Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees

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Endophytes in higher plants

The presence of fungi in healthy plant tissues has been commonly observed (CARROLL, 1988). Such fungi are termed endophytes and have been isolated from a diverse range of plants from all over the world (Tab. 1), ranging from the skins of edible fruits in India and the Tropics (DASTUR, 1916; WARDLAW, 1931; WARDLAW & al., 1939), to the needles and petioles of conifers in Europe and the USA (CARROLL & al., 1977; CARROLL & CARROLL, 1978) and the blades of grasses in the USA (CLAY, 1988). The tissues of healthy hosts are extremely specialised habitats and fungi existing within them must be adapted to cope with the low nutrient availability, high water content and associated high CO_2 and low O_2 levels, and the various defensive mechanisms employed by the host to prevent fungal colonisation. Specific adaptions by endophytic fungi to these pressures might include the ability to penetrate host tissues by various means, for example the production of enzymes such as pectinases and suberases (VERHOEFF, 1974), and the possession of a latent (metabolically inactive) phase in their life cycles (see pages 56–57).

Certain specific genera of fungi have been commonly found to be well represented in the endophytic floras of a wide variety of plants whilst the majority of fungal genera, particularly Basidiomycotina, are conspicuously absent (Tab. 1). Common endophytic genera include *Diaporthe* (and its anamorph *Phomopsis*), *Pezicula* (and its anamorphic form *Cryptosporiopsis), Cryptocline, Phoma, Phyllosticta, Alternaria, Aureobasidium, Cladosporium, Nodulisporium* (anamorphs of *Hypoxylon* and *Daldinia* sp.), *Geniculosporium* (anamorphs of *Xylaria* and *Hypoxylon* spp.) and *Phialophora*. The genus *Phomopsis* is particularly widespread having been found in

hosts as diverse as conifers, ericaceous shrubs, soybean plants and *Coffea arabica* L. (RAYNER, 1948; KILPATRICK, 1957; CARROLL & CARROLL, 1978; PETRINI & al., 1982).

Endophytic floras of different hosts vary in their floristic composition particularly at the species level. Some endophytic species are confined almost exclusively to specific hosts. For example, *Rhabdocline parkeri* occurs only on Douglas fir and not on any related conifer in the Pacific Northwest (CARROLL, 1988). Most endophytic species are, however less host specific but rather are confined to broader taxonomic groupings of hosts. For example, *Phyllosticta vaccinii* EALER, *Physalospora arctostaphili* B. ERIKSS. and *Pleospora herbarum* (FR.) RABENH. are common endophytes in a variety of ericaceous hosts in both the USA and Western Europe (PETRINI & al., 1982), and *Phomopsis occulta* TRAV. is a common endophyte in a wide variety of coniferous hosts and beech (*Fagus sylvatica* L.: CARROLL & CARROLL, 1978; PETRINI & FISHER, 1988). A few endophytic species are host-neutral including *Epicoccum nigrum* LINK and *Aureobasidium pullulans* (DE BARY) ARNAUD (Tab. 1).

Many endophytic species are not only restricted to certain hosts but also to specific tissues within them. A well known example is provided by conifer needle or petiole endophytes. In the conifer *Pinus nigra* (LAMB.) ENDL., *Lophodermium pinastri* (SCHRAD.: FR.) CHEV. is confined to needles and *Geniculosporium serpens* CHESTERS & GREENHALGH to petioles (CARROLL & al., 1977). It is thought that petiole associated fungi are also associated with twigs, particularly with the cortex. Another example of confinement to specific regions of plants includes localisation of endophytes around the veins, especially the midrib of leaves (LUGINBÜHL & MÜLLER, 1981).

Small-scale geographic differences between the endophytic floras of the same host species or group of host species have been reported by various authors. These differences can be explained by a combination of several factors including site moisture levels, elevation, degree of disturbance and openess of stands. The degree of endophyte infection seems to decrease with declining habitat moisture and elevation (CARROLL & CARROLL, 1978; PETRINI & CARROLL, 1981), which may be related to the fact that many endophytes produce masses of slimy spores (e. g. *Phyllosticta* spp.) which are often associated with rain dispersal (BERNSTEIN & CARROLL, 1977; CARROLL, 1988). This would not however apply to Xylariaceous fungi (A. J. S. WHALLEY, pers. comm.). Infection rate also tends to be lower in hosts on disturbed or open sites (PETRINI & al., 1982). It is also noteworthy here that certain hosts show an innate tendency to be less susceptible to endophyte colonisation, e. g. *Tsuga heterophylla, Abies amabilis* and *Pinus contorta*, perhaps related to host preferences for dry habitats (PETRINI & al., 1982; CARROLL & CARROLL, 1978).

Tab. 1. Distribution of common endophytes amongst various different hosts.

Hosts	*Araceae*	*Bromeliaceae*	*Orchidaceae*	*Ilex* spp.			*Buxus* spp.			*Hedera* spp.			*Ruscus* spp.			*Arctostaphylos* sp.	*Gaultheria* sp.	*Mahonia* spp.	*Umbellularia* sp.	*Ulex* spp.	*Salicornia* sp.	*Juniperus* sp.			*Abies* spp.	*Picea* spp.		*Pinus* spp.			*Pseudotsuga* spp.		*Sequoia* spp.	*Taxus* spp.	*Tsuga* spp.		*Fagus* spp.		Other hosts (see legend)
Tissue	Leaves & green tissues			Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves				Spines	Stems	Stems	Needles	Petioles	Twigs	Needles	Petioles	Needles	Petioles	Needles	Petioles	Whole stems	Twig xylem	Needles	Petioles	Needles	Needles	Needles	Petioles	Whole stems	Leaves	Various tissues
Endophytes Ascomycetes *Balansia* spp.																																							A, Ce, Cy
Chaetomium spp.				I			I	I			I									C	I			I													C	I	
Coniochaeta spp.							I			I			I									I	I	I						C									
Diaporthe spp.					I	I		I	I	I	I		I											I															Y, So
Naemacyclus spp.																									I		I	C	C		I								
Pezicula spp.					I		C	I								C	C					I	C	C					I									*	
Sordaria spp.				I	I		I	I		C	I		I	I								C	C	C					C										
Sporormiella spp.				I			I													C	C	C	C	I					C										
Sporormiella australis																					C		I		I					I										
Pleospora spp.																C				C	I	C																		
Anthostomella spp.	I			I																		C	C	I															
Coelomycetes *Colletotrichum* spp.	C	C	C								I													I															Grap, Ora, Man, Pap, Cac, T
Coniothyrium spp.	I			I	C		I	I		I	C		I	C						C	C	C								I									
Coryneum spp.																							I	I		I	I		I								C		
Cryptocline spp.	I	I	I	C	I					I	C							C	C			I	C	C	C	C		C			I	I	I	I		I	C	I	
Cryptosporiopsis spp.	I	I	I								I					C		C				I	I	I	C	C				I	I		C		I		I		Vm
Diplodia spp.										I	I											I	C	I														I		
Epicoccum nigrum				I	I		I	I		I		I	I	C	I							I	I	I		I												C		
Gloeosporium spp.																																								Mop, Ban, Cac, T, App.

Tab. 1 (cont.)

Hosts	*Araceae*	*Bromeliaceae*	*Orchidaceae*	*Ilex* spp.			*Buxus* spp.			*Hedera* spp.			*Ruscus* spp.			*Arctostaphylos* sp.	*Gaultheria* sp.	*Manonia* spp.	*Umbellularia* sp.	*Ulex* spp.	*Salicornia* sp.	*Juniperus* sp.			*Abies* spp.			*Picea* spp.			*Pinus* spp.			*Pseudotsuga* spp.	*Sequoia* spp.	*Taxus* spp.	*Tsuga* spp.		*Fagus* spp.		Other hosts (see legend)
Tissue	Leaves & green tissues			Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves				Spines	Stems	Stems	Needles	Petioles	Twigs	Needles	Petioles	Needles	Petioles	Needles	Needles	Petioles	Whole stems	Twig xylem	Needles	Petioles	Needles	Needles	Needles	Petioles	Whole stems	Leaves	Various tissues
Leptostroma spp.																									C	C	C	C	C	C										
Microsphaeropsis spp																						C	C	C	I			C							I					
Phoma spp.	I	I	I	I	I		C	C		C	C			I	I							C	C	C	C							I							C	
Phoma cava				C	C		I	C		I	I			I								C	C	C																
Phomopsis spp.	I			I	C		I	C		I	I	I		C		C	C	C	C	C	C		I	I	I		C	C	C			C	I	I	I		I			Ceq, Ca, C, Abt, Ama, Les, Len, E
Phyllosticta spp.	I			I	C								C	C		C						C			C	C						C	C		C		C	C	C	
Sclerophoma spp.																									I	I		I	I	I		I								
Hyphomycetes *Acremonium* spp.							I	I					I				C					I		I	I	I					I								I	M, St, L, F
Alternaria alternata				I	I						I		I							I		C	I	I	C			I			I								C	
Alternaria tenuissima				C			I	I		I	I	I	C	I	I							C	I	I	C															
Aureobasidium spp.	I			I	I		I	I					I			C						C	C	C																B, W
Aureobasidium pullulans				C	I					C	I		C	I								C	C	C							I			I	I				C		E
Cladosporium cladosporioides				C	I		I						I				C					I	C	C	C							I							C		E
Cladosporium spp.				C	I		I						I				C	C	I	I	C	I	I	I	I	I			I	I									C		E
Fusarium lateritium				I							I		I						C	I	I																			
Fusarium spp.	I	I	I	I							I		I						C	I	I			I															C	I	Oc, CO
Hormonema spp.																						I	I	C							C	I						C	C	
Hypoxylon spp. (anamorph)																	C		C	C	C										C	I						C	C	B, W, Oak bark
Hypoxylon fragiforme (anamorph)	I	I	I	I			I													C											C	I						C	C	

Tab. 1 (cont.)

Hosts	*Araceae*	*Bromeliaceae*	*Orchidaceae*	*Ilex* spp.			*Buxus* spp.			*Hedera* spp.			*Ruscus* spp.			*Arctostaphylos* sp.	*Gaultheria* sp.	*Mahonia* spp.	*Umbellularia* sp.	*Ulex* spp.	*Salicornia* sp.	*Juniperus* sp.			*Abies* spp.			*Picea* spp.			*Pinus* spp.			*Pseudotsuga* spp.		*Sequoia* spp.			*Taxus* spp.		*Tsuga* spp.		*Fagus* spp.	Other hosts (see legend)
Tissue	Leaves & green tissues			Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves	Twigs	Fruit	Leaves				Spines	Stems	Stems	Needles	Petioles	Twigs	Needles	Petioles	Needles	Petioles	Needles	Petioles	Whole stems	Twig xylem	Needles	Petioles	Needles	Petioles	Needles	Petioles	Needles	Petioles	Whole stems	Leaves	Various tissues
Nodulisporium spp.	I	I	I	C	I		C	I		I	I		C	I			C				I		C	C	C																C	*
Daldinia spp. (anamorph)																				C											C	C									C	C	
Geniculosporium spp.																	C	C	C	I	C		I	I	I		I	C	C			I	I	I							I	*
Xylaria spp. (anamorph)				I			I	I					I	I		C	C	C	C						I	I	I	I		I	C	C										*
Nigrospora spp.				I			I			I			I									I		I																	I		
Botrytis spp.													I	I									I	I																			R, S, G, T, O
Phialophora spp.	I			C			C						I				C					I	I	I									I								I		
Phialophora hoffmanni				I			I						I	I						C		I	I	I																			
Ramularia spp.																C	C				C																				I		
Rhizoctonia spp.					I		I	I			I		I								I		I	C	C						C										I		
Principal Sources	Petrini & Dreyfuss (1981)			Luginbühl & Müller (1981)												Petrini & al (1982)				Fisher & al. (1986)	Petrini & Fisher (1986)	Petrini & Müller (1980)			Bernstein & Carroll (1977); Carroll & al (1977); Carroll & Carroll (1978); Petrini & Fisher (1988)																	Petrini & Fisher (1989); Scheel & Hildebrandt (1987)		See legend

Abbreviations

A, *Andropogon;* Abt, *Abutilon theophrasti;* Ama, *Amaranthus spinosus;* App, *Malus* sp. (Apples); B, *Populus* sp. (Blackcotton wood); Ban, *Musa* spp. (Bananas); C, *Gossypium* sp. (Cotton); Ca, *Coffea arabica;* Cac, *Theobroma cacao* (cacao); Ce, *Cenchrus;* Ceq, *Casuarina equisetifolia;* Cy, *Cyperus;* E, *Ulmus* sp. (Elm); F, *Festuca;* G, *Vitius* sp. (Grapes); Grap, *Citrus* sp. (Grapefruit); L, *Lolium;* Len, *Leonotis nepetaefolia;* Les, *Leonurus sibiricus;* M, *Melica;* Man, *Mangifera indica* (Mango); Mup, *Musa paradisiaca* (Plantain); O, *Allium cepa* (Onion); Oc, *Oxalis corniculata;* Ora, *Citrus* sp. (Oranges); Pap, *Asimina triloba* (Papaw); R, *Rubus* sp. (Raspberry); S, *Fragaria* sp. (Strawberry); So, *Glycine* sp. (Soybean); St, *Stipa;* T, *Lycopersicon esculentum* (Tomato); Vm, *Vaccinium myrtillus* (Ericaceae); W, *Salix* sp. (Willow); Y, *Betula* sp. (Yellow Birch); *, Cupressaceae.-Other symbols: C, common; I, present.

Sources

Asawi & Lorbeer, 1972; Arnold, 1967; Bassett & Fenn, 1984; Bakes, 1938; Bier & Rowat, 1962; Bose, 1947; Brayford, 1983; Cerkauskas & al., 1983; Clay, 1988; Dastur, 1916; Edney, 1964; Fisher & al., 1984; Hepperley & al., 1980, Kessler, 1971; Kilpatrick, 1957; Kmetz & al., 1974, 1978; McClellan & al., 1973; Petrini & Carroll, 1981; Powelson, 1960; Rayner, 1948; Roy & Miller, 1983; Tichelaar, 1967; Verhoeff, 1968; Wardlaw, 1931; Wardlaw & al., 1939.

Scope of the article

Most of the aforementioned studies concern endophytes in fleshy plant parts. However, recent studies have shown that endophytes are also common in woody tissues. Because of the large dimension of woody tissues and the relatively extended periods over which decomposition occurs, it has been possible to perform detailed analyses of the spatial structure and dynamics of early stages in the development of fungal decay communities. In this paper we will consider the role of endophytes and latent invasion in the development of such communities. Emphasis will be placed on colonisation of sapwood of apparently healthy twigs and branches; colonisation of heartwood or of sapwood following major wounding will not be considered.

Structure and composition of endophytic and early decay communities

Attached branches

Fungi usually begin to colonise branches soon after death (or loss of functionality), during death or even contribute to death. However, time of death is difficult if not impossible to assess, hence it is difficult to determine when colonisation begins and which fungi are primary colonisers.

In the detailed studies of Boddy & Rayner (1981, 1982, 1983 a, b, c, 1984 a) on colonisation of oak [*Quercus robur* L. and *Q. petraea* (Mattuschka) Liebl.], many attached branches were found to have dead sectors running from proximal to distal regions (Fig. 1). The lengths of time since functionality of these sectors was lost could be estimated by comparing the number of annual rings in these regions with those in adjacent living tissues. Frequently, loss of functionality in all but the most distal regions had occurred within less than one growing season and often the most proximal regions of the otherwise dead sectors were still living. Consequently, analysis of such branches provided a valuable insight into early stages of colonisation and community development.

Twelve Basidiomycotina characterised the decay communities, and of these six appeared to act as pioneers colonising recently functional sapwood: *Phellinus ferreus* (Pers.) Bourd, *Stereum gausapatum* Fr. and *Vuilleminia comedens* (Nees: Fr.) Maire were found in distal, proximal and middle regions of branches and resulted in extensive white rot but little cambial loosening. *Peniophora quercina* (Fr.) Cooke behaved similarly but was confined to middle and distal regions. *Phlebia rufa* (Fr.) M. P. Christ. also resulted in white rot and acted as a pioneer but typically in obviously weakened branches. *Exidia glandulosa* Fr. seemed principally to

cause cambial death and losening, but sometimes extended into the wood where it caused white rot.

These fungi except perhaps *E. glandulosa,* all frequently produced extensive (sometimes greater than 4–5 m length) individuals in less than one growing season (Fig. 1). Even if the fungi had effected entry halfway along a column it seemed very unlikely that, based upon knowledge of growth rates in the laboratory, they could have achieved such rapid spread by mycelial extension. Hence other mechanisms for such rapid extension were sought, and the possibility that colonization was effected by endophytes latent in the sapwood was investigated (see pp. 56–57).

Fungal communities have also been examined in attached branches of other deciduous trees and have revealed some similarities and some interesting differences. In ash (*Fraxinus excelsior* L.) the Basidiomycotina species *Peniophora limitata* (Fr.) Cooke caused white rot, was thought probably to be a primary coloniser and, like *P. quercina* in oak, was found mainly in middle and distal regions (Boddy & al., 1987). The other primary colonisers were the Ascomycotina *Daldinia concentrica* (Bolt.: Fr.) Ces. & De Not. and *Hypoxylon rubiginosum* (Pers.: Fr.) Fr., which were found throughout the branches, but decayed wood only slowly (Boddy & al., 1985; Boddy & al., 1987). All these primary colonisers formed longitudinally extensive individuals. However, the whole wood cylinder was usually colonised rather than a segment, hence rate of development was unclear, but on a few occasions segments were colonised and decay column development again appeared to have occurred in less than one growing season.

In addition to these primary colonizers, two Deuteromycotina, *Phomopsis platanoidis* Died. and a sterile unidentified species – termed Sp. 12. – were isolated on a number of occasions, and there was some evidence that they may colonise early in community development. *P. platanoidis* was usually found in peripheral regions close to the bark and resulted in little decay, whilst Sp. 12. sometimes occupied large volumes of decayed wood. *Exidia thuretiana,* although never isolated from the wood, was frequently found fruiting on the bark and may have a similar role to *E. glandulosa* in oak.

Preliminary analysis of hazel (*Corylus avellana* L.) branches has revealed longitudinally extensive individuals of *Stereum rugosum* (Pers.: Fr.) Fr. rapidly forming in sectors adjacent to living wood, and producing similar patterns to those of *S. gausapatum* in oak (L. Boddy, unpublished). Another similarity between hazel and oak is that *Exidia glandulosa* again appears to cause cambial death and sometimes to extend into the wood where it produces white rot.

Clearly, these three tree species exhibit similar patterns of early colonization although the primary colonizing fungal species were

different on each host. Such selectivity is often said, by plant pathologists, to result from the strong selection pressures imposed on the host and specialised pathogen, respectively, for resistance and virulence. However, RAYNER & BODDY (1988) have pointed out that these fungi have essentially saprotrophic nutrition and there is presently little evidence to suggest that pathogenic mechanisms are major determinants of sapwood colonization. Abiotic factors, such as differences in the physical, chemical and microclimatic regimes may be of more relevance here (BODDY & al., 1987). Nonetheless, mode of entry may involve intimate associations between fungus and living bark cells of the host (CHAPELA & BODDY, 1988b; see pp. 56–57) which may perhaps involve some degree of cell-cell recognition which would act as a selective mechanism.

By contrast with the above three tree species early stages of colonization of beech is not usually typified by the production of extensive individual mycelia (CHAPELA & BODDY, 1988a) although, as in oak, *Vuilleminia comedens* is sometimes a primary invader colonizing in this manner (L. BODDY, unpublished). Decay often begins in distal regions with fungi associated with a staining of the wood (CHAPELA & BODDY, 1988a). This stained region is usually only a few mm in longitudinal extent, but can sometimes be many cm. Frequently each small chip of wood sampled yielded several different species including the Basidiomycotina *Coniophora puteana* (FR.) P. KARST., the Ascomycotina *Nectria coccinea* (PERS.: FR.) FR. and *Hypoxylon fragiforme* (SCOP.) KICKX, and the coelomycetes *Asterosporium asterospermum* (PERS.: GRAY) HUGHES, *Dichomera saubinettii* (MONT.) COOKE and *Libertella faginea* DESM.

The colonization front in partially living branches was close to the most distally positioned living, side branch (Fig. 6). These fronts

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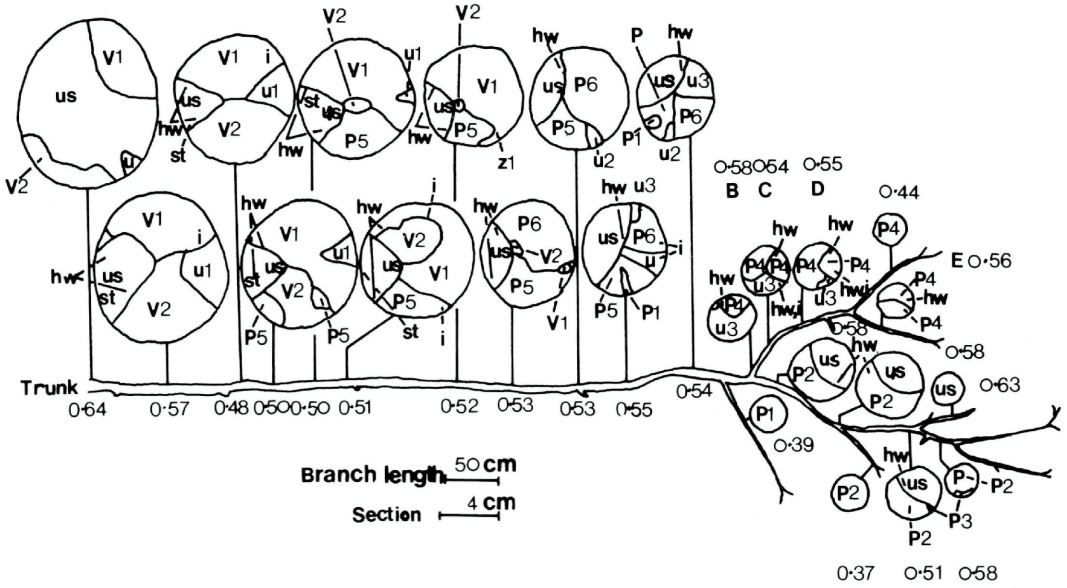
Fig. 1. Decay community structure in an attached oak (*Quercus robur*) branch. Different basidiomycete individuals were demarcated by interactive zone lines (i) in the wood. The predominant species were *Vuilleminia comedens* (two individuals; Vc 1–2) and *Peniophora quercina* (six individuals; Pq 1–6). Three other unidentified basidiomycetes (u 1–3) occupied relatively smaller volumes. Both *V. comedens* and one of the *P. quercina* individuals occurred in the main stem possibly originating from small branch stubs, or by direct entry. It is likely that Pq 2 originated in the branchlet from which section A was taken and progressed to the main fork whence it grew in both available directions. In the distal sections B–E, heartwood wings (hw) were present in the absence of living tissue (us) and separated decay columns occupied by either the same, or two different individuals. In the latter case, an interactive zone line was also present. Presumably the fungi were initially confined to regions between the heartwood wings, with living tissue outside. Subsequently, possibly following some traumatic event, the fungi were able to penetrate or bypass the wings, gaining access to the living wood and colonizing it without further wing formation. Values given are for relative density (g cm^{-3}). Reproduced from BODDY & RAYNER (1983c), by permission of the British Mycological Society.



had apparently often remained static for several years, as evidenced by extensive weight loss and the fact that the proximal living regions often had several additional annual rings.

The large number of small individuals in beech presumably reflects different modes of dispersal and initial establishment compared with the species colonizing oak, ash and hazel, and the confinement of decay initially to distal regions may reflect different branching patterns, patterns of side branch death and water flow (see pp. 56–57).

Attached twigs

Little attention has been paid to fungal communities in attached twigs (< 1 cm diam.), exceptions being studies by BRAYFORD (1983) on elm *(Ulmus)*, BODDY & RAYNER (1984b) on oak, PETRINI & FISHER (1988) on beech and pine *(Pinus)* and GRIFFITH & BODDY (1988; unpublished, manuscript in preparation) on ash, beech and oak.

With regard to healthy bark, several of the fungi commonly isolated in these studies belonged to typical endophytic genera (cf. Tab. 1), including *Aureobasidium, Cladosporium, Epicoccum, Nodulisporium, Phomopsis* and *Xylaria*. Some endophytic genera were, however, represented only in certain hosts. For example, in the study of GRIFFITH & BODDY (1988; unpublished), *Botrytis* sp. was found only in ash and *Trichoderma* sp. in oak (Tab. 2). At the species level certain endophytes were host specific including *Phomopsis platanoidis, Phomopsis glandicola* (LÉV.) GROVE and *Phomopsis quercella* DIED., which were restricted to ash, oak and beech respectively. More typically, species were not restricted to any of these hosts; the same *Nodulisporium* sp. and *Epicoccum nigrum* and *Phoma macrostoma* MONT were common to all three hosts. *Fusarium lateritium* NEES and *Aureobasidium* cf. *pullulans* were also found and, indeed, are known to be present in a taxonomically diverse range of hosts (cf. Tab. 1).

Not only are there some differences in floristic composition of different tree species but site differences are also apparent. For example, *Melanconium atrum* LINK, *Periconiella* sp., *Cylindrocarpon album* (SACC.) WOLLENW., *Hormonema* sp. and *Hypoxylon bipapillatum* BERK. & CURT. were common endophytes of beech bark at a site in Devon (PETRINI & FISHER, 1988) but were absent from a site in Wales (GRIFFITH & BODDY, unpublished; Tab. 2). By contrast *F. lateritium* and *Aureobasidium* cf. *pullulans* were common at the Welsh site but apparently absent from the Devon site. Notable similarities, however, included the common occurrence of species in the genus *Phomopsis* and *Nodulisporium*.

Within site differences have also been found. For example, BRAYFORD (1983) isolated *Phomopsis oblonga* (DESM.) TRAV. at a

Tab. 2. Frequency of isolation of common twig endophytes and decay fungi. (From GRIFFITH & BODDY, 1988; GRIFFITH, 1989).

Species	%isolF* from wood	%isolF* from bark	Host
Basidiomycetes			
Peniophora sp.	9	–	beech
Peniophora lycii (PERS.) V. HOHN & LITSCH.	14	–	ash
Peniophora quercina (FR.) COOKE	18	–	oak
Vuilleminia comedens (NEES: FR.) MAIRE	13	–	oak
Ascomycetes			
Xylaria sp. A	11	71	beech
Xylaria sp. B.	–	12	ash
Xylaria sp. C	–	48	oak
Coelomycetes			
Coniothyrium fuckelii SACC.	–	2	oak
	1	4	ash
	1	17	beech
Cryptosporiopsis sp.	25	12	beech
Cryptosporiopsis fasciculata (TODE) PETRAK	18	–	beech
Cryptosporiopsis quercina PETRAK	19	–	oak
Cystospora ambiens SACC.	6	21	oak
Libertella fraxinea OGANOVA	20	2	ash
Melanconium elevatum CORDA	1	11	oak
Phoma macrostoma MONT.	–	29	oak
	–	31	beech
	2	65	ash
Phomopsis quercella RIED.	8	45	beech
Phomopsis sp. B	–	28	oak
Phomopsis glandicola (LEV.) GROVE	3	20	oak
Phomopsis platanoidis DIED.	33	29	ash
Sp. D	–	5	ash
Sp. 13	–	17	ash
Hyphomycetes			
Acremonium sp. A. (section simplex of GAMS)	13	–	ash
Aureobasidium cf. *pullulans*	1	39	beech
	4	42	ash
	2	53	oak
Botrytis cinerea PERS.: FR.	–	9	ash
Cladosporium spp.	–	21	oak
	–	4	ash
Epicoccum nigrum LINK	–	20	oak
	–	15	beech
	–	40	ash

* isolF: Isolation Frequency, percentage of isolations yielding species.

Species	%isolF* from wood	%isolF* from bark	Host
Nodulisporium sp. (Anamorph of *Daldinia* cf. *concentrica*)	3	10	ash
	–	21	oak
	–	34	beech
Fusarium lateritium NEES	9	22	ash
	–	16	beech
	2	17	oak
Penicillium spp.	1	7	oak
	3	–	ash
	1	3	beech
Trichoderma spp.	4	29	oak
	1	1	beech
Sterile Mycelia spp.			
BB3	–	9	oak
	–	50	beech
BW1	41	–	beech
U9	27	2	oak
Sp. B	–	9	ash
Sp. 12	45	29	ash

* isolF: Isolation Frequency, percentage of isolations yielding species.

higher frequency from twigs lower down rather than higher up in elm canopies, and suggested that this results from *P. oblonga* conidia being dispersed by rainsplash from dead twigs and hence accumulating at lower regions. At the same height in the canopy, however, levels of endophyte infection appeared to be similar. In ash, beech and oak subjective assesment clearly indicated that floristic composition was also similar (GRIFFITH & BODDY, 1988, unpublished).

Differences in the endophytic mycofloras within hosts also result from differences in host tissue age, the degree of colonisation sometimes increasing with tissue age as for example in *Ulex* spp. stems (FISHER & al., 1986). In elm twigs, by contrast, the degree of colonisation by *P. oblonga* was highest in young green twigs and declined in increasingly older twigs (BRAYFORD, 1983). In ash, beech and oak bark (GRIFFITH & BODDY, 1988, unpublished) age apparently had little affect upon the degree of endophyte colonisation; in all cases almost 100% of the plated samples yielded isolates.

Finally, variations can occur within tissues of the same age at similar locations due to the presence of a species which is antagonis-

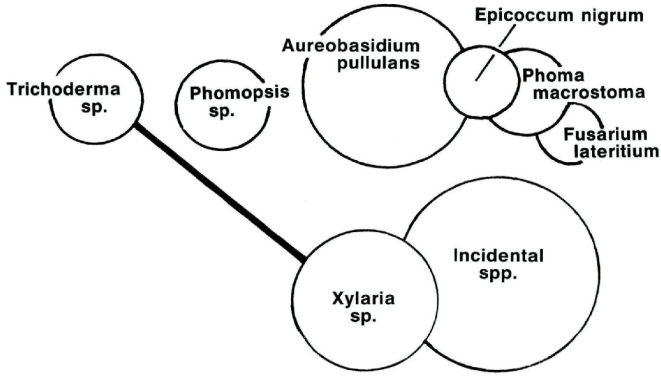


Fig. 2. Association plexus of endophytic community in oak *(Quercus)* twig bark. Diameter of circles is proportional to isolation frequency; diameter of *Aureobasidium* c. f. *pullulans* represents an isolation frequency of 50%. Associations between all species pairs measured by Chi squared statistic. Overlapping circles represent positive associations. (–), very strong negative association ($P \leq 0.001$).

tic against others. In oak bark *Xylaria* sp. C and *Trichoderma* spp. were very strongly negatively associated (GRIFFITH & BODDY, unpublished; Fig. 2). *Trichoderma* spp. are well known to produce antifungal compounds and *Xylaria* sp. C. may be sensitive to these.

Many endophytes commonly isolated from healthy bark were also frequently isolated from dead attached twig wood of the respective hosts, including *Phomopsis platanoidis, Fusarium lateritium* and sp. 12 from ash, *Xylaria* sp. A, *Cryptosporiopsis* sp. and *Phomopsis quercella* from beech and *Cytospora ambiens* SACC. from oak (GRIFFITH & BODDY, 1988, unpublished; Tab. 2).

Not all endophytes present at high levels in the healthy twig bark were, however, common in dead twig wood. Some were present in the wood decay communities only infrequently if at all, for example *Epicoccum nigrum* and *Aureobasisium* cf. *pullulans*. Some endophytes common in the bark (of ash, beech and oak) were often important in the decay communities of twig wood of only one of the host species, for example *F. lateritium* in dead attached ash twigs and *Xylaria* sp. in dead attached beech twigs.

Analysis of the structure of fungal communities in recently living attached ash twigs has revealed large volumes of wood colonized principally by *Phomopsis platanoidis* and Sp. 12, with small pockets of *Fusarium lateritium* (Fig. 3). Whilst all three were iso-

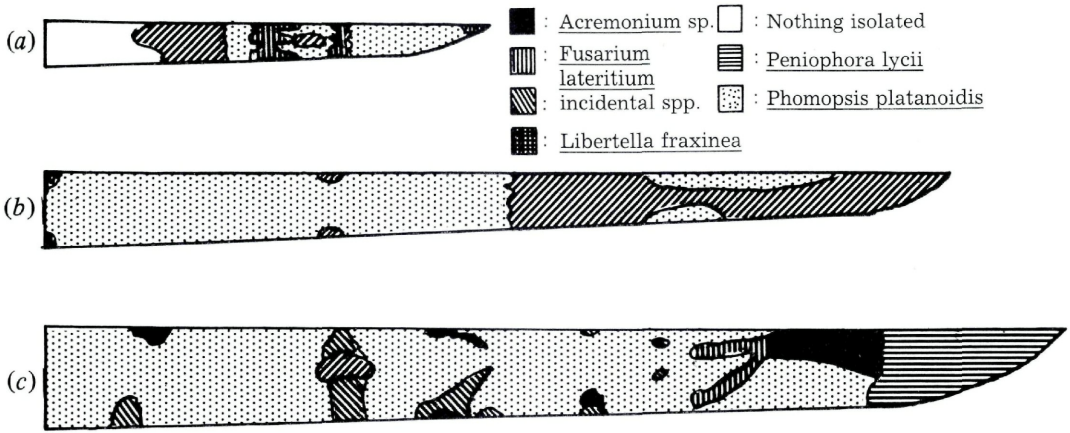
lated in low numbers from living wood they were all very common endophytes in bark. They are suggested as being primary colonizers which effected entry to dead wood predominantly from the bark. Likewise in beech and oak, recently living twigs usually contained species which were common endophytes in bark which presumably act in a similar manner (GRIFFITH, 1989). In a study by BODDY & RAYNER (1984b) on oak, *Colpoma quercinum* (FR.) WALLR. appeared to behave in a similar manner to *Phomopsis platanoidis* in ash, although this species was not found by GRIFFITH & BODDY (unpublished) on their site.

Felled logs

Whilst there have been a number of studies on early stages of colonisation of seasoned and preservative treated timber and of felled logs at later stages of decay (RAYNER & BODDY, 1988), little attention has been paid to early stages of fungal community development in freshly felled trunks and branches. This is unfortunate since this is another type of situation in which endophytes might be expected to develop, since they are positionally advantaged over other fungi by already being present. Some recent studies on beech *(Fagus sylvatica)* have, however, examined primary colonization in detail (COATES & RAYNER, 1985a, b, c; CHAPELA & BODDY, 1988c).

CHAPELA & BODDY (1988c) studied early colonisation at nine different woodland sites, and although there were a few minor differences in species present on different sites the numerically dominant species were similar. *Graphium* sp. was particularly abundant and other species with abundance (i. e. percentage of total chips colonized) of greater than 10% and frequency (i. e. percentage of logs colonized) of greater than 60% included the Ascomycotina *Cryptosporiopsis fasciculata* (TODE) PETRAK, *Hypoxylon fragiforme, Nectria coccinea* and unidentified "Sp. 25", and the Basidiomycotina *Stereum hirsutum* (WILLD.: FR.) S. F. GRAY. The four Ascomycotina species together with the less frequently isolated *Coniophora puteana, Biscogniauxia nummularia* (BULL.: FR.) O. KUNTZE (previously known as *Hypoxylon nummularium* BULL.: FR.) and *Libertella*

Fig. 3. Maps of community structure of recently living, attached ash *(Fraxinus excelsior)* twigs with bark removed. Maps show whole area of twig's surface. – (a) Twig containing some living wood in proximal areas. Dead wood colonised almost entirely by *Phomopsis platanoidis* and Sp. 12, with occasional small pockets of *Fusarium lateritium*. – (b) As (a) but no living wood present. – (c) Twigs with large volumes still occupied by *P. platanoidis* or Sp. 12, but with small pockets of incidental and dominant secondary colonizers; *Libertella fraxinea, Peniophora lycii, Acremonium* sp. From GRIFFITH & BODDY (1988). Reproduced by permission of the British Mycological Society.



faginea have all been found in healthy attached branches or at early stages of decay in the canopy (CHAPELA & BODDY, 1988a, b). In addition to these species COATES & RAYNER (1985a, b, c) commonly found *Corticium evolvens* FR. and *Chondrostereum purpureum* (PERS.: FR.) POUZ. The latter is again found commonly in attached branches in the canopy (RAYNER & BODDY, 1988) and causes silver leaf disease of *Prunus* (DYE & WHEELER, 1968).

Origin and development of early colonisers in wood

Attached branches and felled logs

To account for the rapid development of extensive individuals in recently functional sapwood of attached branches (see earlier pp. 46 ff.), BODDY & RAYNER (1982, 1983a, b and c) suggested that colonization occurred by a process of latent invasion whereby genetically identical fungal propagules (endophytes) were distributed widely but sparsely in functional sapwood, overt development occurring only when the stress of high water content was removed. Recently, CHAPELA & BODDY (1988b) examined experimentally the hypothesis that early colonization was by fungal propagules within functional sapwood and living bark, and that water controls development of mycelium from latent propagules. Freshly felled, healthy beech (*Fagus sylvatica* L.) branches were cut into 10 cm lengths which were divided into five batches each of which was subjected to a different drying regime, ranging from a regime which maintained water saturation to rapid drying which allowed water content to drop from 0.45 g cm^{-3} to less than 0.1 g cm^{-3} in 40 days. Other treatments included debarking, autoclaving or inoculation with ascospores or conidia respectively of *Hypoxylon fragiforme* and *Biscogniauxia nummularia*. Lengths were sampled at various times up to 92 days from the start of the experiment.

Maintaining water saturation prevented any fungal development in the wood, but active mycelium was isolated within 14 days after drying had begun. Increasingly faster drying regimes were accompained by a faster initial fungal growth into the wood, but under the most rapid drying regimes growth was arrested before the end of the experiment because conditions soon became too dry (Fig. 4). The fungi developing in the sections were all found at early stages of colonisation in the field (see earlier pp. 46 ff.), *Hypoxylon fragiforme, Biscogniauxia nummularia, Nectria coccinea, Coniophora puteana* and some coelomycetes being dominant. Direct observations and isolations with time indicated that most colonizers developed inwards from the bark (Fig. 5). However, *H. fragiforme* and *B. nummularia* also grew out from pockets within the wood (Fig. 5a, b), each pocket containing a genetically different individual. These

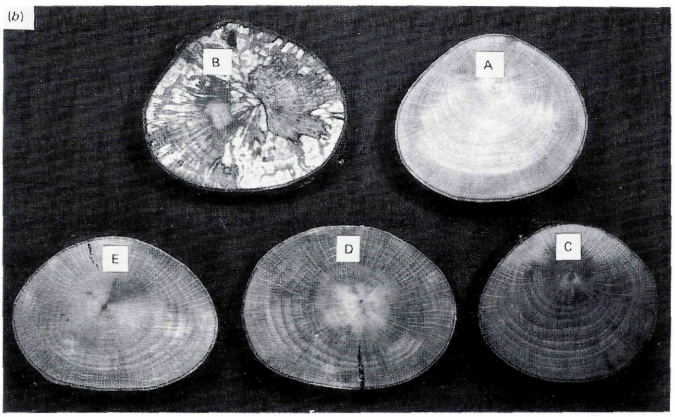


Fig. 4. Straining and decay resulting from development of fungi present as latent propagules in bark and functional sapwood. Transverse sections of beech *(Fagus sylvatica)* branch units cut after 12 weeks incubation under various drying regimes (A. maintained under water saturation, B–E. increasingly faster drying regimes). Notice extensive development of decay due to *Hypoxylon fragiforme* and *Biscogniauxia nummularia* under gentle drying regime (B), absence of staining and decay under water saturation (A), and light staining and mottling unter rapid drying regimes (C–E). From CHAPELA & BODDY (1988b). Reproduced by permision of New Phytologist Trust.

pockets were particularly abundant in certain annual rings (Fig. 5a), which suggests that some year to year variation in infection courts occurs.

These typical patterns of colonization did not occur in autoclaved controls, the only species present being laboratory contaminants. Also, all attempts at inoculating with spores of *H. fragiforme* and *B. nummularia* failed. Thus, taken together, these experiments clearly demonstrated that water content is a major determinant of the development of fungi in beech sapwood, and that these early colonizers derive from the endophyte inoculum latent in the sapwood and bark of healthy, living branches. The patterns of stain and decay in attached branches can be explained in these terms (CHAPELA & BODDY, 1988a) and the course of events is depicted schematically in Fig. 6. Explanation is easiest if main branches are considered to consist of "fields" of sapwood which supply actively transpiring side branches with water (Fig. 6a). Fields active in water conduction would preclude mycelial development, but if a side branch in the vicinity of the colonization front died the field which formerly

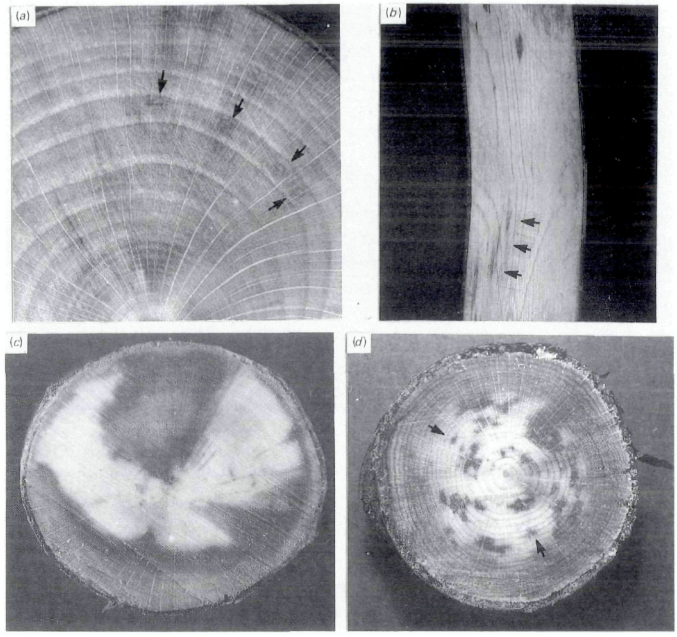


Fig. 5. Transverse (a, c, d) and longitudinal (b) sections 4 weeks (c, d), 6 weeks (b) and 12 weeks (a) after incubation showing stain and decay caused by *Hypoxylon fragiforme* developing from latent propagules in discrete pockets (arrowed) within beech *(Fagus sylvatica)* sapwood (a, b, d) and from bark (c, d). From CHAPELA & BODDY (1988b). Reproduced by permission of New Phytologist Trust.

supplied it would begin to dry out and become available for colonization (Fig. 6b). These latter regions would be colonized by endophytes latent in the wood and the bark but there would presumably be some ingress from mycelium in the old colonization front. Subsequently, secondary colonizers would extend in from decayed regions (Fig. 6c).

Recently, I. H. CHAPELA (pers. comm.) has performed similar experiments with American beech (*Fagus grandifolia* EHRH.) and aspen (*Populus tremuloides* MICHX.). He isolated 18 species from freshly cut wood, mainly Ascomycotina, coelomycetes and slow growing dematiaceous hyphomycetes. Two fungal species,

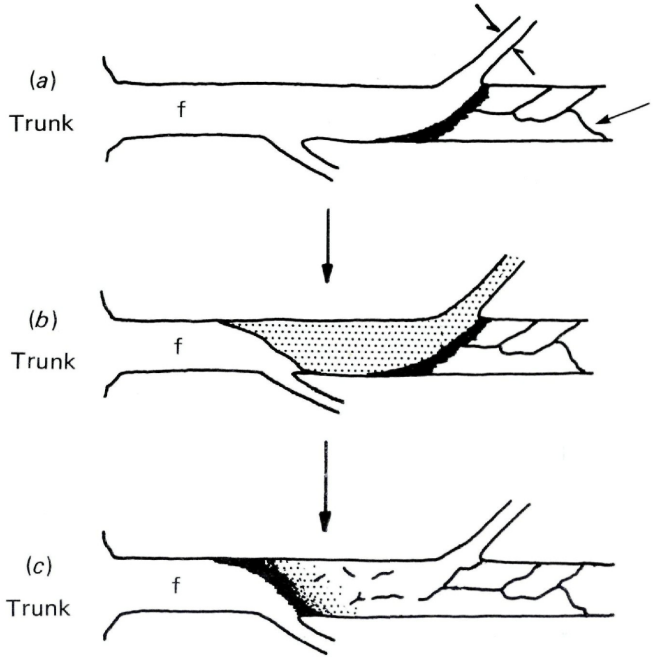


Fig. 6. Schematic representation of proposed course of colonization of a primary, attached beech (*Fagus sylvatica*) branch. Dark shading represents the stained region which separates decayed distal regions containing zone lines (zl) from functional sapwood (f). Stippled region represents zone made available for primary colonization following death of a side branch (arrowed). From CHAPELA & BODDY (1988a). Reproduced by permission of New Phytologist Trust.

Phaeococcus sp. and an unidentified ascomycete, together accounted for 40% and 60% of all isolations from aspen and beech repectively. Fungal propagules were more abundant in healthy aspen than in beech, 14% of aspen chips yielding isolates but less than 3% of beech.

All fungal development was effectively prevented whilst water saturation was maintained. Isolates of species present in healthy wood declined during the first four weeks of drying, but rapid development of others began. Floristically distinct assortments developed in aspen and beech, Basidiomycotina being more abundant

in the former. Over half of all species isolated were Ascomycotina and related groups (including coelomycetes), in particular Xylariaceae comprised 32% and 41% of all species found respectively in aspen and beech. As in the earlier study with *Fagus sylvatica,* some fungi, e. g. *Hypoxylon fragiforme, Phaeococcus* sp. and *Cryptosphaeria populina* (Pers.) Sacc., were isolated from small pockets well within the wood whilst others, including *Hypoxylon mammatum* (Wahl.) Mill. on aspen and *Phomopsis* sp. on beech, grew inwards from the bark as drying proceeded.

Similar experiments have not yet been performed with oak *(Quercus)* and ash *(Fraxinus excelsior),* but a preliminary study with hazel *(Corylus avellana)* also demonstrated that some early colonizers develop from propagules latent in the sapwood (A. P. Banister & L. Boddy, unpublished). In this experiment an unidentified Basidiomycotina species was isolated from three different lengths cut from an healthy branch 98 and 105 days after drying began. Pairing these isolates on malt agar revealed that they were of the same genotype, thus providing evidence that genotypically identical propagules were distributed along the length of the healthy branch prior to felling – i. e. exactly the situation postulated to account for rapid development of longitudinally extensive individuals in attached oak, ash and hazel branches (see earlier pp. 46 ff.).

Attached twigs

In order to determine if the endophytes purported earlier to be primary colonisers of twigs actually take this role, and what controls or triggers establishment by latent endophytes, two experiments were performed (Griffith & Boddy, unpublished). The first involved comparison of community development initiated by girdling (removing a 1 cm wide band of bark from around the circumference of individual twigs, approximately 1.5 m from their distal tips) and removing leaves from ash twigs, with living controls. The second was essentially similar to the other "latent invasion experiment" described for beech branches (pp. 56–57).

The observed sequence of community development in girdled twigs (Fig. 7) was consistent with the hypothesized role of certain endophytes as primary colonizers of wood, in twigs that had died naturally in the field. The moisture content of twigs 12 weeks after girdling did not differ significantly ($P > 0.05$) from that of living control twigs which implied that, unlike in beech branches, drying was not the trigger for development of endophytes from the bark. Other possible triggers include the alleviation of control mechanisms imposed by the host tissue or the presence of substances in the host tissue associated with the senescence process.

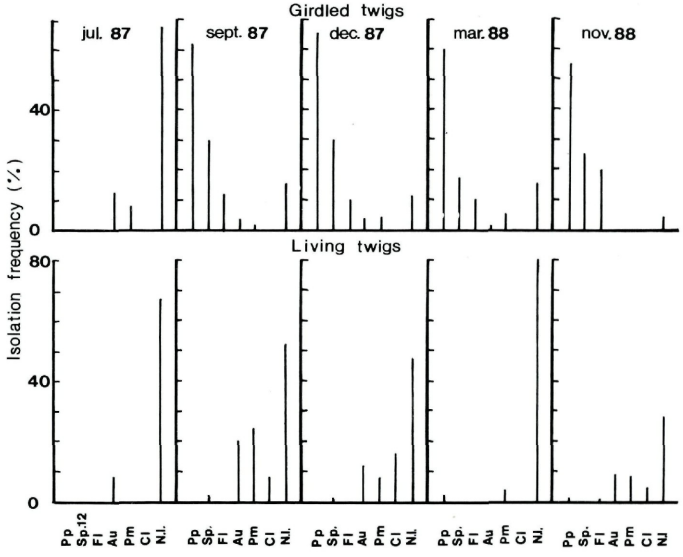


Fig. 7. Isolation frequency of primary colonizers and superficial colonizers from stressed and living ash *(Fraxinus excelsior)* twigs. Abbreviations: Pm: *Phoma macrostoma* MONT.; P p: *Phomopsis platanoidis* DIED.; F1: *Fusarium lateritium* NESS; Au: *Aureobasidium* spp.; C1: *Cladosporium* spp.; N. I.: Nothing isolated. Data for living twigs from 25 samples per time and for girdled twigs from 75. From GRIFFITH (1989).

In living twigs there was a seasonal superficial colonization of tissues lying in close proximity to the cortex by bark endophytes which were infrequently or never isolated from dead twigs. The most frequent superficial colonizers were *Aureobasidium* spp., *Cladosporium* sp. and *Phoma macrostoma*. Superficial colonization was maximal during autumn and winter and minimal during spring and summer. This may be explained by the fact that during the growing period, which in temperate deciduous trees such as ash is limited to a 3 to 4 month period over the summer, the host bark is physiologically active and in an optimum condition for disease prevention (BIER, 1964). Additionally, water potential of healthy twigs varies seasonally being greatest in the spring and summer.

Superficial colonization was observed more commonly in nodal rather than internodal regions particularly during the period of

maximal colonization in the winter. This may be because nodes provide sites of entry via buds and bud scars, as is the case with *Phomopsis oblonga* in elm (BRAYFORD, 1983). The endophytes that were important primary colonisers of dead twigs were not superficially colonizing species which points to the presence of at least two groups of endophytes in ash bark which are distinct in terms of their ecology.

In the latent invasion experiment healthy twigs were subjected to three treatments: (i) bark removed and surface sterilised, (ii) surface sterilised bark intact, (iii) autoclaved (GRIFFITH & BODDY, unpublished). They were then incubated unter a drying regime resulting in a drop in moisture content (% oven dry weight) from 110% to 10% in 15 days. The experiment demonstrated that endophytes present in healthy ash, beech and oak twigs have the capacity to colonize woody tissue after twig death: stripped, surface sterilised ash and beech twig lengths but not oak lengths, incubated at 20° C for 4 wks, remainded uncolonized suggesting that potential colonizers of the former hosts are usually confined to the bark rather than the sapwood or pith of healthy twigs. In oak twigs at least one potential colonizer, *Cytospora ambiens*, was presumably latent in sapwood but as in ash and beech the majority of endophytes were also confined to be bark. This was in agreement with other studies on the endophytic floras of woody stems where, in a similar fashion, most endophytes have been found to be confined to regions in close proximity to the stem cortex (LUGINBÜHL & MÜLLER, 1981; PETRINI & FISHER, 1988).

In ash, endophytes that frequently colonized twig lengths in the latent invasion experiment were not always those that were known to colonize dead twigs in the field (see pp. 50 ff.). *P. platanoidis* was commonly isolated in the latent invasion experiment but both Sp. 12 and *F. lateritium* were not. On the other hand two colonizers commonly isolated from the wood lengths, *Xylaria* sp. B and *Daldinia* cf. *concentrica* (*Nodulisporium* state), rarely colonized ash twigs in the field. Similarly in beech, *Phomopsis quercella* and *Xylaria* sp. A were found both in the field and in the latent invasion experiment; *Cryptosporiopsis* sp. was present in the field but did not develop in the laboratory; and *D. concentrica*, as in ash, was very common in the laboratory experiment but was absent from the field. Again in oak, *Xylaria* sp. C and *D. concentrica* colonized twigs in the laboratory but not in the field, and *Phomopsis quercina* was found in the laboratory and at low levels in the field. Absence of *Xylaria* from oak may at least be partly explained by the fact that representatives of this genus are quite sensitive to tannic and gallic acid, which are found at high levels in this species (A. J. S. WHALLEY, pers. comm.).

These experiments have provided evidence that some of the

endophytes present in bark and wood of healthy twigs are primary colonizers of dead twigs. On the other hand, it is interesting that different endophytes were often selected for in the laboratory to those that developed in dead wood under normal field conditions. This may be because drying in the laboratory was more rapid than that of girdled twigs in the field. As mentioned earlier drying regime can crucially affect the colonizing community in wood (pp. 56–57). It is possible that under certain conditions *Daldinia* cf. *concentrica* might colonise dying twigs in the field, for example if twigs were broken in a storm and remained suspended in the canopy and consequently dried down rapidly.

This also has a bearing on the apparent specificity of *D. concentrica* to ash in the U. K., although it is sometimes found on beech and in northern regions is common on birch (*Betula* spp.) (WHALLEY & WATLING, 1980, 1984). Its predominance in ash may well simply be due to the fact that this wood dries very rapidly, although another possibility is that a different physiological race is found in northern Britain.

Comparison of twigs and branches

As already discussed fungi that are present in a latent form in healthy intact sapwood of beech branches tend to be sparsely but extensively distributed. They also tend to be located in the wood of specific annual rings suggesting that they have been carried into the branch or perhaps left behind within the branch as it has grown. Both these observations indicate that infection may be a rare event but inevitable over prolonged periods of time. This would explain why such endophytes tend not to be present in twig sapwood, since twigs have not been exposed to the inoculum of such fungi for sufficient periods of time and in addition have smaller surface areas exposed to the relevant propagules. It might be expected that occasionally a sapwood endophyte would become established in a twig and in fact *Hypoxylon fragiforme,* one of the sapwood endophytes of beech branches, is occasionaly seen on twigs particularly in woodlands where fruiting bodies of this fungus are very common on fallen branches and hence its inoculum is unusually high (A. J. S. WHALLEY, pers. comm.). The common presence of a sapwood endophyte in oak but not beech or ash twigs can also be explained in this light since oak twigs are slow growing and consequently have been exposed to endophyte inoculum for a considerable period of time.

In dead attached ash branches (BODDY & al., 1987) Sp. 12., *F. lateritium* and *P. platanoidis* (purported primary colonizers of ash twigs) were found to be present in small pockets in close proximity to the cortex (though this was not always so). Possibly, the

ecology of the colonization of tissues lying in close proximity to the cortex of branches may be similar to that of twigs, which is not surprising considering the similarities between these tissues.

The widespread endophyte *Nodulisporium* sp. anamorph of *Daldinia* cf. *concentrica* was common in the healthy bark of ash, beech and oak twigs. This is inconsistent with the distribution of its teleomorphic state which is principally restricted to ash and common only in dead ash branches (Boddy & al., 1987).

Persistence of endophytes until latter stages of decay

Persistence in decomposing tissue of fungal mycelia which developed from endophytes present as latent infections in healthy tissue depends upon their ability to utilise changing energy and nutrient sources, their tolerance of changing microclimatic conditions and their ability to defend territory against ingress of other primary and secondary colonizers.

With regard to changing resources, it has already been mentioned that the Basidiomycotina and Xylariaceous Ascomycotina found in twigs and branches are all capable of decomposing wood, resulting in white rot or brown rot, albeit rather slowly in some cases. Indeed some of the coelomycetes also have enzymes which allow them to utilize wood, although often decay is slow and of the soft rot types (see pp. 68–70). It goes almost without saying that any endophytes capable only of utilising the simple carbon compounds of living plant cells will rapidly decline once these have been used up following death of host tissues.

Following twig or branch death, water content of sapwood usually begins to decline and concomitantly O_2 increases and CO_2 decreases. Different fungi have different tolerances to combinations of these abiotic factors, and even in the absence of other fungi may decline if conditions become unfavourable. In Chapela & Boddy's (1988 b) experiment, which was described earlier, *H. fragiforme* and/or *B. nummularia* showed an increasing abundance with time followed by a decline as drying continued. In wood lengths subjected to a moderate drying regime a peak in abundance (81%) was found after 46 days (water content = 0.314 g cm^{-3}) and then declined to 30% after 92 days (water content about 0.2 g cm^{-3}). Under faster drying regimes maximum abundance was lower and under the most rapid drying regime *H. fragiforme* and/or *B. nummularia* were absent after 46 days (< 0.1 g cm^{-3}). *Coniophora puteana* did not develop at all under the rapid drying regimes; under the moderate regime it developed only after *H. fragiforme* and/or *B. nummularia* had reached a maximum (46 days; water content = 0.314 g cm^{-3}) and

showed maximum abundance after 48 days (water content about 0.25 g cm^{-3}).

In girdled ash twigs within which the stages of decay community development were monitored (see pp. 60–63), moisture content declined only very gradually, notable differences between their moisture content and that of living control twigs not becoming apparent until 6 months after girdling. The gradual drying of these twigs did not apparently correlate with any changes in the decay communities of the twigs.

With regard to replacement by other fungi, all of the primary colonizers of oak branches, except *E. glandulosa*, were good at defensive combat against each other, *Phellinus ferreus* being the least combative (Boddy & Rayner, 1983a). They could, however, be replaced in agar culture by at least one of the major combative secondary colonizers – *Coriolus versicolor* (L.: Fr.) Quel., *Phlebia radiata* Fr., and *Stereum hirsutum*. Nonetheless, attached branches were found in which the primary colonizers had brought about loss of over 50% of the original dry weight, indicating that they can be persistent and/or very active decayers.

In ash branches *Daldinia concentrica, Hypoxylon rubiginosum* and *Peniophora limitata* were all found at least once in attached branches that had lost more than 50% of their original dry weight (Boddy & al. 1987). *D. concentrica* was a particularly aggressive combatant and was not replaced by any of the ash inhabitants in culture, although there was occasionally evidence that it could be replaced in branches. Indeed, *D. concentrica* has been found in tree trunks over 20 years after felling (A. D. M. Rayner & L. Boddy, unpublished). On the other hand, *H. rubiginosum* was replaced by a number of species including *D. concentrica,* although under increased CO_2 regimes (such as would occur in wood of high moisture content at early stages of colonization) *H. rubiginosum* was more successfull at defensive combat; *H. rubiginosum* is often found in well rotted, water soaked wood on the forest floor.

In attached beech branches the main primary colonizers were never found in well decayed wood. None of them appeared to be particularly combative, although *H. fragiforme* did deadlock with the aggressive secondary colonizers *Coriolus versicolor* and *Phanerochaete velutina* (DC.: Pers.) Parmasto in agar culture. In a study of the fate of early colonizers in felled beech logs on the forest floor fungi which established from latent propagules rapidly colonized, showing frequencies of close to 100% by 16–32 weeks, after which time frequency and abundance began to decline, but even after 72 weeks frequency of isolation of this group was greater than 50% (Chapela & Boddy, 1988c; Fig. 8).

In dead attached ash twigs evidence from the previously de-

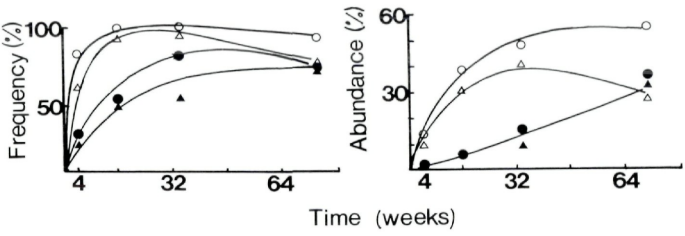


Fig. 8. Temporal changes in frequency and abundance of isolation of latently established colonizers (open symbols) and basidiomycetes (not latent in healthy branches; closed symbols) in freshly felled branches on the forest floor at two different sites (●, site 1; ▲, site 2) in S. W. Britain. From CHAPELA & BODDY (1988c). Reproduced by permission of FEMS Microbiology Ecology.

scribed girdling experiment revealed that once the primary colonizers had established themselves in twigs they could maintain themselves within them for a considerable period of time (for up to 2 years). This correlated with their good defensive ability on agar and in wood lengths particularly at low water potentials, a condition which is common in dead twigs in the field (G. S. GRIFFITH & L. BODDY, unpublished). Despite their good defensive ability these primary colonizers did not have the capacity to replace either each other or common secondary colonizers of dead ash twigs, such as *Peniophora lycii* and *Libertella fraxinea*.

The preliminary stages in the eventual replacement of primary colonizers were observed in some dead attached ash twigs and apparently resulted from the establishment of secondary colonizers in small pockets of wood from which primary colonizers had possibly died-back due to changes in resource quality in these regions (GRIFFITH & BODDY, 1988; unpublished; Fig. 3c).

It is clear from the foregoing that primary colonizers of dead attached ash twigs, like those of branches, derive considerable benefit from their endophytic habit which allows them to respond rapidly to twig death and establish themselves in the resource before the arrival of secondary colonizers, which are presumably distributed via the air spora. The persistence of proposed primary colonizers of beech and oak twigs is at present unclear.

Role of endophytes

Mutualism and pathogenicity

The ecological roles of endophytes are often obscure (CARROLL & PETRINI, 1983) but some are known to be pathogens or mutualistic.

With regard to the former role, *Diaporthe* sp. and *Phomopsis* spp. are known to be latent pathogens of soybean plants. These fungi infect young soybean tissues but only cause disease symptoms such as seed decay, top dieback and stem cankers in mature plants (KMETZ & al., 1974, 1978). Similarly *Rhabdocline* spp. cause disease symptoms in Douglas fir *(Tsuga heterophylla)* needles only after a one or two year latent phase. Many stem and leaf pathogens may have latent endophytic stages in their lifecycles (CARROLL, 1988).

The pathogenicity of most endophytes is less marked and, indeed, the majority are rarely associated with disease symptoms in their hosts. There is a range of endophytes which have been termed weak or facultative parasites only causing disease symptoms in stressed hosts (BRAYFORD, 1983). Stress in this instance implies one or more factors, e. g. low light intensity, frost, drought and low nutrient levels in the hosts environment are unfavorable for growth (LONSDALE, 1983). For example, *Phomopsis casuarinea,* which causes a seed borne infection of *Casuarina equisetifolia* FORST., has been noted actually to invigorate young healthy plants but causes lethal infections of stressed seedlings (BOSE, 1947). *Phomopsis oblonga* has been associated with disease symptoms such as twig dieback and cankering in stressed elm trees and *Phomopsis* spp. in general have been associated with similar disease symptoms in other stressed hosts (BRAYFORD, 1983). Many *Hypoxylon* spp. cause cankers in stressed hosts and these are known to be common in endophytic floras (LONSDALE, 1983; ROGERS, 1979). *Hypoxylon mammatum* (synonym of *Hypoxylon pruinatum* (KLOTZSCH) CKE.), for example, causes cankers in drought stressed willows (BIER, 1964) and *Biscogniauxia atropunctata* (SCHW.) POUZAR (previously known as *Hypoxylon atropunctatum* (SCHW.: FR.) CKE.), which is an endophyte of oak trees (*Quercus alba* L., *Q. marilandica* MUENCHH. and *Q. velutina* LAM.) in the USA, causes sloughing off of bark and extensive stromata formation in drought stressed trees (BASSETT & FENN, 1984).

It is clear that endophytes are ideally suited to be pathogens in that they are adapted to exist within healthy plant tissues, but even when they are found in dying tissues it is often unclear whether they are the cause of the disease symptoms or merely acting saprotrophically. In oak, whilst the primary colonizers only became established in small pockets when inoculated into attached branches, there was evidence that *Stereum gausapatum* and *Vuilleminia comedens* hastened cambial death in the vincinity of the inoculum wound (BODDY & RAYNER, 1984a). Such cambial death, which was accompanied by blackening of tissues, has also sometimes been found in proximal regions of branches which have recently lost or begun to lose functionality (L. BODDY, unpublished).

Simple mutations could give rise to pathogenic varieties of common endophytes by inducing biotrophic characteristics in certain strains. This phenomenon may explain why for example *Phomopsis* spp. have been associated with short lived outbreaks of serious diseases in apple trees (GROVE, 1935, 1937) and non-pathogenic varieties of the Witches broom fungus, *Crinipellis perniciosa* (STAHEL) SINGER, are present in wild Lianas in Equador (G. W. GRIFFITH & J. N. HEDGER, pers. comm.).

Over periods of evolutionary time it might be expected that pathogenic traits in such saprotrophic endophytes would be selected against since this would involve a measure of resistance to their infection being developed by their host. Conversely any mutualistic benefits the endophytes confer on their host during their latent phase would be selected for. It is not surprising to see that such associations have arisen but they are probably not an absolute prequisite for endophytic associations.

In many instances the presence of specific endophytes has been shown to confer benefits upon the hosts, which has lead some workers to conclude that many endophyte/host relationships are highly evolved mutualistic associations (CARROLL, 1988). The presence of *Phomopsis oblonga* in dying elm bark has various antagonistic affects upon Scolytid beetles which are the vectors of the Dutch elm disease fungus *Ophiostoma ulmi*. Hence, *P. oblonga* may provide a natural biological control of the spread of the disease (WEBBER, 1981; BRAYFORD, 1983; WEBBER & GIBBS, 1984). Also various endophytes in dormant willow bark have an antagonistic affect upon the canker forming fungus *Hypoxylon mammatum* (BIER & ROWAT, 1962).

Perhaps the most clear cut mutualistic association that has been found to date is that of *Balansia* spp., which produce substances which reduce the palatability of the grasses to various herbivores (CLAY, 1988). Similar substances are assumed to be produced by conifer needle endophytes (CARROLL, 1988).

Saprotrophy

Not withstanding the foregoing the majority of endophytes are not associated with disease symptoms. These have usually been assumed to be saprotrophs, although in most cases evidence for this has not been produced. In *Ulex europaeus* two of the common endophytes, *Phomopsis ligulata* and *Pleospora herbarum* are known to be involved in the decomposition of dead spines (STRINGER, 1974: FISHER & al., 1986).

In the past fungi that form latent infections of fruits were considered to be essentially parasitic (VERHOEFF, 1974), however, this

is rather an anthropocentric view since under natural conditions fruits when ripe fall from their producers into the litter layer and can no longer be considered as healthy plant organs. The fungi under consideration here only become active after or whilst the fruits ripen and hence are in fact displaying a type of specialised opportunism which is very similar in essence to that shown by fungi that form latent infections of healthy twig bark.

It has been shown that a variety of endophytes from coniferous foliage have an ability to utilise complex substrates including cellulose, hemicelluloses, lipids and pectin, and that petiolar endophytes could utilize a wider range of substrates than needle fungi, suggesting that the latter are dependant upon the host for simple carbon sources whereas the former are more active decomposers (CARROLL & PETRINI, 1983).

The presence of some of the more common endophytes of healthy ash, beech and oak twig bark in the decaying woody tissue of dead attached twigs indicate that these particular endophytes have a saprotrophic capacity. Recent investigations (G. S. GRIFFITH & L. BODDY, unpublished) have shown that the primary colonizers of ash, *Phomopsis platanoidis, Fusarium lateritium* and Sp. 12, can bring about a 20 to 30% loss in dry weight of ash twigs over a period of 5 months. Decay by these species was more active at low water potentials than that of typical white rotters such as the secondary coloniser *Peniophora lycii,* which is important since dry conditions tend to prevail within twigs. Scanning electron microscopy of twig wood decayed by these species demonstrated that *P. platanoidis* produced a type 2 soft rot which in certain regions was more akin to a slow white rot, *F. lateritium* produced a more typical type 2 soft rot and Sp. 12 apparently produced a brown rot. Substrate utilisation tests indicated that *Xylaria* sp. A, *Cryptosporiopsis* sp. and *Phomopsis quercella,* all of which were primary colonizers of dead beech twigs, also had the potential to utilise lignocellulose and hence produce active wood decay.

By contrast the capacity of several other endophytes common in healthy twig bark but not in dead wood of ash, beech and oak, to utilise complex substrates, in particular lignocellulose, was often, but not always, more limited. The role of such endophytes remains a mystery but they are presumably dependant upon their host for simple carbon compounds.

With regard to primary colonizers of attaches branches all have been observed to cause decay in the field (pp. 46–47). In the case of the Basidiomycotina, colonized wood was usually white rotted.

In conclusion it is clear that several of the common endophytes of the sapwood and bark of deciduous trees are primarily saprotrophic being specifically adapted to colonize and utilise dying host

tissue. This does not, however, rule out the possibility that the presence of these endophytes in healthy tissues confers some benefit(s) on their hosts. In view of what has been said it is possible that the association of some endophytes with disease symptoms in their hosts is a result of their tendency rapidly to colonize dying tissues rather than any pathogenic traits on their part. This also raises the possibility that they are in some way involved in the natural pruning of stressed tissues.

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