved from the field along with many non-cellulolytic fungi possibly present as secondary colonizers.

ii. Particular cellulolytic fungi were specific to certain soils.

iii. Soil characteristics had a marked effect on the expression of cellulolysis. Some fungi shown to be cellulolytic in one soil were non-cellulolytic in other soils, although showing good growth in the latter.

iv. More cellulolytic fungi were present in a podzol soil than were isolated from the cotton strips, suggesting some selection by species. The community of cellulose decomposers in a soil appears to be adapted, or selected, to grow and decompose cellulose in the environmental conditions of that soil.

v. Pigmentation of cotton strips inserted in soils can be caused by fungi, but does not appear to be a useful indicator of particular fungi.

2 *Introduction*
Since the cotton strip assay was developed as an ecological test to examine cellulose decomposition rates in the field, it has generally been used without regard for the organisms involved, although data are available in the literature on the organisms colonizing cotton (Thaysen & Bunker 1927; Siu 1951; Nigam *et al.* 1960; Desai & Pandey 1971). However, cotton strips inserted into soils can be of considerable value, not only in determining the level of cellulolytic activity, but also to determine which organisms are responsible for cellulose decomposition in soil, if it can be shown how the flora developing on the cotton is related to the population of soil fungi (Widden *et al.* 1986). Degradation of the cotton in different soils is likely to be affected by different organisms, and characteristics of the soil environment may determine whether a given organism, though present, actually degrades the substrate.

The various pigments seen, when cotton strips have been retrieved from the field, were known to vary according to the soil type, vegetation or management of the site. It was of particular interest to know whether such pigments could be used as indicators of the presence of specific, and possibly cellulose-degrading, micro-organisms.

A laboratory study was therefore designed, using fungi that had been isolated from soil and from buried cotton strips in upland soils, to answer the following questions.

i. What proportion of the fungi found on cotton strips are cellulolytic on that cotton?

ii. Are species of cellulolytic fungi specific to certain soils?

iii. To what extent does the soil type affect the degree of cellulolysis generated by a given fungus?

iv. Are the strips selective for particular fungi?

v. Is the pigmentation seen on strips caused by fungi, and can it be used to indicate the presence of a particular species?

3 *Method*
The soils from which the non-basidiomycete fungi were isolated were located at the Moor House National Nature Reserve in the Pennines, Cumbria (brown earth under upland grassland); Gisburn Forest, Lancashire (peaty gley under Sitka spruce (*Picea sitchensis*)); and at Glen Dye, Kincardineshire (podzol under heather (*Calluna vulgaris*) moorland). They differed considerably in pH, organic matter, calcium (Ca), phosphorus (P) and potassium (K) contents (Figure 1).

Fungal communities in the Moor House site and at Banchory are described elsewhere (Widden 1987; Widden *et al.* 1986; P Widden & G Howson pers. comm.). Most of the microfungi used in this study were isolated as part of a study of the community ecology of microfungi in upland soils by one author (Widden). The fungi were isolated either from the upper 5 cm of the soil, using the soil washing method (Widden 1979), or from threads taken from the side of retrieved cotton strips as described by Widden *et al.* (1986). The general procedure is illustrated in Figure 2. Fifty soil and cotton isolates (Table 1) were chosen to include dominant species from the 3 distinctive upland soils, together with some cultures of hymen-

omycetes, isolated from other sites and supplied by Dr J C Frankland (Institute of Terrestrial Ecology, Merlewood Research Station).

Cellulolytic ability and pigment formation by fungi on cotton overlying each of the 3 soil types were tested using the following procedure. Soil from the upper 0–15 cm level at each study site was separately sieved through a 5 mm mesh screen and dried to about 25% of moisture-holding capacity for storage; 14 g of soil from each site was placed separately into a 9 cm glass petri dish, and a piece of cotton cloth (Shirley Soil Burial Test Fabric, 1981 batch), 5 cm x 7 cm, was pressed on to the soil surface. Distilled water was

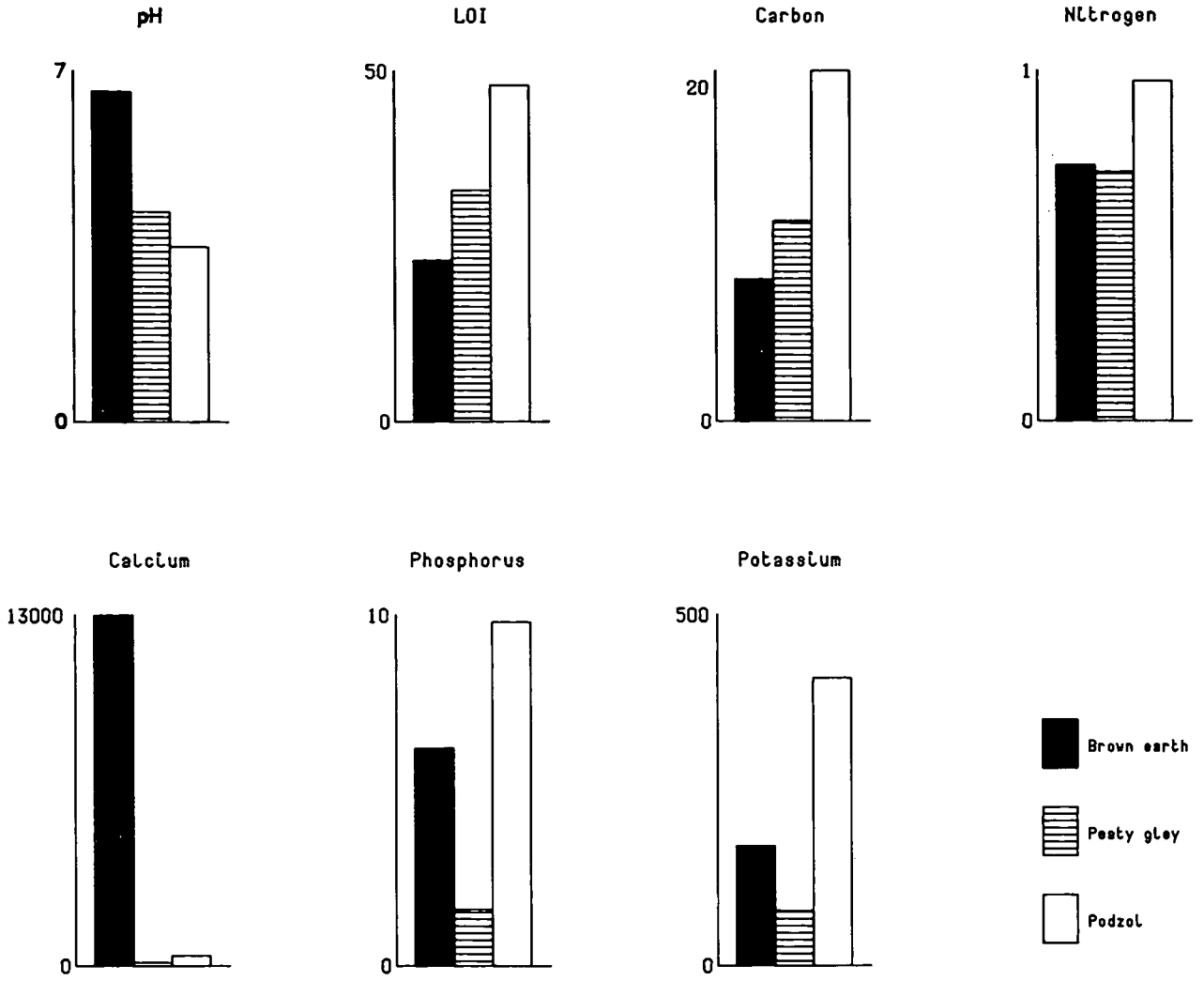

Figure 1. Characteristics of the 3 soil types used to isolate fungi and to test cellulolysis. LOI, C and N as % and Ca, P and K as μg g^{-1}

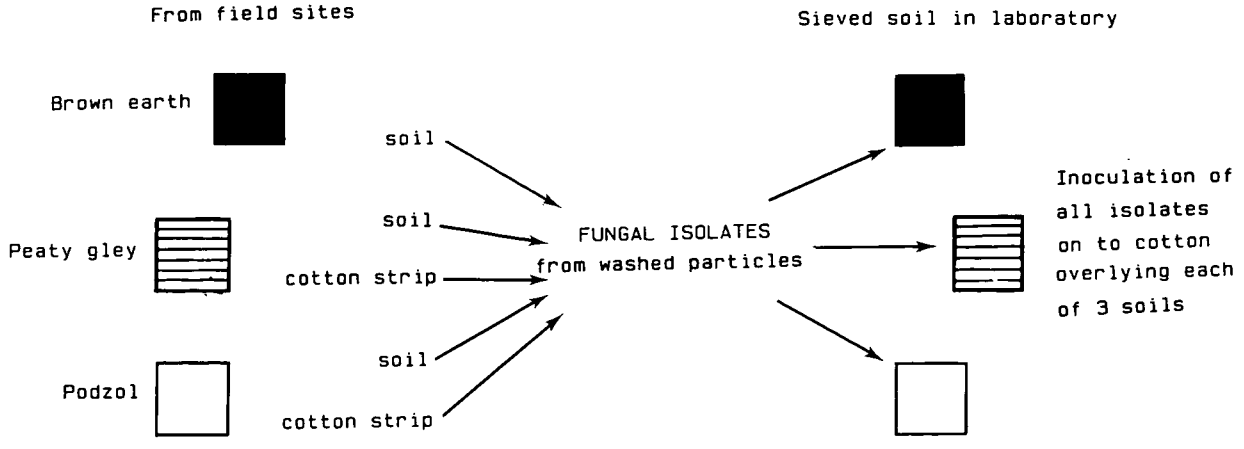

Figure 2. Diagram illustrating the isolation of fungi from soil and cotton, and their inoculation on to cotton overlying the soil

Table 1. List of fungi used in this study, with sources of isolates and occurrence on cotton. Cellulolytic ability is given for cotton strips and cotton cellulose plates (where tested), and as reported by Domsch *et al.* (1980), Domsch and Gams (1970) (D); Flanagan (1981) (F); or Gochenaur (1984) (G) on acid-swollen cellulose. Different isolates of the same species are indicated (i, ii)

		Source of isolates	Occurrence on cotton	Cellulolytic ability: Cotton strips	Cotton plates	Reported by: D	F	G
HYMENOMYCETES								
Armillaria mellea		CBS culture	os	—				
Collybia dryophila		DW litter	os	+				
C. peronata		DW litter	os	+				
Flammulina velutipes		DW rotten wood	os	+	—			
Laccaria amethystea		DW litter	os	+	—			
Marasmius androsaceus		SS litter	os	—				
Mycena epipterygia		DW litter	os	—				
Nolanea staurospora		Moorland litter	os	—				
ASCOMYCETES								
Allescheria sp.[1]		UG soil		+	+			
Pseudeurotium sp.		SS soil	*	+				
PHYTOMYCETES								
Mucor circinelloides		UG soil	—	—	—			
FUNGI IMPERFECTI								
Ceuthospora lauri	i & ii	SS cotton	**	—				
Chloridium chlamydosporis		SS soil	—	+		+		
Chrysosporium merdarium		UG soil	—	+				
C. pannorum		SS soil	*	+		+	+	
Chrysosporium sp.		UG soil	—	+	+			
Cylindrocarpon obtusisporum	i	SS soil	*	+				
	ii	UG soil		+				
Gilmaniella humicola		SS soil	*	+		+		
Humicola fusco-atra[1]		SS soil	*	+		+		
Oidiodendron tenuissimum	i	UG soil	*	+				
	ii	SS soil		—				
Paecilomyces carneus		UG soil	—	—		+		
Penicillium daleae	i	SS soil	**	+	—			+
	ii	HM cotton		+	—			
P. digitatum		SS cotton	*	—	—	+		
P. glabrum	i	SS cotton	**	—	—		—	—
	ii	UG soil		—				
P. janthinellum		SS cotton	**	—		+		+
P. lividum		UG soil	*	—	—	+		
P. melinii		SS soil	**	—				—
P. montanense		HM soil	—	—				
P. spinulosum	i	SS soil	*	—	—	+		+
	ii	HM cotton		—	—			
P. thomii		SS soil	*	+		+	+	—
Penicillium spp.		UG soil	—	—	—			
Phialophora sp.		SS soil	*	—				
Thysanophora penicillioides		SS soil	**	—				
Tolypocladium cylindrosporum		UG soil	—	—				
T. niveum		UG cotton	—	—	—			
Trichocladium opacum		SS soil	*	+	+/—	+		
Trichoderma polysporum		SS soil	*	+	—	+		
T. viride aggr.	i	SS soil	*	+	+/—	+		
	ii	UG soil		+	+/—			
STERILE MYCELIA	i	SS soil	—	—	—			
	ii	SS soil		—	—			

[1] Unconfirmed identification

** indicates major colonizers, ie 10% of isolates on one or more cotton strips from either Sitka spruce or heather moorland sites

SS, Sitka spruce; UG, upland grassland; HM, heather moor sites; DW, deciduous woodland; os, isolated in other woodland studies; CBS, Centraalbureau voor Schimmelcultures, Baarn

added to each dish to bring the moisture close to field capacity, and the dishes were sterilized by autoclaving. Each fungus was then inoculated separately on to the surface of the cotton, using an inoculum cut from the actively growing edge of an agar plate culture. The soil plates were incubated at room temperature for 9 weeks. Three replicates were prepared for each fungus and each soil.

The fungal growth on the strips was photographed at 4 and 9 weeks and the linear spread of mycelium was recorded after 9 weeks. After incubation, the cotton was removed and washed, first in 70% alcohol for one minute (a safety procedure to limit possible fungal infection during subsequent handling of cloth) and then with a jet of water. The strips were dried at room temperature overnight and oven dried at 50°C for 4 hours. The dried strips were then photographed, frayed to 3 cm width, and tested for tensile strength (TS) on a Monsanto Type W tensometer using a jaw distance of 3.5 cm.

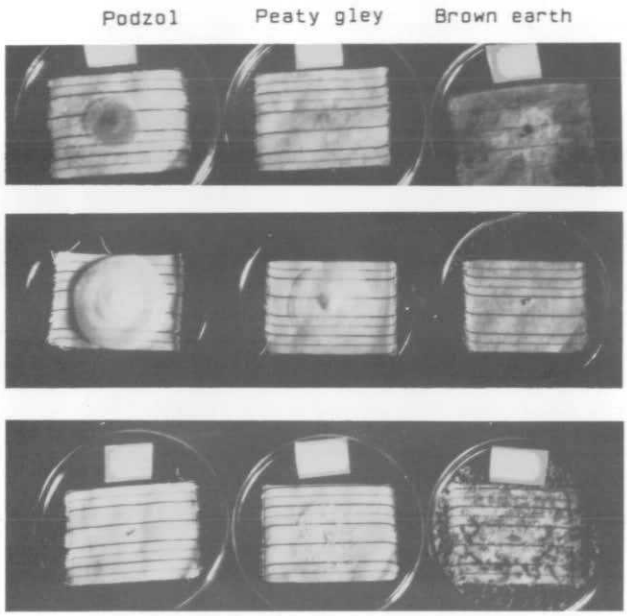

Plate 5. Growth variation of fungi on cotton overlying 3 soils
i. Trichocladium opacum
ii. Phialophora *spp.*
iii. Sterile mycelium
(Photographs J Gillespie)

4 *Results*

Fungal growth on cotton overlying the 3 soils varied markedly with soil type, being of different growth form, extent or colour. For example, *Trichocladium opacum* and a sterile mycelium grew best and produced a black coloration of cotton on the brown earth, but formed more restricted white or grey colonies on the other 2 soils (Plate 5), whereas a *Phialophora* species grew as thick mycelium on the podzol, but sparsely on the brown earth soil. Most of the fungi (74%) grew on all 3 soils, but some grew only on one or 2 soils. More fungi grew best on the brown earth soil (Table 2, groups 1a, b, c) than on the other 2 soils (groups 2 and 3). The brown earth was the most fertile soil in terms of higher pH and calcium, and lower organic matter (Figure 1). Other fungi showed no particular preference for any one soil, but grew equally well on 2 or all of the soils (Table 2, groups 4a–c); these fungi include many of the *Penicillium* spp. There was a tendency for fungal species to show best growth on the soil from which they were isolated.

For any one fungus, the same one of the 3 soils usually showed highest rate of cellulolysis and the most growth, but the degree of cellulolysis, as measured by tensile strength loss of cotton (CTSL), was more dependent on the soil type on which the fungus was incubated (Figure 3). Thus, many fungi which grew well on a particular soil produced no CTSL on that soil. Only one fungus, *Trichoderma polysporum,* was cellulolytic on all 3 soils. As with fungal growth, the highest CTSL values occurred on the brown earth soil among a group of fungi which, although they grew, produced no significant CTSL on the other 2 soils. Another group of fungi produced their highest CTSL on the peaty gley soil, while only 2 fungi, *Laccaria amethystea* and *Oidiodendron tenuissimum,* favoured the podzol (Figure 3). Those fungi producing the highest CTSL on either the peaty gley or podzol soils also produced some CTSL on other soils, and were from the group (Table 2, group 4) which showed no particular growth preference for any soil.

Generally, a fungus gave the highest cellulolytic activity on the soil from which it had originally been isolated (Figure 3). Two fungi, *Penicillium daleae* and *Trichoderma viride,* each had 2 cellulolytic isolates isolated from different soils, but both isolates of each species gave the highest CTSL on the same soil, suggesting that selection for genetically different ecotypes in the different soils of origin had not occurred. Where different species from the same genus were tested, there was also a tendency for these fungi to have the highest activity on the same soil. Thus, *Chrysosporium, Cylindrocarpon* and *Collybia* species were most active on the brown earth, whereas both species of *Trichoderma* were most active on the peaty gley soil.

Twenty-four fungi did not produce any significant CTSL, although some had been isolated originally from cotton strips (Table 1).

Pigmentation of the cotton strips in culture was produced by 23 fungi, but sometimes only on certain soils, and it was usually of indistinct greyish brown colours. *Trichocladium opacum* and *Chrysosporium pannorum* produced a reddish pigment only on the brown earth soil (Plate 8 i), whereas *C. merdarium* produced different coloration on 2 of the soils (Plate 8 ii). Colours can, therefore, indicate fungal colonization, but the colours

Table 2. Relative linear spread of fungi inoculated on to cotton placed on the 3 soils

i. Fungi which grew best on one soil listed and grouped under that soil, with initials after species names indicating where good growth also occurred on other soils

Brown earth		Peaty gley		Podzol	
1a **Allescheria* sp.		2a *Marasmius androsaceus*		3a None	
**Chrysosporium* sp.					
**Flammulina velutipes*					
Mucor circinelloides					
Philalophora sp.					
Tolypocladium cylindrosporum					
1b **Chryosporium pannorum*	P	2b *Armillaria mellea*	B	3b *Thysanophora penicillioides*	G
Sterile mycelium	P				
1c *Nolanea staurospora*	G,P	2c **Chloridum chlamydosporis*	P	3c **Collybia dryophila*	B
Paecilomyces carneus	G,P	**Gilmaniella humicola*	P	**Collybia peronata*	B
Penicillium sp.	G,P	**Oidiodendron tenuissimum*	P	**Laccaria amethystea*	B
Tolypocladium niveum	G,P	i & ii	P		
**Trichocladium opacum*	G,P				
		2d *Chrysosporium merdarium*	B,P		

ii. Fungi which showed no preference for any single soil, but grew equally on 2 or 3 of the soils

Brown earth and peaty gley		Brown earth, peaty gley and podzol		Peaty gley and podzol	
4a **Humicola fusco-atra*		4b **Cylindrocarpon obtusisporum*	i & ii	4c *Ceuthospora lauri*	i & ii
**Pseudeurotium* sp.		*P. digitatum*		*P. montanense*	
**Trichoderma viride*	i	*P. glabrum*		*P. spinulosum*	
		P. lividum		**P. thomii*	
		Penicillium sp.		*P. janthinellum*	
		**Trichoderma polysporum*			
		**T. viride*	i & ii		

* indicates fungi cellulolytic on cotton
B, brown earth; G, peaty gley; P, podzol

are mostly too indistinct and variable to be useful as indicators of colonizing species. Three fungi (*Allescheria* sp., *Humicola fusco-atra, Chrysosporium* sp.), which had shown pigmentation on cotton on soil plates, produced little pigmentation when tested on Perlite in place of soil, again indicating that soil/substrate constituents can influence the colours produced on cotton by fungi.

5 *Discussion and conclusions*

Various cellulolytic fungi isolated from 3 distinct upland soils have been shown to grow on and to decompose cotton strip material incubated *in vitro* on the same soils after autoclaving. Of the 50 fungi tested, 22 produced a significant CTSL of the cotton overlying the 3 soils. A small number of these cellulolytic fungi were recorded at high frequencies from cotton strips buried in the field (notably *Penicillium daleae, Trichoderma polysporum* and *T. viride*), whereas a number of fungi which produced non-significant CTSL (notably species of *Penicillium*) were also frequently isolated from cotton (Table 1). In the case of the podzol, 8 out of 13 cotton isolates tested gave no significant cellulolysis on cotton. These results suggest that successful colonization of the cotton by cellulose decomposers is a competitive process, and that some colonizers may well be secondary colonizers, using glucose or other derivatives released during cellulose decomposition. In this regard, it is worth noting that *P. spinulosum* has been shown to be cellulolytic by Flanagan (1981) and Gochenaur (1984), using acid-swollen cellulose as a substrate. This fungus has been shown by Reese and Levinson (1952) to produce only endo-β-1, 4 glucanases and therefore to be unable to attack native cellulose. It is, therefore, probable that the fungus can attack the acid-swollen cellulose because the partial hydrolysis opens up the cellulose to attack by endo-glucanases.

Chrysosporium pannorum and *Trichocladium opacum* were both cellulolytic only on the brown earth soil (Figure 3), and were not recorded, or had very low frequency, on the cotton strips from the podzol, so the cellulolytic fungi which colonize cotton in a soil may, in part, be limited to those able to decompose cotton in that soil. However, *Chloridium chlamydosporis* and *Gilmaniella* sp., particularly abundant in the podzol, but not recorded on cotton strips, did cause CTSL on this soil in the laboratory tests. Of 8 cellulolytic fungi isolated from the podzol soil, 5 were recorded on cotton strips, indicating some selection of types by cotton in that soil. The fungal community in the soil and on cotton inserted in the podzol soil at the heather moor site is fully discussed in Widden *et al.* (1986). (Information on colonization of cotton strips in the peaty gley soil is yet to be published.) Shawky and

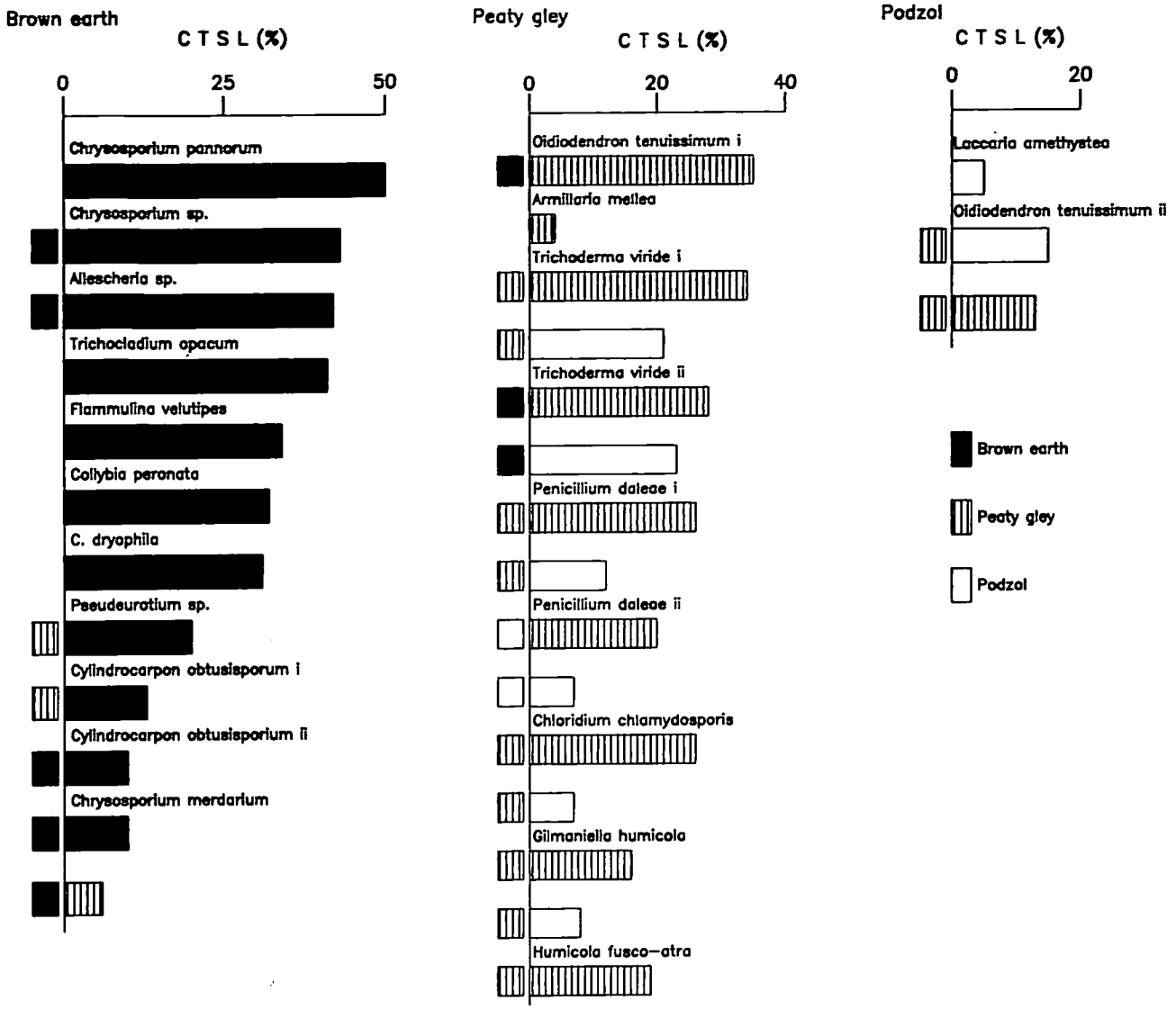

Figure 3. Fungi grouped in 3 columns according to the soil on which they showed highest CTSL. CTSL on other soils is also shown. Smaller hatched blocks indicate origin of isolates obtained from the 3 soils. Different isolates of the same species are indicated (i, ii)

Hickisch (1984) also reported a marked effect of soil type on the decomposition of cotton, and other cellulosic substrates, by *Trichoderma* species.

It is probable that the colonization of a cellulosic substrate is regulated by a selective process, based on an ability to compete for the substrate in a particular environment. In this regard, it is noteworthy that the most successful microfungal colonizers were *Trichoderma* species, which are fast-growing and known to be active soil antagonists. Further studies with a wider range of isolates would be needed to distinguish whether adaptation or selection was the controlling factor.

It must be emphasized that a number of features of this study are at variance with the natural situation. Single pure cultures were used, under laboratory conditions, with soil (not litter) as the substratum, possibly altered chemically and physically by sterilization. Pure cotton is foreign to soil and cannot wholly represent dead vegetation as a substrate for colonization and cellulose decomposition. Depending on the nature of the vegetation type, cotton could be easier or more difficult to attack. The fungi which showed high activity in culture might not do so in the field, and those giving a negative result might be able to attack other cellulosic substrates under field conditions. The competitive element of a mixed microbial population was also eliminated by the test conditions.

The pigmentation of cotton produced by the fungi in these experiments, mostly greyish brown but also red (Plate 8), may be similar to the blackish and reddish coloration of cellulose residues from cellulose decomposition reported by Thaysen and Bunker (1927). However, as the nature of pigment production by an individual fungus varied with soil type, pigmentation cannot be used as an index of either the presence of that fungus or of cellulose decomposition by it.

During this study, a cellulose agar medium was tested

as a possible screening method for cellulolytic ability on cotton, but was not successful. It contained ground cotton passing through a 63 μ sieve, with nitrate as a nitrogen source and 0.1% glucose as an ancillary carbon source. Of 20 fungi tested over a period of 7 weeks, only a few showed any cellulolysis as evidenced by visible clearing of the plates (Table 1). Clearing did not extend outside the colony and was unaffected by added glucose. *Allescheria* and *Chrysosporium* sp. showed clearing after 1–2 weeks, but clearing by *Trichocladium opacum* and *Trichoderma viride* could only be found using a microscope to detect the disappearance of amorphous material from among a larger crystalline form of the material. The earlier loss of amorphous material is also discussed by Smith (1983) in relation to various cellulosic substrates.

Three species which gave positive results on cotton strips gave negative results on the cellulose agar. In many studies where the clearing of cellulose on agar plates has been used to detect cellulolytic activity, acid-swollen cellulose (Aaronson 1970) has been used. Comparison of such results with the cotton strip data presented here suggests that this cellulose agar method was not a satisfactory indication of an ability to attack natural cellulose. Smith (1983) suggests that the differential settlement of unequally sized particulate cellulosic substrates may reduce the availability for attack at the surface of agar plates.

Our data from cotton strips suggest that the cotton strip assay is a sensitive method for detecting the cellulolytic ability of a fungus in soil. The strong influence of soil type on cellulolytic ability (Figure 3) also shows that we should be very careful when laboratory tests are used to evaluate the cellulolytic ability of fungi and then extrapolated back to field conditions. Not only are many fungi that are capable of degrading cellulose likely to give negative results using cellulose clearing, but changes in the mineral composition, pH, or other factors in soil or culture media may markedly change the behaviour of the fungus. Clearly, in this study, some fungi were effective cellulose degraders in the fertile soil, whereas others were effective in the more nutrient-poor soils. These studies support the conclusion of Park (1976), who showed that the influence of nitrogen on the cellulolytic ability of soil microfungi varied from one species to another, some species responding better to low nitrogen levels, whereas others responded better to high levels.

In conclusion, and also in answer to the original 5 questions, the following points can be made as a result of this study.

i. Various fungi isolated from the 3 upland soils were shown to be cellulolytic on cotton overlying the 3 soils, but many non-cellulolytic fungi also occurred on the strips, possibly as secondary colonizers.

ii. Particular cellulolytic fungi were specific to certain soils.

iii. The cellulolytic ability of individual species was greatly affected by soil type, and a group of fungi that gave the highest cellulolysis on the brown earth soil could be separated from a group which favoured the podzol and peaty gley soils for cellulolysis but showed no particular growth preference between the 3 soils. In both cases, there were fungi cellulolytic on one soil which showed no activity on other soils, despite showing good growth on them. Only *Trichoderma polysporum* was actively cellulolytic on all 3 soils.

iv. Cellulolytic fungi recorded on cotton strips from the podzol were apparently selected from a community of cellulolytic organisms present in the soil, as some cellulolytic fungi present in a particular soil were not isolated from cotton retrieved from that soil. Their absence partly reflected their inability to decompose cotton on that soil.

v. Pigmentation on cotton strips can be caused by fungi, but is not recommended as an indicator of the presence of particular fungi.

In general, cellulolytic fungi present in a soil are able to colonize and decompose cotton strips. The native population appears to be adapted to grow and decompose in the prevailing soil conditions of the site, but the study demonstrates a clear separation between growth and cellulolysis in their relative response to soil properties.

6 *Acknowledgements*

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