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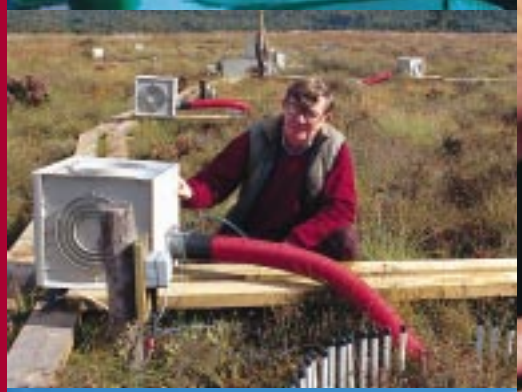

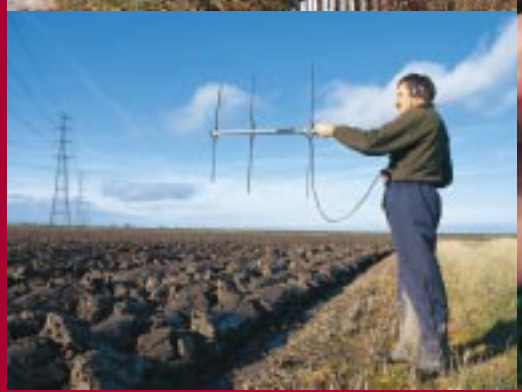

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Preliminary ecological investigation of four wood-inhabiting fungi of conservation concern - oak polypore *Piptoporus quercinus* (=*Buglossoporus pulvinus*) and the tooth fungi *Hericium*/*Creolophus* spp.

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Summary

A PhD project, two undergraduate final year research projects and a short molecular investigation have been carried out over a three year period at Cardiff University. The research has been a preliminary ecological investigation into four wood-inhabiting fungi, including three British tooth fungi - tiered tooth *Creolophus* (=*Hericium*) *cirrhatus*, coral tooth *H. coralloides* and bearded tooth *Hericium erinaceum*, and the oak polypore *Piptoporus quercinus* (=*Buglossoporus pulvinus*). In the UK, over 80% of these tooth fungi have been recorded fruiting on beech *Fagus sylvatica*, while *P. quercinus* is apparently exclusive to oak *Quercus* spp. All species are included in the provisional Red Data List of British Fungi (Ing 1992), but only *H. erinaceum* and *P. quercinus* are UK BAP priority species.

Creolophus cirrhatus, Hericium coralloides* and *H. erinaceum

Firstly the relative growth rates of mycelia of the three tooth fungi were measured under a variety of different abiotic conditions in artificial culture. Petri dishes of malt agar were inoculated centrally to compare growth rates at different temperatures, pH values and water potentials. The optimum temperature for extension was 25°C with slow growth at 5°C and no growth at 35°C. Optimum pH for extension was pH 5 (the pH of beech wood) with no growth at pH 2 and 8. Extension rate decreased with decreasing water potential (ie as it became more difficult to obtain water from the medium) with little or no growth below -5 MPa. These results are similar to those for many wood decay basidiomycetes, and therefore the abiotic variables tested probably do not contribute to the rarity of these species.

The outcome of interactions between the three tooth fungi and over twenty other wood decay fungi commonly found in beech, and covering a range of different ecological functions, was studied under different abiotic conditions. Pairs of fungi were inoculated in close proximity on malt agar media in Petri dishes. *H. coralloides* and *C. cirrhatus* were also paired against seventeen other fungi in beech wood blocks. All three species exhibited a complex range of interactions. *C. cirrhatus* and *H. coralloides* were fairly evenly split between three categories: replaced or partially replaced by other species, deadlock and replacement/partial replacement of other species. *H. erinaceum*, however, was replaced/partially replaced by other fungi slightly less often. Reduced water potential dramatically improved outcomes in favour of *C. cirrhatus* and *H. erinaceum* and to a lesser extent *H. coralloides*. When paired in wood blocks, *H. coralloides* and *C. cirrhatus* both replaced nine species and deadlocked with, or were replaced by, a similar number.

It can be deduced from the above that the three tooth fungi have an average combative ability. They were seldom replaced by heart rot fungi or other early colonizers and were often able to maintain territory against fungi considered to be secondary colonisers in standing wood. Combative late stage colonisers that invade fallen trees and branches from the forest floor, not surprisingly replaced the tooth fungi. However, outcomes of interactions varied dramatically under different regimes. Combative ability probably accounts for the ability of the tooth fungi to remain for a long time in decaying wood, but is unlikely to be a major factor accounting for their rarity.

H. coralloides established well when inoculated into freshly felled wood in the field, extending up to 6 cm from the inoculum wood dowel plug in 6 months, when cut ends of logs were sealed with cheese wax, but less far when unsealed. The ability to grow within beech

wood does not, therefore, seem to be a reason for the rarity of this fungus. Also, it seems likely to be possible to re-establish this species by artificial inoculation should this ever be desirable. Many felled log inoculations remain for sampling in the longer term, to determine how long *H. coralloides* persists in wood. Establishment was often unsuccessful in logs which had been felled six months prior to inoculation, and hence already had a well established mycoflora of early colonizing species, which may indicate that *H. coralloides* establishes at an early stage. Further research is required to see if the answer to why this fungus is rare lies at the stage of colonisation - how exactly do these fungi effect entry into the standing tree? To shed light on this, inoculations were also made into standing, functional trees and are scheduled to be sampled in 2005.

We have taken the first steps towards developing a molecular identification approach to enable detection and determination of the spatial distribution of the tooth fungi at early stages in standing and fallen trees. The tooth fungi can now be identified in the field, with relatively little damage to the standing or fallen tree, with the use of an increment borer and subsequent molecular analysis. One pair of PCR primers was developed that could distinguish *C. cirrhatus* from other wood decay basidiomycetes and ascomycetes. Another primer pair was developed that could distinguish *H. alpestre*, *H. coralloides* and *H. erinaceum* from other wood decay fungi but not from each other.

Single spore isolates have been collected so that, in the future, mating tests can be performed to determine the population structure and as a necessary prerequisite prior to investigating dispersal by spore trapping, using unmated cultures as baits. It should now be possible to address the questions: how abundant are the spores of tooth fungi in nature? where and when do they colonise trees?; are they really rare and declining?

Piptoporus quercinus

Extension rates of *Piptoporus quercinus* mycelium were investigated on 0.5 and 2% malt agar media under a variety of different abiotic conditions. The extension rate of *P. quercinus* was slow in comparison with other wood decay fungi. The optimum growth rate was at a temperature of 25 ^{o}C and a pH of 3.75. Upper limits for growth were at temperatures of 25-30^{o}C and a pH of 6.6. Lower limits for growth were at temperatures of 5-10 ^{o}C, a water potential of -2.5 MPa and there was still growth at a pH of 1.81. *P. quercinus* survived exposure to temperatures as low as -18 ^{o}C for 7 days. *P. quercinus*, like many heart-rot fungi, exhibits host specificity and for survival probably depends on being able to tolerate and/or benefit from very particular environmental conditions; the optimum pH for the growth of *P. quercinus* (a very low value) correlates with that of oak heartwood. Though *P. quercinus* was not very combative, being unable to replace any of the fungi against which it was tested, it was not easily replaced by fungi that it would most commonly encounter in standing trees. Thus once established in heart wood it is likely to be able to hold its territory for a long time.

Again the questions arise as to how, when and where does the fungus enter the tree? Molecular methods to identify *P. quercinus* still need to be developed. Molecular biology techniques may also be able to unravel its population biology/genetics.

Contents

1. Background

There are three wood-inhabiting fungi in Britain with spectacularly conspicuous and distinctive fruit bodies, which liberate spores from white downwardly pointing spines. Indeed, these icicle-like spines are so distinctive that the three species are collectively known as tooth or spine fungi. They have been classified in the genus *Hericium*, although some authors prefer to keep one of the species in the genus *Creolophus*. The species are tiered tooth *Creolophus* (=*Hericium*) *cirrhatus*, coral tooth *H. coralloides* and bearded tooth *Hericium erinaceum*. All three species are included in the provisional Red Data List of British Fungi (Ing 1992), but only *H. erinaceum* is a UK BAP priority species, being one of four fungal species on Schedule 8 of the Wildlife and Countryside Act 1981. *H. coralloides* is probably the rarest of the three species and should be a UK BAP priority species (Boddy & Wald 2003). From the available UK records of fruit bodies, all three species show a predominantly southern or south eastern distribution with very occasional northern or western records. There are many records from semi-natural woodland with a long history of tree coverage, a range of tree age classes and retained dead wood. Hampshire holds the most records and recent surveys have highlighted the importance of a variety of sites in the New Forest (Boddy & Wald 2003; Wicks 1999).

There is evidence of host specificity, over 80% of each species being recorded on beech *Fagus sylvatica*, with a further 14% of *H. coralloides* records on ash *Fraxinus excelsior* (Boddy & Wald 2003). There are reports on standing, fallen and felled trunks, stumps and dead attached branches, including well decayed wood and hollow trunks; *C. cirrhatus* is usually found fruiting on cut surfaces, damaged standing trees, sometimes high off the ground; *H. coralloides* on large fallen logs; and *H. erinaceum* on living trees that have suffered damage from gales or lightning strikes (Boddy & Wald 2003; Marren & Dickson 2000).

Knowledge of the ecology of these species is confined to conditions appropriate for growing the fungi for fruit body production, and to some information on mating behaviour. *H. erinaceum*, at least, is easy to cultivate outdoors by inoculating logs with colonised wood dowels or sawdust, and indoors in logs or containers (eg polythene bags) of sawdust (Stamets 2000). Fruit bodies form in 2–3 weeks at 24^{o}C on agar or sawdust.

Here we report on *Hericium* and *Creolophus* growth, fruiting, germination and their interactions with other wood decay fungi in artificial culture, their associated wood decay rates and ability to establish from mycelial inocula in the field.

The oak polypore *Piptoporus quercinus* is also protected under Schedule 8 of the 1981 Wildlife and Countryside Act (as *Buglossoporus pulvinus*), and is a national Biodiversity Action Plan species (Roberts 2002). It has been most frequently recorded from the south / south east, though also reported elsewhere, eg Derbyshire, Herefordshire, Gloucestershire, Devon, Dorset, Leicestershire, Nottinghamshire and Yorkshire (Roberts 2002). It is "rare but widespread" in Europe, though apparently not found in Spain and Italy, and has been reported from some parts of Asia, but not from North America (Roberts 2002; Ryvarden & Gilbertson 1994).

It has only been reported fruiting on oaks (*Quercus robur*, *Q. petraea* and once *Q. cerris*), though its ability to colonise other tree species has not been experimentally tested. It fruits on old living and dead standing trees, and fallen trunks, including some which have long fallen,

and hence it is assumed to be confined to old oaks. Commonly, old trees sporting fruit bodies are found on relatively open and exposed sites, such as deer parks, commons and mediaeval forests, but sometimes also in more densely wooded areas (Roberts 2002). It is a heart rot fungus causing brown rot.

Little is known of its ecology or general biology. Here we report on the abiotic conditions best suited to the mycelial growth of *P. quercinus*, and its combative ability against other oak wood-inhabiting basidiomycetes.

2. Growth and germination of *Creolophus* (= *Hericium*) *cirrhatus, Hericium erinaceum* and *H. coralloides* in artificial culture

2.1 Materials and methods

To gain an idea of the speed of growth of the tooth fungi relative to others, and determine favourable conditions for growth, the fungi were inoculated centrally onto Petri dishes containing malt agar (MA) and the radius was measured at different times. The inoculum was a 6 mm diam. plug of agar plus fungus, cut from the margin of an actively growing colony. Radial extent was measured, across two diameters, at daily intervals when growth was rapid, but less frequently where growth was slow. Extension rate was estimated over the linear phase of growth. Linear extension rates of several isolates of each species and of *H. alpestre* were measured on 2% MA under a range of different temperatures (5-30^{O}C), at five different water potentials (close to zero and -5 MPa) at 20^{O}C, and at seven pHs (pH 2-8) at 20^{O}C. Water potential was altered by addition of appropriate amounts of KCl (Robinson & Stokes 1959), and pH was altered by addition of phosphoric acid.

Spore prints were obtained from several fruit bodies of each species. Spores were plated onto MA at different dilutions and incubated at 20^{O}C. Germination was then assessed by microscopic examination every 24h for one week for all species, and then after three months for *C. cirrhatus*.

2.2 Results and discussion

Optimum temperature for extension was at 25^{O}C (3.28-6.7 mm d^{-1}), with slow growth at 5 and 10^{O}C, and no growth at 35^{O}C (Figure 1). These cardinal points for temperature are similar to those of many wood decay basidiomycetes (Rayner & Boddy 1988). Optimum pH for extension was at pH5 (7.46 - 8.7 mm d^{-1}), with no growth at pH 2 and 8 (Figure 2), and this pH optimum lies in the region of the pH of beech wood (Gray 1958). Extension rate decreased with decreasing water potential. *H. erinaceum* was unable to grow at -5MPa, but the other two species still showed limited growth (Figure 3). These results are similar to those for many wood decay basidiomycetes (Boddy 1983; Griffin 1981; Koske & Tessier 1986). Clearly, the three species of tooth fungi are not outstandingly different from the majority of wood decay basidiomycetes in respect of effects of pH, water potential and temperature on growth.

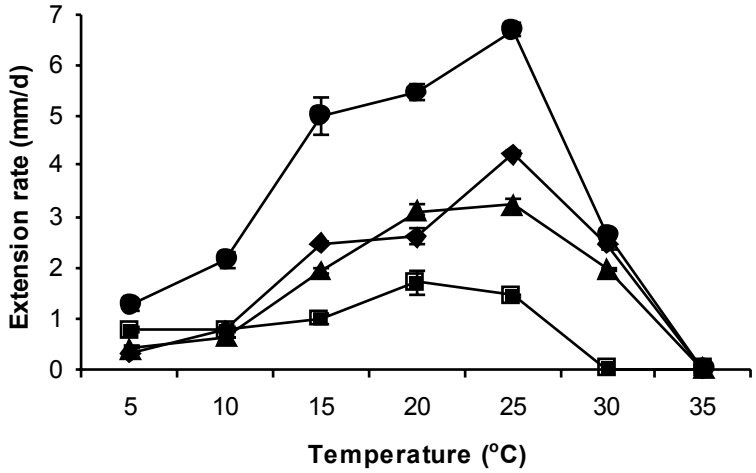


Figure 1. Effect of temperature on linear extension rate of one isolate of each species on MA. Mean and 95% confidence interval.
Symbols: ●, *H. erinaceum*; ◆, *H. coralloides*; ▲, *C. cirrhatus*; ■, *H. alpestre*.

There was high (>90%) germination of *H. coralloides* and *H. erinaceum* within 48 h, but even after three months < 10 % *C. cirrhatus* spores had germinated.

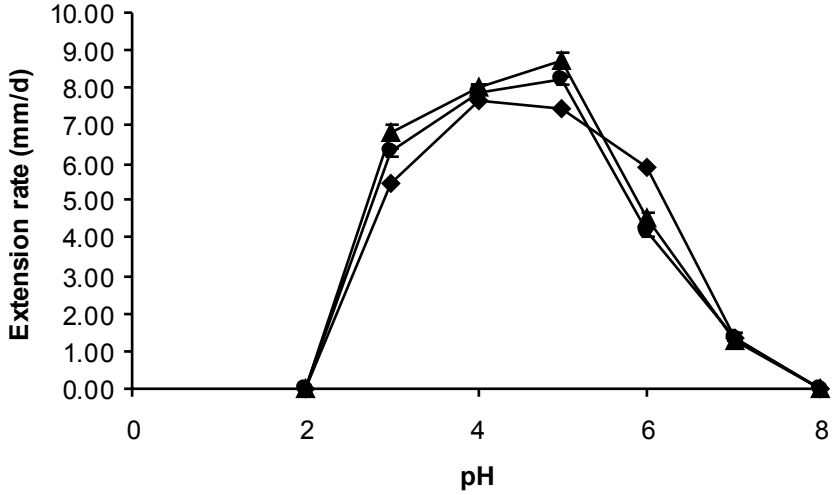


Figure 2. Effect of pH on linear extension rate of one isolate of each species on MA at 20 ^{O}C. Mean and 95% confidence interval. Symbols: ●, *H. erinaceum*; ◆, *H. coralloides*; ▲, *C. cirrhatus*

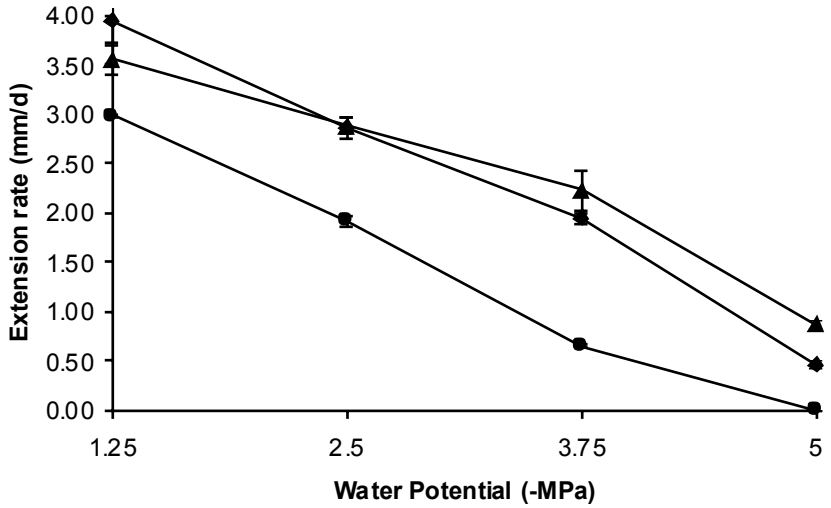


Figure 3. Effect of solute potential exerted by KCl on linear extension rate of one isolate of each species on MA at 20^{O}C . Mean and 95% confidence interval. Symbols: ●, *H. erinaceum*; ◆, *H. coralloides*; ▲, *C. cirrhatus*

3. Interactions between *Hericium* species and other wood decay fungi in artificial culture

3.1 Materials and methods

3.1.1 Interactions on malt agar

Outcome of interactions were studied for one isolate each of *C. cirrhatus* (CcJHC-1, Danish), *H. coralloides* (MA-1, English), and *H. erinaceum* (H-MILL, American) against over 20 other fungi (Table 1), common in beech wood. Interactions were examined by placing two different isolates (as colonised plugs of agar) in close proximity near the centre of Petri dishes containing agar. Mycelia grew out from each inoculum plug, met and interactions were observed macroscopically for several months. Interaction studies were performed on 2% MA (pH 5.5), 0.5% MA (pH 5.9), 0.5% MA adjusted to - 1.25 MPa using KCl (Robinson & Stokes 1959) and on 0.5% MA adjusted to pH 4 (selected as a typical value for beech heartwood) inoculated 30 mm apart, and incubated in the dark at 20 ^{O}C. Plates were observed every 1 - 3 d depending on extension rate; production of pigments, fruit bodies, barrages and invasive fronts were noted. Measurements of colony radii towards and away from opponents were made, and in many pairings perpendicular to this diameter, to the nearest 0.1 mm, using Vernier calipers, every day until the opponents made contact. Finally, after 70 - 84 d, outcomes were recorded as deadlock, replacement or partial replacement. Outcomes were verified by taking agar samples from the top and bottom of plates at the interaction zone and/or on either side of it, as appropriate for the different interactions, plated on 0.5 or 2% MA and subsequently identified. Self pairings of all species served as controls. Four or five replicates were usually made, but occasionally a combination was made again giving nine replicates.

3.1.2 Interactions in beech wood blocks

Hericium coralloides and *C. cirrhatus* were paired against 17 other wood decay species (Figures 4, 5), covering a range of different ecological roles (Table 1) and against self in wood blocks, with 3 - 5 replicates. They were also paired against each other. Wood blocks (3 x 3 x 1 cm) were obtained from freshly felled, sawn then frozen planks of beech *Fagus sylvatica*. Prior to colonization they were defrosted by soaking overnight in distilled water and then autoclaved twice at 24 h intervals, at 121°C for 20 min. Wood blocks were then colonised for 4 weeks at 20°C on 2% MA, in 2 l conical flasks, which had been inoculated 6 weeks earlier with the appropriate fungus. Inoculum blocks were then removed and scraped free of adhering mycelium and agar, and then paired such that surfaces with cut vessels were adjacent to each other, secured with elastic bands and completely covered with Nesco film, to reduce drying.

Paired wood blocks were then incubated at 20°C for 36 - 38 weeks, after which the blocks were separated and cut in half longitudinally. Isolations were made from one half onto 2% MA, to determine the extent of wood occupied by each fungus.

3.2 Results

3.2.1 Outcome of interspecific interactions in agar culture

All three species exhibited the complete spectrum of interactions on MA varying with opponent and abiotic regime (Tables 2-4; Appendix 5-7). Usually interactions followed mycelial contact, but sometimes there was non-contact inhibition (Appendix 5h, 6f, 7f). Overall the outcomes of interactions involving *C. cirrhatus* and *H. coralloides* were fairly evenly split between the categories replacement or partial replacement by other species, deadlock and replacement or partial replacement of other species. *H. erinaceum*, however, was replaced/partially replaced relatively less often. All species were replaced most often on 2% MA; reduced water potential dramatically improved outcomes in favour of *C. cirrhatus* and *H. erinaceum* and, to a lesser extent, *H. coralloides*.

Table 1. Details of *Hericium* and *Creolophus* isolates, and isolates against which they were paired in interactions on agar (A), wood (W) or both (B). (From Wald and others 2005)

Ecological role	Species	Strain	Source	Isolated by	Experiment
Primary colonizer, latently[1] present in attached stems and branches	*Biscogniauxia nummularia**		*F. sylvatica* Ccc	S.J. Hendry	A
	*Eutypa spinosa**	Es1	*F. sylvatica* Ccc	S.J. Hendry	B
	*Diatrype disciformis**	Dd1	*F. sylvatica* Ccc	S.J. Hendry	A
	Stereum gausapatum[4]	Sg1	*Quercus robur* Ccc	L. Boddy	A
	Stereum rugosum	Sr1	*Corylus avellana* Ccc	L. Boddy	A
	Chondrostereum purpureum	Cpur1	*F. sylvatica* Ccc	S.J. Hendry	A
	Coniophora puteana	Cput1	*F. sylvatica* Ccc	S.J. Hendry	B
	Vuilleminia comedens	Vc1	*Quercus robur* Ccc	L. Boddy	A
Attached branch, dry	*Peniophora lycii*	Pl1	*Quercus robur* Ccc	L. Boddy	A
Heart rotter[1]	*Creolophus cirrhatus*	CcJHC1	*F. sylvatica*, Denmark	J. Heilmann-Clausen	B

Ecological role	Species	Strain	Source	Isolated by	Experiment
	Hericium coralloides	MA1	*F. sylvatica,* Windsor Great Park	A.M. Ainsworth	B
	Hericium erinaceum	H-MILL	USA	Helen Millar**	A
	Hericium alpestre	FBSS1	Conifer, probably *Abies*, Switzerland	A.M. Ainsworth	W
	Fomes fomentarius	Ffl	*F. sylvatica* Denmark	J. Heilmann-Clausen	B
	Inonotus obliquus	Io1	*Betula,* Windsor Great Park	A.M. Ainsworth	B
	Piptoporus quercinus	MA-1BQ	*Quercus robur*, Windsor Great Park	A.M. Ainsworth	A
	Ganoderma applanatum	JHC-GA	*F. sylvatica* Denmark	J. Heilmann-Clausen	B
Early secondary colonizers[1] on standing trees and fallen wood	*Bjerkandera adusta*	Ba1	*F. sylvatica* Ccc	L. Boddy	A
	Coriolus versicolor	Cv1	*Quercus robur* Ccc	L. Boddy	B
	*Nemania serpens**	JHC00-2059	*F. sylvatica* Denmark (polyspore)	J. Heilmann-Clausen	W
	Phlebia radiata	Prad1	*Quercus robur* Ccc	L. Boddy	A
	Stereum hirsutum	Sh1	*Quercus robur* Ccc	L. Boddy	B
Later secondary colonizing cord-formers[1]	*Hypholoma fasciculare*	Hfas1	Ccc	D.P. Donnelly	B
	Phanerochaete velutina	Pv1	Ccc		B
Other later stage fungi whose ecological strategies have been little studied[123]	*Antrodiella semisupina*	AsJHC1	*F. sylvatica* Denmark	J. Heilmann-Clausen	A
	Ceriporiopsis gilvescens	CgJHC1	*F. sylvatica* Denmark	J. Heilmann-Clausen	B
	Lycoperdon pyriforme[5]	JHC00-078	*F. sylvatica* Denmark	J. Heilmann-Clausen	A
	Phlebia livida	JHC00-082	*F. sylvatica* Denmark (polyspore)	J. Heilmann-Clausen	B
	Coprinus micaceus[6]	Cm1	*F. sylvatica* UK	Louise Owen	W
	*Mollisia cinerea**	JHC00-2059	*F. sylvatica* Denmark	J. Heilmann-Clausen	W
	*Peziza micropus**	JHC00-081	*F. sylvatica* Denmark	J. Heilmann-Clausen	W
	*Camarops polysperma**	CpJHC1	*F. sylvatica* Denmark	J. Heilmann-Clausen	W

*Ascomycota, others are all Basidiomycota, Ccc- Cardiff culture collection; [1]Rayner & Boddy (1988), [2]Heilmann-Clausen (2001), [3]personal observations, [4] also causes pipe-rot of heartwood in oak, [5]forms cords but does not appear to migrate over large distances through soil, [6]colonizes well decayed wood and forms cords in basic rich soils (Owen 1997), **supplied not isolated by

H. coralloides and *H. erinaceum* often produced fruit body primordia in controls and during most interactions, being particularly prolific at pH 4 (Appendix 5 & 6). *C. cirrhatus* produced primordia much less readily, but they were present in all controls and interactions at pH4 (Appendix 7). Morphological changes sometimes occurred during interactions, the most common being the production of a barrage of mycelium at the interaction front (eg Appendix

7k), *Hypholoma fasciculare* produced mycelial cords, as did *Lycoperdon pyriforme* on 0.5% MA. Pigment production occasionally occurred in colonies of the tooth fungi, but was particularly common when they were involved in interactions and usually occurred post-contact, but occasionally before (eg Appendix 7 b, c).

Table 2. Effect of abiotic environment on outcomes of interactions between *Hericium coralloides* and other beech and oak decay fungi, after 70 - 84 d. (From Wald and others 2005)

	2% MA	**0.5% MA**	**0.5%MA - 1.25MPa**	**0.5% MA pH4**	**0.5% MA, 5% O_2 30% CO_2**
Biscogniauxia nummularia	pr	pr		r	
Diatrype disciformis	pr				
Eutypa spinosa	PR	D	PR(2)pr(2)	D(3)pr(1)	D
Antrodiella semisupina	pr				
Bjerkandera adusta	R	R		PR	
Ceriporiopsis gilvescens	R				
Chondrostereum purpureum	PR				
Coniophora puteana	R				
Coriolus versicolor	D(3)PR(2)	D	D	D(1)PR(3)	D(3)PR(2)
Creolophus cirrhatus	D	D			
Fomes fomentarius	r	PR	D(3)r(1)	r	D(1)pr(3)
Ganoderma applanatum	r	pr	r	r	
Hypholoma fasciculare	R	D(3)PR(2)	D(2)PR(3)	PR	D
Inonotus obliquus	r	r	pr	r	r(2)pr(3)
Lycoperdon pyriforme	D	R	D(3)pr(1)	D	
Peniophora lycii					
Phanerochaete velutina	PR				
Phlebia livida	PR				
Phlebia radiata	R(1)PR(1)	D	PR(1)D(3)	D(2)pr(2)	
Piptoporus quercinus	D(1)pr(3)	pr			
Stereum gausapatum	D	PR	PR(4)D(5)	D	
Stereum hirsutum	D	D	D(4)pr(1)		
Stereum rugosum	pr			D	
Vuilleminia comedens	r	pr		D	

Abbreviations: D, deadlock, R, replacement of *H. coralloides* by opponent; PR, partial replacement of *H. coralloides* by opponent; r, replacement of opponent; pr, partial replacement of opponent; figures in brackets indicate distribution of interaction types among the replicates.

Table 3. Effect of abiotic environment on outcomes of interactions between *Hericium erinaceum* and other beech and oak decay fungi, after (From Wald and others 2005)

	2% MA	**0.5% MA**	**0.5%MA - 1.25MPa**	**0.5% MA pH4**	**0.5% MA, 5% O_2 30% CO_2**
Biscogniauxia nummularia		r		r	
Diatrype disciformis					D(2)pr(3)
Eutypa spinosa		r	D	r(3)pr(1)	
Antrodiella semisupina					
Bjerkandera adusta		R		PR	
Ceriporiopsis gilvescens					
Chondrostereum purpureum					
Coniophora puteana					
Coriolus versicolor	R(3)D(1)	D	D(3)pr(2)	PR	D
Creolophus cirrhatus		D(4)pr(1)			
Fomes fomentarius		r	r	D(1)r(3)	pr
Ganoderma applanatum		r	r	r	
Hericium coralloides			D		
Hypholoma fasciculare		PR	D	PR	D
Inonotus obliquus		r	D(1)pr(3)	r(2)pr(2)	r
Lycoperdon pyriforme		D	D(3)pr(1)	D	
Peniophora lycii	PR(1)D(3)				
Phanerochaete velutina					
Phlebia livida					
Phlebia radiata	R(2)PR(2)	D	D(3)r(1)pr(1)	D	
Piptoporus quercinus		pr	pr		
Stereum gausapatum		D	PR(7)pr(2)	PR	
Stereum hirsutum	D	D	D	D	
Stereum rugosum					
Vuilleminia comedens		r		r	

Abbreviations: D, deadlock, R, replacement of *H. erinaceum* by opponent; PR, partial replacement of *H. erinaceum* by opponent; r, replacement of opponent; pr, partial replacement of opponent; figures in brackets as in Table 2

Table 4 Effect of abiotic environment on outcomes of interactions between *Creolophus cirrhatus* and other beech and oak decay fungi (After Wald and others 2005)

	2% MA	0.5% MA	0.5%MA - 1.25MPa	0.5% MA pH4	0.5% MA, 5% O_2 30% CO_2
Biscogniauxia nummularia	pr	pr		r	
Diatrype disciformis	pr				
Eutypa spinosa	PR	PR	D	D	D
Antrodiella semisupina	pr				
Bjerkandera adusta	R	R		PR	
Ceriporiopsis gilvescens	R				
Chondrostereum purpureum	PR				
Coniophora puteana	R				
Coriolus versicolor	R(3)D(2)	D	D	PR	D
Fomes fomentarius	r	PR	r	r	pr
Ganoderma applanatum	r	r	r	r	
Hericium coralloides	D	D			
Hypholoma fasciculare	R	R	PR(1)D(4)	PR	D
Inonotus obliquus	r	r	r	r	pr
Lycoperdon pyriforme	D	PR	D	D	
Peniophora lycii	PR(1)D(2)				
Phanerochaete velutina	PR				
Phlebia livida	PR(3)D(1)				
Phlebia radiata	PR(2)R(3)	D	D	D(2)pr(2)	
Piptoporus quercinus	D pr(4)	pr	pr		
Stereum gausapatum	D	D	PR(4)D(5)	PR	
Stereum hirsutum	D	D	D	D	
Stereum rugosum	D(2)pr(3)				
Vuilleminia comedens		pr		D	

Abbreviations: D, deadlock, R, replacement of *C. cirrhatus* by opponent; PR, partial replacement of *C. cirrhatus* by opponent; r, replacement of opponent; pr, partial replacement of opponent; figures in brackets as in Table 2

3.2.2 Inhibition and stimulation of extension during interactions

Overall (ie including growth towards and away from opponent) extension rates of the colonies of both *C. cirrhatus* and *H. coralloides* frequently increased in the presence of opponents, though occasionally (eg against *L. pyriforme*) it decreased (Table 5). Usually, there was no significant difference in extension rate towards or away from an opponent, but occasionally there was a decrease in extension rate towards an opponent, and there was a single instance of increase in extension rate towards compared with away from the opponent (*C. cirrhatus* versus *C. versicolor*). With *C. cirrhatus* versus *I. obliquus* although there was an overall increase in extension rate, extension towards the opponent was significantly decreased. Extension rate of *H. coralloides* was affected in 3 out of 7 combinations (Table 5).

The extension rate of opponent wood decay species was influenced less often than that of the tooth fungi, and when effects were significant they were always decreases in extension rate (Table 5). Extension rates of *B. nummularia*, *E. spinosa* and *V. comedens* were reduced towards and overall against all three tooth fungi.

Table 5. Effect of opposing colonies of wood decay fungi on mean (±SEM) extension rate (mm d^{-1}) of tooth fungi on 0.5% MA compared with control pairings, and also growth towards (T) compared with growth away (A) from opposing colony on interaction plates. (From Wald and others 2005)

	C. cirrhatus				*H. coralloides*				*H. erinaceum*			
	towards	**away**	**diam.in interaction < control**	**T<A**	**towards**	**away**	**diam.in interaction < control**	**T<A**	**towards**	**away**	**diam.in interaction < control**	**T<A**
B. nummularia	0.65±	0.80±	***	ns	1.43±	1.39±	S***	ns	2.33±	1.95±	ns	ns
E. spinosa	1.14±	0.96±	ns	ns	1.45±	1.30±	S***	ns	2.59±	2.60±	ns	ns
C. versicolor[1]	1.40±0.04	1.24±0.04	S***	S**								
F. fomentarius	1.03±	0.99±	ns	ns	1.24±	1.31±	S*	ns	1.74±	2.24±	*	**
G. applanatum	1.44±	1.60±	S***	ns	1.03±	1.06±	S*	ns	2.38±	2.70±	ns	ns
H. fasciculare[1]	1.24±0.03	1.26±0.02	S**	ns								
I. obliquus	1.15±	1.39±	S*	*	0.97±	0.96±	ns	ns	2.66±	2.41±	ns	ns
L. pyriforme	0.65±	0.96±	*	ns	0.34±	0.84±	*	***	0.42±	1.48±	*	*
P. radiata[1]	1.09±0.10	1.21±0.08	S*	ns								
S. hirsutum[1]	1.14±0.07	1.06±0.06	ns	ns								
S. gausapatum[1]	1.00±0.06	1.13±0.05	ns	ns								
V. comedens	1.31±	1.53±	S**	*	0.92±	1.09±	S*	ns	2.46±	2.79±	S*	*

[1], performed at a different time, control extension rate 0.84±0.04 mm d^{-1}; rates are only directly comparable with interactions performed at the same time
significant inhibition compared with control pairings: *, $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns not significant
S, significant stimulation.

T<A, significantly slower growth towards compared with away from opponent with t test or Wilcoxon test, when data were non-normally distributed

3.2.3 Outcome of interspecific interactions in wood

Hericium coralloides gained territory from nine species (completely replacing five of these), lost territory to six species (being completely replaced by two species), and deadlocked with two species neither gaining any of the other species' territory (Figure 4). However, it must be noted that *Coniophora puteana*, *Peziza micropus*, *Hericium alpestre* and *Inonotus obliquus* were not successfully re-isolated from control wood blocks, suggesting that they may have died even when growing alone.

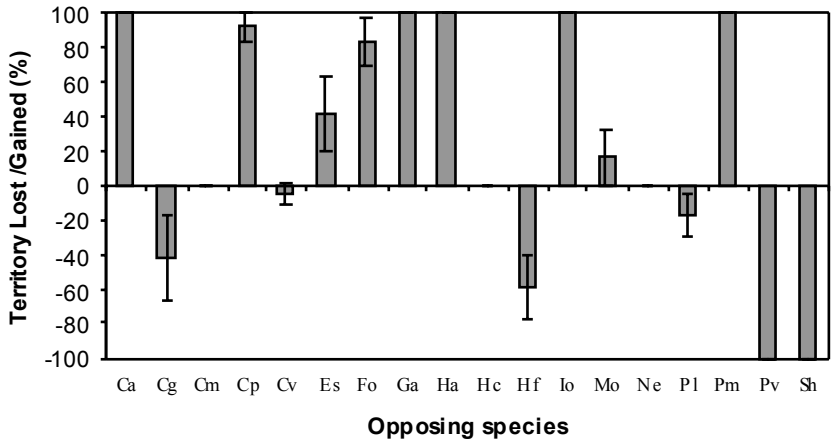


Figure 4. Territory lost and gained by *H. coralloides* when paired against other wood decay fungi in wood blocks at 20 ^{O}C in the laboratory. (After Wald and others. 2005)

Abbreviations: Ca, *Camarops polysperma;* Cg, *Ceriporiopsis gilvescens*; Cp, *Coniophora puteana,* Cm, *Coprinus micaceus*; Cv, *Coriolus versicolor*; Es, *Eutypa spinosa*; Fo, *Fomes fomentarius*; Ga, *Ganoderma applanatum*; Ha, *Hericium alpestre*; Hc, *Hericium coralloides*; Hf, *Hypholoma fasciculare*; Io, *Inonotus obliquus*; Mo, *Mollisia cinerea*; Ne, *Nemania serpens*; Pl, *Phlebia livida*; Pm, *Peziza micropus*; Pv, *Phanerochaete velutina*; Sh, *Stereum hirsutum*.

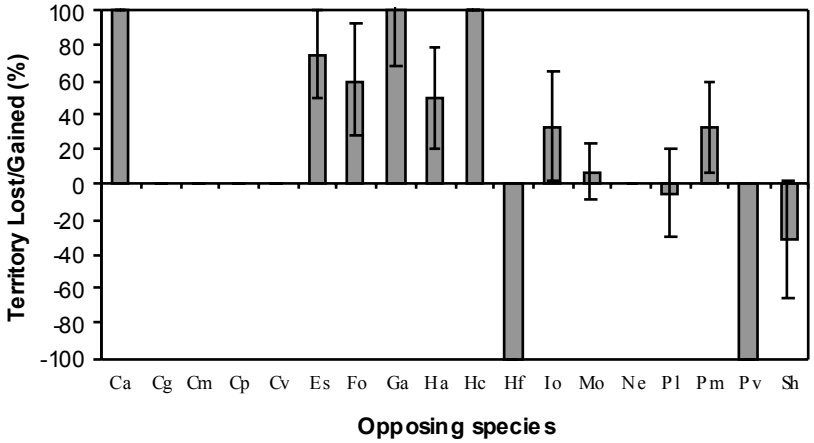


Figure 5. Territory lost and gained by *C. cirrhatus* when paired against other wood decay fungi in wood blocks at 20 ^{O}C in the laboratory. (After Wald and others 2005)

There were some differences in outcome with *Creolophus cirrhatus* (Figure 5). It gained territory from nine species (completely replacing three of these), lost territory to four species (being completely replaced by two species) and deadlocked with five species. It replaced *H. coralloides*.

3.3 Discussion

All three species had average combative abilities, deadlocking, replacing or being replaced by other species in fairly similar proportions in agar culture, *H. erinaceum* being slightly more combative than the other two. They were rarely replaced by heart-rot fungi or primary colonizers latently present in sapwood. Indeed, they often replaced *Ganoderma applanatum* and *Fomes fomentarius*, both of which occupy large volumes of beech trunks in Britain. Likewise they sometimes replaced, and were only infrequently replaced by the ascomycete *Eutypa spinosa*, which is an early colonizer occupying large volumes of beech trunks, especially when trees have been drought stressed (Boddy 2001). The tooth fungi were often able to maintain territory against fungi considered to be combative secondary colonizers of standing wood (Boddy 2001; Boddy & Rayner1983; Rayner & Boddy 1988), eg *C. versicolor*, *S. hirsutum* and *P. radiata*, though not against *B. adusta*. Combative later stage fungi (Boddy 2001; Rayner & Boddy 1988), which invade from the forest floor, eg the cord-former *H. fasciculare*, not surprisingly, replaced the tooth fungi on MA and in wood blocks. However, outcome of interactions varied dramatically under different regimes (Tables 2 - 4; Boddy 2000). In view of the foregoing, prolonged occupancy by these tooth fungi is likely to be at least partly related to combative ability. Moreover, since volume of wood occupied can have a large effect on outcome (Holmer & Stenlid 1993, 1997), the large volume occupied by these fungi in standing trees might further increase their combative ability under field conditions.

Further, the tooth fungi seem to produce compounds inhibitory to some fungi, though there could be other explanations of decrease in mycelial extension rate. Since the tooth fungi occupy large volumes of wood any toxic compounds produced may be even more effective in wood than in agar culture.

Increase in extension rate, particularly by *C. cirrhatus* and *H. coralloides*, when paired against fungi covering a range of ecological roles is particularly interesting. Though such stimulation might be viewed as indicating that the fungus is doing well, especially in pairings in which it wins territory, this may not always be the case. On the contrary, it may be indicative of an attempt to escape a competitor, for example, in interactions against *H. fasciculare*, which is more combative under ambient conditions on MA.

4. Inoculation and growth of *Hericium erinaceum* and *H. coralloides* in wood in the field

4.1 Methods

Inoculations were made into wood at three different sites: Windsor Great Park, Forest of Dean and Bangor. Experiments were slightly different on each site. At Windsor Great Park the logs were felled six months prior to inoculation, at the Forest of Dean the fungi were inoculated into freshly felled logs half of which were sealed at each end with cheesewax, and at Bangor the fungi were inoculated into standing, living trees.

At Windsor (Nat. Grid Ref. SU 935 740, see Appendix 1 & 2), beech logs were inoculated with 8.5 x 25 mm oak dowels (Anne Miller Cultivation, Aberdeen) which were either sterile or had been precolonized by *H. coralloides* (isolate MA-1-Windsor) by incubation on a MA culture in the laboratory for 8 weeks at 20 ^{O}C in the dark. The logs were inoculated between

Nov 26 and Dec 2 2001 and had been felled 6 months previously (June 2001). Inoculations were made by drilling (with a 12v electric hand drill) a hole (9 mm diam and 30 mm length) and then hammering in the dowels. Dowels were sterilized between each inoculation in100% methylated spirit. Logs were inoculated in six ways: (1) dowels colonized by *H. coralloides* inoculated in two longitudinal lines on the upper surface and two along the lower surface, 15 cm apart with alternate spacing in the two rows (16 logs); (2) as 1 but dowels were uncolonized (16 logs); (3) as 1 but logs had 65 cm length of wood removed from both ends at the time of inoculation leaving 125 cm lengths (16 logs); (4) as 3 but dowels were sterile (16 logs); (5) upright logs, length 65cm and 15-20cm diameter inoculated with *H. coralloides* on exposed ends (32 logs); (6) as 5 but with sterile dowels (32 logs). Six of the logs were collected in Sept 2003, leaving the remainder for longer term study.

At the Forest of Dean (Nat.Grid Ref. SO 552 170, Appendix 4), *H. coralloides* was inoculated (on precolonized dowels as above) into beech logs (100 cm length by approx. 12 cm diam., 26 of which still had identification labels at 2nd sampling), within 24 - 48 hours of felling, along upper and lower surfaces in July 2002. Others (24 still tagged at 2nd sampling) were inoculated with sterile dowels (as above). Half of the logs in each treatment were sealed by melting cheese wax (Anne Miller Cultivation, Aberdeen) onto the cut ends. Inoculation points were also sealed with cheese wax in all treatments. Six logs were harvested in January 2003, six (three sealed, three unsealed) in July 2003 and 11 in February-March 2004, leaving the remainder for longer term study.

At Coed Gorllwyn, Bangor (Nat. Grid Ref. SH 589 422, Appendix 3), colonised dowels (as above) of *H. coralloides* were inoculated into 20 standing beech and 4 ash trees (about 10 cm d.b.h.) at four heights (0.5, 1.0, 1.5 and 2.0 m above ground), in November 2002. At each height two inocula were inserted at 180^{O} to each other. Inocula were at 90^{O} from those above and below. Uncolonized dowels were inserted into other trees as controls, in the same way as for colonized dowels. Fifteen agar discs of *H. coralloides* and of *H. erinaceum* were inoculated into the sapwood of standing beech trees, and taped over with Nesco film, at 0.5, 1.0 and 1.5 m above the ground. Some of the trees will be harvested in summer 2004 leaving the remainder for longer term study.

4.2 Results

Hercium coralloides was successfully inoculated into freshly felled beech logs in the Forest of Dean (Figures 6, 7), with decay columns in excess of 6cm in either direction sometimes developing after 6 months. There was also radial and tangential spread, but this was more limited. Extension was significantly ($P<0.05$) greater in logs which had been sealed with wax than in those which had not been sealed. In contrast, extension was limited from inoculum dowels into beech logs which had been felled 6 months prior to inoculation at Windsor (Figure 8).

4.3 Discussion

Clearly, under appropriate conditions it is easy to inoculate *H. coralloides* into logs in the field, and establish viable mycelia. This is no surprise, in view of the ease with which these tooth fungi are artificially cultivated for production of edible fruit bodies (Stamets 2000). However, what is interesting is the considerable difference in establishment between logs at Windsor and those in the Forest of Dean. Although there are many differences between the two experiments, including size of logs, time of inoculation and prevailing weather, the most

likely explanation for differences in colonization lies in the fact that the logs at Windsor were felled six months prior to inoculation. This allowed latently present fungi and early airborne colonisers to become established, typical patterns of decay by xylariacious ascomycetes being evident (Figure 8a). These latter fungi presumably inhibited establishment of *H. coralloides* from the dowel inocula, whereas in freshly felled wood *H. coralloides* was able to establish at an earlier stage.

Establishment in logs that were sealed was probably better because of more favourable microclimatic conditions. Sealing would reduce water loss and gaseous exchange with the external atmosphere, maintaining conditions closer to those found in standing trees. In this respect, the results of inoculations into standing trees will be valuable.

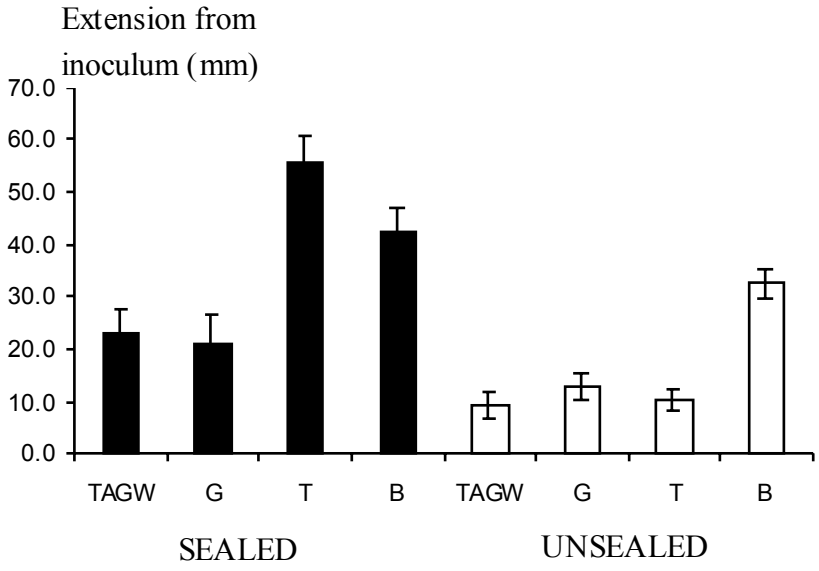
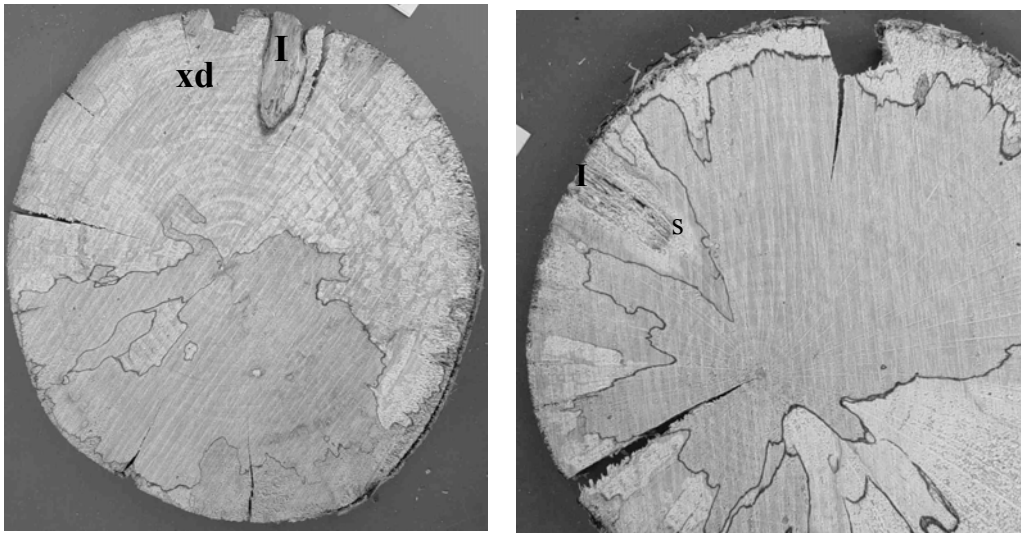


Figure 6. Mean longitudinal extension (mm) (and 95% confidence intervals) of decay columns from dowel inocula, 6 months after insertion into freshly felled beech logs in the Forest of Dean. Dowels were inserted into the top, ends or underside of the logs: TAGW, log end facing woodland ; G, log end facing garden ; T, top; B, bottom.

Figure 7. Longitudinal section through a decay column (D) extending from a dowel (I) inoculated into the upper surface of freshly felled beech logs in the Forest of Dean, 18 months after inoculation. Cut surfaces and inoculation points were sealed with cheese wax. Length of section 14 cm.



a b

Figure 8. Sections through logs inoculated with *Hericium coralloides* 6 months previously at Windsor Great Park. a. 15 cm diam., b. 14 cm diam.. I, indicates the inoculum dowel; xd, decay by xylariaceous ascomycetes; s, slight spread into the surrounding wood.

5. Growth of *Piptoporus quercinus* in artificial culture

5.1 Methods

Extension rate of three strains (Table 6) isolated from fruit body tissue was investigated on 0.5% and 2% (w/v) malt agar (MA; 5 g or 20 g Munton & Fison spray malt; 15 g Lab M agar no. 2 agar l^{-1} distilled water) at 5, 10, 15, 20, 25 and 30 ^{O}C in the dark. After 77 d of incubation at 5 ^{O}C, plates of two strains (KC1623 and 8.1) were transferred to 20 ^{O}C and extension was measured for the next 31 d at 5-6 d intervals. Additionally five replicates of KC1623 were incubated at - 18 ^{O}C for 7 d, and the plugs were then transferred to fresh MA plates and incubated at 20 ^{O}C for 8 wks, and subsequent growth noted.

Table 6. Details of *Piptoporus quercinus* isolates used

Strain reference	Source	Isolated by
KC1627	Bradgate Park, Leics	Kew culture collection
KC1623	Richmond Park, Surrey	Kew culture collection
8.1	Windsor Great Park	Paul Wald from a specimen collected by A. Martyn Ainsworth

Extension rate was also quantified on MA plates adjusted to six different water potentials ranging from close to 0 to -5 MPa using KCl (Robinson & Stokes 1959), incubated at 20 ^{O}C in darkness.

Effect of six different pH values (altered using KOH) between 3.1 and 6.8 (measured after autoclaving) was determined on MA, incubated at 20^{O}C in darkness. The effect of the fungus on the pH of the growth medium was determined after 32 d.

Decay rate of oak *Q. robur* heartwood (2 x 2 x 1 cm blocks cut from a freshly felled trunk) was determined on 2% MA, at 20^{O}C, after 34, 60 and 151 d.

5.2 Results

The optimum temperature for extension of all three isolates was 25^{O}C, but upper and lower temperature limits varied between strains (extension rate ranging between 1.9 and 3.15 mm d^{-1}; Figure 9). There were dramatic differences in colony morphology at different temperatures.

After exposure to -18 ^{O}C KC1623 had a lag of 37d followed by extremely limited growth. Preincubating plates at 5 ^{O}C for 77d and then incubating at 20 ^{O}C resulted in a significantly ($P \leq 0.001$) faster extension (2.89 mm d $^{-1}$) than when incubated continuously at 20 ^{O}C (1.70mm d^{-1}) for KC1623, though not for 8.1 (1.47 mm d^{-1} at 20 ^{O}C; 1.11 mm d^{-1} when raised from 5 to 20 ^{O}C). Colony morphology of both strains was different between these two temperature regimes.

All isolates grew at a water potential of -1.75 MPa but none were able to grow at -2.5 MPa, even after 70 d (Figure 10).

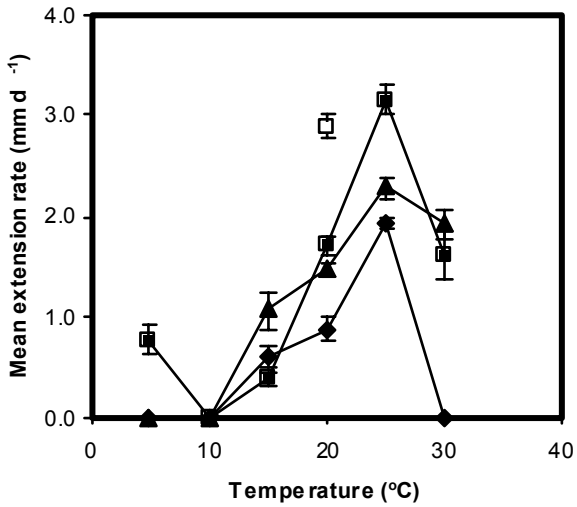


Figure 9. Effect of temperature on radial extension rate of *Piptoporus quercinus* isolates: ■, KC1623; ◆, KC1627 ; ▲, 8.1; □, KC1623 incubated at 5 °C then raised to 20 °C. (After Wald and others 2004)

The optimum pH for mycelial extension (2.23 and 2.21 mm d^{-1} for KC1627 and KC1623 respectively) was at a starting pH of around 4 (Figure 11). Growth occurred at pH 6.6 but not at pH 6.8. The lower limit for growth could not be determined; there was still growth at pH 1.8 and this was as rapid as at pH 3.75.

P. quercinus dramatically altered the pH of the growth medium, lowering it from a starting value of pH 5 or above in the central regions of plates (Figure 12). There was no lowering from pH 6.8, as under this regime there was no growth.

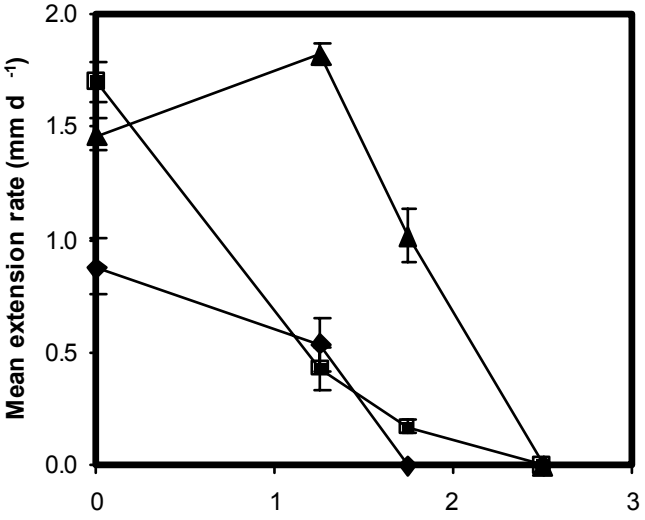


Figure 10. Effect of solute potential (KCl) on radial extension rate of *Piptoporus quercinus*: ▲, 8.1; ■, KC1623 and ◆, KC1627 at 20^{O}C on MA. (From Wald and others 2004)

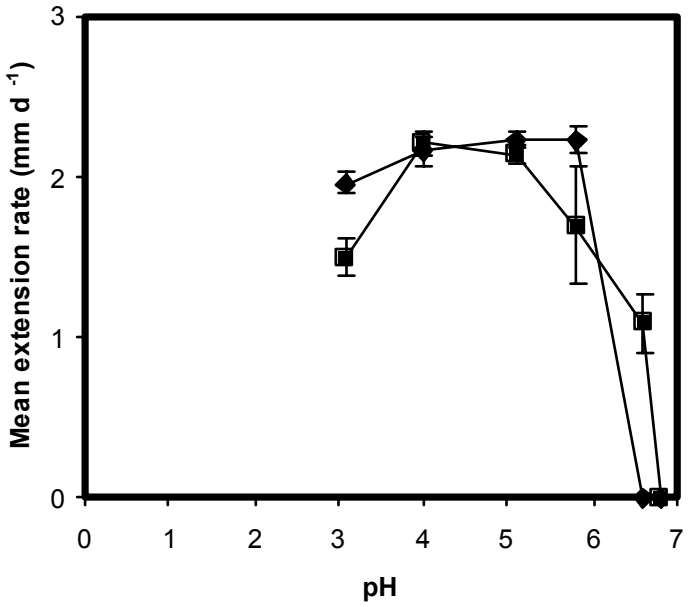


Figure 11. Effect of pH on radial extension rate of *Piptoponus quercinus* ■, KC1623 and ◆, KC1627 at 20^{O}C on MA. (From Wald and others 2004)

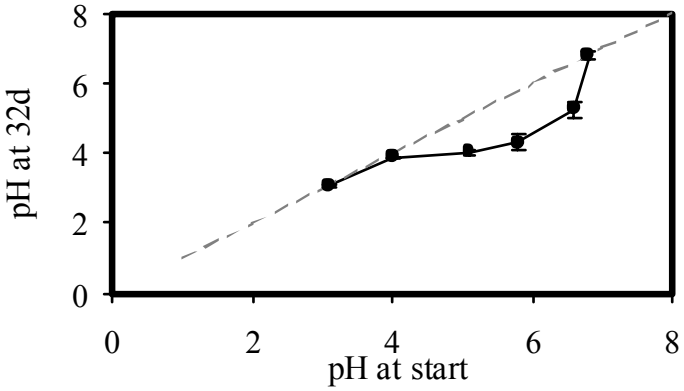


Figure 12. Alteration of medium pH in central regions after 32 d growth by *Piptoporus quercinus* KC1623 at 20^{O}C. Dashed line is pH of medium in the absence of *P. quercinus*. N.B. there was no growth at pH 6.8. (From Wald and others 2004)

In the decay rate experiment, *P. quercinus* brought about 4% weight loss after 151d.

5.3 Discussion

P. quercinus, like many heart rot fungi (Rayner & Boddy 1988), exhibits host specificity – to *Quercus*, which probably results from the highly selective abiotic environment and the way in which the fungus enters and establishes itself (Boddy 2001; Rayner & Boddy 1988). It is unlikely to be due to gaseous regime as there does not appear to be a difference in tolerance of heart rot fungi to elevated CO_2 and reduced O_2, compared with sap rot fungi (Highly and others 1983). Adaptation is more likely to be related to other environmental constraints, eg wood volatiles, extractives and pH. Oak heartwood has a high content of polyphenols (tannins) and a low pH (Gray 1958, Rayner & Boddy 1988), and the optimum pH for extension of *P. quercinus* correlates with that of oak heartwood. Some heart rot fungi, eg *Fistulina hepatica*, *Daedalea quercina* and *Laetiporus sulphureus,* which are found in similar locations to *P. quercinus*, are relatively tolerant of tannins (Cartwright 1937; Cartwright & Findlay 1958; Hintikka 1969, 1971); this may also be the case for *P. quercinus*, though we do not yet have information on effect of polyphenols. Like most brown rot fungi, *P. quercinus* has a low upper pH limit, presumably correlating with the susceptibility to pH of their cellulases and hemicellulases (see references in Rayner & Boddy 1988). The ability to lower substratum pH is a common feature amongst wood decay basidiomycetes, though brown-rot species lower pH more than white rot species (Rayner & Boddy 1988), and *P. quercinus* is no exception.

Cardinal points for temperature are similar to those of many basidiomycetes, though the ability to grow well at 30 ^{O}C or more may be advantageous in exposed wood. Also, survival at -18 ^{O}C is not unusual amongst wood decaying basidiomycetes (Rayner & Boddy 1988; Yakovlev and others 2000). However, exposure to sub zero temperatures may not be very common as heart wood is well buffered (Rayner & Boddy 1988), and it is not of adaptive significance for growth specifically in oak. The difference in extension rate when grown at constant temperature compared with when grown at a lower temperature and then raising that temperature, highlights the organism's ability to withstand fluctuating environmental conditions. Constant conditions are usually used in experiments on growth and physiology, but in another study using realistic temperature fluctuations, there was an increase in growth of *Laetiporus sulphureus* and *Stereum gausapatum* under fluctuating conditions compared to what would have been expected from data on extension under constant conditions (Jensen

1969). Clearly, much more research is needed using environmentally realistic fluctuating temperature regimes.

P. quercinus is even less tolerant to reduced water potential than most wood decay basidiomycetes, with respect to mycelial extension (Boddy 1983; Griffin 1981; Koske & Tessier 1986). It should, however, be remembered that using KCl reduces solute potential, rather than matric potential, and that both the K^+ and Cl^- ions may be toxic to the fungus (Boddy 1983; Griffin 1981). Further, ability to grow at low water potential is only one way of being successful in xeric conditions, survival is another. *P. quercinus* produces chlamydospores which may be crucial to survival under dry conditions, allowing the mycelium to grow again when conditions improve. This same strategy seems to be adopted by other wood decay fungi, for example, *Schizopora paradoxa* which is commonly found in attached oak wood prone to desiccation (Boddy & Rayner 1983).

Rate of wood decay by *P. quercinus* is slow, though a lot of other wood decay fungi are equally slow (Rayner & Boddy 1988). This certainly reflects the unfavourable conditions in oak heartwood, but may also be part of the ecological strategy of this fungus, ie slow growth, slow decay, and long occupancy of the substratum. There is some evidence, albeit not very strong, of long occupancy by *P. quercinus*; Roberts (2002) reported it fruiting on trees fallen for over 40 years, and it had presumably colonized while the tree was standing.

6. Interactions between *P. quercinus* and other wood decay fungi in artificial culture

6.1 Methods

Outcome of interactions with ten other decay fungi from oak wood (which have different ecological roles), and effects on extension rate were studied for two isolates (KC1627 and KC1623) (Table 7). Interactions were examined on 2% MA (pH 5.5), 0.5% MA (pH 5.9), 0.5% MA adjusted to - 1.25MPa using KCl, 0.5% MA adjusted to pH 3.75 (selected as a typical value for oak heartwood) in the dark at 20 ^{O}C. Control plates of each *P. quercinus* strain against itself were also made. Plates were observed every 1 – 2 d depending on extension rate; after 100 d, outcomes were recorded as deadlock, replacement or partial replacement.

During interaction between the heart rot fungi there was sometimes stimulation or inhibition in the presence of an opponent. Thus, pairings of *P. quercinus* were again made against the other species, and all were paired against self as above on 0.5% MA, for measurement of extension in different directions every 1 - 2d, on a line towards each other, away from each other, and along a diameter parallel with the line of interaction.

Table 7. Details of isolates against which *P. quercinus* was paired in interactions on agar

Ecological role	Species	Strain reference	Source	Isolated by
Primary colonizer, latently[1] present in attached stems and branches	*Stereum gausapatum*[4]	Sg1	Cardiff culture collection	L. Boddy
	Vuilleminia comedens	Vc1	Cardiff culture collection	L. Boddy
Heart rotter[1,2]	*Fistulina hepatica*	Fh1	Ebernoe Common	Paul Wald
	Piptoporus quercinus	KC1627	Bradgate Park, Leics	
	Piptoporus quercinus	KC1623	Richmond Park, Surrey	
	Piptoporus quercinus	8.1	Windsor Great Park	Paul Wald from a specimen collected by A. Martyn Ainsworth
Standing and fallen trunk colonizer (rare), more commonly on *F. sylvatica*[3]	*Hericium erinaceum*	H-MILL	USA	Anne Miller
	Creolophus cirrhatus	JHC1	Denmark	Jacob Heilmann-Clausen
	Hericium coralloides	MA-1	Windsor Great Park	A. Martyn Ainsworth
Secondary colonizers[1]	*Coriolus versicolor*	Cv1	Cardiff culture collection	L. Boddy
	Phlebia radiata	Prad1	Cardiff culture collection	L. Boddy
	Stereum hirsutum	Sh1	Cardiff culture collection	L. Boddy
Late secondary colonizing[1] cord-former	*Hypholoma fasciculare*	Hfas1	Cardiff culture collection	D.P. Donnelly

[1]Rayner & Boddy (1988), [2]Roberts (2002), [3]Boddy & Wald (2003), [4] also causes pipe-rot of heartwood

6.2 Results

P. quercinus was frequently replaced by the opposing fungus, though outcome sometimes varied depending on abiotic regime, and occasionally depending on strain (Table 8; Appendix 8 a -f). Under optimum conditions for growth (pH 3.75) *P. quercinus* was replaced or partially replaced (initial replacement followed by deadlock) by all opponents, except *Vuilleminia comedens* and *Fistulina hepatica* with strain KC1623. There was sometimes obvious non-contact inhibition (Appendix 8d, 8f, see below), often pigment production in the interaction zone extending into both opponents, and this was sometimes obvious before as well as after contact (Appendix 8d, 8e). Mycelium at the interaction front sometimes became massed into a raised barrage (Appendix 8e).

Table 8. Effect of abiotic environment on outcomes of interactions of *P quercinus* (KC1627 and KC1623) with other oak decay basidiomycetes after 85 - 100 d. Outcomes in brackets are for KC1623 when it differed from KC1627. (From Wald and others 2004)

	2% MA	**0.5% MA**	**-1.25 MPa 0.5% MA**	**pH 3.75 2% MA**
Stereum gausapatum	R	R	R	R
Vuilleminia comedens		D		D
Fistulina hepatica		D		PR(D)
Hericium coralloides		PR(D)		R
Hericium erinaceum		D/PR		
Coriolus versicolor	R	R	PR	R
Phlebia radiata	D/PR	R(D/PR)	D/PR(D)	R
Stereum hirsutum	R	R	PR(R)	PR
Hypholoma fasciculare	R	R	D(PR)	PR(R)

Abbreviations: D, deadlock, R, replacement of *P. quercinus* by opponent; PR, partial replacement of *P. quercinus* by opponent

Growth of *P. quercinus* appeared sometimes to be inhibited by self (data not shown), and it was occasionally inhibited by opponents, eg *F. hepatica* against KC1627. There was, however, also some significant stimulation of extension rate: *P. quercinus* KC1623 was stimulated when paired against *H. fasciculare*, *P. radiata*, *S. hirsutum* and *H. erinaceum*. Significantly, extension of several of the opponents was inhibited in the direction of *P. quercinus* (Table 9).

6.3 Discussion

P. quercinus appears to be a very poor combatant, at least on agar under the conditions used in the present study, including pH 3.75. However, outcome of interactions can vary dramatically under different regimes (Table 8; Boddy 2000), and there were noticeably more deadlocks on a medium with lower nutrient status. Volume of wood occupied can also have a large effect on outcome (Holmer & Stenlid 1993, 1997). Thus in the standing tree the large volume of low nutrient wood occupied by *P. quercinus* might considerably increase its antagonistic ability against other oak decay fungi. Moreover, *P. quercinus* was not easily replaced by fungi which it would most commonly encounter in standing heartwood of trunks, eg *F. hepatica*, and it certainly produces compounds inhibitory to other fungi (Table 9), which might again be even more effective in the huge volumes assumed to be occupied by this fungus in standing trees.

Table 9. Effect of *P. quercinus* on mean (±SEM) extension rate (mm d^{-1}) of an opposing colony compared with control pairings, and in the final column growth towards compared with growth away from *P. quercinus* (From Wald and others 2004)

	towards	away	diam.	T<A
C. cirrhatus v KC1623	0.43±0.18*	0.59±0.25**	2.85±1.40	
C. cirrhatus v KC1627	0.69±0.03	1.20±0.09*	1.69±0.13***	**
H. coralloides v KC1627	1.13±0.11^{S}**	1.21±0.07	2.62±0.31 ^{S}**	
F. hepatica v KC1623	0.25±0.05***	0.59±0.37	0.91±0.45*	
F. hepatica v KC1627	0.23±0.07***	1.19±0.17	1.39±0.51	***
H. erinaceum v KC1623	1.63±0.08**	2.78±0.31	6.46±0.27 ^{S} ***	**
V. comedens v KC1623	0.66±0.05***	1.24±0.07	2.42±0.64	***
P. radiata v KC1623	3.23±0.21	4.09±0.22	6.84±0.32	*
P. radiata v KC1627	1.10±0.08***	2.20±0.44	2.98±0.18***	ns
H. fasciculare v KC1623	0.98±0.24	0.74±0.32	1.64±0.57	ns
H. fasciculare v KC1627	0.84±0.21	0.99±0.29	1.77±0.41	ns
C. versicolor v KC1623	3.28±0.84	4.89±1.46	12.00±1.91	ns
C. versicolor v KC1627	1.39±0.05***	1.82±0.03***	3.14±0.09***	***
S. hirsutum v KC1623	2.92±0.07	2.45±0.08	7.54±0.37**	**S
S. hirsutum v KC1627	2.22±0.08***	2.36±0.11***	4.74±0.20***	ns
S.gausapatum v KC1623	3.28±0.26**	3.98±0.38	8.56±1.05**	ns
S.*gausapatum* v KC1627	1.83±0.30***	1.87±0.47	3.65±0.84	ns

significant inhibition compared with control pairings: *, $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$
^{S}, significant stimulation.
T<A, significantly slower growth towards than away from opponent with t test or Wilcoxon test, when data were non-normally distributed.

7. Molecular identification of *Creolophus cirrhatus*, *Hericium erinaceum* and *H. coralloides*

7.1 Introduction

To discover the spatial distribution of tooth fungi in standing trees and fallen trunks and limbs, cores could be taken with an increment borer, leaving most of the wood undisturbed. The presence of a particular fungus could then be determined by making isolations on agar media and subsequently identifying outgrowing mycelia, or by extracting DNA/RNA from wood and identification using modern molecular approaches. The former, though inexpensive to perform suffers from the inherent difficulties of finding sufficient discriminating characters in white mycelial cultures, and problems of lack of current activity by the mycelia in the wood. Molecular approaches have the advantage that even if the fungus is dormant or 'mixed' with another species identification would still be possible. However, they can be expensive to develop and finding discriminatory molecular sequences is not always easy. A PCR-based method for detecting *Hericium* species using ribosomal internal transcribed spacer (ITS) sequences has been reported (Lu and others 2002). In this paper there is confusion between the terms strain, species and genus, but the authors are probably reporting on identification to genus. The work clearly needs checking. As well as finding appropriate discriminatory molecular sequences, methods need to be developed for extracting DNA/RNA from wood.

7.2 Method and approach

7.2.1 Testing primers reported in the literature

Firstly, the pair of primers, HT-U1 and HT-L1 (termed here HERF and HERR respectively), designed by Lu and others (2002) were tested against two strains each of *Creolophus cirrhatus, Hericium erinaceum* and *H. coralloides*, one strain of *H. alpestre*, and single strains of a range of wood decay fungi common in beech and oak (Table 10). The DNA extraction protocol, PCR mixture and PCR conditions were as listed below. The primers used were:

Forward HERF	AGGCATGTGCACGCTCCAATCTC
Reverse HERR	CAAGACCTCCAAGTCCAAGCCCA

Protocol for extraction of DNA from samples grown on agar

1) Scrape mycelium from 20 cm^2 of agar into a 1.5 cm^3 Eppendorf tube.
2) Add 300µl of extraction buffer (200mM Tris HCl, 250mM NaCl, 200mM EDTA , 0.5% w/v SDS, pH 8.5) and 300µl of 0.4% w/v dried skimmed milk.
3) Vortex and microcentrifuge at 13000rpm for 5 min.
4) Transfer supernatant to a fresh 1.5 cm^3 Eppendorf tube.
5) Add 150µl 3M sodium acetate.
6) Stand at –20˚C for 10 min.
7) Microcentrifuge at 13000rpm for 5 min.
8) Transfer supernatant to a fresh 1.5 cm^3 Eppendorf tube.
9) Add an equal volume of isopropanol.
10) Stand at room temperature for 5 min.
11) Microcentrifuge at 13000rpm for 5 min.
12) Remove supernatant and wash pellet with 500µl of 70% v/v ethanol.
13) Microcentrifuge at 13000rpm for 5 min.
14) Air dry and re-suspend in 50µl of water.

PCR amplification of extracted DNA

96˚C for 15 min
35 cycles of 94˚C for 1 min, 57˚C for1 min, 72˚C for 1 min.
72˚C for 10 min.

PCR mix (per sample)

Water	18.875µl	
10X buffer	2.5µl	
10mM dNTPs	0.5µl	
HERF primer (100ng/µl)	1.0µl	
HERR primer (100ng/µl)	1.0µl	
HotStar Taq	0.125µl	
Template	1.0µl	(10% dilution of agar extract or 1% dilution of wood extract)

Different PCR conditions were tested to determine which specifically amplify DNA from *Hericium* species. Annealing temperatures, template concentrations, Ca^{2+} concentrations, primer concentrations and polymerase concentrations were varied, and reagent Q tried, to optimise specificity. PCR products were run on 1.5% agarose gel with ethidium bromide and bands visualised using UV.

7.2.2 Testing new primers for specificity

New primers were designed from database sequences. These were tested to determine whether the primer pair HER2F/HER3R is specific for *C. cirrhatus* and whether the primer pair HER2F/HER2R is specific for *H. erinaceum, H. coralloides* and *H. alpestre*.

HER2F	ATCTCATCCATCTTACACC
HER2R	CTCATAACAAGAGGATTGA
HER3R	CATATGACAAGAGGATCGA

DNA was extracted and cleaned as described in section 8.2.1., using the treatment conditions and mixture listed below.

PCR treatment

96˚C for 15 min
35 cycles of 94˚C for 1 min, 50˚C or 53˚C for1 min, 72˚C for 1 min.
72˚C for 10 min.

PCR mix (per sample)

Water	18.875µl
10X buffer	2.5µl
0mM dNTPs	0.5µl
HER2F primer (100ng/µl)	1.0µl
HER2R or HER3R primer (100ng/µl)	1.0µl
HotStar Taq	0.125µl
Template	1.0µl (10% dilution of agar extract or 1% dilution of wood extract)

DNA extracts from *C. cirrhatus H. erinaceum, H. coralloides, H. alpestre, Eutypa spinosa, Diatrype disciformis* and *Stereum gausapatum,* grown on agar, were used as templates for PCRs using HER2F/HER3R or HER2F/HER2R at annealing temperatures of 50˚C or 53˚C. The PCRs at 53˚C annealing temperature were repeated with both primer pairs and all 36 DNA extracts (Table 10). PCR products were run on 1.5% agarose gel with ethidium bromide and bands visualised using UV light.

Table 10. Total DNA concentration ($\mu g\ ml^{-1}$) extracted

Tube	Species	DNA
1A	*H. coralloides (i)*	1
2	*H. erinaceum (ii)*	21
3	*C. cirrhatus (ii)*	3
4	*H. alpestre*	2
5	*Eutypa spinosa (i)*	9
6	*Diatrype disciformis (i)*	14
7	*Stereum gausapatum (i)*	6
8	*Stereum rugosum (i)*	6
9	*Chondrostereum purpureum*	14
10	*Coniophora puteana*	18
11	*Vuilleminia comedens*	12
12	*Biscogniauxia nummularia (i)*	16
13	*Inonotus obliquus*	21
14	*Bjerkandera adusta (i)*	16
15	*Phlebia radiata (i)*	26
16	*Ganoderma applanatum*	12
17	*Fomes fomentarius*	19
18	*Fistulina hepatica*	42
19	*Biscogniauxia nummularia (ii)*	55
20	*Piptoporus quercinus*	36
21	*Peniophora lycii*	8
22	*Coprinus picaceus*	9
23	*Ceriporiopsis gilvescens*	36
24	*Lycoperdon pyriforme*	8
25	*Phanerochaete velutina*	46
26	*Antrodiella semisupina*	45
27	*Hypholoma fasciculare*	7
28	*Stereum hirsutum*	19
29	*Phlebia radiata (ii)*	69
30	*Bjerkendera adusta (ii)*	7
31	*Eutypa spinosa (ii)*	
32	*Diatrype disciformis (ii)*	
33	*Stereum gausapatum (ii)*	
34	*Stereum rugosum (ii)*	
35	*H. erinaceum* from wood	
36	*Heterobasidion annosum*	

7.2.3 DNA extraction from wood

A protocol (see below) for extracting DNA from wood colonized by *H. erinaceum* (artificially cultured on 0.5% malt agar for several months at room temperature in the laboratory) was also tested.

Protocol for extraction of DNA from samples grown on beech wood blocks

1) Wipe all external fungus from block.
2) Cut block open and scrape 50mg from freshly exposed surface (no pieces bigger than 0.5mm cubes) and wrap in foil.
3) Immerse in liquid nitrogen for at least 5 min.

4) Transfer to a mortar and, using liquid nitrogen additions, grind to a fine dust.
5) Add 300µl of extraction buffer and grind to a fine dust.
6) Thaw and transfer to a 1.5 cm^3 Eppendorf tube.
7) Wash mortar with 300µl of 0.4% w/v dried skimmed milk and transfer washings to the Eppendorf tube.
8) Proceed as extraction from agar from step 3.
9) Clean DNA using QIAquick spin method (handbook p18).

7.3 Results

7.3.1 Testing primers reported in the literature

Extraction techniques were successful. Concentrations are listed in Table 11.

With an annealing temperature of 57^O C (as used by Lu and others 2002) not only was there a strong positive response for the tooth fungi but also weak or moderate bands for most of the other wood decay fungi (Table 11). Raising the temperature to 58^O C weakened the response of some of the other wood decay species in one attempt, but in another attempt there was no response from any of the species. Increasing the annealing temperature to 60˚C to increase specificity led to failure of PCR to amplify except for one anomalous positive, of *Stereum rugosum* (Table 11).

Band strengths with different template concentrations, Ca^{2+} concentrations, primer concentrations, polymerase concentrations and reagent Q, using *H. erinaceum* and *E. spinosa* at 57˚c annealing temperature are shown in Figure 13.

7.3.2 Testing new primers for specificity

PCRs using primer pair HER2F/HER3R were specific for *C. cirrhatus* at annealing temperatures of both 50˚C and 53˚. PCRs using primer pair HER2F/HER2R were specific for but could not discriminate between *H. erinaceum, H. coralloides* and *H. alpestre* at an annealing temperature of 53˚C but not at 50˚C.

7.3.3 DNA extraction from wood

Using *HERF* and *HERR*, running a PCR with DNA extracted from *H. erinaceum* grown on beech wood, at a 1% dilution, produced a strong band. Using undiluted template, 0.1% and 10% dilution did not produce a band. A positive band was detected using DNA extracted from *H. erinaceum* cultured on wood with *HER2F/HER2R* primers, but this was much weaker than those from *Hericium* species cultured in agar (section 8.3.2).

7.4 Conclusions

The HERF/HERR primers designed by Lu and others (2002) do not appear to discriminate between tooth fungi and other wood decay species. With HERF/HERR primers, an annealing temperature of 57˚C appears too low giving poor specificity, but an annealing temperature of 60˚C appears too high with no amplification. An annealing temperature of 58˚C appears borderline between the two. The theoretical Tm for these primers is ~ 72˚C. This suggests that there are no conditions in which these primers will be completely specific for *Hericium* species.

Table 11. PCR checks of fungi grown in culture medium or beech wood blocks with HT-U1/ HT-L1 primers

Tube number	Species	DNA Conc^{n} µg ml^{-1}	PCR check (+ve/-ve) at annealing temp of:-			
			57˚C	58˚C	58˚C	60˚C
2A	*H. erinaceum (i)*	2	Strong +ve			-ve
3A	*C. cirrhatus (i)*	3	Strong +ve			-ve
1	*H. coralloides (ii)*	20	-ve	+ve	-ve	-ve
1A	*H. coralloides (i)*	1	Strong +ve	Strong +ve	-ve	-ve
2	*H. erinaceum (ii)*	21	Strong +ve	Strong +ve	-ve	-ve
3	*C. cirrhatus (ii)*	3	Strong +ve	Strong +ve	-ve	-ve
4	*H. alpestre*	2	Strong +ve	Strong +ve	-ve	-ve
5	*Eutypa spinosa (i)*	9	+ve	+ve	-ve	-ve
6	*Diatrype disciformis (i)*	14	+ve	+ve	-ve	-ve
7	*Stereum gausapatum (i)*	6	+ve	Weak +ve	-ve	-ve
8	*Stereum rugosum (i)*	6	-ve	Weak +ve	-ve	+ve
9	*Chondrostereum purpureum*	14	+ve		-ve	
10	*Coniophora puteana*	18	-ve		-ve	
11	*Vuilleminia comedens*	12	+ve		-ve	
12	*Biscogniauxia nummularia (i)*	16	+ve		-ve	
13	*Inonotus obliquus*	21	+ve		-ve	
14	*Bjerkandera adusta (i)*	16	+ve		-ve	
15	*Phlebia radiata (i)*	26	-ve		-ve	
16	*Ganoderma applanatum*	12	-ve		+ve	
17	*Fomes fomentarius*	19			-ve	
18	*Fistulina hepatica*	42			-ve	
19	*Biscogniauxia nummularia (ii)*	55			+ve	
20	*Piptoporus quercinus*	36				
21	*Peniophora lycii*	8				
22	*Coprinus picaceus*	9				
23	*Ceriporiopsis gilvescens*	36				
24	*Lycoperdon pyriforme*	8				
25	*Phanerochaete velutina*	46				
26	*Antrodiella semisupina*	45				
27	*Hypholoma fasciculare*	7				
28	*Stereum hirsutum*	19				
29	*Phlebia radiata (ii)*	69				
30	*Bjerkendera adusta (ii)*	7				
31	*Eutypa spinosa (ii)*				-ve	
32	*Diatrype disciformis (ii)*				-ve	
33	*Stereum gausapatum (ii)*				-ve	
34	*Stereum rugosum (ii)*				-ve	
35	*H. erinaceum* from wood				-ve	

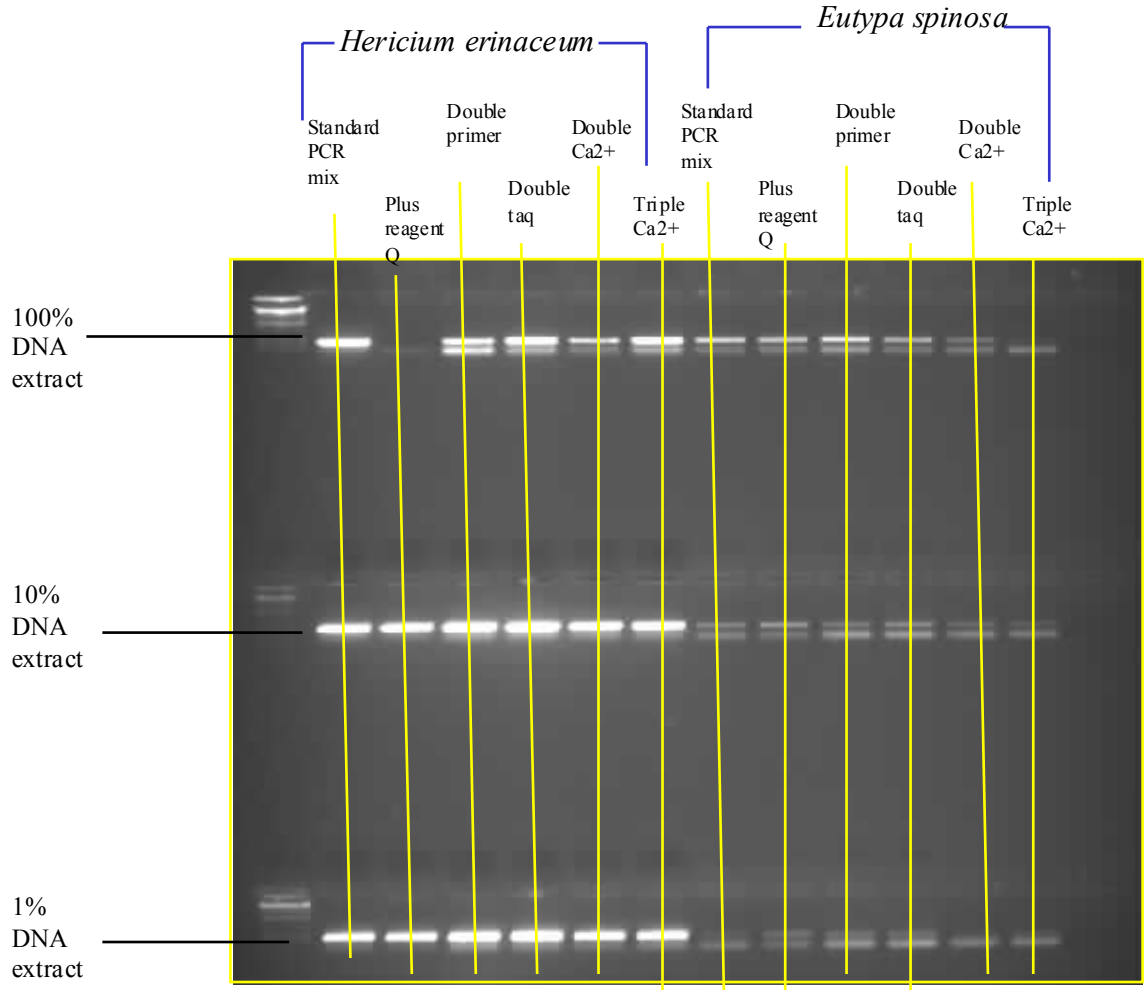


Figure 13. PCR optimisation

The biggest differences between *H. erinaceum* and *E. spinosa* were at 1% template dilution and with increased Ca^{2+} concentration. Decreasing the number of PCR cycles from 35 to 33 almost removed the control, *E spinosa,* bands. Further reducing the cycles, perhaps in conjunction with 1% template dilution and with increased Ca^{2+} concentration could make the PCR *appear* specific for *Hericium* species. This might be acceptable if the template DNA concentration was kept constant for every sample, but this is not possible if DNA is to be extracted from field samples. The maximum template concentrations used were similar to those used by Lu and others (2002).

HER2F/HER2R primers designed in the present study are specific for, but do not discriminate between, *H. erinaceum, H. coralloides* and *H. alpestre* at an annealing temperature of 53˚C. they were not specific at 50˚C. *HER2F/HER3R* primers designed here are specific for *C. cirrhatus* at an annealing temperature of 53˚C and could well be at 50˚C although only three control species were used at this temperature. Thus, there may be scope to reduce the annealing temperature if band strengths of *C. cirrhatus* from wood are too weak. These primer sets can, therefore, be used to discriminate between unidentified fungal samples.

Hericium DNA extracted from wood is clean enough to allow PCRs to work but the reaction seems very dependent on template concentration. Possibly it is still too contaminated to work at higher concentrations and a second cleanup or an improved cleanup method might help.

This needs to be investigated further if the method is to be successfully employed with field samples. Using *HER2F/HER2R* primers, the band from *H. erinaceum* extracted from wood was relatively weak, perhaps indicating that these primers are more sensitive to impurities than HERF/HERR and a more rigorous cleanup of DNA from wood may be necessary.

8. Conclusions and future research priorities

In conclusion, we now know that these three species of tooth fungi have similar cardinal temperatures, water potential relationships and extension rates to many wood decay basidiomycetes, and prefer a low pH. Basidiospores may be the main dispersal mode: all species fruit readily and prolifically in agar culture, in the presence and absence of other fungi; in the field, *H. coralloides* and *H. erinaceum* fruit repeatedly on the same host, though we do not yet know how soon this occurs after initial colonization; *C. cirrhatus*, however, seems not to fruit repeatedly on a host. Basidiospores of *H. coralloides* and *H. erinaceum* germinate readily, though those of *C. cirrhatus* do not. All three species are average to good combatants which helps to explain their long residency in wood. However, we do not yet know how, when or where colonization of wood occurs. Nor do we know about the breeding biology and population structure of UK tooth fungi. These questions need answering before we can understand why these species are rare.

P. quercinus grows and decays wood slowly, prefers a low pH, and is a poor combatant in agar culture but does inhibit some fungi at a distance. We have, however, no knowledge of how the fungus arrives and establishes in oak heartwood, and no knowledge of its population structure. Again, basidiospores may be the main dispersal mode, but we know nothing of their production, spread, survival, or germination. Nor do we really know how the fungus remains for what we assume to be a long time in heartwood, other than that oak heartwood is inimical to the growth of most fungi, and that *P. quercinus* produces chlamydospores. Clearly much more ecological, physiological and genetical work is urgently needed before we can begin to understand why this fungus is so rare, whether it always has been rare, and whether it is likely to be possible to conserve it.

- **Spore germination.** Knowledge of effect of abiotic variables, including temperature, pH, polyphenols, water potential and gaseous regime, is important not only for understanding how and under what circumstances colonization is likely to occur, but also to determine suitable conditions for making single spore isolations for mating experiments, and for spore dispersal studies (see below). Using gradient plates (Boddy and others 1989) allows multiple conditions to be tested on single plates. Information is needed for basidiospores of all of the tooth fungi and of *P. quercinus*, and also for chlamydospores of the latter.
- **Population structure and breeding biology.** No information is available on UK populations. Molecular approaches need to used to investigate heterogeneity in populations within and between sites. Some studies have already been performed, by other authors, on the breeding biology of the wood-inhabiting tooth fungi. *H. coralloides*, *H. erinaceum*, *H. abietis* and *H. americanum* probably have a bifactorial mating system (ie there is a 25 % chance of successful mating in pairwise combinations of mycelia obtained from spores produced by a single fruit body) (Ginns 1985). With inbreeding success thereby reduced to 25%, the mating system of these fungi favours outbreeding, ie mating between mycelia obtained from spores produced by different fruit bodies. However, it has also been suggested that *H.*

coralloides is amphithallic (=homothallic) (Hallenberg 1983), ie sexual reproduction can occur on a mycelium derived from a single basidiospore, but this was largely based on observing single spore isolates with clamp connections (structures associated with mating in some, but not all, species) obtained from a fruit body which had developed in culture. Germlings that were small tended not to have clamps, whereas faster growing germlings were clamped. Haploid fruiting (ie fruiting of a culture that has not mated) was observed in single spored cultures of *H. coralloides* and *H. alpestre*, resulting in bi-sterigmate basidia formed directly on the mycelium. Crossing tests indicated tetrapolarity (ie there is a 25% chance of successful mating with mycelium formed from spores taken from a single fruit body), though only a few crosses were made (Hallenberg 1983). It would be useful to determine the mating systems of the British species.

Mating tests need to be performed for all species. Information on mating genetics is not only important for understanding population structure, but is a necessary prerequisite prior to investigating dispersal by spore trapping using unmated cultures as baits. Single spore isolates have been obtained and are maintained in culture from several fruit bodies of *H. coralloides* and can be used for mating studies. Single spore isolates of *H. erinaceum* have not yet been made, but this is not likely to be difficult. However, since spores of *C. cirrhatus* do not germinate readily, few single spore isolates are available, and it is likely that it will only be possible to investigate its population biology using molecular approaches. Sets of single spore isolates of *P. quercinus* have not yet been obtained.

- **Spore dispersal.** The extent to which basidiospores of the tooth fungi and oak polypore are disseminated from fruit bodies in nature could be determined using traditional spore sampling devices. A better option would be to use agar plates baited with unmated cultures (Adams and others 1984), which would then be selective for a specific species. These could be placed at various distances from fruit bodies. Modelling approaches similar to those employed by Stenlid (pers. comm.), investigating a rare wood decay fungus in Sweden, could be used to determine which factors influence distribution.
- **Entry and establishment in wood in the field.** We successfully inoculated *H. coralloides* into uncolonized felled logs in the field, but not into logs already colonized by other fungi. No information is yet available on the other two tooth fungi although a small number of logs were inoculated and should be harvested within six months. Information on how these fungi enter and become established naturally in standing trees is urgently needed. The successful development of PCR primers for identifying the tooth fungi will be an invaluable tool in detecting small foci of latent propagules within functional tree tissues.
- Information is urgently needed on entry and establishment of *P. quercinus* in standing trees, and whether it enters fallen parts. Inoculations (mycelia, basidiospores and chlamydospores) should be made into different locations within standing oaks, including heartwood exposed by wounds, limb pruning and large broken branches, branch and trunk sapwood, twigs and scars. Colonization progress could be followed by removing small samples for identification (by traditional isolation and molecular techniques - which must be developed), and subsequently by destructive harvesting. A survey of heartwood samples from oaks, some of which have and others which have not borne fruit bodies of *P. quercinus*, in different locations could help to determine the distribution and abundance of this species.

- **Survival and interspecific interactions in wood in the field.** The inoculation experiments already set up for *H. coralloides* can be harvested after a further 2, 5 and more years to determine longevity, extent of replacement and by what species. There will also be a few logs colonized by *H. erinaceum* available. It is now necessary to inoculate logs with *C. cirrhatus* and, more importantly, *P. quercinus*. It is likely to be necessary to colonize wood with the latter and/or to obtain some naturally colonized wood, eg from wind blown oak.

9. References

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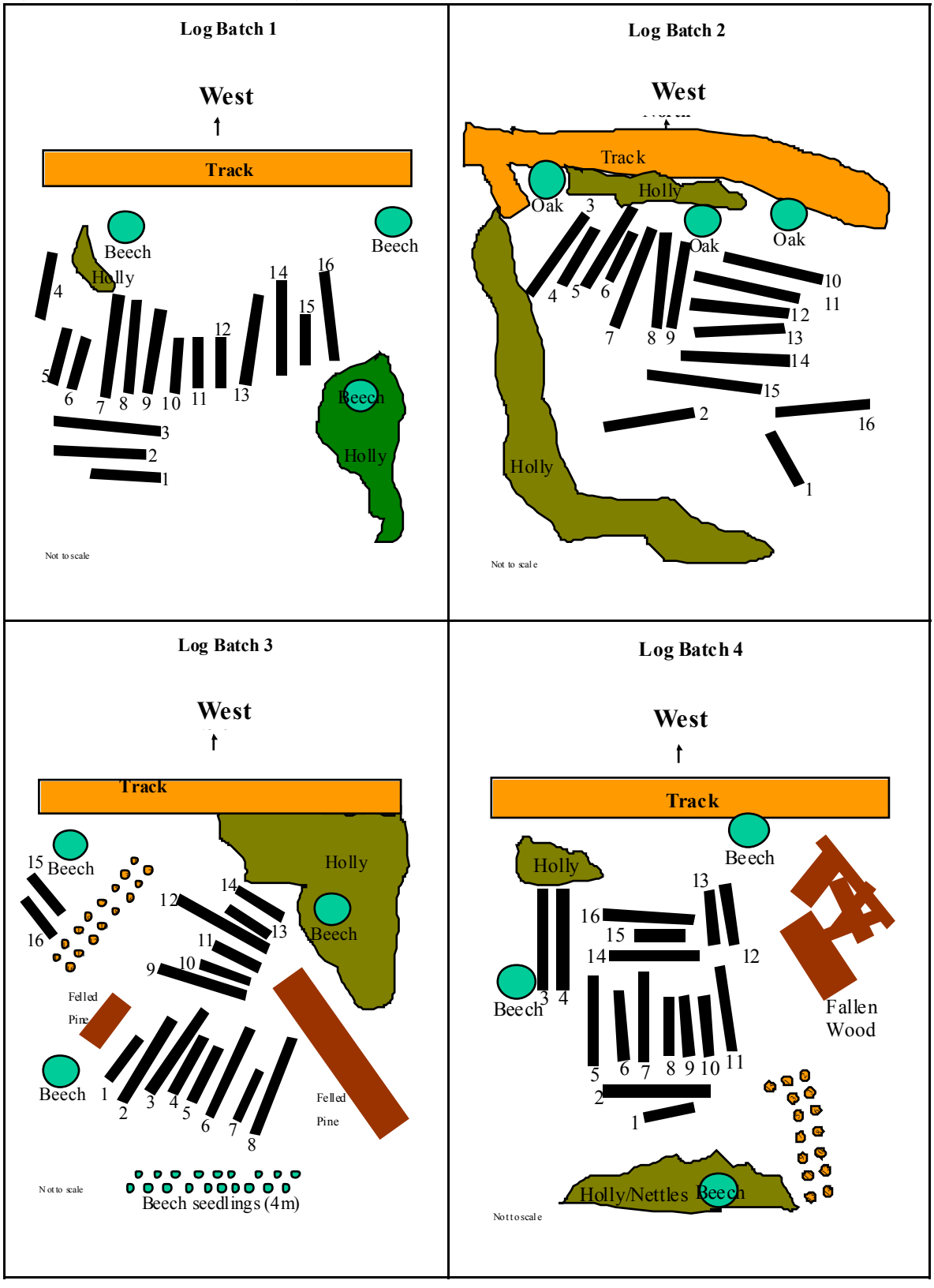
Appendix 1. Locations of logs inoculated at Windsor Great Park

(Nat. Grid Ref. SU 935 740)



Appendix 2. Details of beech logs into which *H. coralloides* was inoculated at Windsor Great Park

(Nat. Grid Ref. SU 935 740)

Key to treatments
EC, Inoculated into cut logs with *Hericium coralloides*
EU, Inoculated into uncut logs with *Hericium coralloides*
CC, Inoculated into cut logs with sterile dowels
CU, Inoculated into uncut logs with sterile dowels
SLGE, Upright logs of 65cm length and 15-20cm diameter inoculated on exposed ends
SLGC, as for SLGE, but dowels were uncolonized

Batch No.	Log No.	Treatment	Length (cm)	Diam.1 (cm)	Diam.2 (cm)	Age. Diam	% Bark cover	Fruit bodies along length	Fruit bodies on ends
1	1	EC	226.5	31	22	26.5	85	Nc	Rud.
1	2	EU	230	18.5	20.5	19.5	85	Nc	Cp
1	3	EU	220.75	16.5	19	17.75	90	Nc	Cp
1	4	CC	233	17	19	18	55	Nc	Rud.
1	5	EC	210	18.5	27	22.75	90	Nc	Rud.
1	6	EC	233	18	22	20	75-80	Nc	Rud.
1	7	CU	211.5	18.5	17	17.75	80-85	Nc	Rud.
1	8	CU	220	18	17.5	17.75	85-90	Nc	Cp
1	9	CU	216	22	16	19	80-85		Cp
1	10	CC	227	17.5	17.5	17.5	65-70		Rud.
1	11	CC	215	21	28	24.5	70-75	Nc	Rud.
1	12	EC	223	22	27	24.5	65	Nc	Rud
1	13	EU	226	25	18	21.5	90	Nc	
1	14	EU	223	19.5	29.5	24.5	85	Nc	Cp
1	15	EC	220	19	16.75	17.875	80		Rud.
1	16	EU	229	19.5	19.5	19.5	85	Nc	
2	1	EC	232	14.5	17	15.75	85-90	Nc	
2	2	EU	231	15.5	20	17.75	75-80	Nc	Cp
2	3	CU	224	19	22.5	20.75	65-70	Nc	
2	4	CC	227	23.25	18	20.625	90		Rud.
2	5	CU	227	19.5	22.5	21	80-85	Nc	Cp
2	6	EU	230	18	16.5	17.25	75-80		Rud.
2	7	CC	227	20.5	18	19.25	75-80		Rud.
2	8	CU	224	23.5	18.5	21	75	Nc	Cp
2	9	EU	235	18.5	15.5	17	80	Nc	Cp
2	10	EC	231	31	20	25.5	85	Hf	Rud.
2	11	EU	233	23	17	20	80		Cp
2	12	EC	220	19.75	16	17.875	70-75	Hf	Rud.
2	13	EC	215	19.75	16	17.875	70-75	Nc	Rud.
2	14	EC	215	17.5	15.75	16.625			
2	15	E	228	17	21.5	19.25	70-75	Nc	Cp
2	16	EC	230	19.5	17	18.25	75-80		Rud.
3	1	CC	225	18.5	23.5	21	60	Hf, Nc	Rud.
3	2	EU	217	22	28	25	75-80	Nc	Cp

Batch No.	Log No.	Treatment	Length (cm)	Diam.1 (cm)	Diam.2 (cm)	Age. Diam	% Bark cover	Fruit bodies along length	Fruit bodies on ends
3	3	EU	224	29	25	27	50-60	Nc	Cp
3	4	EC	230	25.75	31	28.375	70	Nc	Rud.
3	5	EC	220.5	32	28	30	70	Nc	Rud.,Cp
3	6	EU	220	26	32	29	75	Nc	Cp
3	7	EC	218	29.5	30	29.75	75-80	Nc	Rud.
3	8	CU	228	21	19	20	85	Nc	
3	9	EU	207	18	30	24	85-90	Nc	Rud.
3	10	EC	222	15	19	17	80	Nc	Rud.
3	11	EC	220.5	20	20	20	85	Nc	Rud.
3	12	EU	217	20	17.5	18.75	85-90	Nc	Cp
3	13	CC	227	19	21.25	20.125	70	Nc	Rud.
3	14	CC	225.5	18.75	21	19.875	90		Cp
3	15	CU	230	23	20	21.5	65-70		Rud.
3	16	CU	227.5	30	27	28.5	65-70	Nc	Cp
4	1	EC	225	18.5	23.5	21	65	Nc	
4	2	EU	217	22	28	25	90	Nc	Cp
4	3	EU	224	29	25	27	80	Nc	Cp
4	4	EC	230	25.75	31	28.375	85-90	Nc,Hf	Trich
4	5	CU	220.5	32	28	30	50-60		Cp
4	6	EC	220	26	32	29	70	Nc	
4	7	EU	218	29.5	30	29.75	85	Hf	
4	8	EU	228	21	19	20	70	Nc	Cp
4	9	EC	207	18	30	24	70-75	Nc	Rud
4	10	EC	222	15	19	17	75	Nc	Rud
4	11	EU	220.5	20	20	20	85-85	Nc	Cp
4	12	CC	217.5	20	17.5	18.75	80	Nc	Rud.
4	13	CC	227	19	21.25	20.125	90	Nc	Rud.
4	14	CU	225.5	18.75	21	19.875	80		
4	15	CC	230	23	20	21.5	50	Nc	
4	16	CU	227.5	30	27	28.5	80		
3	1	SL GE	65-70			20-25			
3	2	SL GC	65-70			20-25			
3	3	SL GE	65-70			20-25			
3	4	SL GC	65-70			20-25			
3	5	SL GE	65-70			20-25			
3	6	SL GC	65-70			20-25			
3	7	SL GE	65-70			20-25			
3	8	SL GC	65-70			20-25			
3	9	SL GE	65-70			20-25			
3	10	SL GC	65-70			20-25			
3	11	SL GE	65-70			20-25			
3	12	SL GC	65-70			20-25			
3	13	SL GE	65-70			20-25			
3	14	SL GC	65-70			20-25			
3	15	SL GE	65-70			20-25			
3	16	SL GC	65-70			20-25			
4	1	SL GE	65-70			20-25			
4	2	SL GC	65-70			20-25			

Batch No.	Log No.	Treatment	Length (cm)	Diam.1 (cm)	Diam.2 (cm)	Age. Diam	% Bark cover	Fruit bodies along length	Fruit bodies on ends
4	3	SL GE	65-70			20-25			
4	4	SL GC	65-70			20-25			
4	5	SL GE	65-70			20-25			
4	6	SL GC	65-70			20-25			
4	7	SL GE	65-70			20-25			
4	8	SL GC	65-70			20-25			
4	9	SL GE	65-70			20-25			
4	10	SL GE	65-70			20-25			
4	11	SL GE	65-70			20-25			
4	12	SL GE	65-70			20-25			
4	13	SL GE	65-70			20-25			
4	14	SL GE	65-70			20-25			
4	15	SL GE	65-70			20-25			
4	16		65-70			20-25			

Fructification codes:
Hf= *Hypoxylon fragiforme*
Nc= *Nectria cinnabarina*
Trich= *Trichoderma* sp.
Rud. = unidentified ruderal species
Cp = *Chondrostereum purpureum*

Appendix 3. Details of trees into which *H. coralloides* and *H. erinaceum* were inoculated at Coed Gorllwyn, Bangor

Nat. Grid Ref. SH 589 422. Inoculations were performed during 9-11 November, 2002.

Tree Code	Treatment	Inoculum	Dbh (cm)	Moss Cover Height (cm)	Notes
6	EXPDWL	Hc	6.25	30	Butt Wound
8	EXPDWL	Hc	9	38	Butt Wound
13	EXPDWL	Hc	9.5	600	
14	EXPDWL	Hc	6.75	68	
9	EXPDWL	Hc	7.5	48	
4	EXPDWL	Hc	8	400	
10	EXPDWL	Hc	10.5	85	
3	EXPDWL	Hc	7.5	25	
12	EXPDWL	Hc	8	48	
11	CONDWL	Hc	6.75	94	
5	CONDWL	Sterile	9	500	
7	CONDWL	Sterile	5	43	
2	CONDWL	Sterile	10	400	
9	CONDWL	Sterile	7.5	48	
15	CONDWL	Sterile	6.5	44	
16	EXPAG	Hc,He,c	8.5	46	
17	EXPAG	Hc,He,c	6.25	80	
18	EXPAG	Hc,He,c	5	34	
19	EXPAG	Hc,He,c	8.5	39	
20	EXPAG	Hc,He,c	9.25	150	
21	EXPAG	Hc,He,c	7	400	
22	EXPAG	Hc,He,c	8	28	
23	EXPAG	Hc,He,c	4.5	30	
24	EXPAG	Hc,He,c	6.25	54	
25	EXPAG	Hc,He,c	9.25	20	
26	EXPAG	Hc,He,c	9.5	38	
27	EXPAG	Hc,He,c	10.5	18	
28	EXPAG	Hc,He,c			
29	EXPAG	Hc,He,c			
30	EXPAG	Hc,He,c			
ASH 31	EXPDWL	Hc	11	10	
ASH 31	EXPDWL	Hc	8	10	
ASH 31	EXPDWL	Hc	7	10	

Key: **EXPDWL**, trees inoculated at four heights (50, 100, 150 and 200cm) with dowels colonised with *Hericium coralloides*
CONDWL, as for **EXPDWL**, but inoculated with sterile dowels
EXPAG, Trees inoculated with agar discs
Hc, *H. coralloides*; He, *H. erinaceum*; c, *C. cirrhatus*

Appendix 4. Details of beech logs (freshly felled) into which *H. coralloides* (treatments ES and EU logs 1 - 59) and *H. erinaceum* (logs 60 - 71) were inoculated in the Forest of Dean

Nat. Grid Ref. SO 552 170. Inoculations were performed during July, 2002.

Log No.	Treatment	Length	Diam.1	Bark	Fructifications
		(cm)	(cm)	Cover (%)	Length
1	CS3	88	15.5	50	
2	CS4	86	10	70	Bn/Hf
3	CS5	87.5	11.5	85	Hf/Nc
4	CS6	83	14	80	Bn
5	CU?no tag	88	13	55	Bn/Hf
6	CS8	88	13.5	65	Hf/Bn
7	CU?no tag	90	16	75	Bn/Hf
8	CS10	90	14	70	Bn
9	CU?no tag	89	11	85	
10	CS12	90	15	85	Hf/Nc
11	EU16	89	9.5	65	Bn
12	EU15	88	15	75	Bn/Hf
13	ES6	89	11.5	95	Bn/Hf/Nc
14	EU14	91	11	65	Hf
15	CU2	89	16.5	55	Bn
16	CS2	88	12.5	85	Bn/Hf
17	CU12	90	14	60	Bn
18	CU10	86	11	65	Hf
19	EU11	89.5	13	65	Hf,Bn
20	CU8	92	8	85	Bn
21	EU10	90	11.5	70	
22	?U?	87	16.5	45	
23	ES5	87.5	11.5	60	Hf
24	ES14	86	14	75	Cv,Hf
25	ES13	89	10	75	Hf
26	CS?	93	11	75	Hf
27	CU14	96	15	60	Hf
28	CU?no tag	90	12.5	35	
29	CU?no tag	94	8	80	
30	CU4	90	13	40	
31	CU5	90	11	70	
32	CU7	92	12.5	65	Hf/Bn
33	CU1	89	10	85	Cv/Hf
34	CU11	90	12	85	
35	CU12	92	11	75	
36	CS?	88	11	75	
37	CU13	92	12	75	Bn/Hf
38	CS14	93.5	10.5	85	Hf
39	CS15	95.5	11.5	95	Hf
40	CU15	89	14	60	
41	EU8	89	11.5	95	

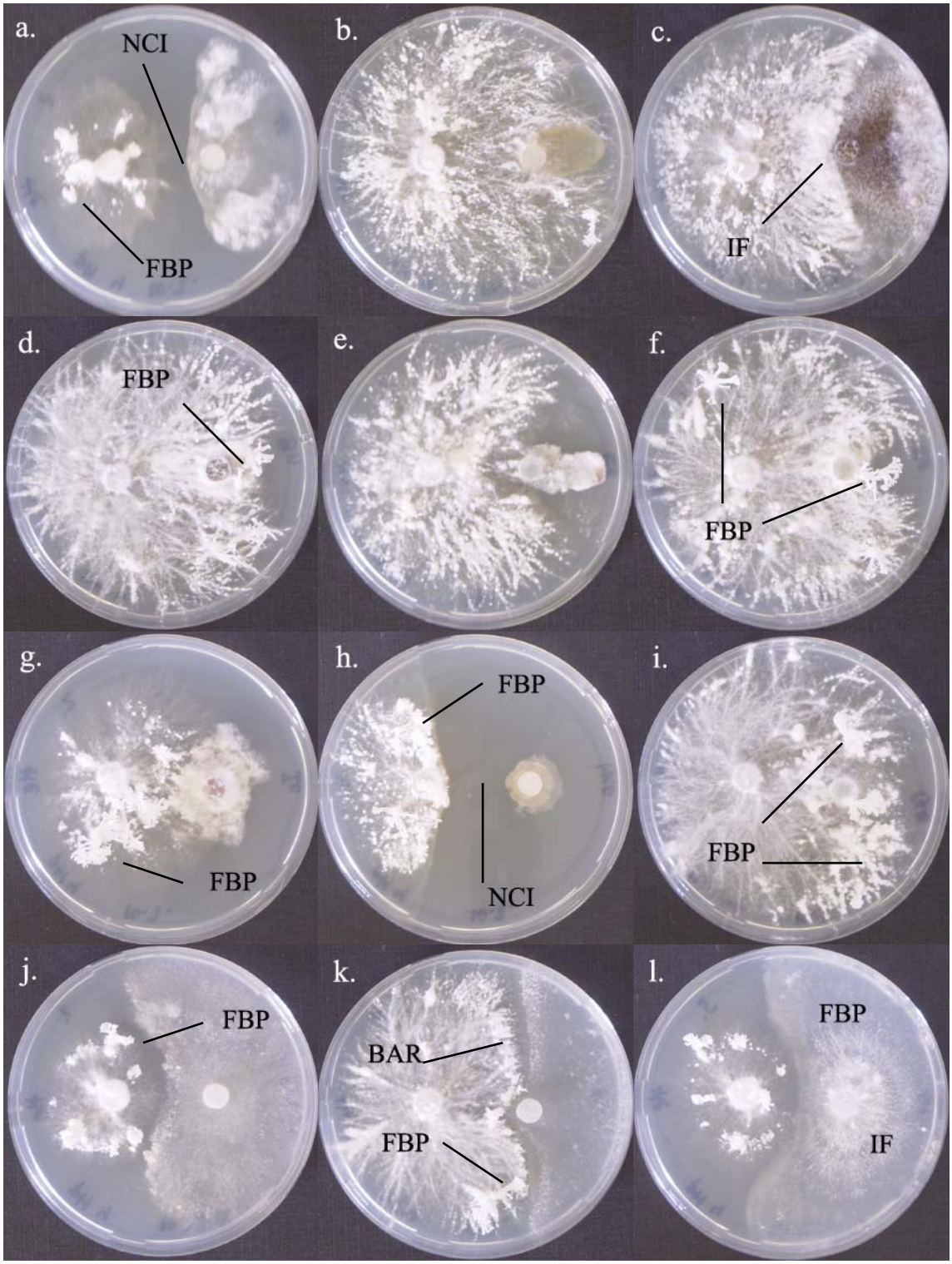
Log No.	Treatment	Length	Diam.1	Bark	Fructifications
42	EU7	88	15	85	
43	EU6	85	12	85	
44	ES?	89	14	80	Hf
45	EU5	89.5	13	70	Cv
46	ES10	90	13.5	75	Cv
47	ES9	90	10	80	Bn
48	CS?	87	11	85	Hf/Bn
49	CU3	88	15	50	
50	EU2	93	85	85	
51	ES6	88.5	13	85	Hf
52	ES8	85	15	90	Hf
53	ES?	90	12.5	90	
54	ES5	87	12	90	Hf
55	ES?	90	13	90	Hf/Bn
56	ES3	89	10	85	Hf
57	ES2	84	11.5	80	Bn/Hf
58	EU1	87	15	90	Hf
59	ES?	91	13	90	Hf
60	EU	93	14	75	Cv
61	ES	94	15	85	
62	EU	92	14	85	Cv
63	ES	93.5	13.5	90	Cv
64	EU	89	14	90	
65	ES	90	12.5	85	Cv
66	ES	93	14	90	
67	EU	94	15	90	Cv
68	EU	88.5	16.5	85	Cv
69	ES	92.5	14	80	
70	EU	93.5	15	85	
71	ES	90	11	90	

Key: ES = Logs inoculated with test species and sealed with cheese wax
EU = Logs Inoculated with test species and left unsealed
CS = Logs inoculated with sterile dowels and sealed with cheese wax
CU = Logs inoculated with sterile dowels and left unsealed

Fructification codes:
Bn= *Biscogniauxia nummularia*
Hf= *Hypoxylon fragiforme*
Nc= *Nectria cinnabarina*
Cv= *Coriolus versicolor*

Appendix 5. *H. erinaceum* interactions on agar

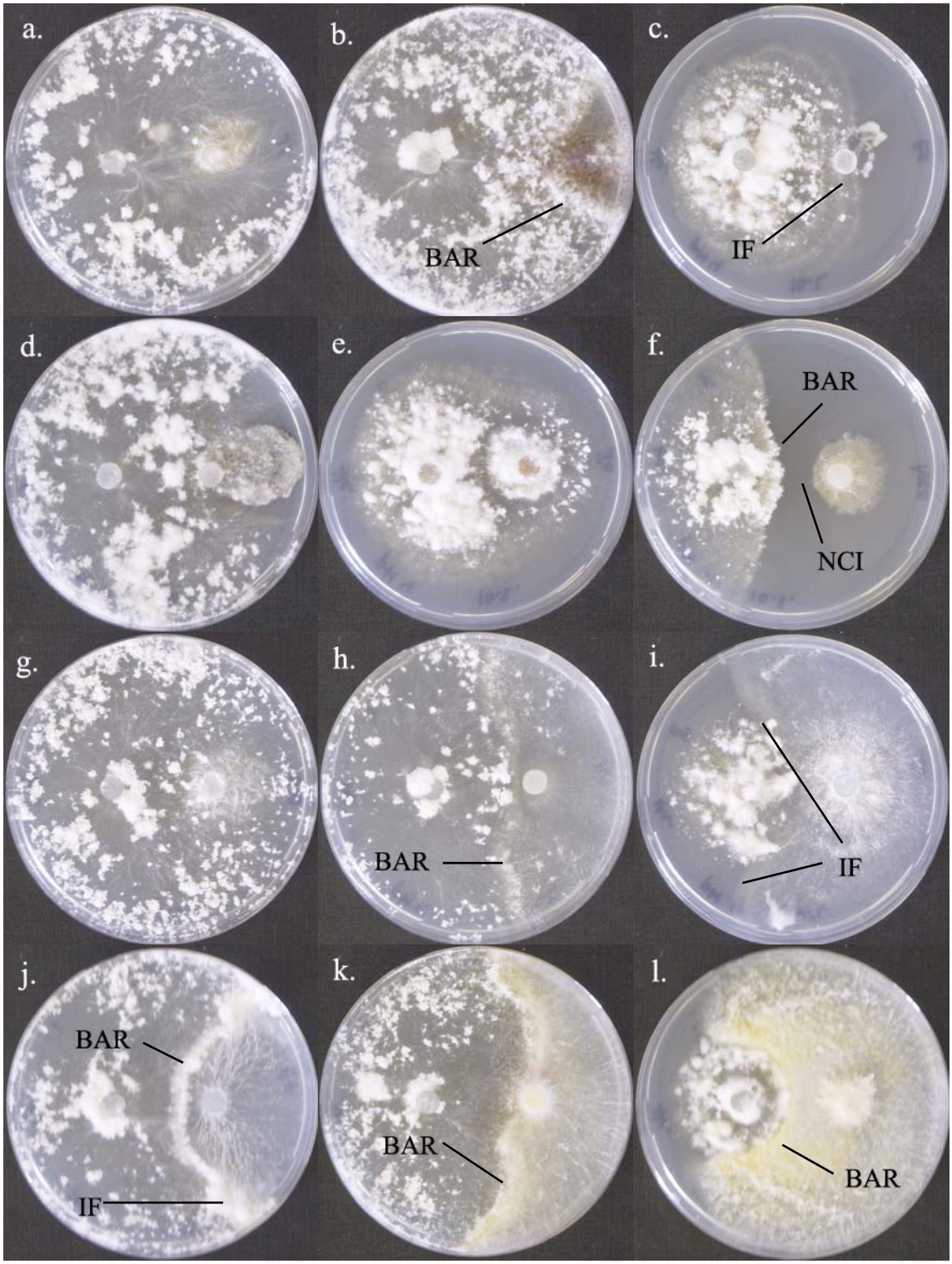
H .erinaceum (left hand side species on all photos) paired against various species on 0.5 % MA pH 4 adjusted plates: *H. erinaceum* against (a) and (b) *F. fomentarius*; (c) and (d) *B. nummularia*; (e) and (f) *E. spinosa*; (g) *I. obliquus*; (h) *L. pyriforme*; (i) *G. applanatum*; (j) and (k) *P. radiata*; (l) *C. versicolor*.



IF = invasive front; FBP = fruit body primordia; BAR = barrage; NCI = non-contact inhibition.

Appendix 6. *H. coralloides* interactions on agar

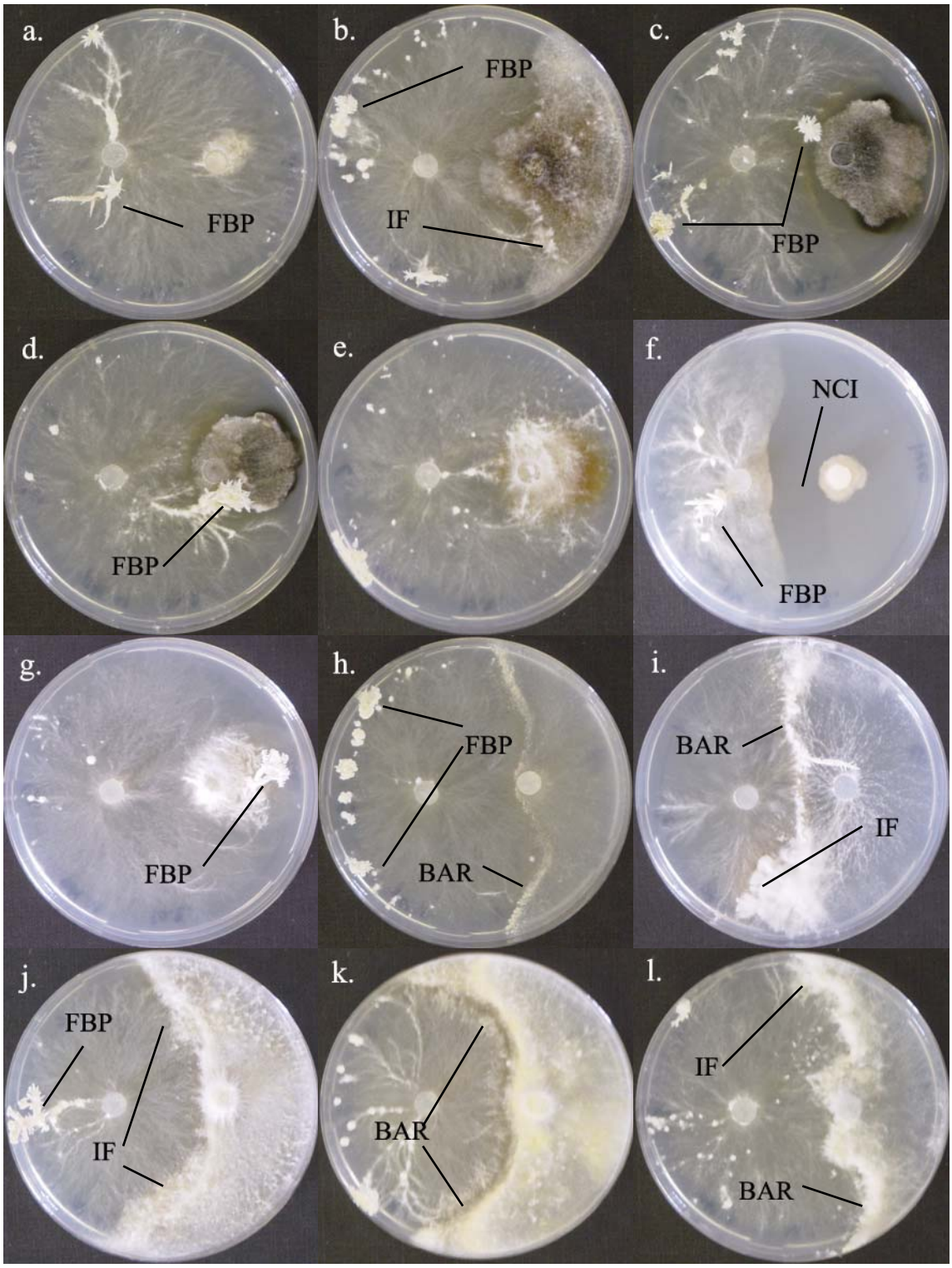
H. coralloides (left hand side species on all photos) paired against various species on 0.5 % MA pH 4 adjusted plates: *H. coralloides* against (a) *F. fomentarius*; (b) *B. nummularia*; (c) and (d) *E. spinosa*; (e) *I. obliquus*; (f) *L. pyriforme*; (g) *G. applanatum*; (h) *P. radiata*; (i) and (j) *C. versicolor*; (k) and (l) *S. gausapatum*.



IF = invasive front; BAR = barrage; NCI = non-contact inhibition.

Appendix 7. *C. cirrhatus* interactions on agar

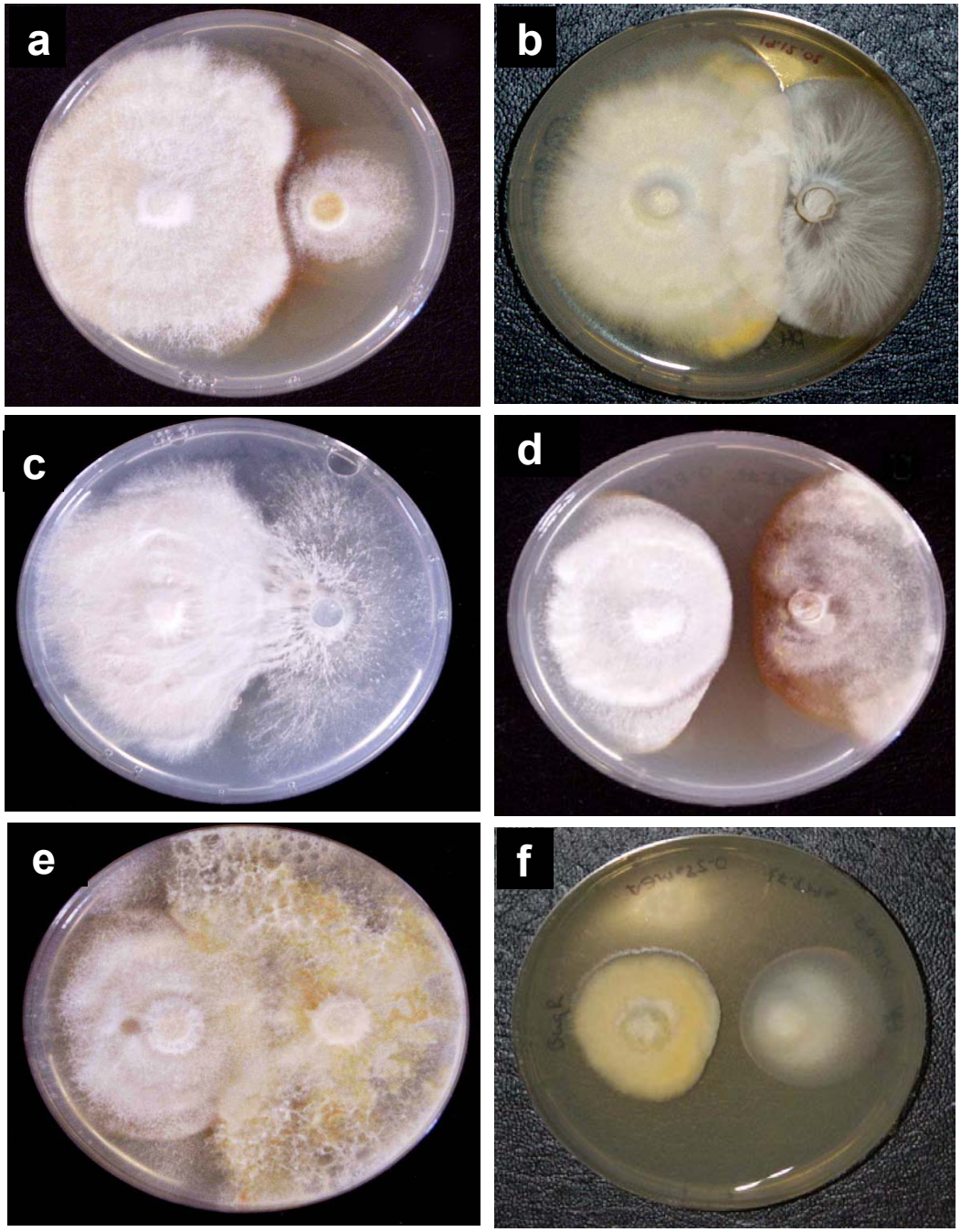
C. cirrhatus (left hand side species on all photos) paired against various species on 0.5 % MA pH 4 adjusted plates: *C. cirrhatus* against (a) *F. fomentarius*; (b) *B. nummularia*; (c) and (d) *E. spinosa*; (e) *I. obliquus*; (f) *L. pyriforme*; (g) *G. applanatum*; (h) *P. radiata*; (i) *C. versicolor*; (j) *S. gausapatum*; (k) *S. hirsutum*; (l) *B. adusta*.



IF = invasive front; FBP = fruiting body primordia; BAR = barrage; NCI = non-contact inhibition.

Appendix 8. *P. quercinus* interactions on agar

Interactions between *P. quercinus* (left) and other wood decay fungi (right) on malt agar at pH 3.75. a – e, isolate KC1627 after 80d; f, KC1623 after 21d. (a) *Fistulina hepatica*; (b) *Hypholoma fasciculare*; (c) *Coriolus versicolor*; (d) *Vuilleminia comedens*; (e) *Stereum gausapatum*; (f) *Fistulina hepatica* . Photographs taken by Martha Crockatt



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Peter Wakely/English Nature 21,792
Bottom left: Radio tracking a hare on Pawlett Hams, Somerset.
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Paul Glendell/English Nature 24,888