Competitive Interactions between Wood Decay Basidiomycetes

Sally Caroline Fryar B.Sc. (Hons).

Flinders University of South Australia
Faculty of Science and Engineering
School of Biological Sciences

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Summary:

The coexistence of species competing for limited resources is a common problem within ecological communities. Although a number of mechanisms have been proposed to explain the coexistence of competing species, none have been tested in a wood decay community.

The community of organisms which decay fallen wood generally consist of fungi and are mainly basidiomycetes. These organisms compete for fallen wood and coexist within habitats. There are likely to be many mechanisms of coexistence of wood decay basidiomycetes, however, two were tested in this thesis: the presence of (1) indirect effects and interactions modifications and (2) intransitive competition.

Indirect effects and interaction modifications are the effects that one species has on another via a third (or more) species. They occur in many ecological communities and can affect community structure. Their occurrence has never been reported between competing wood decay basidiomycetes. This thesis reports for the first time (1) the presence of indirect effects and a possible interaction modification and (2) the increased chance of coexistence in the presence of indirect effects between competing wood decay basidiomycetes. It is proposed that indirect effects and interaction modification can act as a mechanism for the coexistence of competing wood decay basidiomycetes.

Intransitive competition occurs when species A outcompetes species B, species B outcompetes species C but species C outcompetes species A. This thesis reports the presence of intransitive loops embedded within a competition hierarchy of 19 species. Previous studies on other communities have identified intransitive loops as a mechanism for the coexistence of species. It is proposed that the intransitive loops

found in this thesis could also act as a mechanism aiding the coexistence of competing wood decay basidiomycetes.

A field survey of fruiting bodies of wood decay basidiomycetes was conducted to test two hypotheses: (1) that many species of wood decay basidiomycetes coexist within the same habitat and; (2) that a moister site with a continuous overstory would have a higher species diversity than a site with less moisture, patchy overstory. The survey found 32 species at the moister site and 21 species at the drier site. In addition species diversity was higher at the moister site. Both hypotheses are therefore accepted.

The homokaryotic stage of a wood decay basidiomycete life cycle is often considered to be less important than the heterokaryotic stage. However, there is very little empirical data to support this opinion. In this thesis the relative competitive abilities of mycelial homokaryons and heterokaryons of four species of wood decay fungi (*Peniophora* sp.1, *Peniophora* sp.2, *Pereniporia medulla-panis*, *Aleurodiscus lividocoeruleus*) were assessed. It was found that there was no simple relationship between nuclear status and competitive ability. The homokaryon of *Peniophora* sp.2 was competitively superior to it's heterokaryon, whereas the homokaryon of *Peniophora* sp.1 was inferior to it's heterokaryon. This experiment showed that homokaryons as well as heterokaryons have the potential to influence community structure through competitive effects.

This thesis identifies two possible mechanisms for the coexistence of wood decay basidiomycetes; indirect effects/interaction modifications and intransitive competition. Both mechanisms require field testing.

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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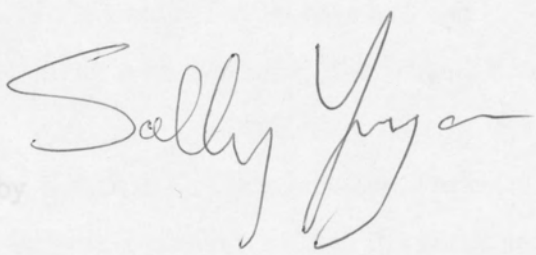

Sally Caroline Fryar

I believe that this thesis is properly presented, conforms to the specifications of thesis presentation in the university and is *prima facie* worthy of examination.

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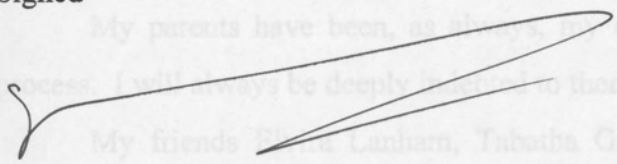

Dr G.C. Kirby

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1. General Introduction:

The problem of the coexistence of competing species has been the focus of many ecological studies (eg Vincent and Vincent, 1996; Tilman, Lehman and Yin, 1997; Holt and Polis, 1997; Turner, Souza and Lenski, 1996; Morris, 1996; Hulme, 1996). Gause's law of competitive exclusion states that complete competitors cannot coexist (Hardin, 1960). Ever since this principle was developed (Gause, 1934) many ecologists have faced the problem that many competitors do coexist (eg Jeltsch *et al*, 1996; Schwimming and Parsons, 1996; Connolly and Wayne, 1996; Wolff, 1996; Crook and Vuren, 1995; Blossey, 1995; Basset, 1997). This thesis is concerned with discovering mechanisms by which many competing species of wood decay fungi may coexist on the same substrate. The main theme is on testing for (1) indirect effects or interaction modifications between triplets of species and (2) intransitive competitive networks which have been shown to enhance species diversity (Karlson and Jackson, 1981). The influence of indirect effects and interaction modifications on the coexistence of species is observed.

Indirect effects and interactions modifications are the effects that one species has on another via a third (or more) species. These are discussed in more detail in section 1.8. Intransitive competition occurs when species A outcompetes species B, species B outcompetes species C and species C outcompetes species A.

The model organisms in this study are fungi that decay wood. The decomposition of a tree branch is a complex, multidimensional process which may follow a diverse array of optional pathways (Boddy, 1992). The process may involve many species of fungi and other organisms performing many different functions.

Interactions between species may be as diverse as the species themselves including mycophagy (Dowding, 1973), mutualism (Tanesaka 1993), parasitism (Hutchinson and Barron, 1996), competition (Holmer, Renvall and Stenlid, 1997) and predation (Tzean and Liou, 1993). Competition has been identified as a major factor in the structuring of wood decay fungal communities (Rayner *et al*, 1981; Coates and Rayner, 1985a, 1985b, 1985c). However almost all studies on fungal competition have only looked at pairwise interactions (eg Holmer *et al*, 1997; Rayner and Hedges 1982; Owens, Reddy, and Grethein, 1994; Holmer and Stenlid, 1993). In more general ecological literature there is a growing concern that undue emphasis has been placed on direct two-species interactions. Ecologists have been turning more towards interactions within the context of the whole community such as indirect effects (eg Menge, 1995; Van Buskirk, 1988; Walters and Moriarty, 1993). Some negative interactions such as competition or predation, once placed into the context of the community, may actually be advantageous to coexistence (Stone and Roberts, 1991).

1.1 Wood Decay Basidiomycetes:

Fungi play an essential role as decomposers in terrestrial ecosystems. They decay dung (Webster, 1970), leaves (Attili and Tauk-Tornisielo, 1994), twigs (Boddy and Rayner, 1984), logs (Marra and Edmonds, 1994) and branches (Scheu and Schauermann, 1994). In addition, fungi are pathogens, mutualists and predators. The functioning of terrestrial ecosystems is dependent upon the recycling of nutrients and carbon recycling by wood decay fungi. Wood decay fungi are abundant and diverse in

terrestrial ecosystems all over the world and play an essential role in ecosystem functioning.

The basic units of a fungal individual are the hyphae, which are collectively called a mycelium (see Figure 1.1). The mycelium grows through a substrate (eg soil, wood). Individual hyphae secrete enzymes in front of the growing hyphal tip. These extracellular enzymes break down macromolecules into soluble and absorbable molecules. The hyphae grow through the zone of digestion, ingesting the degraded material. Free water must be present as a medium for diffusion of enzymes and soluble nutrients (Alexopoulos, Mims and Blackwell, 1996). Even the dry rot fungus (*Serpula lacrimans* (Wulf. ex Fries) Shröt) which degrades wood in dry environments transports water to the growing hyphal tips (Hawksworth, 1995).

The sexual reproductive structures of a fungus are called fruiting bodies. These structures produce sexual spores for dispersal. Fungi also produce asexual spores on very small, simple structures (conidiophores) as in Figure 1.2.

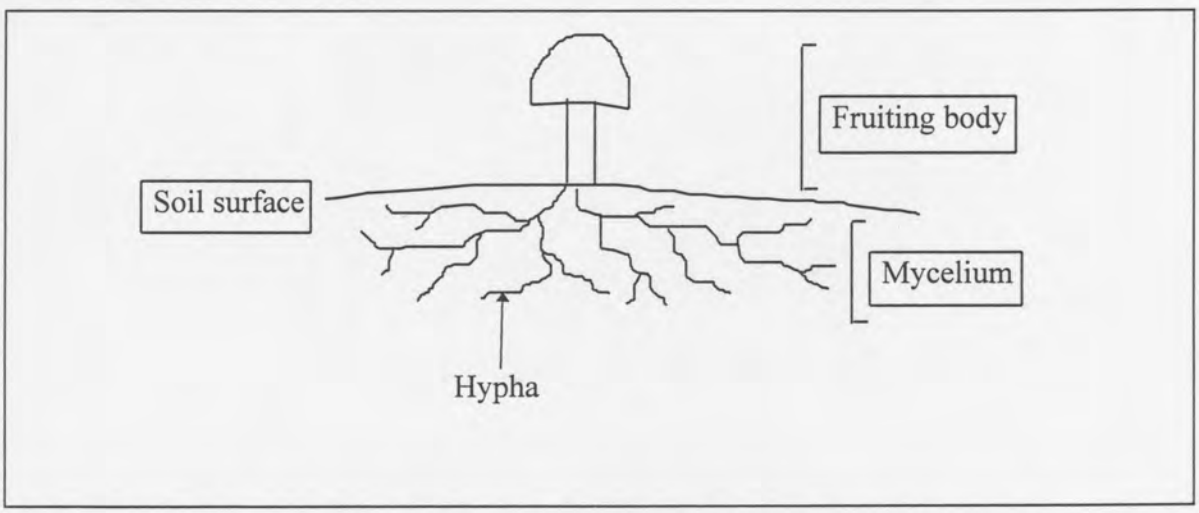

Figure 1.1: Diagram of a fruiting body and mycelium of a basidiomycete

1.1.1 Classification:

In the current classification (Barr, 1992) the kingdom 'Eumycota (true fungi)' contains four phyla as shown in Figure 1.2. The phylum Chytridiomycota is a group with a motile stage at some point in their life cycle. Chytrids are microscopic and their hyphae are mostly coenocytic and simple. Members of the phylum Zygomycota are also relatively simple. They produce small, simple structures for reproduction and their hyphae have no septa. The ascomycetes are more complex, with septa in their hyphae and often larger fruiting bodies. Basidiomycetes have the most complex fruiting bodies which can weigh over 2.5 kg (Stamets, 1993). The mycelium of a basidiomycete in the heterokaryotic phase of the life cycle is characterised by the presence of clamp connections (see Figure 1.2), however, not all basidiomycetes possess these structures. Until recently there was another phylum called the Deuteromycota in which were placed fungi for which the sexual stage was not known. Consequently this group was polyphyletic, with most fungi put in it belonging to the ascomycota or basidiomycota (Alexopoulos *et al,* 1996).

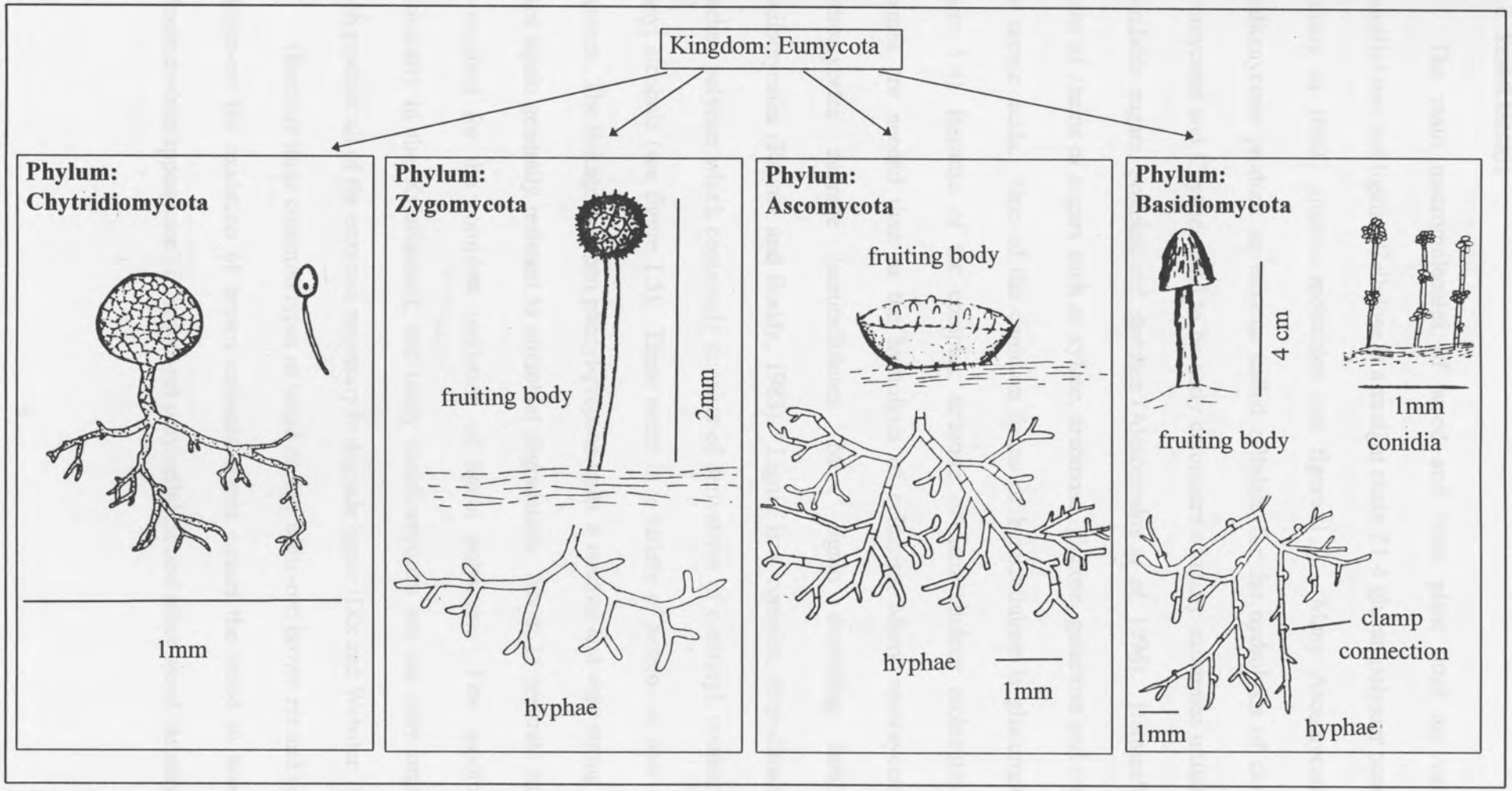

Figure 1.2: Diagram of the kingdom Eumycota. Classification according to Alexopoulos *et al*, 1996.

1.1.2 Wood Decay:

The main macromolecules of wood and other plant litter are cellulose, hemicelluloses and lignin. Cellulose is a straight chain β1-4 glucan polymer containing as many as 10000 glucose molecules (see figure 1.3). Many Ascomycetes and Basidiomycetes produce an enzyme called cellulase for the hydrolysis of cellulose. Zygomycetes and Chytrids tend to be early colonisers of many substrates utilising the assimilable sugars, proteins and starches (Alexopoulos *et al,* 1996). Hemicelluloses consist of chains of sugars such as xylose, arabinose, glucose, galactose and mannose with uronic acids. One of the common forms of hemicellulose is glucuronoxylan (figure 1.4). Because of the complex structure of hemicellulose molecules more enzymes are needed than in the hydrolysis of cellulose. Many ascomycetes and basidiomycetes degrade hemicelluloses. Most lignin degrading fungi are Basidiomycetes (Rayner and Boddy, 1988). Lignin is a complex three-dimensional branched polymer which commonly consists of derivatives of coniferyl, coumaryl and sinapyl alcohols (see figure 1.5). These occur in a variety of hetero- or near mono-polymers. The linkage between phenyl-propane units is random and very strong which makes lignin generally resistant to microbial degradation. About 15 separate enzymes are required for the complete oxidation of lignin polymers. Few ascomycetes (particularly in the Xylariaceae), and many basidiomycetes are the only organisms which produce all of the enzymes necessary to degrade lignin (Dix and Webster 1995).

There are three common types of wood decay: white-rot; brown rot and soft-rot. In white-rot the oxidation of brown coloured lignin causes the wood to take on a diagnostic white appearance. In brown-rot only cellulose and other wood carbohydrates

are utilised causing the decaying wood to remain brown. Soft-rot is caused mostly by ascomycetes and some members of the group which were in the Deuteromycetes. Although fungi that cause soft-rot can also degrade cellulose and lignin they are mostly associated with decay in wood that has a high moisture content. They are often early colonisers of woody substrates, and outcompeted by brown- or white-rotting fungi (Dix and Webster, 1995).

Because basidiomycetes are the main group involved in the decomposition of wood (Rayner and Boddy, 1988), this thesis will focus on wood decay basidiomycetes (WDB's).

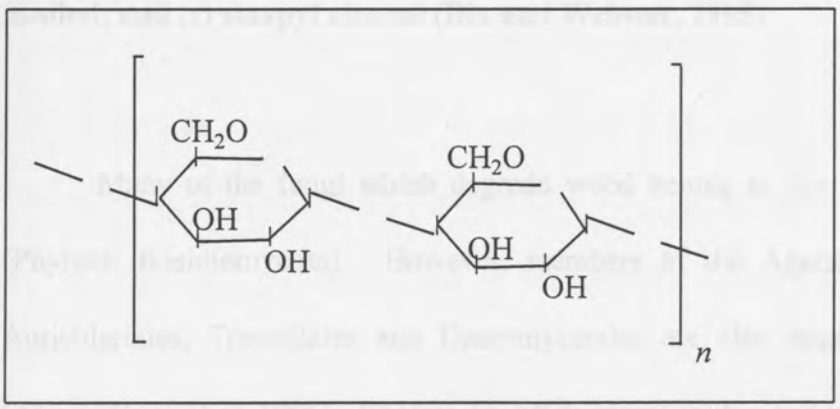

Figure 1.3: Diagram showing the molecular structure of cellulose (Dix and Webster, 1995)

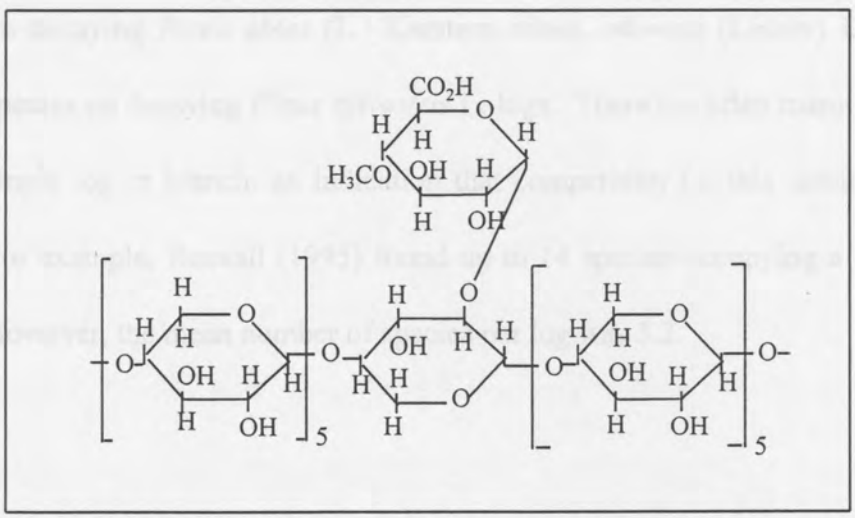

Figure 1.4: The structure of methyl glucuronoxylan (one form of hemicellulose) (Dix and Webster, 1995)

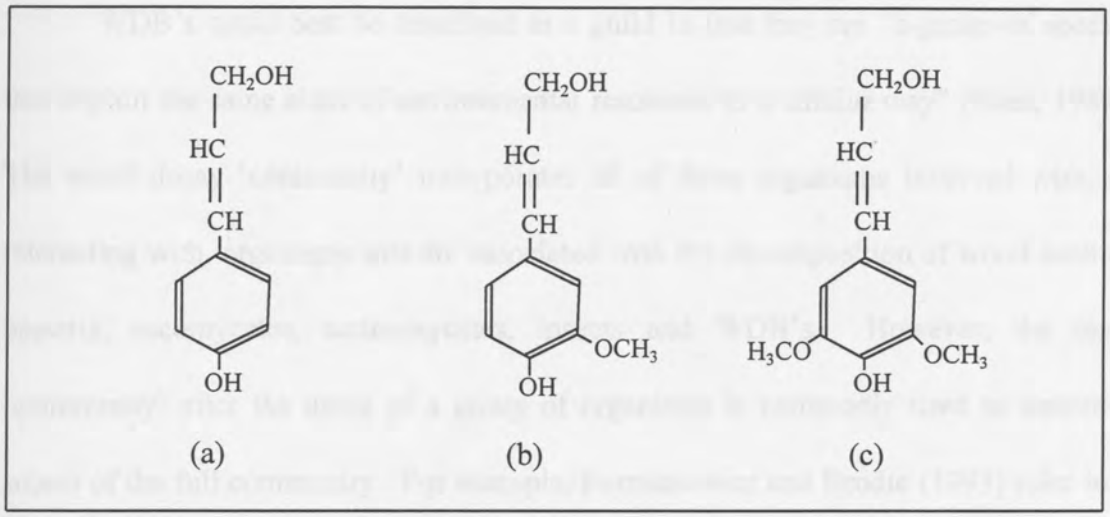

Figure 1.5: The structure of lignin alcohols (a) p-coumaryl alcohol; (b) conferyl alcohol; and (c) sinapyl alcohol (Dix and Webster, 1995)

Many of the fungi which degrade wood belong to the order Aphyllophorales (Phylum: Basidiomycota). However, members of the Agaricales, Gasteromycetes, Auriculariales, Tremellales and Dacrymycetales are also important in wood decay (Alexopolous *et al,* 1996). Species are often cosmopolitan and in any one habitat there are many species. For example in Northern Finland Renvall (1995) found 120 WDB's on decaying *Picea abies* (L.) Karstern subsp. *obovata* (Ledeb.) Domin logs and 104 species on decaying *Pinus sylvestris* L. logs. There are often many species occupying a single log or branch, an indication that competition for this resource might be fierce. For example, Renvall (1995) found up to 14 species occupying a single decaying log. However, the mean number of species per log was 3.2.

1.1.3 Terminology:

WDB's could best be described as a guild in that they are "a group of species that exploit the same class of environmental resources in a similar way" (Root, 1967). The wood decay 'community' incorporates all of those organisms involved with, or interacting with, organisms that are associated with the decomposition of wood such as bacteria, ascomycetes, actinomycetes, insects and WDB's. However, the term 'community' after the name of a group of organisms is commonly used to denote a subset of the full community. For example, Formanowicz and Brodie (1993) refer to a salamander community and Hill *et al* (1993) refers to shorebird communities. In this same way a guild of fungi is usually referred to as fungal community (eg Boddy, 1992). For consistency, the term WDB community will be used in this thesis to mean the subset of the full wood decay community that are basidiomycetes.

Community structure refers to the patterns of distribution and abundance of species within a community.

1.1.4 Genetics and reproduction:

There are a number of mating systems in WDBs. The most common mating system (90%) is heterothallism (Burnett, 1975) in which uninucleate haploid spores germinate to produce primary or homokaryotic mycelium (see figure 1.6). Homokaryons grow in wood and form territories. When two homokaryons of the same species meet hyphal anastomoses (fusions) may occur, and if they are sexually compatible, nuclei of one migrate into the mycelium of the other. The new growth and much of the pre-existing mycelium now have binucleate cells, with one nucleus from

each homokaryotic parent, and this mycelium is called a heterokaryon. The sexual compatibility of homokaryons is determined by mating type factors. In bipolar species the mating types are determined by a single factor, *A*, with two or more alleles in the population (eg A1 and A2). Two homokaryons are compatible when they carry different alleles. In tetrapolar species, there are two factors, *A* and *B*, each with two or more alleles. For example, with only two alleles for each factor, the four mating types are *A1B1*, *A1B2*, *A2B1*, *A2B2*. The homokaryons are compatible only when both *A*s and *B*s differ (eg A1B1 and A2B2). The *A*s and *B*s are best referred to as factors as their determinants are genetically complex (Elliott, 1994).

Following nuclear migration the resultant heterokaryon occupies the territories of the parent homokaryons. The heterokaryon continues to grow and decay the wood. Eventually, the heterokaryon can form a fruiting body (or basidiocarp) usually on the surface of the wood within which many basidia form. Karyogamy and meiosis occurs within the basidia, and they usually produce four haploid uninucleate spores (Alexopoulos *et al,* 1996).

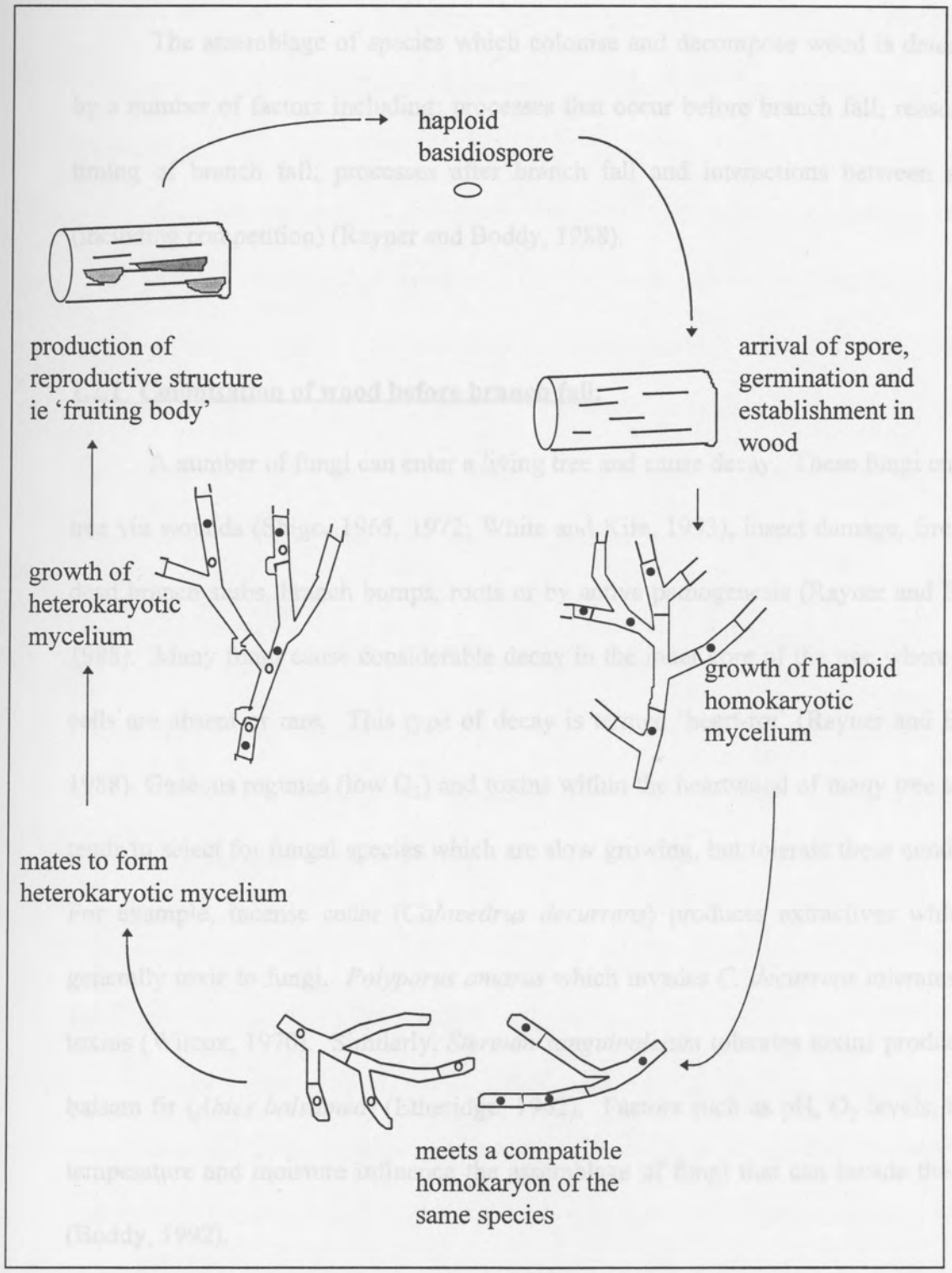

Figure 1.6: Life cycle of a diaphoromictic (multiple allelic heterothallic) wood decay basidiomycetes

1.2 Colonisation and Decomposition of Wood:

The assemblage of species which colonise and decompose wood is determined by a number of factors including: processes that occur before branch fall; reasons and timing of branch fall; processes after branch fall and interactions between species (including competition) (Rayner and Boddy, 1988).

1.2.1 Colonisation of wood before branch fall:

A number of fungi can enter a living tree and cause decay. These fungi enter the tree via wounds (Shigo, 1965, 1972; White and Kile, 1993), insect damage, fire scars, dead branch stubs, branch bumps, roots or by active pathogenesis (Rayner and Boddy, 1988). Many fungi cause considerable decay in the inner core of the tree where living cells are absent or rare. This type of decay is termed 'heart-rot' (Rayner and Boddy, 1988). Gaseous regimes (low O_2) and toxins within the heartwood of many tree species tends to select for fungal species which are slow growing, but tolerate these conditions. For example, incense cedar (*Calocedrus decurrens*) produces extractives which are generally toxic to fungi. *Polyporus amarus* which invades *C. decurrens* tolerates these toxins (Wilcox, 1970). Similarly, *Stereum sanguinoletum* tolerates toxins produced by balsam fir (*Abies balsamea*) (Etheridge, 1962). Factors such as pH, O_2 levels, toxins, temperature and moisture influence the assemblage of fungi that can invade the wood (Boddy, 1992).

Pathogenic fungi such as *Armillaria* species (Kile, 1981; Legrand and Guillaumin, 1993; Entry *et al*, 1991) and *Heterobasidion annosum* (Swedjemark and Stenlid, 1993) infect healthy trees, causing considerable decay and sometimes tree or

branch death. Although secondary colonisers such as *Coriolus versicolor* are able to replace pathogens such as *Armillaria luteobubalina* (Pearce, 1990), the incidence of pathogens is likely to influence the assemblage of fungi that can subsequently colonise the wood.

Branches are usually colonised by a range of fungi before the branch or log falls to the ground. Uncolonised wood may fall to the ground due to large storms, strong winds or hot weather (Rayner and Boddy, 1988). Uprooted healthy trees tend to be quickly colonised by competitive secondary colonisers such as *Stereum sanguinolentum*, and species of *Trametes* (Niemelä, Renvall, and Penttilä, 1995). Pre-colonised wood which falls to the ground is later colonised by more combative species. Niemelä *et al* (1995) describe relationships between preceding species which colonise wood before it falls to the ground and successor species which colonise wood after it falls. Examples are *Skeletocutis* sp. 1 following *Phellinus ferrugineofuscus*, *Skeletocutis* sp. 2 following *Phellinus chrysoloma*, *Gleoporus dichrous* following *Inonotus obliquus* and *Antrodiella semisupina* following *Fomes fomentarius* (Niemelä *et al,* 1995).

1.2.2 Colonisation of branches after fall:

Bacteria and ascomycetes are often reported to be the first invaders of fresh, intact wood (Roll-Hansen and Roll-Hansen, 1979; Eriksson *et al,* 1990; Hallaksela and Salkinojo-Salonene, 1992; Hallaksela, 1993). The presence of these organisms enhance mycelial growth of fungi in the wood, increase weight loss of the branch (Blanchette and Shaw, 1978) and are likely to alter the colonisation pattern of basidiomycetes. Ascomycetes and some members of the group which were the Deuteromycetes (soft

rots) are capable of considerable decay (Tanaka, Fuse and Enoki, 1992). These organisms usually only break down cellulose and leave untouched the bulky lignin molecules. However, under high humidity some soft rots (eg *Aspergillus flavus* Link) are capable of degrading some lignin (Betts, Dart and Ball, 1988).

1.2.3 Establishment of mycelium in wood:

A fungal species may be either unit restricted or non-unit restricted (Rayner and Boddy, 1988). Unit restricted fungi produce spores to disperse to other branches (or resource "units"), while non-unit restricted fungi can produce spores (see figure 1.7) and also produce cord-like filaments (see figure 1.8) which are aggregates of hyphae. These "cords" can travel between branches and are hence non-unit restricted (Boddy, 1993). When a mycelial cord arrives at a fresh branch it must change modes from transportation to utilisation of resources. A mycelium must either penetrate the outer bark to enter the branch or forage along the branch to find a hole in the branch where once inside a mycelium can grow out three-dimensionally.

Individuals form territories in the branch, separated by demarcation zones or barrages (see figure 1.9). If one individual is competitively superior to another it will push the barrier forward and take over the territory of the inferior competitor (Rayner and Boddy,1988).

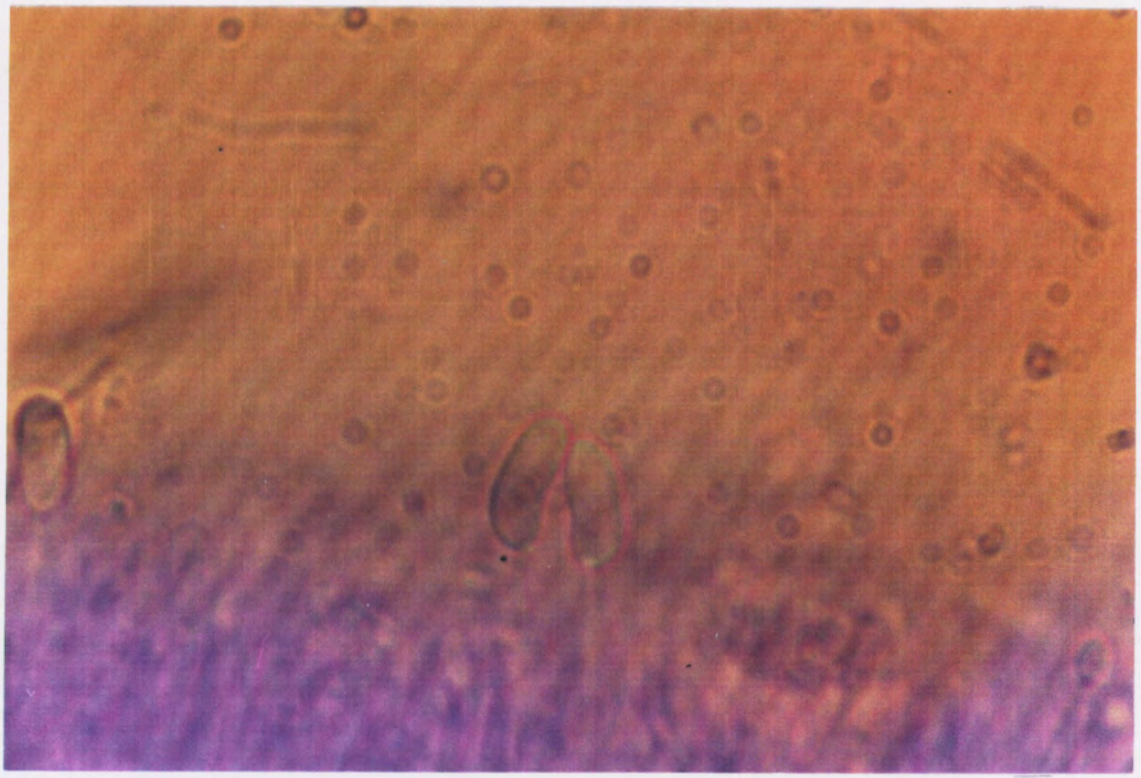

Figure 1.7: Photograph of a basidiospore under light microscope 400x 1 μm

Figure 1.8: Photograph of some mycelial cords on a fallen *Eucalyptus* branch 1cm

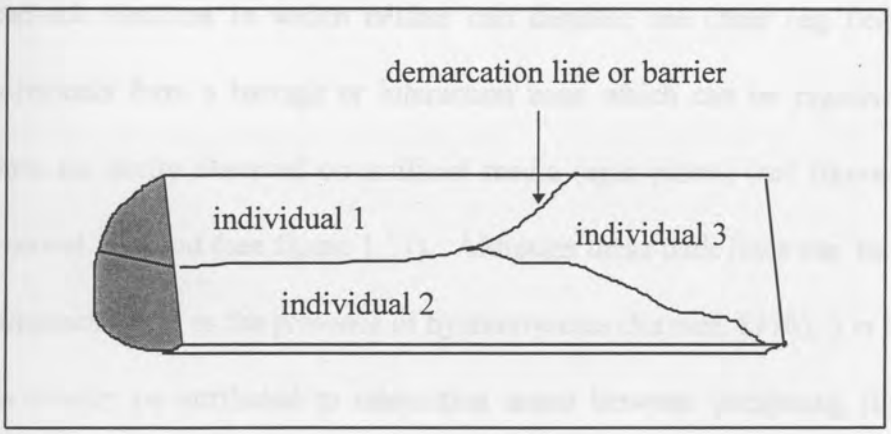

Figure 1.9: Diagrammatic representation of a branch colonised by three WDB's

1.3 Competition between WDBs:

Competition is defined as the negative effects which one organism has upon another by consuming or controlling access to a resource that is limited in availability (Keddy, 1989). There are two types of competition: exploitation and interference (Lockwood, 1992). Exploitation competition occurs when one organism or population depletes limiting resources used by another organism or population without reducing the access of the other organism or population to the same resource pool. Interference competition includes behavioural or chemical mechanisms by which access to a resource is influenced by the presence of a competitor (McNaughton and Wolfe 1973).

Interactions between WDBs are almost always antagonistic (Rayner and Boddy, 1988). Exceptions are when two compatible homokaryons meet (Biggs, 1938), when two identical genotypes (same individual) meet (Fischer and Bresinsky, 1992) or when a heterokaryon and one of its' parent homokaryons meet (Coates, Rayner and Boddy,

1985). Usually when two WDBs meet either one displaces the other or they form a deadlock situation in which neither can displace the other (eg Pearce, 1990). The individuals form a barrage or interaction zone which can be pigmented. Interaction zones are easily observed on artificial media (agar plates) (see figure 1.10) and often observed in wood (see figure 1.11). Although these dark lines can be caused by other phenomena such as the presence of hyphomycetes (Rayner, 1976), it is thought that they can usually be attributed to interaction zones between competing fungal individuals (Rayner and Boddy, 1988).

Figure 1.10: Photograph of an interaction zone between two wood decay basidiomycetes on a 9 cm diameter agar plate

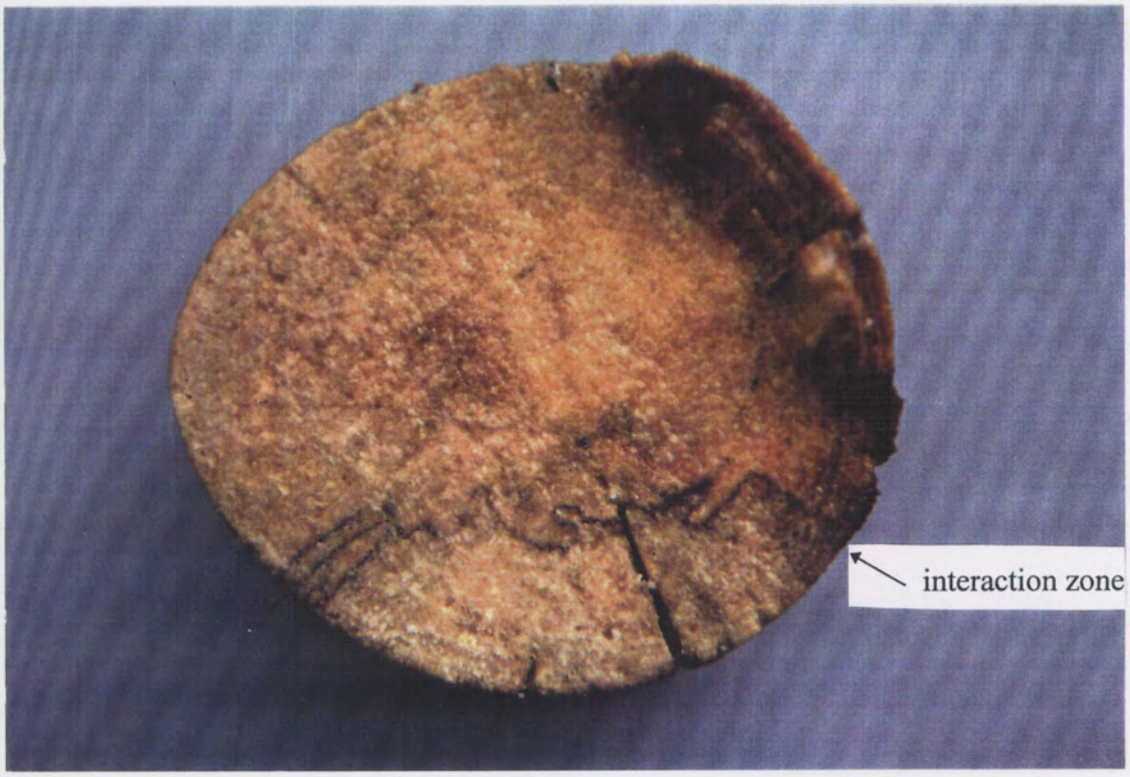

Figure 1.11: Photograph of an interaction zone in a cut section of a branch

1.3.1 Interference competition:

Interference competition between fungi involves either hyphal interference or release of chemical substances which inhibit other fungi. Ikediugwu and Webster (1970 a, 1970b) first described hyphal interference between coprophilous fungi. Since then it has been reported in a number of basidiomycete competition studies (Kellock and Dix, 1984; Ikediugwu 1976a, 1976b). This interaction involves either the direct contact or near contact between two individual hyphae of the opposing species. The hypha of one or both of the individuals ceases to grow, becomes permeable and loses turgor.

Repeated hyphal contact between the two individuals results in either the death of the mycelium of one of the individuals or a deadlock between the two individuals. There have been no reported cases of penetration of the victim and no toxins or destructive enzymes have been isolated (Dix and Webster, 1995). This type of interaction is common between WDB's (Rayner and Boddy, 1988).

Chemical defence strategies are also quite common amongst wood decay basidiomycetes (Rayner and Boddy, 1988). One individual may be able to suppress another by releasing a toxin. For example when *Trichoderma* spp. and *Lentinus lepidus* were grown together on an agar plate, the former overgrew the latter. Volatile substances which were released by *Trichoderma* spp. had the same effect as the whole organism on *Lentinus lepidus* (Bruce, Austin and King, 1984).

1.3.2 Exploitation competition:

While exploitation competition has been extensively studied in soil and phylloplane fungal communities (Lockwood, 1981) it has not been explicitly investigated in WDB communities. Exploitation competition can be studied at two levels: individuals and populations. Between populations of WDBs exploitation competition would almost certainly take place. Primary colonisers first utilise the resource. Secondary colonisers can displace primary colonisers. However, the resource would have already been depleted of many nutrients. The effect that this initial depletion of nutrients has on the secondary colonisers has not been investigated, but is likely to be exploitation competition.

Cooke and Rayner (1984) advise against using this terminology for fungal competition whereas Wicklow (1986) and Lockwood (1992) defend it.

Cooke and Rayner (1984) suggest that it is inadvisable and misleading to use the terms exploitation and interference competition when considering competition among fungi. They argue that during resource depletion many mycelial fungi restrict the access of others as a result of either efficient nutrient uptake through densely branching hyphal system or via antagonism. However, this only occurs between individuals. A fungus with a higher dispersal rate would generally arrive at resources before others. If this fungus uses part of the resource, then it has potentially affected any secondary colonisers (ie exploitation competition).

1.3.3 Interspecific and intraspecific competition:

In addition to interspecific competition, intraspecific interactions also result in an antagonistic reaction whenever two incompatible individuals meet such as two different heterokaryons or two incompatible homokaryons. For example, Williams, Todd and Rayner (1981) inoculated various combinations of homokaryons and heterokaryons of *Coriolus versicolor* into birch logs. The heterokaryons formed territories of roughly equal size when inoculated at the same time. The homokaryons found mates and quickly formed heterokaryons and territories. Incompatible homokaryons also have antagonistic reactions (Rayner and Todd, 1977).

1.3.4 Measuring competition:

A number of methods have been used to measure the competitive effect that one individual fungus has on another. Firstly, and most simply, is to classify pairwise interactions into three categories: (1) one individual fully overgrows another ("overgrowth"); (2) they meet and form a barrage in which the two individuals hold their territory ("deadlock") or (3) they intermingle (intraspecific competition, compatible individuals) (eg Owens *et al*, 1994; Boddy and Rayner, 1983b; Pearce, 1990; Rayner and Hedges, 1982). While this method is good for gaining a broad picture of competition, it does not quantify the strength of the deadlock interactions. If one individual takes 80% of the resource before deadlock, then it has had a significant negative influence on the other individual compared with the case where they each take 50% of the resource.

A second method which was initially used by Fokkema (1973) utilises the radial extension of the mycelium towards and away from the competitor taken from the point of inoculation. Percentage inhibition is calculated by equation 1.1.

$$\%Inhibition = 100x\ (r_1-r_2)/r_1 \qquad \text{Equation 1.1}$$

where r_1 = radius of colony away from the competitor

r_2 = radius of colony toward the competitor

While this method has the advantage that measurements are only one dimensional and relatively easy to obtain, these measurement become confusing when observing competition between more than two species.

A more direct measure of overall competitive effect on an agar plate is to measure the area covered by each fungal colony. The area (or volume if dealing with fungi actually in a piece of wood) covered by a fungal mycelium is a reasonable measure of competitive success. Since the more area or volume that an individual fungus gains, the more energy that it can put into growth and reproduction, a measure of area or volume is more likely to be closely related to the success of that individual including the number of propagules that it produces. With recent advances in image analysis programs, the measurement of the area of a colony is easy. Access to a suitable image analysis system enabled me to use area covered by a mycelium for the calculation of competitive effects in this thesis.

1.3.5 Competitive ability and displacement ability:

As discussed above, an appropriate measure of overall competitive ability is the area that one individual covers when paired with another individual. The two common types of interactions "deadlock" and "overgrowth" reveal two different strategies of combat. Within a deadlock interaction, the faster growing species gains more territory initially and because neither species displaces the other, this faster growing species retains that territory acquired by a faster growth rate. In these cases we would expect to find a correlation between growth rate of an individual and competitive ability. If however, one individual can displace the other, then irrespective of growth rates, the individual with the greater "displacement ability" may gain more territory and therefore also have greater competitive ability. The displacement activity in each interaction was therefore recorded.

1.3.6 Correlation between field and laboratory studies:

In vitro pairwise competition studies often do not correlate with observed fungal community patterns in the field. For example, Webber and Hedger (1986) discuss the observation that several saprotrophs from elm bark that successfully antagonised *Ceratocystis ulmi* by antibiotic production in culture were usually ineffective as competitors in the natural substratum. Magan and Lacey (1985), and Widden and Hsu (1987) also found little correlation between laboratory and field studies. However within WDB communities there is often a correlation between observed patterns in the field and interactions on agar plates (Rayner, 1977, Carruthers and Rayner, 1979). For example, Rayner (1977) and Carruthers and Rayner (1979) reported the replacement of *Bjerkandra adusta* by *Pseudotrametes gibbosa* in pairwise competition studies on agar plates and that this was also observed in the field.

1.4 Ecological strategies of WDBs:

The ecological strategies of wood decay fungi can be generally split into three extremes:

(1) ruderal strategists (R selected) which are often primary colonisers of fresh fallen timber with high dispersal rates, fast mycelial growth rates and low combative ability;

(2) combative strategists (C selected) which are secondary colonisers of wood and outcompete ruderal strategists. They are generally slower growing and have lower dispersal rates than ruderal strategists;

(3) stress-tolerant strategists (S selected) are capable of causing decay in highly selective conditions such as the presence of toxins, and different gaseous regimes (Rayner and Boddy, 1988).

This categorisation of ecological strategies comes from the theory of r- and k-selection developed mainly by MacArthur and Wilson (1967) and further development into R-, C- and S- strategies (Grime, 1974, 1977). These theories have been widely criticised (eg Grubb, 1985; Menges and Waller, 1983). However, there is some evidence to support the R-, C- and S-selection theory within WDB communities and the ecology of WDBs has been largely discussed in terms of R-, C- and S-selection (eg Rayner and Boddy, 1988). For example, in section 1.2, WDB which invade living tissue tend to be stress tolerant (S-selected). They are able to grow in conditions of low O_2 and in the presence of toxins produced by the plants (see section 1.2.1). These species are often outcompeted by secondary colonisers (C-selected) (see section 1.2.2 and 1.2.3). Fresh substrate is often initially colonised by primary colonisers such as bacteria and ascomycetes (R-selected) (see section 1.2.2).

The main criticisms have come from the oversimplification of the theory. There are many different forms of stress or disturbance that can be consolidated into a single parameter (Andrews, 1992). By reducing the multidimensional properties of organisms into a two-dimensional triangle of ecological strategies, much of the detail is lost (Andrews, 1992). However, the initial use of the concept can be of some benefit. While the theory does not comprehensively cover the details of ecological trade-offs, it allows us to broadly categorise the ecological strategies used by organisms at a particular time. It is important to note that the concept as used in WDB communities is used to define an

organisms behaviour at a particular time, but not to classify an organism *per se* (Boddy, 1992).

The study of community dynamics of WDB is still in its infancy. Until we know more details about the ecological trade-offs of WDBs, the use of a concept such as the R-, C- and S-selection theory is useful and will therefore be used in this thesis.

1.5 Influence of competition on WDB communities:

There are several ways to test the influence of competition on community structure. Two common methods are the addition or removal of a species from an area and subsequent observations in the changes in community structure (Connell, 1983; Goldberg and Barton, 1992; Heske, Brown and Mistry, 1994). To test the influence of WDB community structure it would be necessary to inoculate a target species into fallen logs and branches in an area, then observe the resultant change in WDB community structure in that area. Unfortunately, since the decay process can take many years (15 to 20 years for small branches (<5 cm diameter) and over 300 years for large trunks (Boddy and Swift, 1984; Grier, 1978)), the generation time could potentially also be many years. Since changes in community structure are likely to take a few generations, the length of time required for such a study means that it is not feasible. Therefore, to examine the influence of competition on WDB community structure other methods must be used. Although there have been only a few detailed ecological investigations of fungal colonisation and community structure of natural wood substrates (Thompson and Boddy, 1983), they all indicate that competition occurs and that it influences community structure. For example, in a study by Coates and Rayner (1985 a, 1985 b, 1985 c)

basidiospores of known fungi were inoculated onto cut beech logs while other (control) logs were uninoculated. The resultant colonisation patterns in inoculated and uninoculated logs were markedly different, demonstrating the influence of competition.

Observations from other studies show a trend of primary resource capture by relatively non-combative species, then secondary resource capture by more combative species (Rayner, 1977; Carruthers and Rayner, 1979; Rayner and Boddy, 1985). For example, Carruthers and Rayner (1979) found that *Phlebia merismoides* and *Hypholoma fasciculare* were highly combative, replacing most other species in both laboratory and field experiments. This demonstrates that competition does occur in the natural situation and is likely to influence community structure.

1.6 Coexistence of species:

The coexistence of competing species is a problem that has been studied by community ecologists for many years (eg Gause, 1934; Hutchinson, 1959). That two species in static conditions competing for the same resource should not be able to coexist indefinitely is an intuitive concept and is the basis of Gauses' law of competitive exclusion (Aarssen, 1983).

The competitive exclusion principle can be simply stated as "complete competitors cannot coexist" (Hardin, 1960). Unfortunately this intuitive statement is too vague and ever since Gauses' experiments many ecologists have worked to give detail to the theory. Aarssen (1983) summarises the generalised concept of coexistence of competing species as:

Competitive exclusion (lack of coexistence) should occur if:

(1) resources are in sufficiently limited supply;

(2) their resource requirements (or the number of niche dimensions in which they interact) overlap beyond a certain critical point, and;

(3) one of them must be a superior competitor for these common resource requirements.

These three criteria predict the conditions under which competitive exclusion should take place in a static, closed system. They serve as a basis for a generalised theory of coexistence of species. When moving from a static, closed system to a natural environment many variables, including other species, are added to the system. These additional parameters in the system create some conditions under which complete competitors can coexist.

1.6.1 Some conditions under which two or more competitors can coexist:

(1) Patchiness of environment:

If there are at least *n* patches for *n* species and the environment favours different species in different patches then *n* species will be able to coexist. For example Brown (1982) reported the coexistence of two species of snails, *Physa gyrina* and *Lymnaea elodes* in a habitat. *L. elodes* was competitively superior to *P. gyrina*. However, *P. gyrina* had smaller offspring and was able to reproduce earlier. In ponds which dried up early, only *P. gyrina* was able to reproduce. The two species were therefore able to coexist in the area.

(2) Neighbourhood models:

For many sessile species the number of organisms with which they interact is limited to neighbours. Due to differential mortality and dispersal each neighbourhood may be different. Models of competition that assume a homogeneous mixture of species predict that the dominant species will eventually outcompete all others, whereas coexistence of species may be allowed in a model of neighbourhood competition where the spatial structure of the community is considered (Tilman, 1994).

(3) Environmental variation/disturbance:

Environmental variation can occur on either a temporal or spatial scale. On a temporal scale environmental variation or disturbance events may allow the coexistence of species. Changes in environmental conditions may reverse the order of competitive superiority among species. If these changes occur at the right frequencies then the species will be allowed to coexist (Chesson and Case, 1986). Likewise, if disturbance events occur at intermediate levels, then it can be a mechanism for the coexistence of competing species (eg Sousa, 1979). Chesson and Case (1986) and Chesson (1986) provide thorough reviews of non-equilibrium coexistence of species.

Spatial variation in the environment (or environmental heterogeneity) can also facilitate the coexistence of competitors. For example, McLachlan (1993) found that two midge species, *Chironomus pulcher* and *Chironomus imicola* could coexist in some rain puddles. Yet, in the laboratory, *C. pulcher* showed total competitive exclusion of *C. imicola*. McLachlan (1993) found that coexistence of the two species required both sunny and shady refuges.

(4) Predation/herbivory/disease:

The superiority of one competitor over another can be nullified by adding a predator, herbivore or pathogen to the system. If the predator prefers to prey upon the superior competitor, then the inferior competitor will be able to gain an advantage and it is possible for the two species to then reach a stable equilibrium of coexistence. For example, Werner and Anholt (1996) reported competition between tadpoles of small bullfrogs (*Rana catesbeiana*), large bullfrogs of the same species and green frogs (*R. clamitans*) in the presence of a non-lethal (caged) predator *Anax junius*. Without the predator small bullfrogs had a higher per-unit-biomass competitive effect than the larger bullfrogs or green frogs. However, in the presence of the predator the growth and survivorship of the small bullfrogs and green frogs was reduced. In contrast, large bullfrogs increased in growth and survivorship relative to the non-predator treatment. It was concluded that the presence of the predator had altered the behaviour of the small bullfrogs such that they would retreat from the predator and forage less. This allowed the usually inferior large bullfrogs to utilise more of the resource and hence increase growth and survivorship. The predator had an indirect positive effect on the large bullfrogs. This type of indirect effect could potentially allow the coexistence of species.

(5) Intransitivity of competitive interactions

If species A is a superior competitor to species B, and species B outcompetes species C, then the three-way interactions is called intransitive if species C then outcompetes species A (A>B, B>C but C>A). If A outcompetes C then the three species form a transitive hierarchy of competitive abilities (A>B>C). When

intransitivities are embedded in a larger competition matrix then they tend to increase the species diversity (Karlson and Jackson, 1981).

(6) Indirect competitive effects

Indirect effects can be defined as the effect that one species has on another as mediated through a third species. For example, if species A reduces the density of both species B and C, and species B also has a negative effect on the density of C, then species A would also have a positive indirect effect on species C because it reduces the density of species B. So the occurrence of indirect effects reduces the impact of the direct effects of species A and B on C therefore increasing C's chance of coexisting with A and B.

Lawlor (1979) proposed a model of a competition matrix which incorporated both direct and indirect effects of all species on each other. It was found that in the community context, some indirect positive effects may outweigh the direct negative effects.

1.7 Coexistence of wood decay basidiomycete species

Using the three criteria for competitive exclusion outlined in section 1.6, it can be seen that many species of WDB's should not be able to coexist under static conditions. The criteria are explored in more detail:

(1) Resources in sufficiently limited supply.

The mycelium of a fungus is potentially limitless. *Armillaria bulbosa* is an aggressive tree pathogen that can also utilise woody debris. An individual of this species can form cords to link resource units. Smith *et al* (1992) discovered an individual *A. bulbosa* which covered 15 hectares, weighed in excess of 10000 kg and had remained genetically stable for more than 1500 years. While not all species extend this far, it does make the point that once an individual is established within a resource it can colonise the whole resource unit.

In addition, it is rare to find a branch on the forest floor that doesn't have some indication of either mycelium or fruiting body of WDB or both (Pers. obs.; Renvall, 1995).

2) Overlapping resource use.

Most WDB's at the later, more competitive stages of decay are specialised in utilising fallen wood (Rayner and Boddy, 1988). This means that there are many species utilising the same resource.

3) Asymmetric competition.

Fungal literature is replete with examples of one species outcompeting the other. Competition between WDB species is usually asymmetric. For example, in *in vitro* studies *Coriolus versicolor* replaced *Exidia glandulosa*, *Peniophora quercina*, *Stereum gausapatum* and *Vuilleminia comedens* (Boddy and Rayner, 1983b) and *Phlebia merismoides* replaced *Coriolus versicolor* and *Stereum hirsutum* (Carruthers and Rayner, 1979). Although there are fewer examples of asymmetric competition in the

field, Coates and Rayner (1985c) demonstrated that asymmetric competition does occur in the natural situation. For example, *Armillaria bulbosa* was replaced by *Hypoxyloma fasciculare*, *Phallus impudicus*, *Phanerochaete velutina* and *Tricholomopsis platyphylla* (Coates and Rayner, 1985c). Also, *Chrondostereum purpureum* was replaced by *Bjerkandera adusta* and *Coriolus versicolor* (Coates and Rayner, 1985c).

1.7.1 Do competing WDBs Coexist?

Study of the three criteria show that in a WDB community, resources are limited, species use of these resources is overlapping and that interspecific competition is asymmetric. Therefore WDB species should not be able to coexist with one another in the same habitat. Many studies have reported the occurrence of many species of WDB within a habitat (eg Niemela et al, 1995; Rayner, 1977; Renvall, 1995). The fact that WDB guilds do occur leads us to then examine how it is that these competitors coexist.

1.7.2 Conditions under which WDB have been known to coexist:

1) Patchy landscape

Whether a WDB species must produce spores to disperse to a new branch or whether it produces cords, it must still go through the process of producing those propagules and colonising the new substrate. This implies that each branch can be considered to be a single resource unit, much the same as a single patch of a resource. If different WDB species have different ecological strategies such as ruderal (R selected), combative (C selected) or stress-tolerant (S selected), then it may be possible for these

species to coexist within the same habitat. By ruderal strategists arriving early at a branch, and quickly growing and escaping before more combative species arrive and displace the other species, the species with these two different strategies would be able to coexist. This is the traditional hypothesis proposed to explain community structure of WDBs (Cooke and Rayner, 1984; Rayner and Boddy, 1988). There is some evidence to suggest that there are some trade-offs between quick dispersal and combative strategies. These were discussed in section 1.4.

It is possible that the coexistence of some species may be due to R, C and S strategies. However, this still does not explain the coexistence of many secondary colonisers (C selected) of wood. For example, *Hypholoma fasciculare*, *Phallus impudicus*, *Phanerochaete velutina* and *Tricholomopsis platyphylla* are highly combative species which often invade pioneer decay communities (Rayner and Boddy, 1988). At this highly combative stage, how do so many species which often form a hierarchy of competitive abilities (Rayner and Boddy, 1988) coexist?

2) Environmental variation/disturbance.

Although non-equilibrium theories have not been widely studied in fungal systems, the potential importance has been recognised (Strong, 1992). Examples of physiological optima are abundant in mycological literature. For example Boddy (1983) reported the different optimal temperatures for growth of 11 WDB species. In a variable temperature environment, the growth rates of the fungi would presumably vary with the temperature and therefore not allow a single species to outgrow the others. The chance of these species coexisting would be higher in a variable environment than under

static conditions. Unfortunately this concept has not yet been tested in a fungal community.

3) Predation/herbivory/disease

Newell (1984a, 1984b) reported the competition between two litter decomposing fungi. In the laboratory *Marasmius androsaceus* outcompeted *Mycena galopus*. However when a collembolan *Onychiurus latus* which selectively grazed *M. androsaceus* was added to the system, *M. galopus* became more abundant. It is therefore quite possible that WDB species may be able to coexist due to the number of links to other species in the community such as bacteria, collembolans, termites etc.

4) Intransitivity

The occurrence of intransitive loops in competition matrices of WDB is highly likely given the variation in the modes of competition such as diffusion of toxins and hyphal interference (see section 1.3). Species A may be able to repel species B by releasing a toxin and species B may be able to outcompete species C by using another toxin, but it does not necessarily mean that A will be able to repel C with it's toxin as C may be immune to this substance. If so then it is quite possible that C could outcompete A by hyphal interference.

Intransitivities have not been reported in WDB communities and their occurrence is dealt with in Chapter 6.

5) Indirect competition

Culver (1992) suggested that indirect competition may be very important in fungal communities but their occurrence has not yet been demonstrated. Indirect effects in a WDB community are reported in Chapter 4.

Indirect effects and interaction modifications can be very influential in community dynamics (eg Dungan, 1986; Creed, 1994; Abrams and Matsuda, 1996; English-Loeb, Karban and Hougen-Eitzman, 1993). It has been suggested that indirect effects can allow the coexistence of many species competing for the same resource (Lawlor, 1979).

1.8 Indirect effects, interaction modifications and non-additivity

Indirect effects and interaction modifications can be very influential in community dynamics (eg Dungan, 1986; Creed, 1994; Abrams and Matsuda, 1996; English-Loeb, Karban and Hougan-Eitzman, 1993). It has been suggested (Lawlor, 1979) that indirect effects can allow the coexistence of many species competing for the same resource.

An indirect effect is the effect that one species (A) has on another (B) via a change in density of a third species (C) (Billick and Case, 1994). Indirect effects occur as a logical sequence of chains of direct interactions between species. For example, in pairwise interactions, species A outcompetes both species B and C, and species B also outcompetes C. When these three species are put together, species A directly reduces the population size of C. It also has an indirect effect on C, because it reduces species B.

Since B is reduced it has less effect on C. Therefore the effect of both species A and B on C may not simply be a sum of the effects found in pairwise interactions.

An interaction modification occurs when the nature of the interaction between two species is altered by the presence of a third species (Abrams, 1983). Interaction modifications cannot be predicted from pairwise, direct interactions. There have been a number of studies which have used interaction modification terms to explain discrepancies between predicted and observed data. However, many of these studies have been criticised because of problems with the model chosen or experimental faults (Billick and Case, 1994). Wissinger and McGrady (1993) provide a clear demonstration of an interaction modification in a study of interactions between a migratory dragonfly (*Tramea lacerata*), a common resident dragonfly (*Erythemis simplicollis*) and their shared prey (damselfly larvae). The two dragonfly species also preyed on each other. The predatory effects of the two dragonfly species were not additive on the damselfly larvae. They conducted experiments where they removed the mouth parts of one of the dragonfly species so that they could not consume prey. In these experiments *T. lacerata* reduced the consumption rates of *E. simplicollis* to less than half of that observed when *E. simplicollis* foraged alone. This is a clear demonstration of a change in behaviour resulting in an interaction modification.

Non-additivity refers to a situation where the effects calculated from pairwise interactions cannot be simply summed to predict the result of the three-way interactions. The non-additivity could be due to either indirect effects or interaction modifications. For example, the effect of species A and B on species C is not simply the sum of the effect that species A has on C in pairwise interactions and the effect that species B has on C in pairwise interactions. In the experiment described above by Wissinger and

McGrady (1993) the predatory effects of the two dragonflies were non-additive. They discovered that this non-additivity was due to both an interaction modification caused by a change in the behaviour of the dragonflies and to an indirect effect mediated through *T. lacerata*'s predation on *E. simplicollis* larvae.

In order to separate indirect effects from interaction modifications, either the measurements have to be taken over a time period which is short enough to negate the indirect density effects, or the indirect effects have to be accounted for in model to be tested, or the change in behaviour needs to be directly observed (Billick and Case, 1994).

Indirect effects and interaction modifications have been known under various names. Indirect effects are also known as trophic linkage, ecological, abundance, population or species interactions indirect effects. Interaction modifications are also termed higher order interactions or behavioural or trait-mediated indirect effects (Menge, 1997). Much of the discussion on interaction modifications in the literature has been carried out under the term higher order interactions (Wootton, 1994b). This had led to some confusion because higher order interactions can also refer to non-linear direct effects (Wootton, 1994b). Therefore the term interaction modification is used in this thesis.

1.8.1 Testing for non-additivity

There have been several different approaches to detecting indirect effects and interaction modifications. Firstly, interaction modification terms were invoked to explain a deviation from the predicted dynamics of a multispecies community based on

Lotka-Volterra equations. Vandermeer (1969), Levine (1976) and Pomerantz (1981) all tested for interaction modifications by looking for departures from the Lotka Volterra equations (equation 1.2) for multispecies competition.

$$\frac{dN_i}{dt} = \frac{r_i N_i}{K_i}\left\{K_i - N_i - \sum_{j=1}^{m} \alpha_{ij} N_j\right\} \qquad \textbf{Equation 1.2}$$

$j \neq i$

where there are m species in the community, α_{ij} is the effect of the *j*th species on the *i*th species, r is the intrinsic rate of natural increase, N is the number of individuals, t is time and K is the saturation density.

This approach was strongly limited by the assumptions of the Lotka-Volterra equations which have been more broadly criticised (Neill, 1974; Tilman, 1987), and also by a basic problem that equilibrium densities need to be calculated (Neill, 1974).

A more general approach is the use of two-way ANOVA to test for non-additivities (see figure 1.12). A significant interaction term indicates that the response variable is not additive (Morin, Lawler and Johnson, 1988; Fauth and Resetarits, 1991; Worthern and Moore, 1991; Huang and Sih, 1990, 1991; Blaustein, Kotler and Ward, 1995). For example, Morin *et al* (1988) reported a significant interaction between the presence of aquatic insects and the tadpoles of the frog *Bufo woodhousei fowleri* on the body mass at metamorphosis of the frog *Hyla andersonii*. The combined competitive effect of insects and *Bufo* on *Hyla* was less than the sum of the separately measured effects of both groups of competitors. They discuss a possible mechanism underlying this interaction. The reduction have been caused by insects competing with and reducing the size of *Bufo* tadpoles. Because small tadpoles tend to be weaker

competitors than their larger counterparts there could have been a reduction in the per capita competitive effect of *Bufo*.

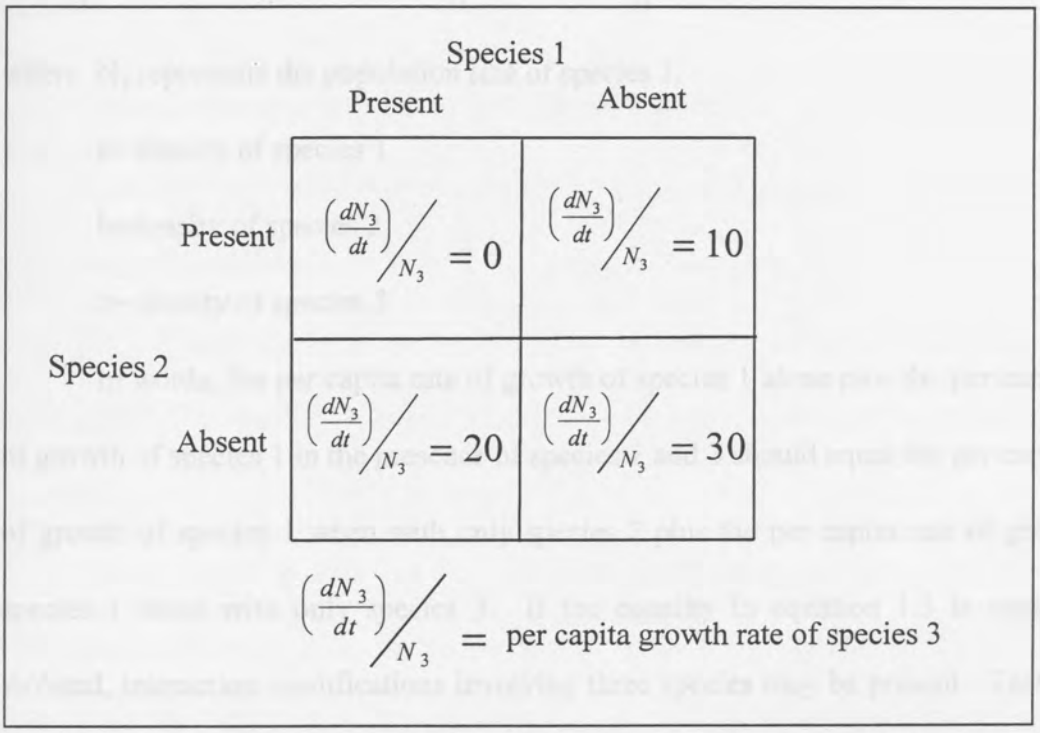

Figure 1.12: Diagram of the additive effect of two species on a third species demonstrating the model behind a two-way ANOVA testing-for non-additivity in per capita growth rate

Use of ANOVA is convenient and has the potential to reveal non-additivities. However, to test for non-additivities using ANOVA an experimenter must be extremely cautious about the parameters chosen, the response variable, the underlying model and assumptions of the ANOVA and the interpretation of the results. As Billick and Case (1994) point out, an ANOVA may have a significant interaction term if an inappropriate response variable is used or an extra unforseen parameter is within the system, but not accounted for (such as an extra species).

Case and Bender (1981) and Billick and Case (1994) determined a general model for testing for non-additivities in per-capita interactions terms (see equation 1.3).

$$\frac{\frac{dN_1}{dt}(a,0,0)}{a} + \frac{\frac{dN_1}{dt}(a,b,c)}{a} = \frac{\frac{dN_1}{dt}(a,b,0)}{a} + \frac{\frac{dN_1}{dt}(a,0,c)}{a} \qquad \textbf{Equation 1.23}$$

where N_1 represents the population size of species 1.

a= density of species 1

b=density of species 2

c= density of species 3

In words, the per capita rate of growth of species 1 alone plus the per capita rate of growth of species 1 in the presence of species 2 and 3 should equal the per capita rate of growth of species 1 when with only species 2 plus the per capita rate of growth of species 1 when with only species 3. If the equality in equation 1.3 is statistically violated, interaction modifications involving three species may be present. Testing for non-additivities using two-way ANOVA is equivalent to the test in equation 1.3 when per-capita growth terms are used as the response variable in the ANOVA (Billick and Case, 1994).

This model is designed to detect indirect effects and higher order interactions in systems where the response variable is per capita rate of population growth after starting off at a set density. However, as Billick and Case (1994) point out, effects could be mediated through other variables (eg body size) and the target variable should be body size. The problems of using a model based on population size in the WDB system on agar plates is discussed in section 1.8.2.

Other models for testing for indirect effects and interaction modifications (eg Wootton, 1994a; Adler and Morris, 1994) have a similar difficulty because they utilise

population size as the response variable. Therefore these models cannot be used to test for indirect effects and interaction modifications between fungal individuals. However, some of the principles on which the models are based can be used to study fungi.

1.8.2 Non-additive competition in fungal communities

A fungal individual interacts with only a few other fungal individuals over one generation. For example Renvall (1995) found that there were an average of 3.2 individuals in each log. This means that when we test interactions between species in the laboratory, we need to replicate the natural situation by having just a few individuals interacting.

Most competition studies on WDB's are therefore conducted on the scale of a few individuals rather than at the population level. By reducing the scale of the experiment from population to individual, we are making important assumptions that; (1) there is no variation between individuals, and (2) that generations occur as discrete time periods. Obviously in a natural situation these assumptions are violated. However, in order to study the mechanisms of competition at the simplest level it is sufficient to conduct experiments using individuals as representatives of species. For this reason there will be no inferences or generalisations made from the interactions between these representatives of their species to the whole population or species. The experiments in this thesis are designed to test mechanisms at the simplest level. The next step will be to investigate intraspecies or intrapopulation variation and observe the effects of these on community dynamics.

Since the models for detecting interaction modifications are based on populations, is it relevant to talk about indirect effects and interaction modifications between individuals instead of populations in fungal communities? The argument for using individuals is that this is the scale at which interactions occur therefore if we are to look for indirect effects and interaction modifications then surely we must look for them at the level at which they are interacting. Unfortunately, by focusing on the individuals we may be losing some detail of the interactions under which community dynamics occur. In addition it means that we cannot generalise from the results of this experiment to a WDB guild in nature. The purpose of this study is to detect the occurrence of indirect effects and interaction modifications at the level of the individual.

A problem might arise if indirect effects or interaction modifications somehow act at the population level i.e. through the variation between individuals or the non-discrete time periods. These possibilities can be tested at a later date. For now it is enough to look for indirect effects and interaction modifications at the level of the individual.

A fungal individual has potentially limitless growth. In this sense then, a fungal individual behaves much like a population does. One individual does not have a constant effect on another individual. The effect changes with the size of the individuals (Holmer and Stenlid, 1993). Per capita interaction terms in general ecology models can be translated in fungal competition to per unit area or per unit length of contact between two individuals.

1.8.3 Testing for indirect effects and interaction modifications amongst fungal individuals:

How do we test for indirect effects or interaction modifications between individuals? We cannot use the same models that were based on populations. However, we can use some of the same principles. It is important at this stage to state explicitly the hypothesis to be tested. Broadly the aim is to ask 'are there indirect effects or interaction modifications present between individuals of WDBs? Initially the aim is to combine both of these effect and look for non-additivities. If these are present, then at a later stage we can separate out indirect effects from interaction modifications. To use the essence of Case and Benders' (1983) model of additivity, we can then test generally for non-additivities. That is, do the effects of species B on species A and species C on species A combine in an additive fashion when both species B and C are in the presence of species A?

Most studies on indirect effects and interaction modifications have used additive designs whereas many plant ecologists use a replacement design almost exclusively. Both designs have their advantages and disadvantages. In a standard replacement (or substitutive) design, mixtures are formed by replacing a given number of individuals of one component by the same number of the other component (see figure 1.13). As a result, the density of each component is less in the mixture than it's pure stand, but the total stand density is the same in the mixture as in each pure stand (Snaydon, 1991). In a standard additive design, mixtures are formed by adding individuals of J to the number of individuals of I present in the pure stand. As a result the total stand density is greater in the mixture than in the pure stand, but the density of each component is the same in the mixture as in the pure stand.

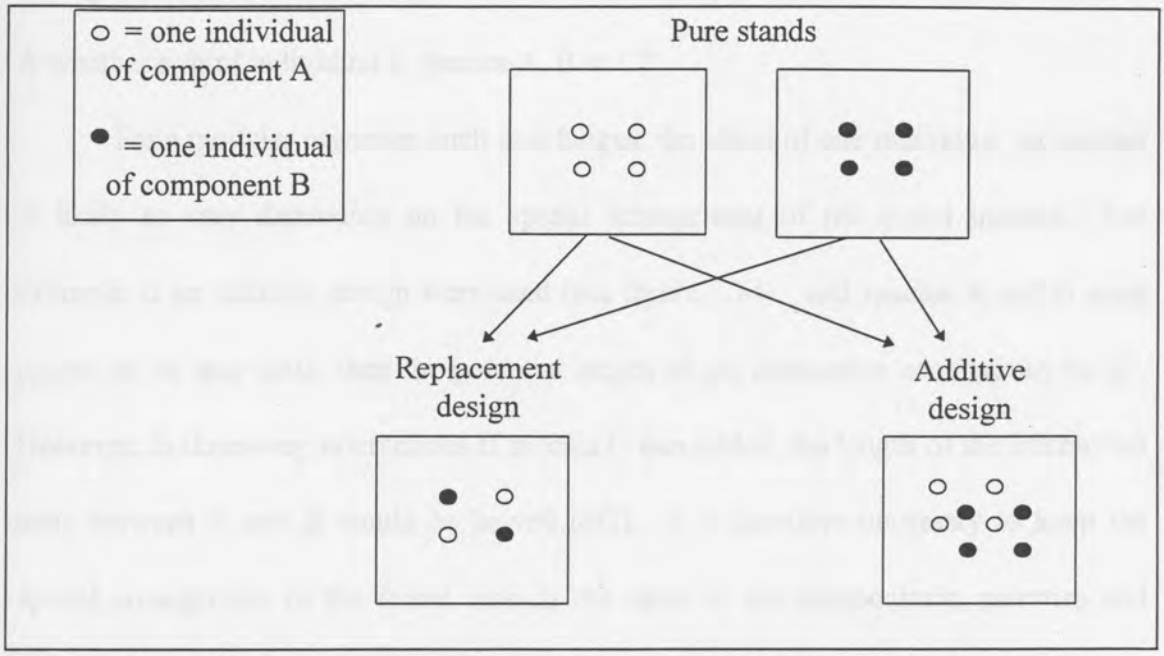

Figure 1.13: Diagram demonstrating additive and replacement experimental designs for competition

An additive design has the advantage that there is no confounding effect of changing density of a component, however it has a disadvantage that it has a confounding effect on overall density. The question arises: is the effect due to the different component or to the increased density? For this reason plant ecologists studying competition have tended to use the replacement design where there is no confounding effect of overall density. However, there is a problem in that a different component density is added to the target species. This last problem can however be overcome by measuring per capita effect.

The two different experimental designs ask two different questions. In terms of testing for indirect effects and interaction modifications the additive design is asking: does one individual of species B have the same effect on an individual of species A whether an individual of species C is present or not? Whereas a replacement design is

asking, does an individual of species B have the same effect on an individual of species A whether a third individual is species A, B or C?

For a modular organism such as a fungus, the effect of one individual on another is likely to vary depending on the spatial arrangement of the initial inocula. For example, if an additive design were used (see figure 1.14), and species A and B were paired on an agar plate, then the potential length of the interaction zone would be 'd'. However, in three-way interactions if species C was added, the length of the interaction zone between A and B would be halved (d/2). It is therefore necessary to keep the spatial arrangement of the initial inocula the same in the monoculture, pairwise and three-way treatments. A replacement design was therefore used in this experiment.

In a replacement design like this, if the effect of the two strains on the third is not the sum of the effects in pairwise competition, then this would indicate some indirect effect or higher order interaction. We can then look for evidence of an indirect effect by looking for alterations in the area of the mediator species.

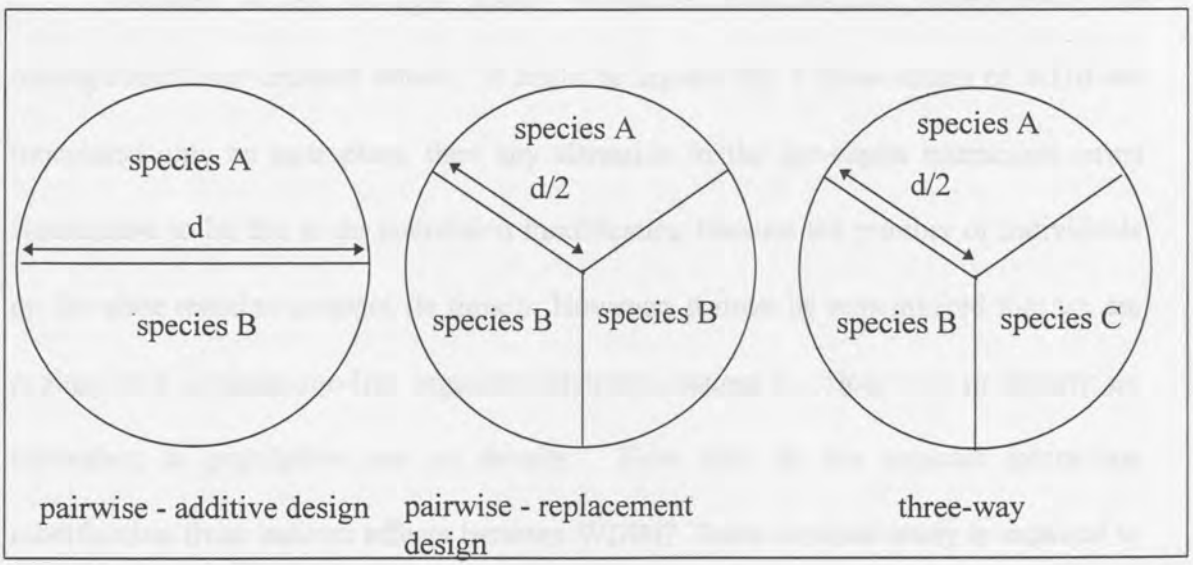

Figure 1.14: Diagram showing the length of the interaction zone between two strains in pairwise and three-way interactions in additive and replacement designs. In a pairwise-additive design there are only two inocula, whereas in the pairwise-replacement design and in the three-species interactions there are three inocula.

1.8.4 Separating interaction modifications from indirect effects in agar plate WDB competition studies:

Classical indirect effects are mediated via changes in population density. Therefore interaction modifications can be separated from indirect effects if: (a) the treatment species are held at constant density or; (b) the interactions terms are insensitive to changes in the treatment species density or; (c) the per-capita interaction terms are measured over a time period which is short enough to negate any density effects or; (d) the mechanism of the change in the interaction between a pair of species on adding a third is identified (Billick and Case, 1994). The strongest demonstration of interaction modifications are from studies that can mechanistically identify how one species modifies the interactions between other pairs of species (Wootton, 1994b). For example, Wootton (1992) found that goose barnacles indirectly affected limpet abundance by changing the efficiency of bird predation, a modification of the predator-prey interaction.

Between WDBs on agar plates, interaction modifications cannot easily be distinguished from indirect effects. It could be argued that if three strains of WDB are inoculated onto an agar plate, then any alteration in the per-capita interaction terms would have to be due to an interaction modification because the number of individuals on the plate remains constant (ie three). However, it must be remembered that we are dealing with a population-like organism whereby changes in colony size or density are equivalent to population size or density. How then do we separate interaction modification from indirect effects between WDBs? More detailed study is required to answer this question. The purpose of this study was to detect non-additivities and gain some insight into how these non-additivities may occur. If non-additivities are present,

then separating interaction modification from indirect effects is a topic of further study. In this study, hypotheses are given about how these non-additivities could occur. For example, if strain 1 negatively effects both strain 2 and 3 in pairwise interactions, and strain 2 also negatively effects strain 3, and if in three-way interactions the combined effect of strains 1 and 2 on 3 were non-additive and 3 was not as reduced as it should have been, then it could be hypothesised that strain 1 had an indirect positive effect on strain 3 by inhibiting strain 2, thereby lessening the effect that strain 2 had on strain 3.

1.8.5 Do non-additivities in ecological communities increase the chance of species coexisting?

The problem of the coexistence of many species which compete for the same resource was discussed in section 1.6. One of the hypotheses proposed to explain coexistence of competing species was the presence of indirect effects and/or interaction modifications within the competition matrix. Lawlor (1979) demonstrated theoretically that by incorporating indirect effects into a model of community dynamics, the species within the community have net mutualistic interactions and are therefore more likely to coexist. Stone and Roberts (1991) elaborated further and term these net mutualistic interactions Advantageous in the Community Context (ACC) interactions and argued that there is considerable empirical evidence of ACC interactions. For example, Davidson (1980) studied a community of granivorous ants which competed for resources. She found that in the context of the whole community 34% of the interactions were advantageous.

The intention in chapter four is to test whether indirect effects and/or interaction modifications between competing WDBs could increase the chance of coexistence.

1.9 Field vs laboratory based experiments:

Ideally, when studying the mechanisms of WDB community dynamics, experiments should be conducted in the field. There are many essential differences between the field situation and controlled laboratory conditions. Results from laboratory experiments can often not be directly applied to understanding mechanisms of community structure in the field. However, laboratory experiments are satisfactory for investigating the possibility of certain mechanisms. For example, we now know how antagonistic interactions occur between pairs of species because laboratory experiments on agar plates discovered diffusible chemicals that inhibit the victim organism (Bruce, Austin and King, 1984). Likewise, we can investigate the basic mechanisms of community ecology in the laboratory. The experiments in this study are therefore conducted in the laboratory.

1.10 Aims and hypotheses:

The main aim of this thesis was to further the understanding of the processes influencing the dynamics of WDB communities. One of the observed features of the structure of WDB communities is that many competing species coexist. The main

theme of the thesis is therefore to discover mechanisms by which many competing WDB species could coexist.

1.10.1 Field survey (chapter 2):

Initially, a field survey was conducted to confirm that many WDB species coexist within a habitat in South Australia. This survey also aimed to reveal the abundance patterns of WDB species and gain some insight into the structure of the community. The fruiting bodies which were collected in the survey were used to isolate cultures of WDBs for competition studies (chapter 3).

1.10.2 Mechanisms of coexistence:

The two hypotheses tested were that indirect effects or interaction modifications (chapter 4) and intransitive competitive abilities (chapter 6) within competition matrices could increase the chance of species coexistence. The occurrence of indirect effects or intransitivities had not previously been tested, although several authors had recognised that they probably do occur (Culver, 1992; Rayner and Boddy, 1988).

1.10.3 Competitive ability of homokaryons vs heterokaryons

Another aspect of community structure of WDB that needs further investigation was the influence of the homokaryotic stage on community dynamics. Little is known about the mycelium of homokaryons, including how long they exist before they find compatible mates and form heterokaryons and if they are combative against other

WDBs. Some previous studies had shown that the growth rate (Simchen, 1966) and degrading ability (Amburgey, 1970; Elliot *et al*, 1979) of homokaryons was not necessarily inferior to heterokaryons of the same species. However, there is a general perception that homokaryons are relatively unimportant in the dynamics WDB communities (I thank the many mycologists who participated in my informal survey of opinion of this topic).

2. Species abundance patterns of two wood decay basidiomycete communities

Summary:

Wood decay basidiomycetes from fallen *Eucalyptus* branches were sampled from two patches of native vegetation over two years. Thirty six species were found at the two sites. Species richness was higher at the site with more dense vegetation and higher moisture levels. Principle components analyses and species abundance curves indicate that the community is influenced by many unknown factors.

2.1 Introduction:

This chapter describes a field survey of wood decay basidiomycete (WDB) fruiting bodies in two patches of Australian native vegetation. This survey was used to detect the coexistence of competing species of WDB (see section 1.6). In addition, log abundance curves, diversity, evenness and species richness measures, Principle Components Analysis and Discriminant Function Analyses were used to gain some insight into the underlying structure of these two communities and to compare the two sites.

A common method for representing species abundance patterns is to plot the rank order of abundance of species against the log number of individuals of each species (Tokeshi, 1993). The resulting curves commonly fall into one of three categories: geometric, broken-stick or lognormal (May, 1975). The ecological meaning of these curves have been the cause of considerable debate as to their ecological meaning (eg May, 1975; Pielou, 1975). Generally, geometric and broken stick distributions are thought to be characteristic of relatively simple communities whose species dynamics are dominated by some single factor. The lognormal distribution is associated with communities in which the species are affected by many variables (May 1975).

2.1.1 Coexistence:

One of the objectives of this thesis was to discover mechanisms by which multiple species of wood decay basidiomycetes may coexist. This concept was discussed earlier in section 1.6. In previous studies of WDB, many species have been found to utilise the same resources in the presence of strong asymmetric competition (eg

Niemelä *et al*, 1995; Rayner, 1977). Under these conditions, multiple species should not be able to coexist. Hence the first aim of this field survey was to see if multiple species of WDB coexist on the same resource within two patches of remnant native vegetation in South Australia.

2.1.2 Species diversity:

The second objective of this survey was to estimate the species diversity of wood decay basidiomycetes in the two patches of remnant native vegetation. The majority of the vegetation on the Fleurieu peninsula of South Australia (see figures 2.2 & 2.3) was cleared for farm land between the 1800's and the 1950's. As a result there are only remnant patches of native vegetation left. Since the objective of this survey was to determine natural species abundance and distribution, it was necessary to sample within these remnant patches. Two sites were chosen because of their contrasting vegetation types (see section 2.2.1) and close proximity to one another. One site (Kyeema Conservation Park) has a continuous overstory of *Eucalyptus baxteri* and *E. obliqua* and has non-sandy (yellow podzolic) soil (Northcote, 1976). The other site (Cox Scrub Conservation Park) has patches of *Eucalyptus baxteri* and *E. obliqua* and sandy soils. The understorey at Cox Scrub is generally more exposed to the weather and the area has slightly less rainfall.

The hypothesis proposed was that Kyeema would have higher species diversity of WDB than Cox Scrub. This was predicted due to the slightly higher rainfall at Kyeema with thicker vegetation and non-sandy soil, would create an overall moister

habitat for fungal growth. Previous studies on fungal diversity indicate a positive relationship between moisture levels and species diversity (Christensen, 1981).

Species diversity has two distinct components; species richness and evenness. Species richness is simply the number of species in the community whereas evenness refers to the relative abundances of species. Measures of heterogeneity (species diversity) are a combination of these two concepts (Krebs, 1989).

Many different diversity indices have been constructed, however there is still no agreement which is the 'best' measure (Krebs, 1989). The two most common indices used are the Shannon-Weiner and Simpson's indices. The Shannon-Wiener index tends to discriminate better between communities with different diversities but it is severely affected by sample size (Brower *et al,* 1977; Krebs 1989). Due to the variability between diversity indices it was necessary to calculate estimates using two types of diversity index, two measures of species richness and two measures of evenness.

Common measures of the precision of species richness are based upon general rarefaction, Jackknife and bootstrap methods. The bootstrap method is more appropriate when a large number of quadrats have been sampled (>20) (Krebs 1989), so only the first two measures were used.

2.1.3 Australian perspective:

Due to the cosmopolitan distribution of many fungal species and their occupancy of apparently the same ecological niches worldwide (Christensen, 1981), it is likely that there are many similarities between the wood decay flora found in studies in the Northern hemisphere and in Australia. However there are some fundamental

differences between broadleaf or conifer forests and Eucalypt forests/woodlands which are likely to influence WDBs, in particular, fire and litterfall.

Fires play an important role in the Australian landscape. Many Australian plants are adapted to bushfire, only regenerating from seed after a fire (Gill, 1981). Although the influence of fire on WDBs is unknown it is expected that a fire would influence the community structure enormously because it removes all litter from the forest floor (see figure 2.1) and replacement litter does not begin accumulating for another 12 months after the fire (pers. obs.: In 1994 there was a bushfire in Kyeema Conservation Park). How the WDB community survives is unknown. Possibly, these fire regimes create a situation whereby this community never reaches an equilibrium.

Figure 2.1: Photograph of a section of Kyeema Conservation Park after a fire

The nature of litterfall in *Eucalyptus* forests/woodlands is quite different to that of the forests studied previously. There are very few large fallen logs on Eucalypt forest floors. The *Eucalyptus* species in South Australian woodlands/forests are characterised by a high rate of self pruning: most branches are shed once the canopy grow above them. The major woody litterfall is of branches between 1 and 10 cm diameter and usually no longer than 2m (pers. obs.). Northern Hemisphere studies have looked either at twigs (<1cm) or large fallen logs and have found a vastly different fungal communities in each (Rayner and Boddy, 1988).

2.1.4 Nomenclature:

Despite the significant contributions of collectors and taxonomists such as Cleland and Mueller the state of fungal taxonomy in Australia remains far behind Europe and North America (May and Pascoe, 1996). Throughout the past 200 years of Australia's colonisation, there has been considerable interest in the fungi, mostly culminating in extensive collections (May and Pascoe, 1996). Unfortunately however, there was a lack of taxonomic expertise in Australia and many specimens were sent overseas. Many overseas taxonomists have studied Australian fungi without actually stepping foot on Australian soil. Mueller was one of the first collectors to actually describe and treat fungal material in Australia (May and Pascoe 1996). Cunningham (1963; 1965) published extensive keys to the polypores and thelephores of New Zealand and Australia but sadly much of the nomenclature is now outdated and many species are not treated (Simpson, 1996).

Today there are only a handful of fungal taxonomists in Australia and the main collections are spread over four herbaria. There are no relevant keys to the main groups of WDBs in Australia. Putting names to specimens is therefore extremely difficult. There are keys to the European and North American species (eg Jülich and Stalpers, 1980; Eriksson and Ryvarden, 1973; Eriksson and Ryvarden, 1975) and many of the fungi appear cosmopolitan. Some taxa fit the morphological descriptions in these keys and can be named in this way: this is not to say that they are identical because critical systematic studies may show the Australian taxa to be distinct. Taxonomic studies of wood decay fungi in Australia are currently challenging and represent a great opportunity in the future.

2.2 Methods:

2.2.1 Field sites

Both sites are located on the Fleurieu Peninsula of South Australia (see map, figure 2.2). Cox Scrub is 10 km south of Kyeema (see map, figure 2.3) and at a lower elevation (~160m vs 350m).

The rainfall in both sites falls predominantly in winter with the highest gaugings in May to August. The average annual rainfall at Kyeema is around 900 mm, that of Cox Scrub is approximately 700 mm. The hottest months are January and February with maximum temperatures averaging 27°C. July temperatures are generally 10 to 15°C below summer temperatures (Jenkins, 1985).

A bushfire swept through most of the Fleurieu Peninsula in February 1983. Both Kyeema and Cox Scrub were entirely burnt out except for one very small patch in Cox Scrub. During the course of this study (February 1995) another fire burnt approximately a third of the area of Kyeema (see figure 2.1). The newly burnt area was not surveyed after the fire.

Kyeema Conservation Park

Kyeema Conservation Park (S 35° 16' E138° 39') covers 348.9 hectares (see figure 2.3). This open woodland forest is dominated by an overstorey of a common stringybark, *E. obliqua* L'Herit. with some patches of a second stringybark, *Eucalyptus baxteri* (Benth.) Maid. and varying amounts of understorey, from sparse, low herbaceous plants to thick understory 2m high. This understorey consists mostly of *Acacia verticillata*, *A. myrtifolia*, *Pultenaea daphnoides*, *Platylobium obtusangulum*,

Hakea rostrata, *Leucopogon concurvus*, *Baeckea crassifolia*, *Davesia benthamii*, *Boronia caerulescens* and *Tetratheca pilosa*. Figure 2.4 shows medium understorey. The overstory of Kyeema is generally continuous but in some areas there is a patchiness of *E. baxteri* or *E. obliqua*. Kyeema has rolling hills with Precambrian schists and gneisses outcropping occasionally in gullies and creekbeds (Jenkins, 1985).

Cox Scrub Conservation Park

Cox Scrub (S 35° 22' E 138° 44') covers approximately 525 hectares. The area is comprised of gently undulating sands formed from Permian fluvioglacial quartz sands overlying ironstone horizons (Jenkins, 1985). Cox Scrub consists of open scrub and tall open shrubland of mainly *Eucalyptus baxteri* with some patches of *E. fasciculosa* or *E. cosmophylla*. One of the main structural differences as compared to Kyeema is the patchiness of the landscape. Where the overstory at Kyeema is mostly continuous (see figure 2.4), the overstory at Cox Scrub occurs in patches (see figure 2.5) which are interspersed with low shrubs, sedges or herbaceous plants such as *Banksia ornata*, *Leptospermum myrsinoides*, *Hakea rostrata*, *Hakea ulicina*, *Xanthorrhoea semiplana*, *Allocasuarina muelleriana*, *Pultenaea canaliculata*, *Platylobium obtusangulum*, *Calytrix tetragona* and *Hibbertia stricta* (Jenkins, 1985).

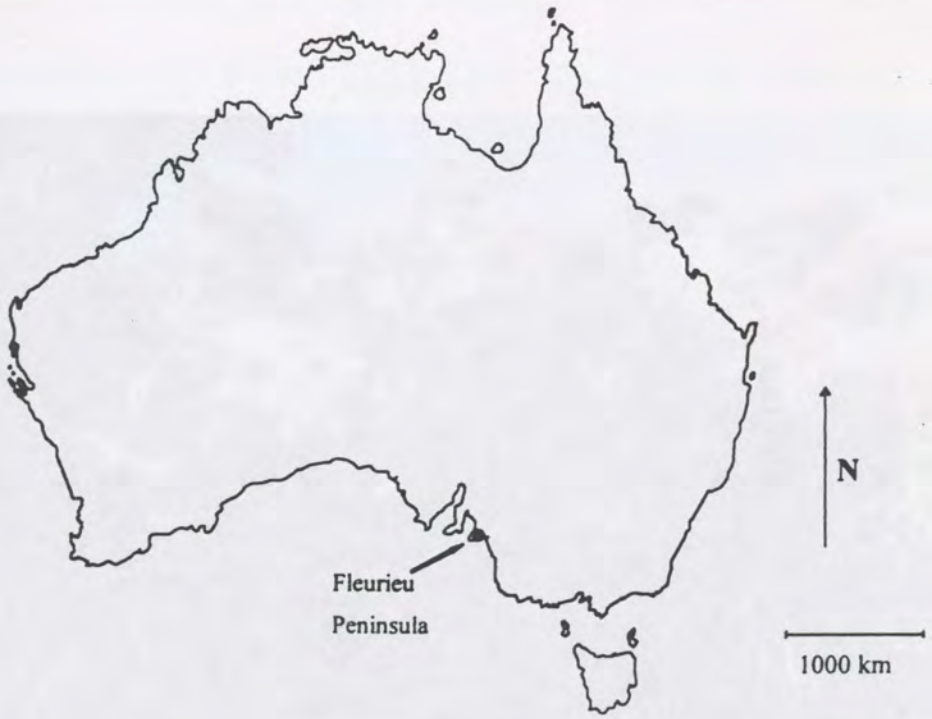

Figure 2.2: Map of Australia showing position of study area.

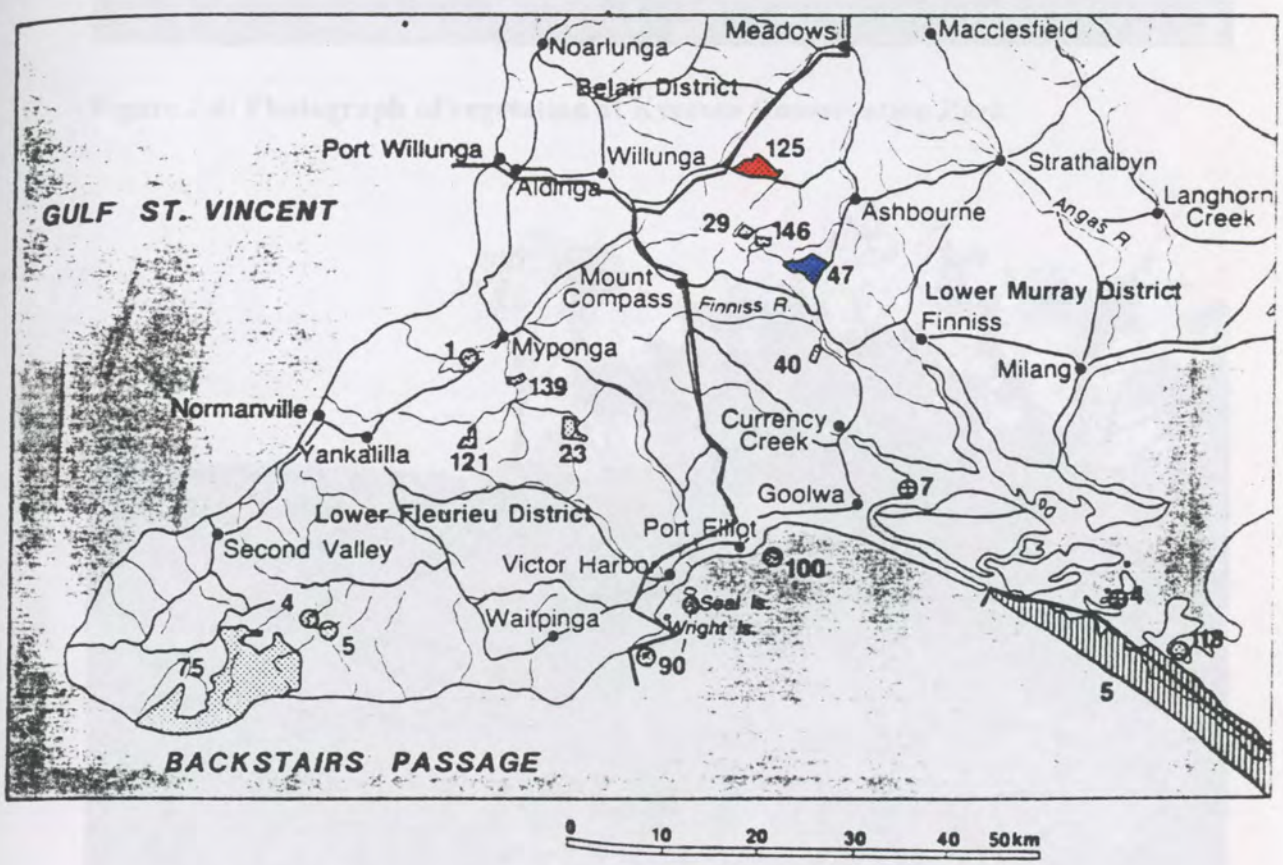

Figure 2.3: Map of Fleurieu Peninsula showing position of Kyeema (■) and Cox Scrub (■)

Figure 2.4: Photograph of vegetation at Kyeema Conservation Park

Figure 2.5: Photograph of vegetation at Cox Scrub Conservation Park

2.2.2 Survey of wood decay basidiomycete fruiting bodies:

The two field sites were sampled using quadrats. The quadrat size was restricted to 2 x 2 m because the clumps of 5-15 plants of *E. baxteri* or *E. obliqua* at Cox Scrub were roughly 3 m wide. The position of these quadrats were randomly selected by entering the coordinates of the edges of the park into a random number generator, and then navigating to a randomly chosen position using a GPS (Geographic Positioning System) receiver (Garmin 75). Once at the position I walked to the nearest clump of trees (usually not necessary in Kyeema) and randomly put down a marker on the edge of the clump. A quadrat was then measured out and sampled. The dimensions of each branch in the quadrat and the fungi fruiting on the outside of these branches were recorded. Figure 2.6 shows a photograph of a branch with a fungal fruiting body.

Branches were included in the survey if they were:

(1) detached from a living tree,

(2) touching the ground,

(3) from either *Eucalyptus baxteri* or *E. obliqua,*

(4) >1 cm or <10cm diameter at any point along the branch.

A quick sketch of each branch was made and the diameter and length were recorded on the sketch. The positions of any fruit bodies were recorded and samples were taken back to the lab to be identified and cultured. Only basidiomycetes were recorded and any dead fruit bodies were disregarded. When a branch crossed the boundary of the quadrat, the branch was sampled but the position along the branch

where it crossed the quadrat was recorded. The location of the quadrats, time of collection and brief descriptions are in appendix A.

Figure 2.6: Photograph of a fallen *Eucalyptus obliqua* branch with a fruiting body of *Ceriporia viridens*

2.2.3 Identification of specimens:

Several taxonomic keys were used for the identification of specimens. Ryvarden and Johansen (1980) or Ryvarden (1991) were used for specimens with pores on the under surface and Ainsworth, Sparrow and Sussman (1973) for the heterobasidiomycetes. The nomenclature of the non-poroid resupinate species follows either Jülich and Stalpers (1980) or the series; Eriksson and Ryvarden (1973), Eriksson

and Ryvarden (1975), Eriksson and Ryvarden (1976), Eriksson, Hjortstam and Ryvarden (1978), Eriksson, Hjortstam and Ryvarden (1981), Eriksson, Hjortstam and Ryvarden (1984), Hjortstam, Larsson and Ryvarden (1988) and Hjortstam, Larsson and Ryvarden (1987).

To identify a specimen, a wet mount was prepared by using a razor blade to cut thin sections (~50-100 um thick) of the fruit body. These sections were then placed onto a microscope slide with a drop of either distilled water, 10% KOH or Melzers reagent and observed under a light microscope at 400x or 1000x (oil immersion). The KOH slide was squashed gently by pressing on the cover slip with the eraser end of a pencil. This allows the hymenium to spread and separate, so that characters such as the clamps at the base of the basidia may be observed. An eyepiece micrometer was used to measure the size of the microstructures. Often a section of the fruit body was sterile, so many sections were taken in order to observe basidia and basidiospores.

The microstructural characters observed initially were:

Hyphal structure: presence or absence of skeletal hyphae, binding hyphae, clamps on the generative hyphae, thickness of hyphae and hyphal walls.

Sterile elements in the hymenium: presence or absence of various types of cystidia, cystidioles, hyphidia.

Basidiospores: shape, size, colour

KOH reaction: whether spores and/or hyphae change colour in 10% KOH

Amyloid or dextrinoid reaction: In Melzers reagent, if spores appear bluish-grey then they are amyloid, it they are reddish brown then they are dextrinoid (these reactions can be difficult to observe at first).

Macrostructures to observe initially were:

Fruit body: shape (eg. resupinate, pileate, or stipitate), colour, size, texture.

Hymenial surface: under dissecting microscope (40x) (eg poroid, hydnoid, odontoid, warted, even).

Once these structures were recorded for a specimen, the appropriate key was used to determine the genus and species name. Often, though, many other characters were used to identify a specimen which were specific to a genus.

There were many specimens which could not be identified because they were sterile (did not have basidia or basidiospores). Therefore, specimens were only included in the survey if they were not sterile.

2.2.4 Statistical analysis:

One way ANOVA's were performed when comparing the two sites with respect to the number of branches per quadrat, the number of species per quadrat, and the number of species per branch, the volume of branches per quadrat, the length of the branches, the largest diameter of the branches. Cochran's Heterogeneity of variance test was also performed in each case. If the data were heteroscedastic then a log transformation was performed. There were no cases in which this transformation failed to result in homogeneity of variances.

One-tailed Student's t-tests were used to test the difference between pairs of indices. T-tests were one-tailed because there was a clear alternative hypothesis. In addition, t-tests were used to test the difference between two slopes of log abundance curves as in Zar (1996).

2.2.4.1 Principle Components Analysis (PCA):

PCA was conducted on five sets of species abundance data. Each analysis dealt with a different question. In each case, the variables were the number of branches on which a species occurred in a quadrat.

1) Are the abundances of species similar between the two sites? That is, are the same patterns of abundance seen in the quadrats surveyed at Kyeema as those surveyed at Cox Scrub? All species except those that were only found on one branch were used as variables, and data from quadrats at both sites were used.

(2) Are the abundances of species similar in different quadrats at Kyeema? Only data from quadrats surveyed at Kyeema were used. All species that were present at Kyeema were used as variables.

(3) Are the abundances of the common species at Kyeema similar in different quadrats at Kyeema? The 10 most abundant species were used as variables. This PCA was conducted to remove any potential bias of species that occurred in only one quadrat which tend to make that quadrat appear quite different, even though that one individual is numerically insignificant. If that individual altered the community structure of the quadrat then this difference should still show up in an analysis of the 10 most common species.

(4) Are the abundances of species similar in different quadrats at Cox Scrub? Only data from quadrats surveyed at Cox Scrub were used. All species that were present at Cox Scrub were used as variables.

(5) Are the abundances of the common species at Cox Scrub similar in different quadrats at Cox Scrub? The 10 most abundant species were used as variables. As with

the PCA on the data from Kyeema, this PCA was conducted to remove any potential bias of species that occurred in only one quadrat.

For each of these analyses, the first two principle components were plotted against each other to observe the euclidean distance between the points (difference in species abundances between quadrats).

2.2.4.2 Discriminant Function Analysis (DFA):

DFA was conducted on species abundances in quadrats (same data as PCA) using four sets of data: (1) all species, (2) only the 10 most common species, (3) all species except those that occurred in only one quadrat (4) all species except those that occurred on only one branch.

The question being asked when conducting these DFA was: Given that there are two sites, is there any pattern in the data that can differentiate the two sites, and if so, which species contribute most to that difference? For each of these analyses, the frequency distributions of the factor scores from each site were plotted to observe the difference between the two sites.

2.2.5 Species diversity, evenness and richness:

The equations used in this study to calculate species diversity, evenness and richness are listed below. All indices are as in Brower *et al* (1990):

Diversity Indices:

Simpson's Index:

$$1-d = 1-\sum_{i=1}^{s}\left[\frac{n_i(n_i-1)}{N(N-1)}\right]$$

$$\text{var}\,iance = 4[\sum p_i^{\,3} - (\sum p_i^{\,2})^2]/N$$

where n_i = Number of individuals of species *i* in the sample.

N = Total number of individuals in the sample = $\sum n_i$

s = Number of species in sample

p_i = proportion of species *i* in the sample

Shannon-Wiener Function:

$$H' = -\sum_{i=1}^{s}(p_i)(\log p_i)$$

$$variance = \frac{\sum n_i \log^2 n_i - (\sum n_i \log n_i)^2 / N}{N^2}$$

Evenness Measures:

Using Simpson's Index of species diversity:

$$\text{Evenness } (E_s) = \frac{1-d}{D_{\max}}$$

$$D_{\max} = s\left(\frac{N-1}{N-s}\right)$$

Using the Shannon-Weiner Index of species diversity:

$$\text{Evenness } (J') = \frac{H'}{H'_{max}}$$

$$H'_{max} = \log s$$

Strictly, evenness measures should only be applied to samples from communities in which the total species number is known (s) (Pielou 1975). However, evenness is commonly calculated where total species number is not known and the species number found in the sample is used as an estimate of 's', but variance is not calculated and t-tests cannot be performed.

Another, more useful measure of evenness is to take the slope of the regression line through the log abundance versus rank species curves and use standard statistical methods to compare the slopes of the two lines from Cox Scrub and Kyeema. The slope of the line is a measure of evenness. The linear regression equation does not have to be a good fit to estimate evenness accurately (Tokeshi, 1993).

Species Richness Estimator:

Jackknife:

$$\hat{S} = s + \left(\frac{n-1}{n}\right)k$$

$$variance = \left(\frac{n-1}{n}\right)\left[\sum_{j=1}^{s}(j^2 f_j) - \frac{k^2}{n}\right]$$

where $\hat{S}$ = Jackknife estimator of species richness

s = Observed total number of species present in n quadrats

n = Total number of quadrats sampled

k = Number of unique species

f_j = Number of quadrats containing j unique species (j = 1, 2, 3, ..., s)

Unique species are species that occurred in only the one quadrat.

t and df were calculated as a standard t-test (Zar, 1996). Indices were compared using a one-tailed t-test because the alternate hypothesis was in one direction (ie that Kyeema had higher diversity, evenness and richness than Cox Scrub).

There were several ways of calculating the indices for this data set. Because multiple branches were collected within quadrats, the individual (n) unit could have either been presence/absence of a species on each branch or within each quadrat. Although it is tempting to use the smallest unit (ie the branch) the quadrat may be a more sensible unit if there are a large number of cord formers (see section 1.2.3) in the community. If one individual in a quadrat is a cord former and spreads to several branches, then fruits, then that one individual would be counted as several. If presence/absence in a quadrat is used, then the individual would only be counted once. Since it was unknown whether or not this would be a problem, both data sets were tested. However, both data sets showed the same trends, so only the per branch data will be shown.

The Jackknife estimate of species richness by definition uses quadrat data only. Rarefaction curves were not used because they are for habitats with same heterogeneity only (Krebs, 1989).

In the calculation of these indices, the whole data set was used. That is, not only the area within the 2 x 2 m quadrat, but also the rest of any branch which crossed the boundary of the quadrat.

2.3 Results:

2.3.1 Species list:

A total of 36 species of wood decay basidiomycetes were found in the two study sites (see table 2.1). There appeared to be considerable overlap in the species present at the two sites. Species which were common at Kyeema, such as *Aleurodiscus lividocoeruleus,* agaricoid sp.1, heterobasidiomycete sp. 1, *Calocera sinensis, Ceraceomyces sublaevis, Ceriporia ferruginicincta, Ceriporia viridens, Heterotextus peziziformis* and *Peniophora piceae* were also common at Cox Scrub. Two exceptions to this were *Gloeocystidiellum convolvens* and *Phanerochaete filamentosa* which were common at Kyeema but were not found at Cox Scrub. Generally, only rare species were unique to a site. This may simply have been a sampling problem where rarer species were less likely to be found and were therefore only found once at one site.

Several species could not be confidently identified. Three agaricoid species (agaricoid sp.1, sp.2 and sp.3), two polypore species (polypore sp.1 and sp.2) and a common heterobasidiomycete species could not be identified and have been sent to various fungal taxonomists for identification. Species within the genus *Peniophora* are notoriously difficult to distinguish (Eriksson *et al,* 1978). In the northern hemisphere, a character which is used to identify *Peniophora* species is the host tree species (Eriksson *et al,* 1978). These tree species do not occur in Australia and so this character could not be used. *Peniophora* sp.1 has affinities with *Peniophora piceae* (Pers.) Erikss., while *Peniophora* sp.2 has affinities with *Peniophora pithya* (Pers.) Erikss. Specimens of these two species have also been sent to fungal taxonomists for identification. Figures 2.7-2.10 show fruiting bodies of four WDBs.

Table 2.1: List of the species found in Kyeema and Cox Scrub with the number of quadrats and branches on which the species were found. Figures within brackets show counts of fruit bodies that were found within the boundaries of the quadrat only.

Species	*Kyeema*		*Cox Scrub*	
	No. of quadrats	No. of branches	No. of quadrats	No. of branches
Aleurodiscus lividocoeruleus (Karst.) Lemke	4 (3)	5 (4)	4 (2)	7 (3)
Antrodia oleracea (David. & Lombard.)Ryv.	1 (1)	1 (1)	0 (0)	0 (0)
agaricoid sp. 1	4 (2)	5 (3)	5 (3)	5 (3)
agaricoid sp. 2	0 (0)	0 (0)	1 (1)	1 (1)
agaricoid sp. 3	1 (1)	1 (1)	0 (0)	0 (0)
Botryobasidium candicans Erikss.	1 (1)	1 (1)	0 (0)	0 (0)
heterobasidiomycete sp. 1	9 (8)	27 (22)	7 (5)	15 (13)
polypore sp. 1	1 (0)	1 (0)	0 (0)	0 (0)
polypore sp. 2	1 (1)	1 (1)	0 (0)	0 (0)
Calocera sinensis McNabb.	4 (4)	13 (9)	5 (3)	6 (3)
Ceraceomyces sublaevis (Bres.) Julich	5 (3)	9 (5)	2 (0)	2 (0)
Ceriporia ferruginicincta (Murr.) Ryv.	6 (5)	7 (5)	4 (4)	5 (5)
Ceriporia purpurea (Fr.) Donk	1 (1)	1 (1)	0 (0)	0 (0)
Ceriporia viridens (Berk.& Br.) Donk.	8 (6)	18 (10)	4 (0)	6 (0)
Fibulomyces fusoides Julich	1 (0)	1 (0)	0 (0)	0 (0)
Gloeocystidiellum convolvens (Karst.) Donk.	4 (4)	7 (4)	0 (0)	0 (0)
Heterotextus peziziformis	5 (5)	6 (6)	11 (10)	28 (21)
Hymenochaete innexa G.H.Cunn	9 (9)	12 (12)	7 (6)	11 (7)
Hymenochaete minuscula G.H.Cunn	1 (0)	1 (0)	2 (0)	2 (0)
Hyphoderma praetermissum (Karst.) Erikss. & Strid	5 (5)	8 (8)	1 (0)	2 (0)
Hyphodontia breviseta (Karst.) Erikss.	1 (1)	1 (1)	0 (0)	0 (0)
Hyphodontia flocosa (Bourd. and Galz.) Erikss.	1 (1)	1 (1)	3 (2)	3 (2)
Hyphodontia subalutacea (P.Karst.) Erikss.	1 (1)	1 (1)	0 (0)	0 (0)
Peniophora sp.1 (Pers.) Erikss.	4 (3)	6 (3)	6 (2)	8 (3)
Peniophora sp.2 (Pers.) Erikss.	1 (1)	2 (2)	0 (0)	0 (0)
Phanerochaete creamea (Bres.) Parm.	1 (0)	1 (0)	0 (0)	0 (0)
Phanerochaete filamentosa (Burk. & Curt.) Burds.	4 (3)	6 (3)	0 (0)	0 (0)
Phlebia lacteola (Bourd.) Christ.	2 (2)	2 (2)	0 (0)	0 (0)
Phlebia radiata Fr.	1 (0)	1 (0)	0 (0)	0 (0)
Radulodon erikssonii Ryv.	0 (0)	0 (0)	1 (1)	1 (1)
Schizopora paradoxa (Fr.)Donk.	1 (1)	1 (1)	2 (1)	2 (1)
Schizopora trichiliae (Van der Byl)Ryv.	1 (1)	1 (1)	1 (1)	1 (1)
Stereum gausapatum (Fr.) Fr.	3 (3)	3 (3)	1 (0)	1 (0)
Tomentellopsis bresadoliana (Sacc. & Trotter) Julich & Stalpers	3 (3)	3 (3)	1 (0)	1 (0)
Trechispora stellulata (Bourd. & Galz.) Liberta	0 (0)	0 (0)	1 (1)	1 (1)
Tremella mesentrica Retz. ex Fr.	0 (0)	0 (0)	3 (2)	3 (2)
Number of species	32 (26)		21 (15)	
Total number of species	36			

Figure 2.7: Photograph of *Heterotextus pezizformis* fruiting body

Figure 2.8: Photograph of *Calocera sinensis* fruiting body

Figure 2.9: Photograph of *Hymenochaete innexa* fruiting body 1cm

Figure 2.10: Photograph of agaricoid sp.1 fruiting body 1cm

2.3.2 Community Structure:

2.3.2.1 Pairwise correlations:

Pearson correlations between pairs of species excluding those species occurring only once showed that there were no negative associations between pairs of species and only seven positive associations. At Kyeema agaricoid sp 1. and *Calocera sinensis* tended to occur in the same quadrats (r=0.78, p=0.005). *Peniophora piceae* was positively associated with heterobasidiomycete sp. 1 (r=0.83, p=0.002), *C. sublaevis* (r=0.742, p=0.009) and *Phlebia lacteola* (r=0.77, p=0.006). However these three species were not interrelated. *P. lacteola* was in turn associated with *Stereum gausapatum* (r=0.77, p=0.006). At Cox Scrub *H. miniscula* and *Schizopora paradoxa* were correlated (r=1.0, p=0.000), also *Calocera sinensis* and *P. piceae* (r=0.91, p=0.000). Although the α value was reduced to 1%, the chance of a type II error is still high since 422 correlations were conducted. These correlations should therefore be treated with caution.

2.3.2.2 Principle Components Analysis (PCA):

A PCA was conducted on the abundance data from both sites of all species except those that occurred in only a single quadrat. The analysis extracted eight factors. The first three factors explained 48.8 % of the variance. Figure 2.11 shows the first principle component plotted against the second. The clumping of the points on the graph (which represent quadrats) shows that the species abundance patterns at the two

sites were fundamentally the same. The two outliers were from Kyeema and were the same as in figures 2.12 and 2.13.

Kyeema: The first PCA conducted on the species abundance from Kyeema (figure 2.12) extracted 10 factors. The first three factors explained 58.3% of the variance. The two outliers were quadrats 1 and 10. The species that contributed the most variance to factor 1 (eigenvalues >0.8) were agaricoid sp. 3, *Fibulomyces fusoides*, *Schizopora trichiliae*, and *Peniophora piceae*. The first three species were unique to quadrat 10 and *Peniophora piceae* was most abundant in quadrat 10. The species that contributed most to factor 2 (eigenvalues >0.8) were *Botryobasidium condicans*, *Antrodia oleracea*, *Schizopora paradoxa*, polypore sp.1 and agaricoid sp.1. The first four species were unique to quadrat 1 and agaricoid sp 1 was most abundant in quadrat 1. These observations explain the distinctiveness of quadrats 1 and 10 in figures 2.11 and 2.13.

To remove the bias of rare species in the analysis another PCA was conducted on Kyeema data using only the 10 most common species. Quadrats 1 and 10 were again outliers (see figure 2.13). The PCA extracted three factors which accounted for 76.8% of the variance. The species that contributed most to the first factor were *Ceraceomyces sublaevis* and *Peniophora piceae*. Factor two was mostly due to *Hyphoderma praetermission* and a negative loading of agaricoid sp. 1. All of these species were abundant in most quadrats and no obvious explanation for the distinction of quadrats 1 and 10 could be detected. However, quadrats 1 and 10 also had an unusually high number of branches and species. Both quadrats were surveyed in mid to early winter as were many of the other plots, so it is unlikely that seasonal differences had an influence on the distinctness of these two quadrats.

Cox Scrub:

The PCA conducted firstly on the species abundance data from Cox Scrub included all species that occurred at Cox Scrub. The analysis extracted seven factors. The first three factors explained 58.7% of the variance. Figure 2.14 shows the first PC plotted against the second PC. There were two outliers which were quadrats 6 and 16. Factor 1 was mostly due to *H. minuscula*, *H. praetermission*, and *S. paraoxa*. The first two species both occurred in quadrats 6 and 16 and no other quadrats. *H. praetermission* occurred only in quadrat 16. The species that contributed the most variance to factor 2 were *S. gausapatum* and *T. stellulata*. These two species occurred only in quadrat 6.

As with the Kyeema data, a second PCA was conducted to remove the bias of the unique species (see figure 2.15). The results were quite different to the first PCA. with a less divided distribution of points. There were three points which were a slightly away from the other points. The most obvious point was quadrat 18 and the other two were quadrats 16 and 4. *H. peziziformis* and *C. viridens* contributed most to the first factor. The distinction of quadrat 18 could perhaps be attributed to the unusually high abundance of *H. peziziformis*. The second factor was mostly due to *P. piceae* and *C. guepinioides*. There were no obvious single factors in the abundances to explain this variance.

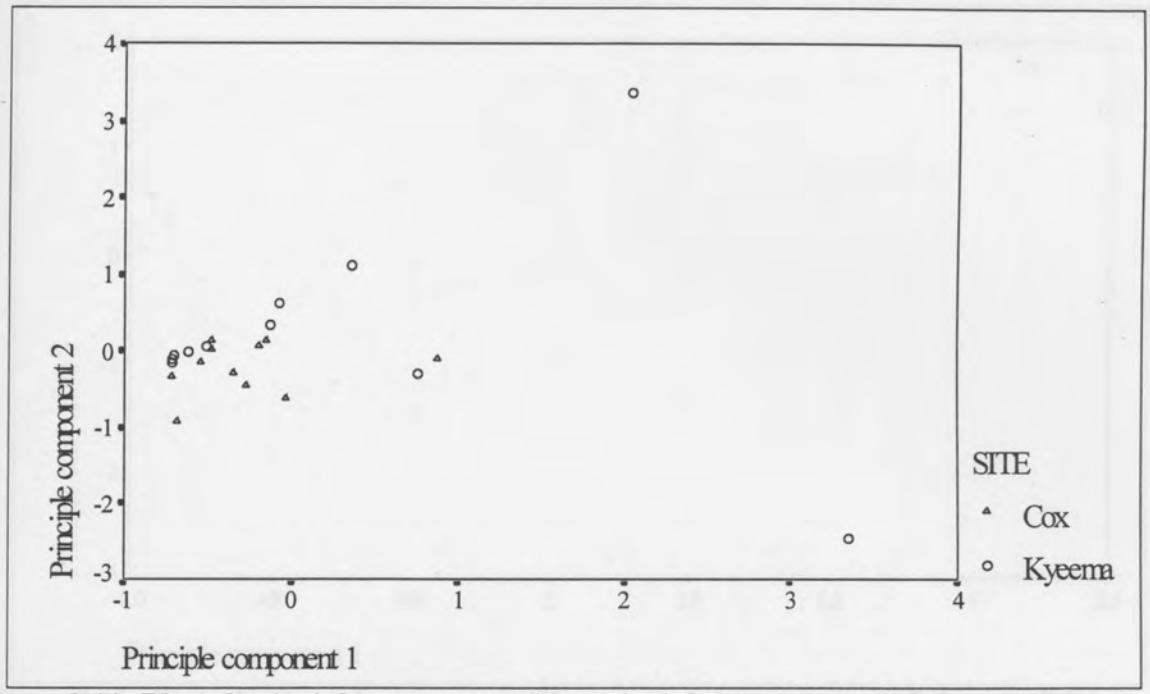

Figure 2.11: Plot of principle component 1 vs principle component 2 from a PCA of quadrats based upon abundances of all species except those that occurred only once. Data included both sites.

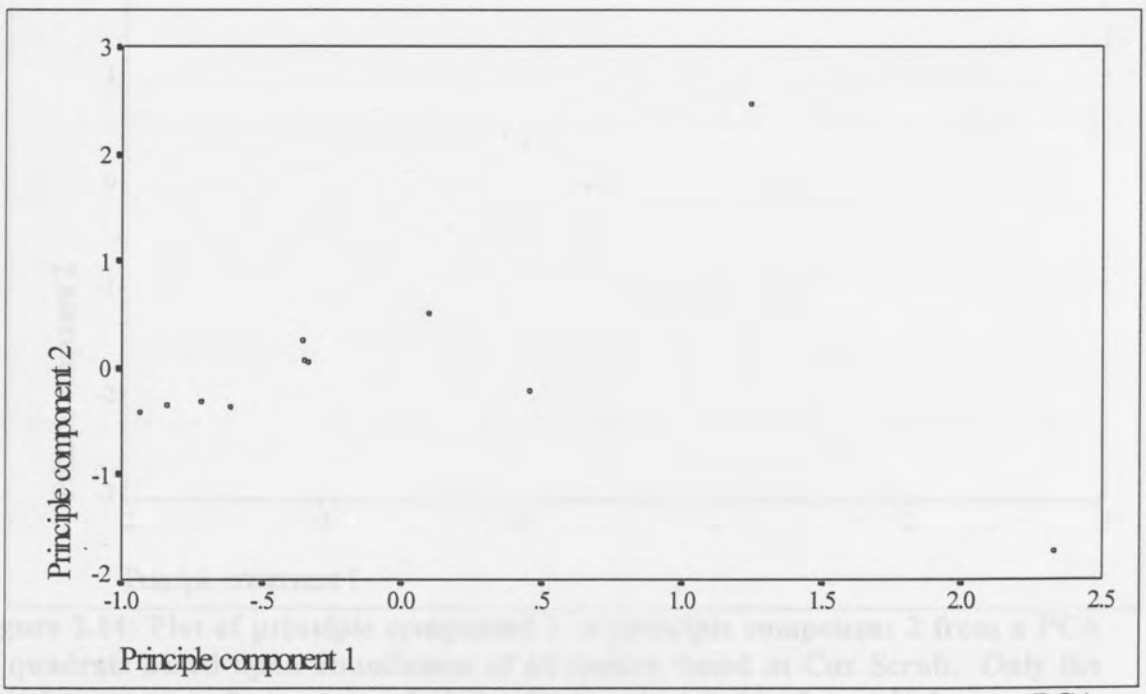

Figure 2.12: Plot of principle component 1 vs principle component 2 from a PCA of quadrats based upon abundances of all species found at Kyeema. Only the data from the quadrats surveyed at Kyeema were used in the analysis.

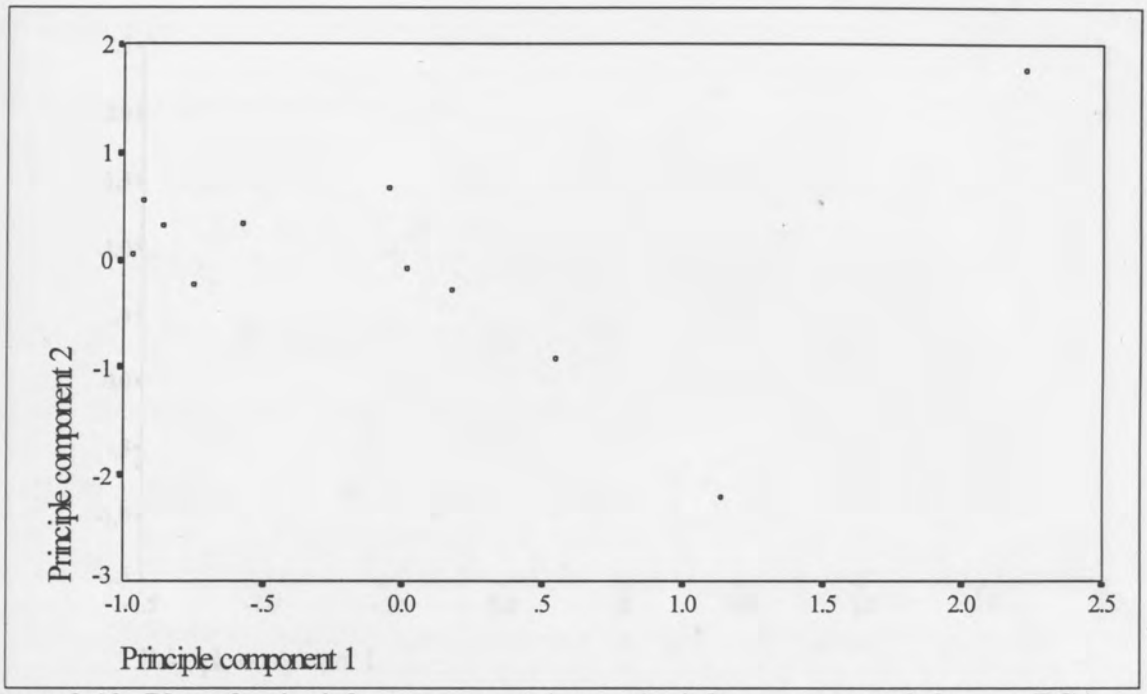

Figure 2.13: Plot of principle component 1 vs principle component 2 from a PCA of quadrats based upon abundances of the 10 most abundant species found at Kyeema. Only the data from the quadrats surveyed at Kyeema were used in the analysis.

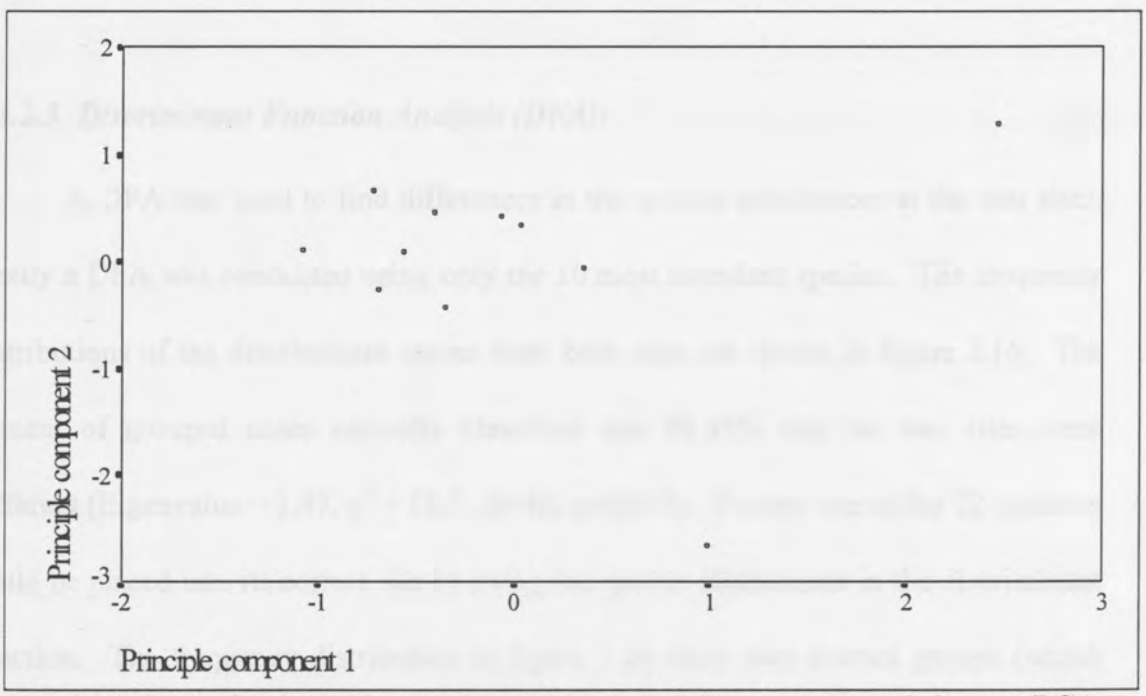

Figure 2.14: Plot of principle component 1 vs principle component 2 from a PCA of quadrats based upon abundances of all species found at Cox Scrub. Only the data from the quadrats surveyed at Cox Scrub were used in the analysis.

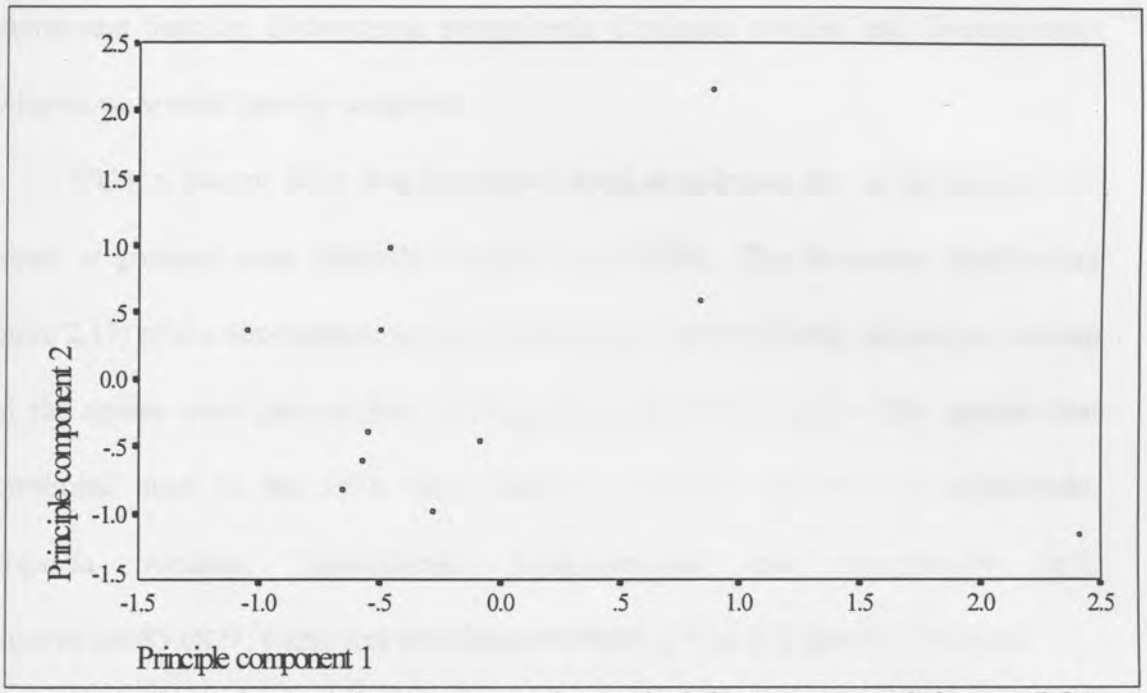

Figure 2.15: Plot of principle component 1 vs principle component 2 from a PCA of abundances of the 10 most abundant species found at Cox Scrub. Only the data from the quadrats surveyed at Cox Scrub were used in the analysis.

2.3.2.3 Discriminant Function Analysis (DFA):

A DFA was used to find differences in the species abundances at the two sites. Firstly a DFA was conducted using only the 10 most abundant species. The frequency distributions of the discriminant scores from both sites are shown in figure 2.16. The percent of grouped cases correctly classified was 95.45% and the two sites were different (Eigenvalue = 2.47, $\chi^2 = 18.7$, df=10, p=0.045). Twenty one of the 22 quadrats could be placed into its correct site by using the species abundances in the discriminant function. The frequency distribution in figure 2.16 show two distinct groups (which represent the two sites) the apices of which were three standard deviations apart. However, there was some overlap between the two frequency distributions. In the

discriminant function, *Heterotextus peziziformis*, *Ceriporia viridens* and *Ceraceomyces sublaevis* were most heavily weighted.

When a second DFA was conducted using abundance data of all species, the percent of grouped cases correctly classified was 100%. The frequency distributions (figure 2.17) of the discriminant scores from Kyeema and Cox Scrub showed no overlap and the apices were greater than 17.4 standard deviations apart. The species that contributed most to the DFA were *Calocera sinensis*, *Ceriporia ferruginicincta*, *Ceriporia viridens*, *Hyphoderma praetermissum* and *Peniophora* sp.1. (Eigenvalue=83.0877, Canonical correlation=0.9940, χ^2=44.319, df=20, p=0.0014).

The third DFA used abundance data of all species except those that were found only at one of the sites. The result was quite similar to the DFA using common species in that 21 of the 22 quadrats were correctly classified by the DFA and the apices of the frequency distributions were 3.9 standard deviations apart (figure 2.18). However, the frequency distributions were not different (Eigenvalue=4.2525, Canonical correlation=0.8998, $\chi^2 = 19.1$, df=17, p=0.32).

The final DFA used abundance data of all species except those that were found only once (figure 2.19). The result was exactly the same as using all species. All quadrats were correctly classified by the analysis and the apices of the frequency distributions were 17.4 standard deviations apart. (Eigenvalue=83.0877, Canonical correlation=0.9940, χ^2=44.319, df=20, p=0.0014). The species that contributed most to this DFA were *Calocera sinensis*, *Ceriporia ferruginicincta*, *Ceriporia viridens*, *Hyphoderma praetermissum* and *Peniophora* sp.1.

These demonstrate that a large amount of the differences in species abundance can be attributed to species which occurred in only one site, but more than one quadrat.

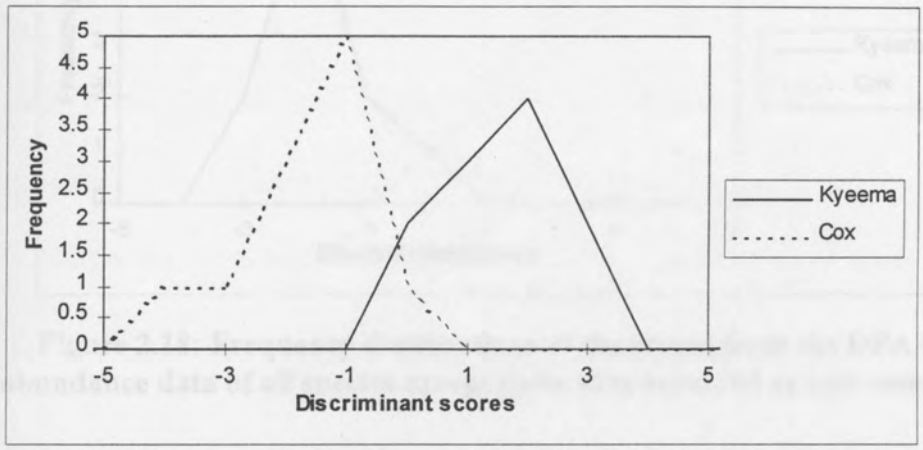

Figure 2.16: Frequency distributions of the scores from the DFA using abundance data of only the 10 most common species

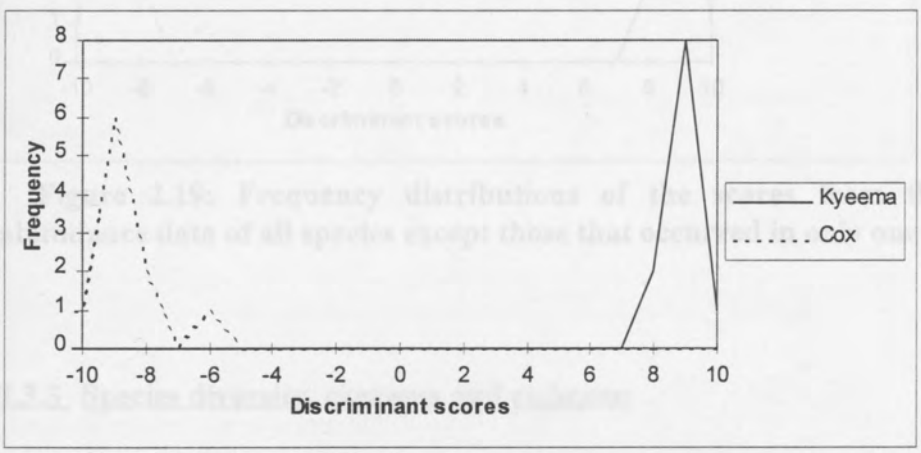

Figure 2.17: Frequency distributions of the scores from the DFA using abundance data of all species

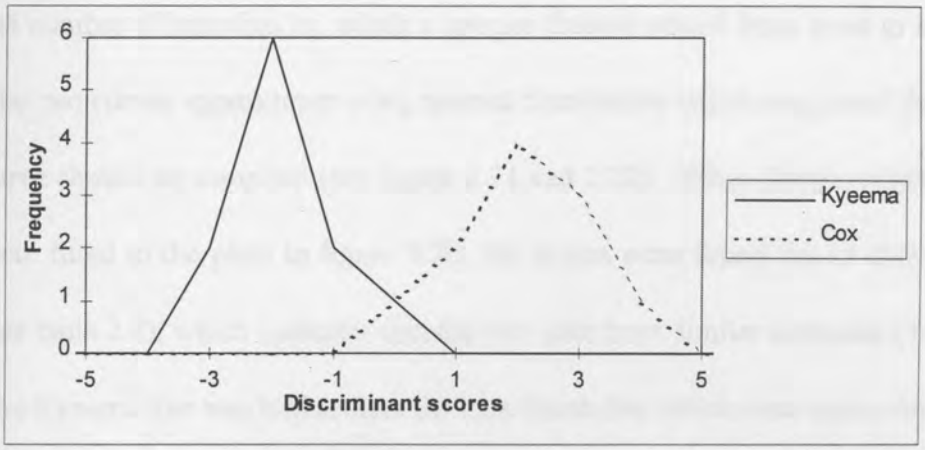

Figure 2.18: Frequency distributions of the scores from the DFA using abundance data of all species except those that occurred at only one site

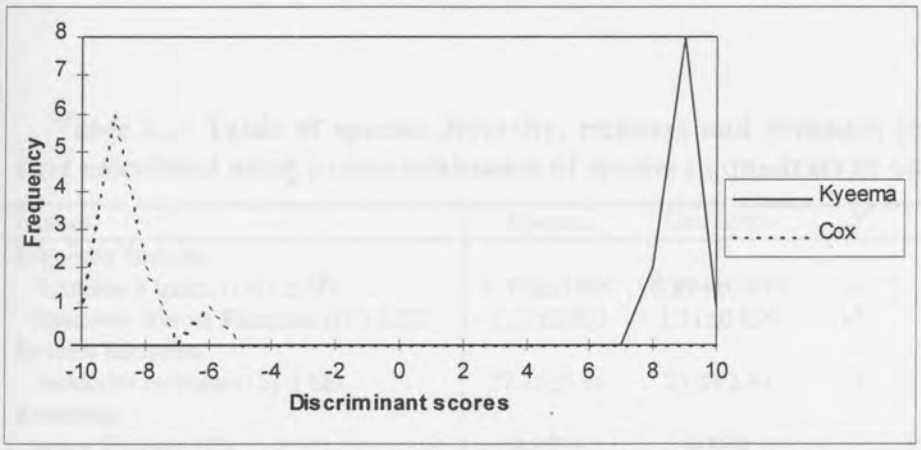

Figure 2.19: Frequency distributions of the scores from the DFA using abundance data of all species except those that occurred in only one quadrat

2.3.3 Species diversity, evenness and richness:

Using the Jackknife estimate, species richness was found to be higher at Kyeema. This is also seen in the absolute number of species counted from quadrats where 50% more species were found at Kyeema (table 2.2). In addition, both diversity indices showed a higher diversity at Kyeema than Cox Scrub.

Evenness was similar at both sites although variances could not be calculated (see section 2.2.5) and statistical tests were not possible. Figure 2.20 shows the log of

the number of branches on which a species fruited ranked from most to least abundant. The two curves approximate a log normal distribution which suggested that a lognormal curve should be compiled (see figure 2.21 and 2.22). When linear regression equations were fitted to the plots in figure 2.20, the slopes were found not to differ significantly (see table 2.2), which indicates that the two sites have similar evenness (Tokeshi, 1993). The Kyeema line was higher than the Cox Scrub line which once again demonstrates the higher species richness at Kyeema.

Table 2.2: Table of species diversity, richness and evenness (± s.d.) at the two sites calculated using presence/absence of species in quadrats or on branches

Indices	*Kyeema*	*Cox Scrub*	*df*	*t*	*p*
Diversity Indices					
Simpson's Index (1-d) ± SD	0.930±0.009	0.894±0.017	∞	1.897	<0.05
Shannon-Weiner Function (H') ± SD	1.27±0.033	1.11±0.039	12	3.51	<0.05
Species Richness					
Jackknife estimates (S) ± SD	27.35±3.66	21.5±2.44	17	3.62	<0.05
Evenness					
using Simpson (E)	0.9536	0.930			
using Shannon (J)	0.8434	0.8359			
slope of regression (figure 2.6)	-0.046649	-0.068500	45	0.687	0.5
Parameter	*Kyeema*	*Cox Scrub*	*df*	*F*	*p*
No. species (S)	32	21			
No. individuals (N)	154	111			
Mean no. branches per quadrat ± SD	10.8±4.9	7.1±3.1	1,20	4.57	0.045
Mean no. species per quadrat ± SD	7.2±3.6	4.0±1.5	1,20	7.25	0.014
Mean no. species per branch ± SD	1.28±1.3	1.44±1.2	1,195	0.71	0.402

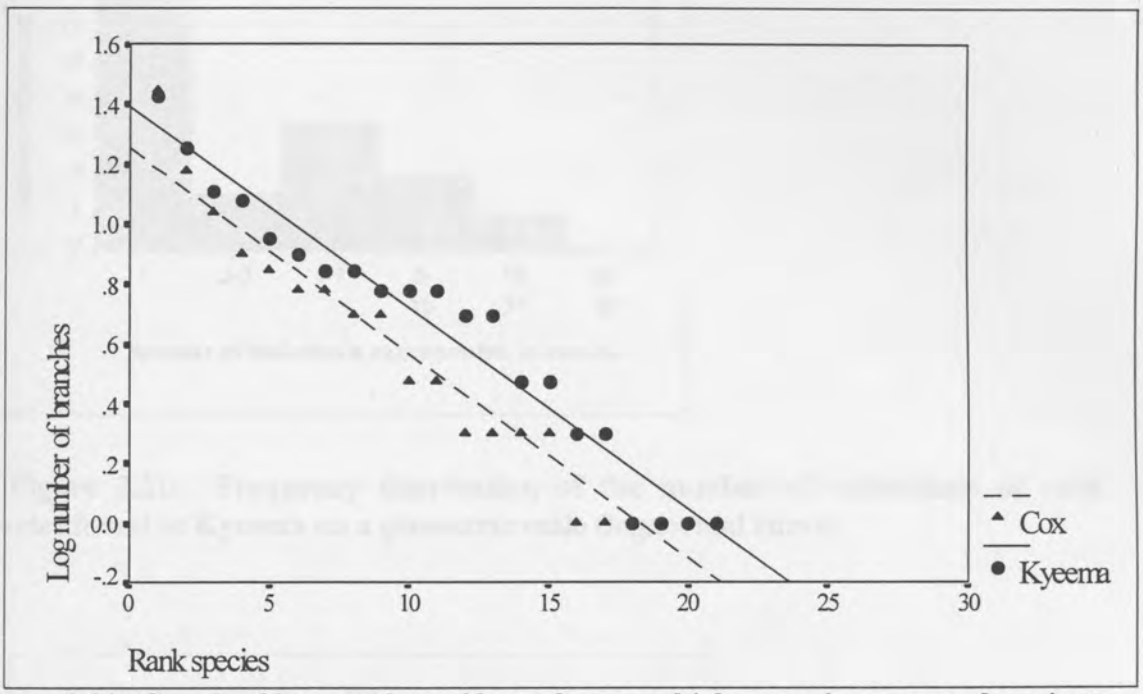

Figure 2.20: Graph of log number of branches on which a species occurred against the rank abundance of the species

The lognormal curves (figures 2.21 and 2.22) of species abundance indicate that there are many more species in the community than has been found in this survey. In a large survey the distribution should show a peak and from this distribution the number of species missing from the survey could then be calculated (Krebs, 1989). However, since in this survey a peak has not been reached, then the only conclusion that can be made is that there are many species missing from the two samples. The Kyeema plot (figure 2.21) did not exactly resemble a bell shaped log normal curve, due to the relatively few number of species with an abundance of two or three. This could either have been due to the curve not being truly lognormal or simply due to sampling variation.

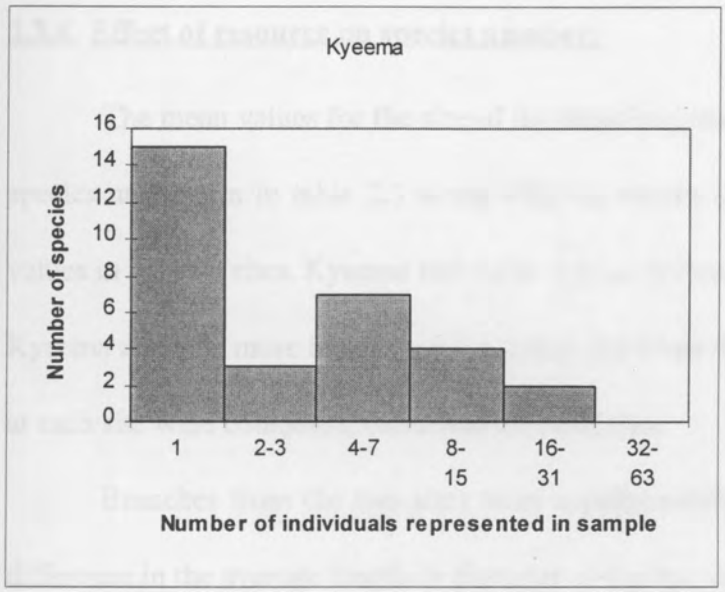

Figure 2.21: Frequency distribution of the number of individuals of each species found at Kyeema on a geometric scale (lognormal curve)

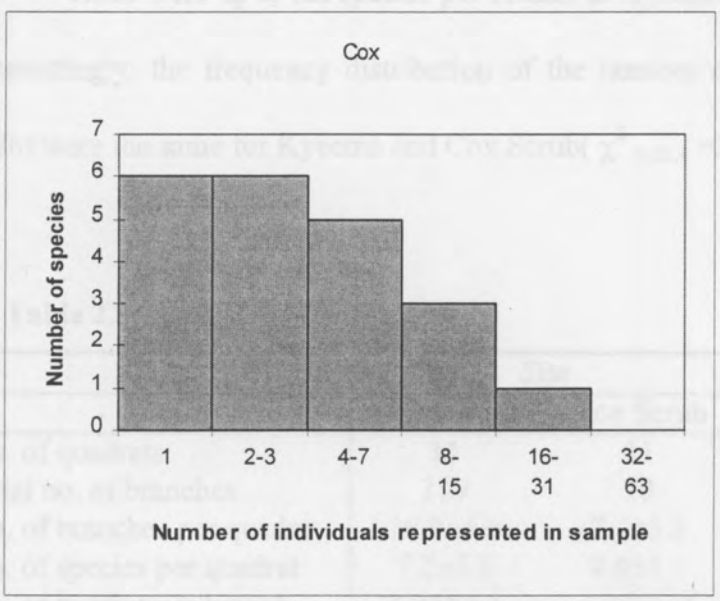

Figure 2.22: Frequency distribution of the number of individuals of each species found at Cox Scrub on a geometric scale (lognormal curve)

2.3.4 Effect of resource on species number:

The mean values for the size of the branches, number of branches and number of species are shown in table 2.3 along with the results of ANOVA tests comparing the values in the two sites. Kyeema had more species per quadrat than Cox Scrub. However, Kyeema also had more branches per quadrat and when the number of species per branch at each site were compared, there was no difference.

Branches from the two sites were approximately the same size. There was no difference in the average length or diameter of the branches from each site. The number of species per branch increased with both the diameter and length of the branches (see figures 2.23 and 2.24). The length and diameter were also correlated (figure 2.25).

There were up to six species per branch at Kyeema and up to five at Cox Scrub. Interestingly, the frequency distribution of the number of species per branch (figure 2.26) were the same for Kyeema and Cox Scrub($\chi^2_{0.05,4}$ =3.2, p>0.25).

Table 2.3: Results of field survey

	Site		*Results of ANOVA*		
	Kyeema	Cox Scrub	df	F	p
No. of quadrats	11	11			
Total no. of branches	119	78			
No. of branches per quadrat	10.8±4.9	7.1±3.1	1,20	4.57	0.045
No. of species per quadrat	7.2±3.6	4.0±1.5	1,20	7.25	0.014
No. of species per branch	1.28±1.3	1.44±1.2	1,195	0.71	0.402
Length of branch	155.9±11.5	149.0±10.1	1,195	0.18	0.675
Largest diameter of branch	3.4±0.2	3.6±0.2	1,195	0.36	0.55

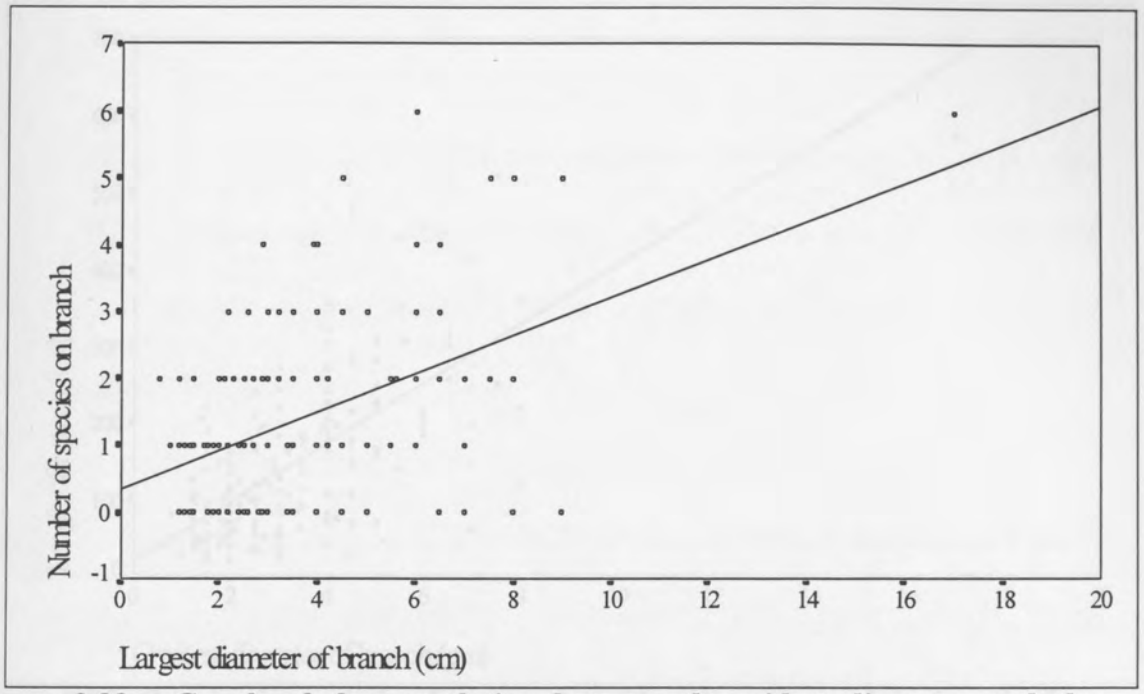

Figure 2.23: Graph of the correlation between the widest diameter and the number of species on a branch r = 0.4444, p = 0.000

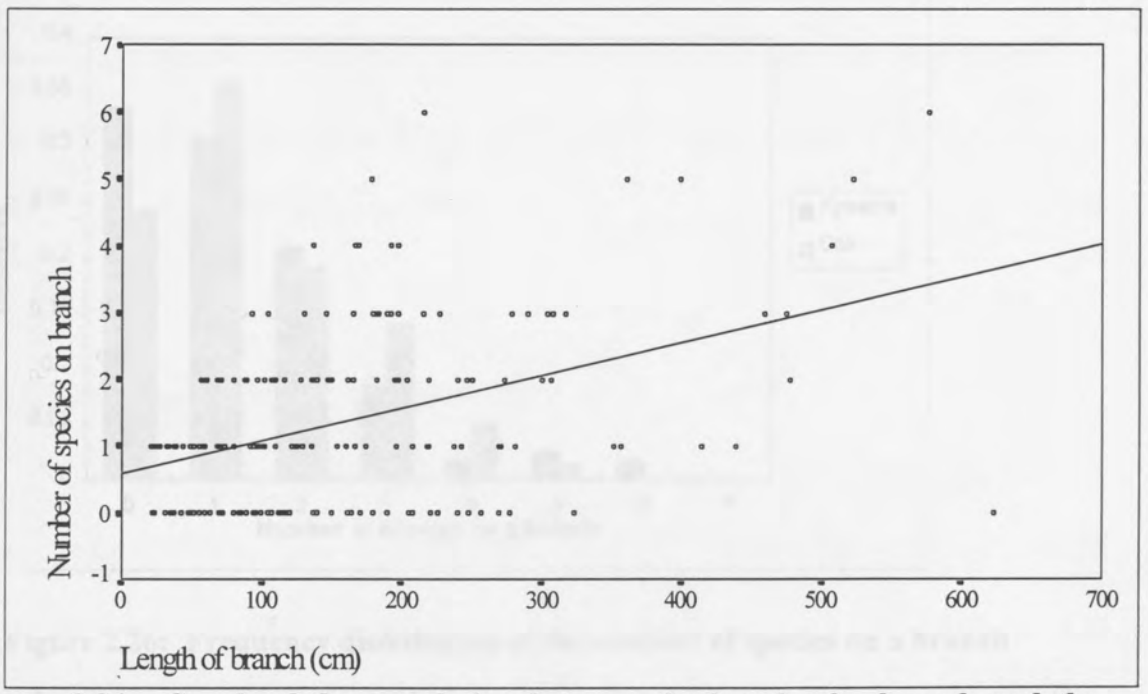

Figure 2.24: Graph of the correlation between the length of a branch and the number of species on a branch r = 0.4295, p = 0.000

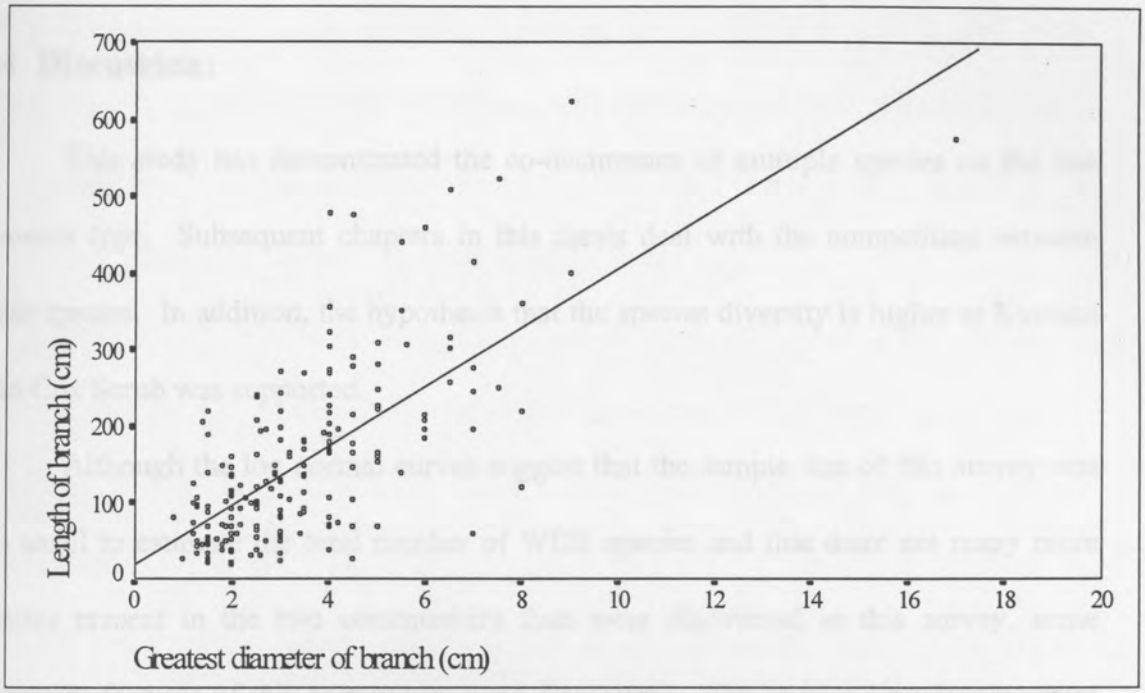

Figure 2.25: Graph of correlation between the widest diameter and the length of the branches from both sites r = 0.6832, p = 0.000

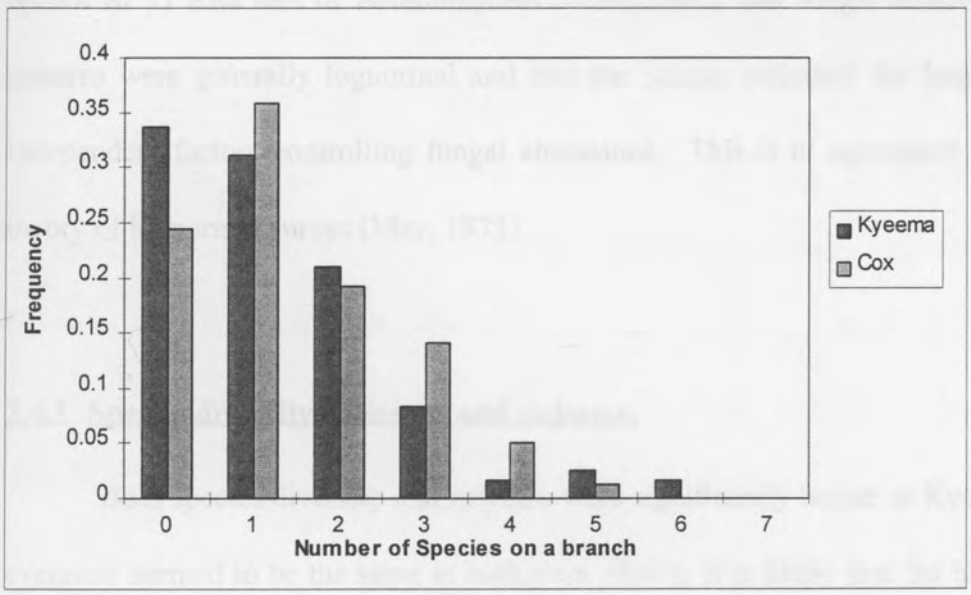

Figure 2.26: Frequency distribution of the number of species on a branch

($\chi^2_{0.05,4}$ =3.2, p>0.25)

2.4 Discussion:

This study has demonstrated the co-occurrence of multiple species on the one resource type. Subsequent chapters in this thesis deal with the competition between these species. In addition, the hypothesis that the species diversity is higher at Kyeema than Cox Scrub was supported.

Although the log normal curves suggest that the sample size of this survey was too small to estimate the total number of WDB species and that there are many more species present in the two communities than were discovered in this survey, some important features of this community were discovered. The species abundance curves indicated a lognormal distribution at both sites. Lussenhop (1981) concluded from a review of 31 data sets of basidiomycete communities, that fungal relative abundance patterns were generally lognormal and that this simply reflected the large number of independent factors controlling fungal abundance. This is in agreement with general theory of lognormal curves (May, 1975).

2.4.1 Species diversity, evenness and richness:

Both species diversity and richness were significantly higher at Kyeema, but the evenness seemed to be the same at both sites. Hence, it is likely that the higher species diversity found at Kyeema is mostly due to the higher species richness. However, the evenness measures must be viewed with caution. Evenness indices calculated from Simpson's or the Shannon-Weiner diversity functions are not reliable when sampling from a community (Pielou, 1975). They should only strictly be applied to samples from communities where the total species number is known. However, total species numbers

are rarely known and these evenness measures are calculated commonly (eg Bulla, 1994; Smith and Wilson, 1996). It is thought that the slope of the regression line from the log abundance curves is a better estimate of evenness (Tokeshi, 1993) and these did not significantly differ between Kyeema and Cox Scrub.

The higher diversity/richness found at Kyeema could be due to a number different factors. Of the abiotic factors which may influence diversity, rainfall alone is not likely to be the only influential factor since Kyeema received only slightly more rainfall than Cox Scrub. However, the sandy soil at Cox Scrub may increase the drainage and therefore reduce the soil surface moisture content. In addition, the soil surface layer at Kyeema is more protected from wind and sun by the continuous overstorey which would reduce the loss of moisture.

The relationship between moisture levels and species richness is complex. Many studies have demonstrated the influence of moisture on fungal growth (eg Gibson *et al* 1994; Kieft *et al*, 1993; Palmer *et al,* 1987), including WDBs (Boddy, 1983; Eamus and Jennings, 1986; Griffin, 1977). These studies mostly show an increase in fungal growth associated with increasing moisture content, up until a point where any further increase in moisture level tends to inhibit fungal growth, presumably due to decreased oxygen levels (Rayner and Boddy, 1988). Similar patterns are seen to occur with species richness of fungi. Christensen (1981) has demonstrated that dry to mesic habitats generally have less species than mesic to wet habitats, whereas bogs with even higher moisture levels have less species. Orpurt and Curtis (1957) demonstrated reduced species diversity of soil fungi in extremely moist conditions. This pattern is very similar to abundance patterns seen in vascular plants (Christensen, 1981).

A second factor which is likely to influence species diversity/richness is the level of resource in each habitat. A cellular automaton model of fungal diversity in different regimes of input levels of resource found that generally the species number increases with increased level of resource (Halley *et al,* 1994). Since Kyeema was found to have more branches than Cox Scrub and also a greater species diversity and richness it is possible that this higher resource level at Kyeema is a factor influencing the number of species found at each site. To remove the confounding effect of moisture and resource levels on species diversity, an experimental manipulation of the resource levels at the two sites would be necessary.

Unfortunately, a major problem in assessing diversity of wood decay communities based on counts of sporophores, is that there is no guarantee that the species diversity of sporophores reflects the true species diversity. For example species with perennial fruiting bodies would have a higher chance of being counted than species which fruit for only three days in a year. Therefore, if a site has predominantly perennial fruiting species then it would show a higher diversity index than a site with predominantly short term fruiting species. In addition, some cord-forming fungi may not form sporophores directly on the wood (Carruther and Rayner, 1979). These fungi would therefore not be counted in a survey.

The only way of truly testing the species diversity of wood decay basidiomycete communities is to sample the mycelia or to observe fruiting over a long period of time as in the study by Renvall (1995).

2.4.2 Community structure:

The pairwise correlations of some species indicated that the WDB species influence the distribution and abundance of one another. However, it is possible that the community patterns observed are only a reflection of fruiting patterns. For example some species may happen to fruit at the same time, and hence will tend to be seen together. This is a matter that can only be resolved by sampling the mycelium rather than fruit bodies.

2.4.3 Coexistence of competing species:

This survey has shown that there are at least 32 species at Kyeema and 21 at Cox Scrub co-occurring in the same habitat. Whether or not this is inconsistent with Gause's law of competitive exclusion depends on whether competition is strong enough and whether there are factors operating to which enhance coexistence between species such as intermediate disturbance. This concept is discussed in section 1.6. Previous studies have found competition to be influential in WDB community structure and this study shows multiple species co-occurring in the same habitat. It will therefore be assumed that multiple species of WDBs compete for the same resource and that these species should not be able to coexist by Gause's Principle. Chapters 4 and 6 explore mechanisms to explain the coexistence of multiple competing species.

2.4.4 Branch size and species richness:

In this study the number of species per branch was correlated with both the length and diameter of the branch. This finding is consistent with studies by Renvall (1995) and Bader, Jansson and Jonsson (1995) where log diameter was also correlated with the number of species. The most obvious explanation is that more fungal propagules (spores or cords) come into contact with larger branches due to the higher surface area. This does raise questions however, on the importance of direct competition between these species. If competition were very strong then only one individual would inhabit a single branch regardless of its size.

2.4.5 Future directions:

There have been a number of studies based on the distribution and abundance of fruiting bodies (Renvall, 1995; Niemelä *et al*, 1995; Pearce and Malajczuk, 1990). They are a convenient indicator of the presence of a species. However, they may not be a good unit of measurement for distribution and abundance patterns. Cotter and Bills (1985) have looked at the association between mycelium and fruit bodies and found it to be adequate when a branch or log is re-sampled over many years. This could not be done in this study because by turning the branch over to look at the fruit bodies, the habitat became too disturbed and the branch occasionally fell apart.

The best way to sample this community is therefore by direct sampling of the mycelium. Kirby, Webster and Baker (1990) devised a method for effectively sampling basidiomycete mycelium from wood. However, while there are good keys to cultures of basidiomycetes (Nobles, 1948; Stalpers, 1978), relatively few species have had their

mycelium characterised. To sample effectively using this method would constitute an enormous project.

In addition, this community cannot be expected to be static. More rigorous sampling on a regular basis is required.

3. Isolation of Wood Decay Basidiomycetes

Summary:

The mycelium of twenty one species of wood decay basidiomycetes were isolated from either fruit bodies or directly from decaying branches. Single spores were isolated from fruit bodies to produce homokaryotic mycelia. These were then paired on MEA plates to produce heterokaryotic mycelia. Cultures were screened by testing for ligninase activity and checking for clamp connections to ensure that they were lignicolous basidiomycetes.

3.1 Introduction:

A suite of cultures from different species were isolated for studies on the interactions between species of wood decay basidiomycetes from a naturally occurring community. Culturing from either fruit body tissue or single basidiospores appears ideal because the identity of the species can be determined from the fruit body, but this was not always an efficient means of isolating cultures. Firstly, uncontaminated heterokaryotic tissue from fruit bodies was difficult to obtain from very thin resupinate species. Secondly, single basidiospores should be gathered from a fruit body and germinated to make single spore isolates (homokaryons), then paired with other homokaryons of that species to form heterokaryotic mycelia. Unfortunately this process can be extremely time consuming and sometimes produces no result. For example, spores of some specimens have very low germination rates on media that are commonly used for the maintenance of basidiomycete cultures (pers. obs.). Presumably there would be some conditions under which these spores would germinate on media, however discovering that method could take appreciable time (eg Booth, 1971). In addition, if the species being cultured does not form clamp connections in the heterokaryotic phase, successful matings between compatible homokaryons cannot be detected.

Culturing directly from wood has the disadvantage that the identity of the species often can not be determined, however, it does have the advantages that it is relatively quick and the cultures are derived directly from wood (whereas cultures from fruit bodies could possibly be from decomposers of substrates other than wood and the species is just using the fallen branch as a convenient place on which to fruit). Since

both methods have their advantages, a combination of the two were used to collect wood decay basidiomycete cultures for the study of interspecific interactions (Chapters 4-6).

To confirm that the cultures isolated using these two methods are wood decay basdiomycetes, the isolates were tested for both lignin degrading ability and evidence that the culture was a basidiomycete. A number of methods have been found to test the activity of ligninases, but they have variable success rates. The best method seems to be the use of guaiacol. A culture growing on wood-guaiacol agar (see appendix B) degrades the wood by releasing ligninases which react with the guaiacol to produce a red pigment in the medium (Nishida *et a,l* 1988).

There are several methods for testing whether or not a culture is a heterokaryotic basidiomycete (Alexopoulos *et al*, 1996), the easiest and most common is to check the culture for clamp connections (Boidin, 1986; Barnett, 1937; Fischer and Bresinsky, 1992; Petersen and Cifuentes, 1994; Nobles, 1937). This does not work for some basidiomycetes that do not possess clamp connections (Campbell, 1937; Campbell and Davidson, 1939; Fischer, 1994) such as *Ceriporia* species in this study.

3.2 Methods:

3.2.1 Media:

All cultures were maintained on MEA or PDA plates and slopes and put into more permanent storage using silica gel. Initially benlate was added to MEA plates to reduce the contamination rate. See appendix B for media preparation.

3.2.2 Single spore isolates:

Fruit bodies were collected as described in chapter 2 and brought into the lab to be cultured. Some additional specimens were collected for culture isolation to increase the number of species in culture. The origin, habitat, and date of collection are shown in Appendix B.

To collect single spores from a fruit body a piece of the specimen (usually about $5mm^2$) was cut away from the wood and placed into sterile distilled water for 10-60 minutes depending on the thickness and density of the specimen. The piece of fruit body was then attached to the lid of a MEA plate using vaseline, with the hymenium facing downwards (towards the agar). Plates were then left overnight to allow the spores to drop to the surface of the agar. Individual spores were then pulled across the surface of the agar using a micro-manipulator until they were in a position away from the other spores. This position was then marked using the micro-manipulator and later cut away using a scalpel and placed onto a fresh MEA plate. Usually 20 spores were isolated in this way per fruit body.

3.2.3 Crosses to form heterokaryotic mycelium:

Heterokaryons of *Aleurodiscus lividocoeruleus* (KQ12B5), *Peniophora* sp.2 (KQ2B1), *Peniophora* sp.1 (KQ14B3), *Pereniporia medulla-panis* (KS1) were artificially synthesised from homokaryons. Single spore isolates from the same fruit body were paired in all combinations. A $5mm^2$ piece of agar and mycelium was cut from the actively growing edge of the homokaryotic mycelium. The different isolates were placed 1cm apart on MEA. Interactions were observed weekly for five weeks and hyphal samples examined under 400x magnification for clamp connections.

3.2.4 Isolation of vegetative mycelium from wood:

Branches collected as part of the field survey (chapter 2) were used to isolate vegetative mycelium. The exterior of a branch was sterilised by dipping it into 70% ethanol for ~ 10 seconds. A coping saw was sterilised by squirting 70% ethanol on the thin blade, then flaming. A 5 cm section of wood was then cut off to expose an uncontaminated flat surface. Five to ten small pieces (~ $1mm^2$) of the wood were then removed from this surface and placed onto separate MEA plates.

Cultures were checked for clamp connections by taking a 5x5 mm piece of agar and preparing a wet mount using cotton blue (see appendix B).

3.2.5 Hyphal identification:

In the competition experiments the hyphae of the different isolates needed to be distinguishable. A set of reference slides were prepared by cutting out 5x5 mm pieces of agar and mycelia (not from the growing edge of the each colony) which were wet

mounted using glycerol. All isolates were easily distinguishable by hyphal morphology using this method.

3.2.6 Testing for ligninase activity:

Cultures were placed onto agar plates containing sawdust from eucalypt branches from the two field sites and guaiacol (see appendix B). Any cultures that did not produce a red pigment in the agar within seven days of inoculation were discarded.

3.3 Results:

3.3.1 List of cultures:

Wood decay basidiomycetes isolated from either spores or wood are listed in table 3.1. The origin of each specimen from which spores came is given in Appendix C.

Table 3.1: List of cultures isolated from spore and wood.

Code	Species	Assigned number	Genetic status
	From Fruit bodies		
25	*Xylobolus illudens*	1	homokaryon
38	*Ceriporia purpurea*	2	homokaryon
kq14b3	*Peniophora* sp. 2	4	homokaryon, heterokaryon
8	*Pycnoporus australiensis*	5	homokaryon
kq2b1	*Peniophora* sp. 1	6	homokaryon, heterokaryon
kq12b7	*Hymenochaete innexa*	7	homokaryon
43	unidentified corticioid 1	8	homokaryon
15	*Stereum* sp.1	9	homokaryon
17.2	*Stereum* sp.2	11	homokaryon
Cq16b12	*Aleurodiscus lividocoeruleus*	13	homokaryon
Kyeema 1	unidentified hydnum	14	homokaryon
ks1	*Pereniporia medulla-panis*	15	homokaryon, heterokaryon
k1 stalked	unidentified agaricoid	17	heterokaryon
kq14b1	*Phanerochaete filamentosa*	18	homokaryon
C1	too old to identify - hydnum	19	homokaryon
kq12b5	*Aleurodiscus lividocoeruleus*	20	homokaryon, heterokaryon
	From wood		
KQ14B2	unknown	21	heterokaryon
KQ14B3	unknown	22	heterokaryon
KQ12B6	unknown	23	heterokaryon
KQ14B7	unknown	24	heterokaryon

3.3.2 Crosses:

Four species were successfully mated: *Peniophora piceae* (code: KQ2B1), *Peniophora pithya* (code: KQ14B3), *A. lividocoeruleus* (code: KQ12B5) and *Pereniporia medulla-panis* (code: KS1). All of them took one to two weeks to form heterokaryons. The two colonies would grow together, form a mild antagonistic reaction then produce a thick mat of hyphae growing from the region where the two colonies met. When these thicker mats were observed under a light microscope (x400), clamp connections were observed. On plates where there was no mating, antagonistic reactions were observed. There was no clear zone between the colonies, but where they touched there was a distinct zone of demarcation.

4. Non-additive competitive effects between wood decay basidiomycetes.

Summary:

Most previous studies on fungal competition have only reported interactions between pairs of species. In many ecological communities, when there are more than two species interacting, indirect effects and interaction modifications can be important in community dynamics. Indirect effects and interaction modifications are effects that one species has on another which are mediated via a third (or fourth etc) species. This chapter investigates the frequency and influence of indirect effects and interaction modifications in three-species combinations of WDBs. This is important because if indirect effects or interaction modifications occur between WDBs, they may increase the chance of species coexistence.

Seven species of WDBs were inoculated in one, two and three-species combinations onto malt extract agar plates using a replacement design. Of the three-species combinations 48.6 % had no indirect effects or interaction modifications, 48.6 % had at least one indirect effect, and 2.8 % had an interaction modification. In most of the cases (89 %) where indirect effects or interaction modifications were found, these effects led to an increased chance of species coexistence.

This study reports indirect effects and a possible interaction modification between WDBs for the first time. In addition, this study demonstrates the potential influence of these non-additive interactions on the community structure of WDBs, in particular, the increased chance of coexistence of competing WDB species.

4.1 Introduction:

This chapter focuses on reporting the presence of indirect effects and interactions modifications between WDBs and demonstrating their influence on WDB community structure. Indirect effects and interaction modification have not yet been recorded in a WDB community and therefore their role in the structure of WDB communities is also unknown. In section 1.7 it was proposed that indirect effects or interaction modifications could increase the chance of the coexistence of competing WDBs. Indirect effects and interaction modifications are introduced in section 1.8.

One of the most fundamental questions that we can ask in community ecology is; how do interactions between species influence their distribution and abundance? Although mycologists have been studying fungal species interactions for many years (eg Fokkema, 1973; Dennis and Webster, 1971; Skidmore and Dickinson, 1976), studies on interactions between WDBs have so far involved only pairwise interactions. While these studies have yielded much information about the ecology of WDBs, they vastly underestimate the complexity of community dynamics such as the presence of non-additive interactions (indirect effects and/or interaction modifications).

In chapter 2 there were up to six species fruiting on a single branch (see figure 2.26). By observing the pairwise interactions between those six species fruiting on a branch, do we gain a clear picture of the dynamics between those six species? This question can only be answered by observing the interactions between more than two species simultaneously. If indirect effects or interaction modifications are present then observations of pairwise interactions will not be able to predict the outcome of competition in multispecies assemblages.

4.1.1 Pairwise interactions:

In addition to observing non-additive interactions this study was designed to reveal several novel aspects to pairwise interactions between WDBs. Firstly, the strength of interactions were observed. As outlined in section 1.3, pairwise interactions between WDBs have been well studied. However, the competitive effects are usually scored as either overgrowth, deadlock or intermingling. Deadlock is a common outcome (Owens *et al*, 1994; Pearce, 1990; Coates and Rayner, 1985c; Carruthers and Rayner, 1979; Boddy and Rayner, 1983; Rayner and Hedges, 1982; Thompson and Boddy, 1983; Boddy, Bardsley and Gibbon, 1987). In ecological terms, the strength of the deadlock interactions is important. Since the area which an individual ends up with decides its allocation of resource, and hence the potential reproductive success, the strength of the deadlock reaction should be observed.

Secondly, because a replacement design was used, size-dependent competitive effects could be tested. That is, does one inoculum of one species have a constant effect on one inoculum of another species, whether there is another inoculum of the same strain present or not?

Thirdly, because a permanent record of the area covered by each strain over time was made, the dynamics of pairwise interactions could be recorded. This is similar to the traditional overgrowth or deadlock, but more detail could be recorded. It is essential to observe the nature of pairwise interactions before attempting to analyse the interactions between three species. If a non-additivity is detected between the three species, then we can look back to see if the nature of the interactions between the pairs of species was altered. For example commonly observed interactions are either one fungal individual overgrows the other or the two individuals "deadlock" with neither

individual being able to impinge on the others territory. If, in three-species competition, one of these strains displaces the other, then this is an obvious change in behaviour (interaction modification).

Most competition studies of WDBs only pair species at the one density. What happens when we double the density of one individual while the other remains the same? Is the effect of one inoculum of species *i* on species *j* the same in different inoculation densities? The reason for suspecting that they may not be constant is if by gaining extra territory (because there were two inocula to the one inoculum of the other individual) and therefore more resources an individual is then able to put more resources into repelling the other individual (ie production of toxic substances). To answer this question, pairs of species were inoculated in 2:1, 1:2, and 1:1 ratios of inocula. Per-inocula interaction terms were then calculated and compared.

4.1.2 Objectives:

The main aims of this experiment were to:

(1) investigate the frequency of non-additivities in competitive effects between species of WDBs;

(2) determine the influence of non-additivities of competitive effects between WDBs on the coexistence of multiple competing species;

(3) test for size-dependent competitive abilities between pairs of WDB strains.

4.2 Methods:

4.2.1 Cultures:

The cultures used in this experiment are listed in table 4.1. The methods used to isolate these cultures were explained in Chapter 3. For convenience each culture was given a letter from A to G and will be referred to by that letter in this chapter. All cultures were heterokaryons with clamp connections and had shown an ability to degrade lignin (see section 3.2.6). These cultures were chosen because they were the only heterokaryons that were available at the beginning of the experiment.

Table 4.1: Table of cultures used in experiment

Species	culture	letter
unknown	KQ14B2 from wood	A
Agaricoid sp.	K1	B
unknown	KQ14B3 from wood	C
Peniophora piceae	KQ12B1	D
Aleurodiscus lividocoeruleus	KQ12B5	E
unknown	KQ14B7 from wood	F
Perreniporia medulla-panis	KS1	G

4.2.2 Media

This experiment was performed on malt extract agar (MEA) plates (see appendix B for preparation of media) which were allowed to set and air dry in a lamina flow for one day to reduce the excess moisture from the plates. This was found to successfully reduce the incidence of fungal contamination. The plates were then kept in polythene bags (10 plates per bag) and sealed with elastic bands to stop additional moisture loss.

4.2.3 Experimental design:

The seven species were inoculated onto agar plates in all possible one, two and three species combinations using both additive and replacement designs. Table 4.2 shows an example of a hypothetical three species combination. An inoculum was made by cutting a 5x5 mm block of agar from the edge of actively growing colonies. Plates with one inoculum had the agar block placed in the centre of the plate. If there were two inocula per plate, they were placed 1 cm apart in the centre of the plate. Plates with three inocula had the agar blocks placed in a triangle in the centre of the plate with each block 1cm apart. Figure 4.1 shows a diagram of three MEA plates with one, two or three inocula per plate.

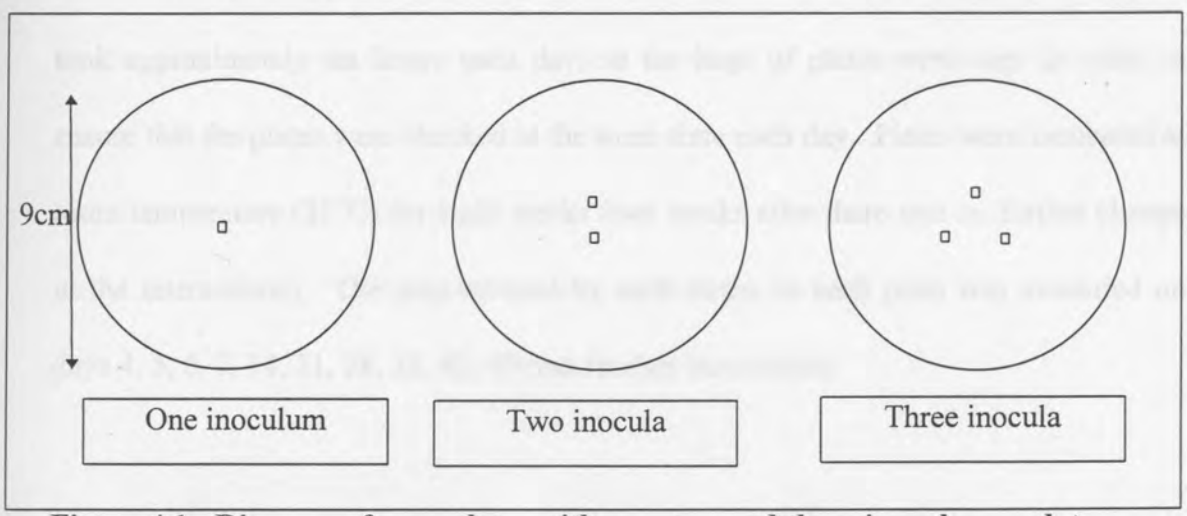

Figure 4.1: Diagram of agar plates with one, two and three inocula per plate

Table 4.2: Table showing an example of one-, two- and three-species combinations using additive and replacement designs where letters i, j and k represent inocula of species i, j and k. The symbolism used to represent the different combinations are given in columns three and five.

Treatment	Additive design	symbol	Replacement design	symbol
species i	1 inoculum of i	i	3 inocula of i	iii
species j	1 inoculum of j	j	3 inocula of j	jjj
species k	1 inoculum of k	k	3 inocula of k	kkk
species i & j	1 inoculum of i and 1 of j	ij	plate 1= 1 inoculum of i and 2 of j	ijj
			plate 2=2 inocula of i and 1 of j	iij
species i & k	1 inoculum of i and 1 of k	ik	plate 1= 1 inoculum of i and 2 of k	ikk
			plate 2=2 inocula of i and 1 of k	iik
species j & k	1 inoculum of j and 1 of k	jk	plate 1= 1 inoculum of j and 2 of k	jkk
			plate 2=2 inocula of j and 1 of k	jjk
species i,j and k	1 inoculum of i, 1 of j and 1 of k	ijk	1 inoculum of i, 1 of j and 1 of k	ijk

There were three replicate plates for each treatment. The measurements from these plates were averaged. The experiment was then repeated and the means from the three plates from each of the two experiments were used as replicates in statistical analyses.

All replicate plates were separated and randomised. Recordings of the plates took approximately six hours each day, so the bags of plates were kept in order to ensure that the plates were checked at the same time each day. Plates were incubated at room temperature (21°C) for eight weeks (two weeks after there was no further change in the interactions). The area covered by each strain on each plate was measured on days 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after inoculation.

4.2.4 Measurement of area:

The outline of each colony was drawn onto a plastic sheet and this was scanned into a computer using a video camera. The area and perimeter of each colony was measured using an image analysis program (Optimus 5.1).

4.2.5 Checking the accuracy of measurements:

On day 56, the final area covered by each strain was recorded. To ensure that the area recorded was accurate, 5 x 5 mm blocks of agar and mycelium were taken from areas on each plate where there could be some uncertainty. On all plates, blocks were cut from either side of an interaction zone to check for hyphae that may be growing under the interaction zone. In some cases, where a distinct barrage was not observed, many core samples were taken to ascertain where each strain grew. A wet mount was prepared of each block and examined under a light microscope at 400x. Samples taken from the plates were compared to a set of reference slides of each of the cultures.

4.2.6 Data analysis:

4.2.6.1 Growth rate:

The one-inoculum plates were used to assess the growth rates of the different strains. The area covered by a strain on day five was used because this was the day with the most variation between strains. The difference in area covered by the strains was tested using one-way ANOVA and contrast tests.

4.2.6.2 Interspecific competition:

Pairwise interactions

To test whether or not competitors had an effect on a target strain one-way ANOVA (and contrast tests) were used to compare the final area covered by a target strain when paired with itself to the area covered when paired with each other strain. To

be able to compare the effect of one inocoulum of another strain with the effect of one inoculum of the same strain, it was necessary to use final area. Calculated effects could not be used because they could not then be compared statistically with the effect of an inoculum of the same strain on the target inoculum. Therefore only one of the pairwise treatments could be used because they are only comparable once they have been converted to an "effect". Hence, only the two-inoculum plates were used for this initial analysis.

4.2.7 Calculation of competitive effects:

For each pair of strains there were three inoculum densities (ij, iij, ijj). The effect per inoculum in each of the three treatments was calculated as below. The measurement used to calculate these effects was final area of the plate covered by mycelium of the target strain.

4.2.7.1 One inoculum of target strain (i) with two inocula of effector strain (jj) (=ijj)

A measure of the effect that two inocula of *j* had on one inoculum of *i* is the deviation from the area covered by one inoculum of *i* when with two inocula of itself (*iii*) and when with two inocula of *j* (*ijj*). The effect must then be halved to estimate the effect of one inoculum of strain *j* on *i* in that inoculum density (see equation 4.1). Figure 4.2 shows a diagram of this calculation.

$$E_{ijj} = \frac{A_{ijj} - \frac{A_{iii}}{3}}{2}$$ **Equation 4.1**

Where E_{ijj} = the effect of one inoculum of strain *j* on strain *i*

A_{ijj} = the final area covered by strain *i* when in competition with two inocula of strain *j*

A_{iii} = the final area covered by strain *i* when there were three inocula of strain *i*

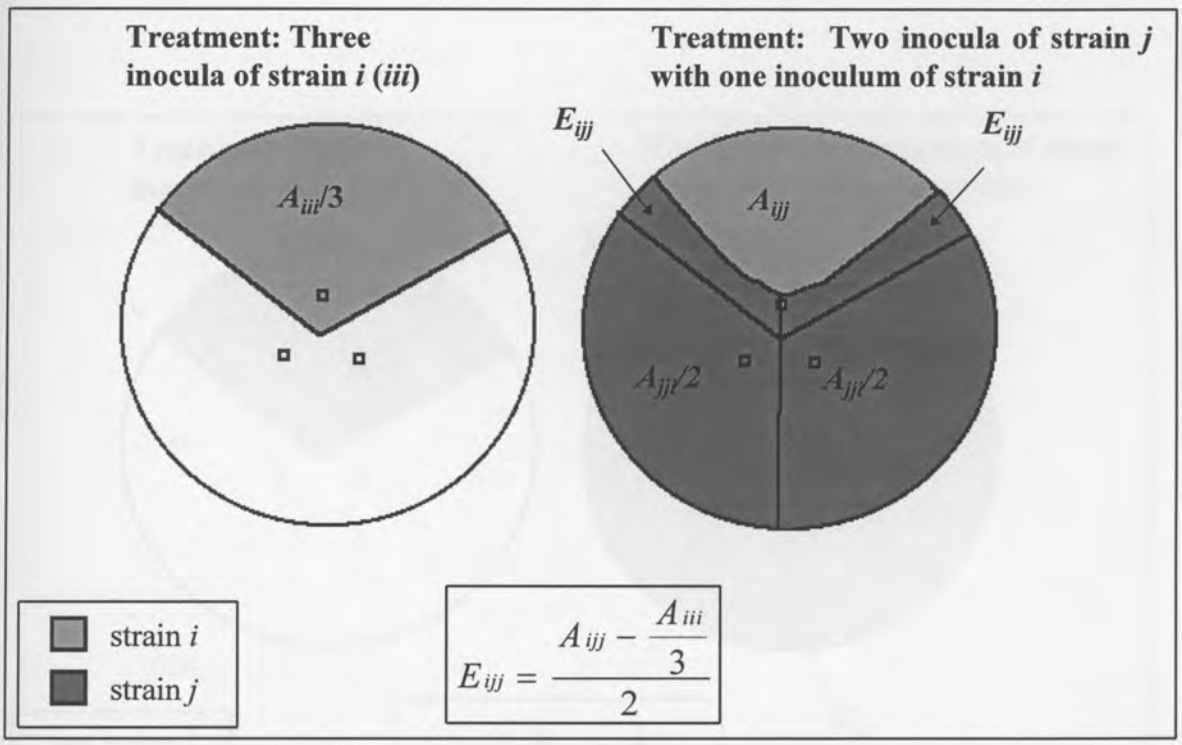

Figure 4.2: Diagram showing the calculation of the effect of the colony formed by two inocula of strain *j* on the colony formed by one inoculum of strain *i*. E_{ijj} is the effect of one inoculum of strain j on one inoculum of strain i in the ijj treatment. A_{ijj} is the area covered by one inoculum of strain i. A_{jji} is the area covered by two inocula of strain j.

4.2.7.2 Two inocula of strain i with one inoculum of strain j (iij):

The area covered by the target strain was halved to give the area covered by one inoculum. The area covered by one inoculum under intraspecific competition at the same inoculum density (three inocula per plate) was then subtracted from this value to give the effect of one inoculum of the competitor on one inoculum of the target species (equation 4.2). Figure 4.3 shows a diagram of this calculation.

$$E_{iij} = \frac{A_{iij}}{2} - \frac{A_{iii}}{3}$$ **Equation 4.2**

Where A_{iij}= the final area covered by species i when there were two inocula of species *i* and one inoculum of species *j*.

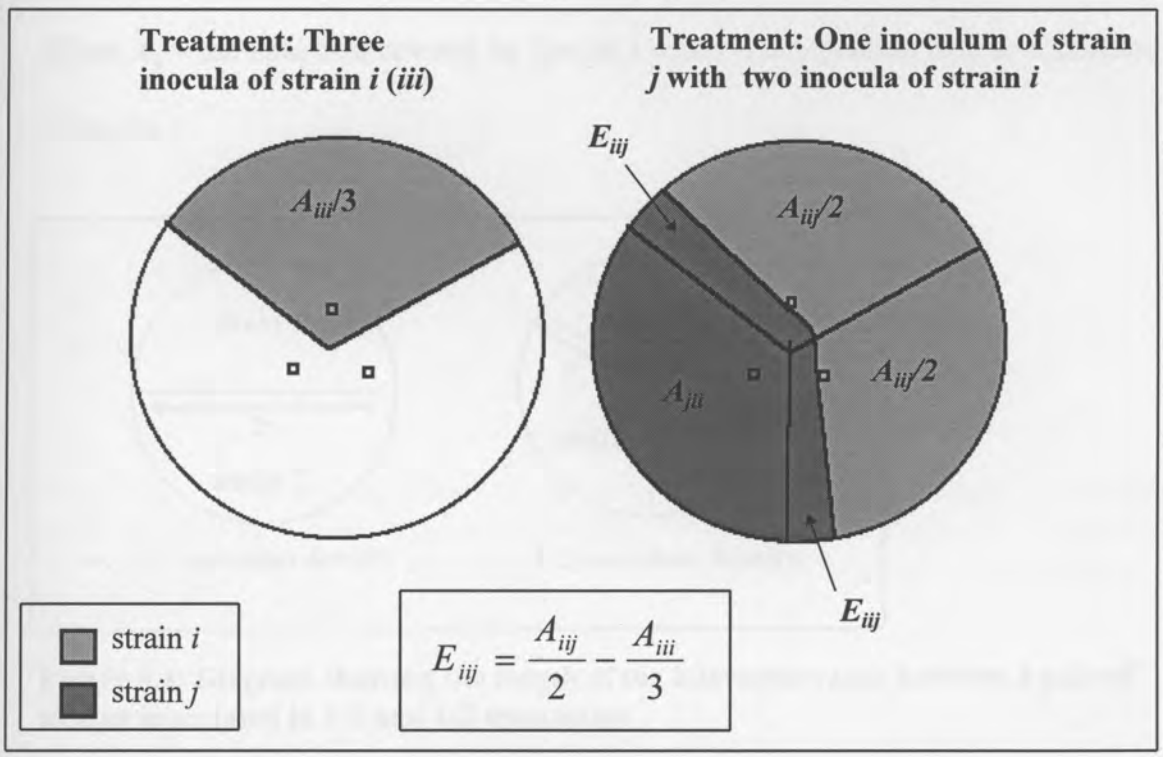

Figure 4.3: Diagram showing the effect of one inoculum of strain *j* on one inoculum of strain *i* in the *iij* treatment, where A_{iij} is the area covered by two inocula of i and A_{jii} is the area covered by one inoculum of j.

4.2.7.3 One inoculum of strain i with one inoculum of strain j (ij):

The area covered by one inoculum of the target species under intraspecific competition at the same plating density (two inocula per plate) was subtracted from the area covered by the target species in the presence of the competitor (*ij*). In many cases the value did not equate with the calculated values E_{ijj} and E_{iij}. Because the growing front along which a competitor can influence a target strain is approximately half in the

three inoculum cases (see figure 4.4), the effect per inoculum was halved in the two inocula cases to equate with the other plates (equation 4.3). Figure 4.5 shows a diagram of this calculation.

$$E_{ij} = \frac{\left(A_{ij} - \frac{A_{ii}}{2}\right)}{2}$$ **Equation 4.3**

Where A_{ij} = the final area covered by species i when in competition with one inoculum of species j.

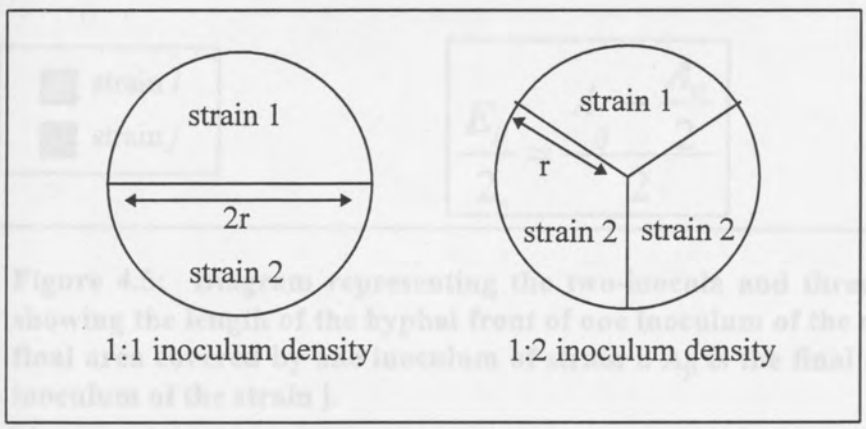

Figure 4.4: Diagram showing the length of the interaction zone between a pair of strains inoculated in 1:1 and 1:2 treatments

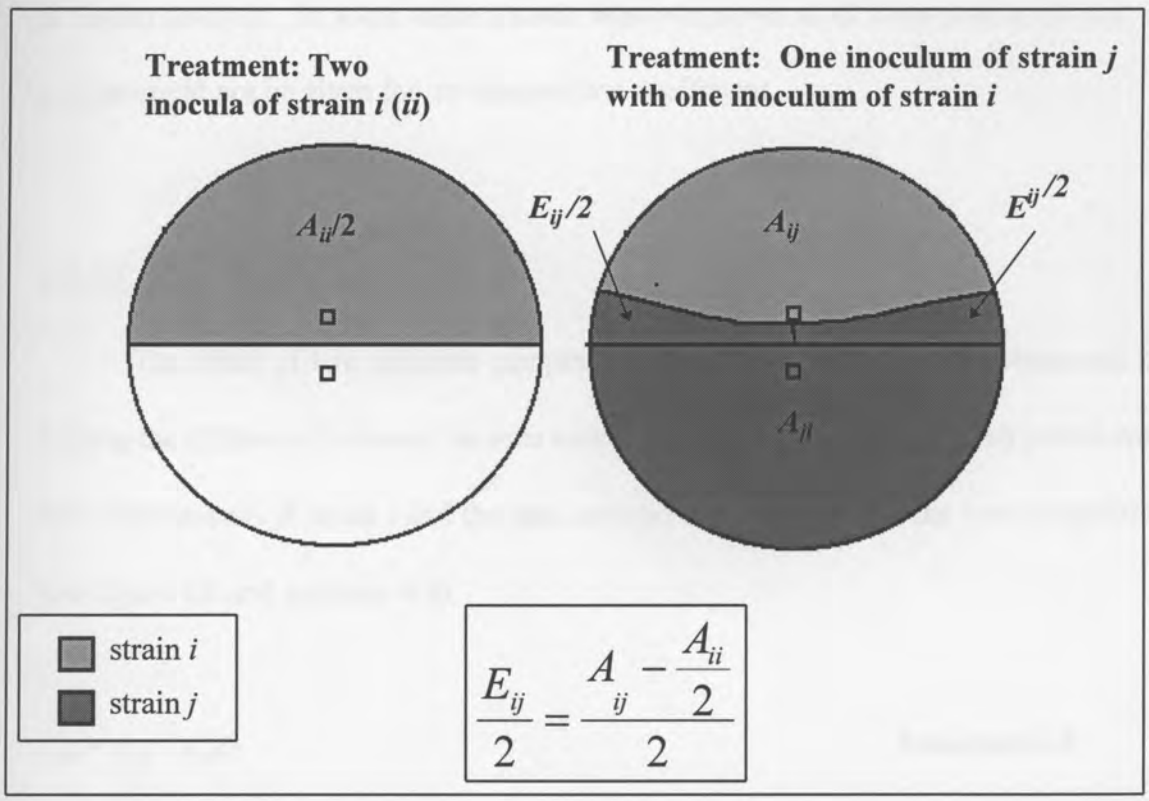

Figure 4.5: Diagram representing the two-inocula and three-inocula treatments showing the length of the hyphal front of one inoculum of the competitor. A_{ij} is the final area covered by one inoculum of strain i. A_{ji} is the final area covered by one inoculum of the strain j.

One way ANOVA was used to test the constancy of competition coefficients between different inoculum density treatments. If there was no difference between the coefficients then they were pooled (mean) for further analyses (equation 4.4).

$$E_{IJ} = \frac{E_{ijj} + E_{iij} + E_{ij}}{3}$$ **Equation 4.4**

Where E_{IJ} = average effect of strain i on strain j per inoculum

If one strain was overgrown then a value for the coefficient could not be calculated. If this occurred in only one of the treatments then the remaining two coefficients were tested for equality. If they were the same then they were averaged. If a strain was overgrown in two of the treatments then the remaining coefficient was used

in further analysis. In some cases a strain was overgrown in all three treatments and so a value could not be given for the competition coefficient.

4.2.7.4 Three-way interactions (ijk)*:*

The effect of two different competitors on a target strain (*i*) was determined by finding the difference between the area covered by one inoculum of *i* when plated with two other inocula of strain *i* and the area covered when plated with the two competitors (see figure 4.6 and equation 4.5).

$$E_{ijk} = A_{ijk} - A_{iii}/3 \qquad \textbf{Equation 4.5}$$

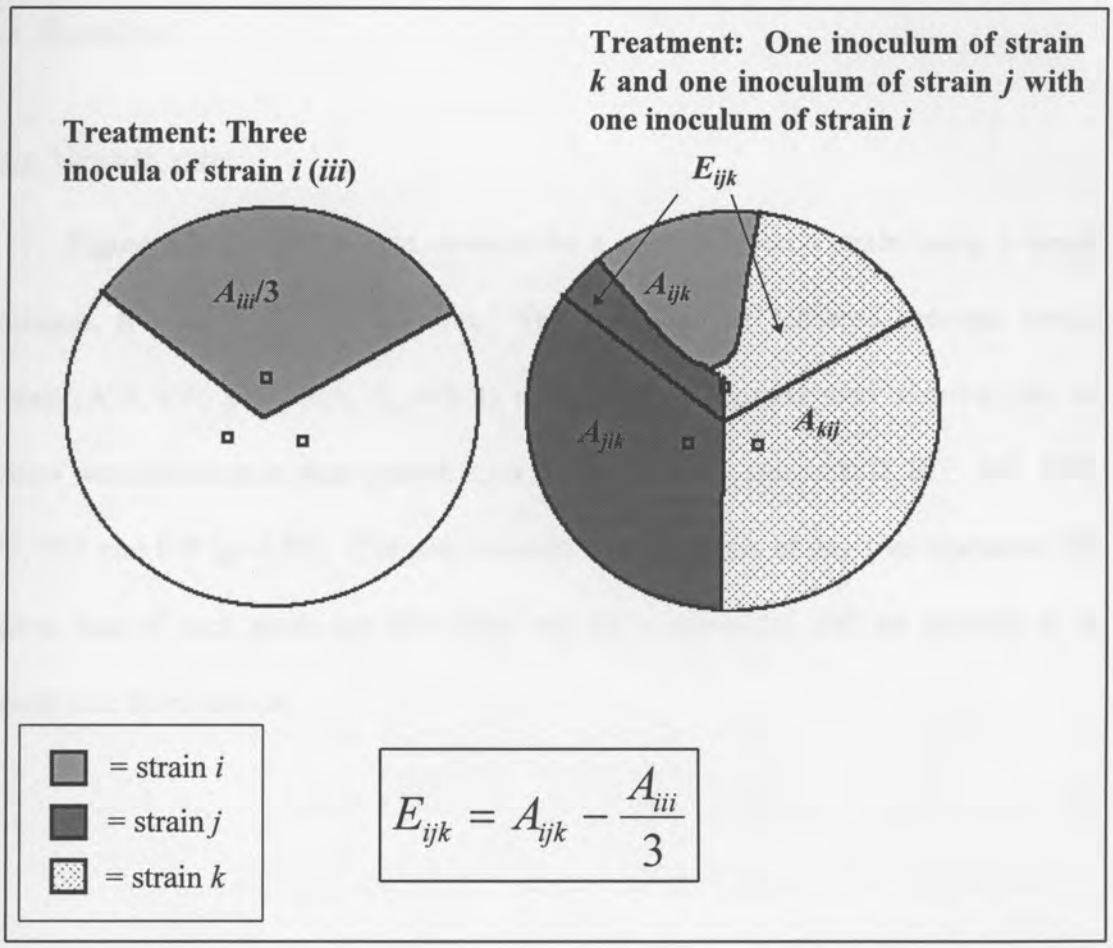

Figure 4.6: Diagram showing the effect of strains *j* and *k* on one inoculum of strain *i*, where A_{ijk}, A_{jik} and A_{kij} are the areas covered by strains i, j and k respectively

4.2.8 Testing for additivity of 3-way interactions:

To test for additivity the effect per inoculum of the two-species combinations were added together and compared with the effect of the two competitors in the three-species treatment. That is, equality of equation 4.6 was tested using a one-way ANOVA:

$$E_{IJ} + E_{IK} = E_{ijk}$$ **Equation 4.6**

4.3 Results:

4.3.1 Growth rate:

Figure 4.7 shows the area covered by a colony of each strain using a single inoculum, five days after inoculation. The areas covered differed between strains overall (ANOVA, $p=0.0001$, $F_{6,7}=28.3$) and post hoc contrast tests showed that all species were different in their growth rates ($p<0.05$) except strains B/D, B/E, B/F, C/G, D/E, D/F and E/F ($p>0.05$). The area covered by each strain at day five represents the growth rate of each strain per five days and for convenience will be referred to as growth rate from now on.

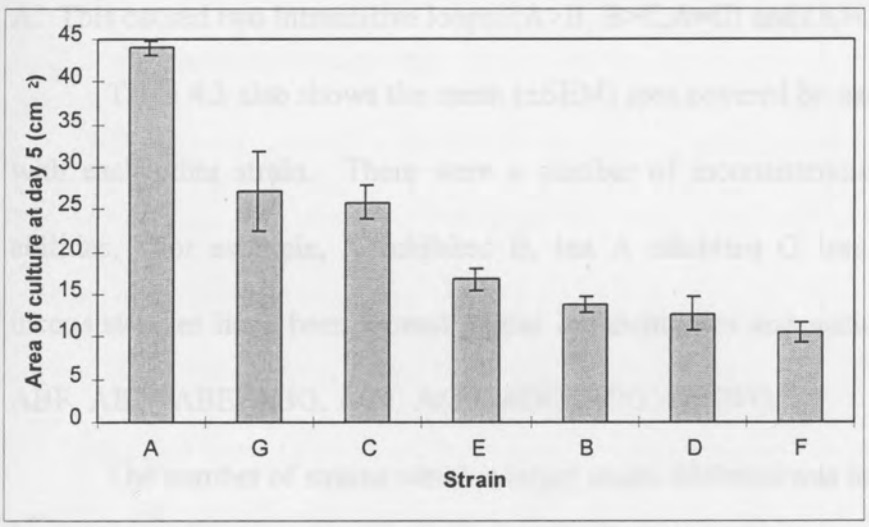

Figure 4.7: Graph of the area covered by a single inoculum each strain after five days of growth on MEA plates. Error bars = ±SEM

4.3.2 Pairwise interactions.

4.3.2.1 Inter- vs Intraspecific Competition:

The competitive abilities of the seven strains were ranked according to how many strains they were able to inhibit (competitive effect) and how many strains they were inhibited by (competitive response) (see table 4.3). It is interesting to note that the highest competitor (A) did not actually fully overgrow any of the other strains, whereas the more inferior competitors such as D, E and G were able to fully overgrow some of the other strains.

The seven strains formed a largely transitive hierarchy of competitive abilities. However there were two intransitive loops. Although strain A was able to inhibit five strains and was not inhibited by any, it was not able to inhibit strain C which could only inhibit three strains and was inhibited by two strains which were lower competitors than A. This caused two intransitive loops: (A>B, B>C,A=C) and (A>G, G>C, A=C).

Table 4.3 also shows the mean (±SEM) area covered by each strain when paired with each other strain. There were a number of inconsistencies in the competitive abilities. For example, A inhibited B, but A inhibited G less than B did. These inconsistencies have been termed partial intransitivities and occurred between strains: ABF, ABD, ABE, ABG, ACF, ACD, ADG, BCG and DFG.

The number of strains which a target strain inhibited was termed the competitive effect. The number of strains which inhibited a target strain was termed the competitive response. These two values were added together to give the competitive score of a strain. The strains were then ranked according to these scores.

Table 4.3: Table showing the area (mean ± SEM) covered by each strain paired with each other strain on the two-inocula plates (ab). -, + and = signs show that the effect on the target species was negative ($p<0.05$), positive ($p<0.05$) or neutral ($p>0.05$) by ANOVA, respectively.

Area covered by strain (cm^2)							
Competitor ↓	*A*	*B*	*G*	*C*	*E*	*D*	*F*
A		- 10.9±4.0	- 11.5±6.0	= 26.5±3.5	- 1.1±0.7	- 7.1±5.6	- 5.3±2.8
B	+ 43.3±4.0		- 0±0	- 7.6±2.5	- 0±0	- 0±0	- 0±0
G	+ 42.8±6.0	+ 54.3±0.1		- 20.6±4.2	- 5.6±4.2	- 0±0	- 4.5±2.9
C	= 27.8±3.5	+ 46.7±2.4	= 33.7±4.2		- 5.7±2.1	- 0±0	- 0±0
E	+ 53.2±0.7	+ 54.3±0.1	+ 48.7±4.2	+ 48.6±2.1		= 25.1±6.1	- 7.8±1.0
D	+ 47.1±5.6	+ 54.3±0.1	+ 54.3±0.1	+ 54.3±0.1	= 29.2±6.1		= 25.6±7.5
F	+ 49.0±2.7	+ 54.3±0.1	+ 49.8±2.9	+ 54.3±0.1	+ 46.1±1.3	= 28.6±7.5	
mean	43.8	45.8	33	35.3	14.6	10.1	7.2
competitive effect	5	5	4	3	1	0	0
competitive response	0	-1	-2	-2	-4	-4	-5
competitive score	5	4	2	1	-3	-4	-5
competitive rank	1	2	3	4	5	6	7

4.3.2.2 Interaction descriptions:

The growth rate and competitive abilities of these strains appeared to be linked. The interactions will therefore be described with reference to growth rates. Most interactions could be placed into three categories (i>j implies that strain i inhibited strain i, i=j implies that strains i and j did not inhibit one another):

(1) One strain grew faster than the other and this faster strain displaced the other while growing. Interaction C>D fell into this category.

(2) There was no displacement of either strain. If one of the strains was faster growing than the other, it ended up with a greater portion of the plate. Interactions A>B, A>C, A>F, C=G, D=E, D=F, E=F fell into this category.

(3) One strain grew faster than the other, but did not displace the other until there was no space left on the plate. The faster growing strain then displaced the other strain either a small amount (~5mm) or entirely. Interactions between A>G, B>E, B>G, C>F, G>D, G>E, G>F fell into this category.

Interactions involving strain B could not be placed in the above three categories. B grew at the same rate as D, E and F. However, B displaced these three strains as it grew as in category 1 above. Although G grew faster than B, B also displaced this strain as it grew. The interaction between B and C resembled category 3 above in that there was no displacement until there was no space left. However, this interaction was slightly different in that B grew slower than C, but was able to displace C.

4.3.2.3 Displacement ability:

Table 4.4 shows the displacement behaviour between pairs of strains. Each strain was given a score for displacement ability. For each other strain which it could displace, it was given a score of one (+1), and for each strain that it was displaced by, it was given a score of minus one (-1). The 'displacement effect' was the number of strains that the target strain was able to displace and 'displacement response' was the number of strains that displaced the target strain. The sum of these two values gave the 'displacement score'. The strains were then ranked according to the scores. The rank displacement abilities of the strains differed from the competitive ability. For example strain E was competitively superior to D, but E was displaced by A whereas D wasn't. Hence D was ranked higher for displacement.

Table 4.4: Table of displacement behaviour of each strain. nd= no displacement, d1= competitor displaced target strain as it grew. d2= competitor displaced target strain after there was no space left on the plates. * = target strain displaced competitor

	Target strain						
Competitor ↓	A	B	G	C	E	D	F
A		nd	nd	nd	d2	nd	nd
B	nd		d1	d2	d1	d1	d2
G	nd	d1*		nd	d2	d2	d2
C	nd	d2*	nd		d1	d1	d2
E	d2*	d1*	d2*	d1*		nd	nd
D	nd	d1*	d2*	d1*	nd		nd
F	nd	d2*	d2*	d2*	nd	nd	
displacement effect	1	5	3	3	0	0	0
displacement response	0	0	-1	-1	-4	-3	-3
score	1	5	2	2	-4	-3	-3
rank	4	1	2.5	2.5	7	5.5	5.5

4.3.2.4 Competitive ability, displacement ability and growth rate:

The competitive ability of the strains as shown in table 4.3 was plotted against the average growth rate of each the strains (see figure 4.8). Spearman correlation showed a correlation (r= -0.786, p=0.036) between growth rate and competitive ability. There was one obvious outlier in figure 4.8 which was strain B.

The displacement ability was not correlated with growth rate (Spearman r= -0.382, p=0.398) (see figure 4.9).

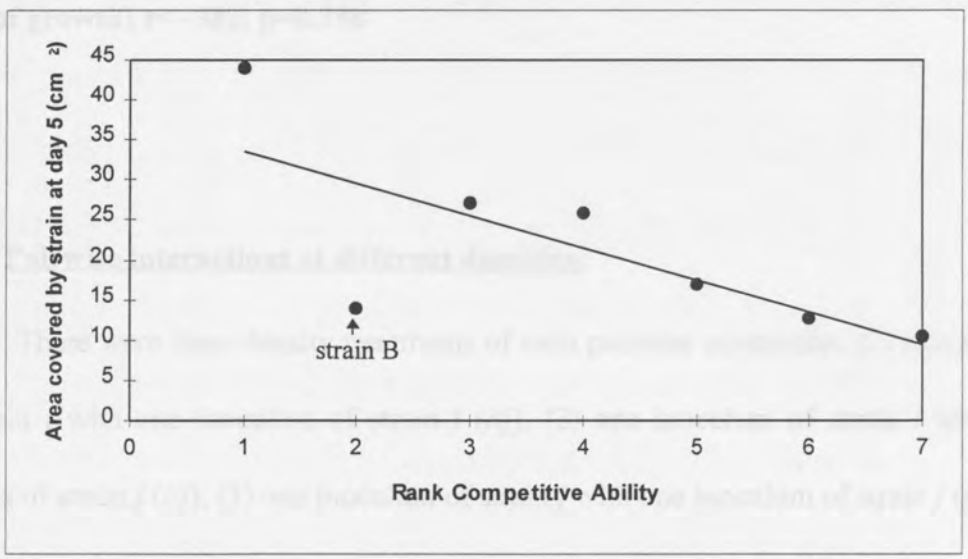

Figure 4.8: Correlation between the competitive ability of the strains and the growth rate of each of the strains (average area covered by the strains after five days of growth) r= -0.786, p=0.036

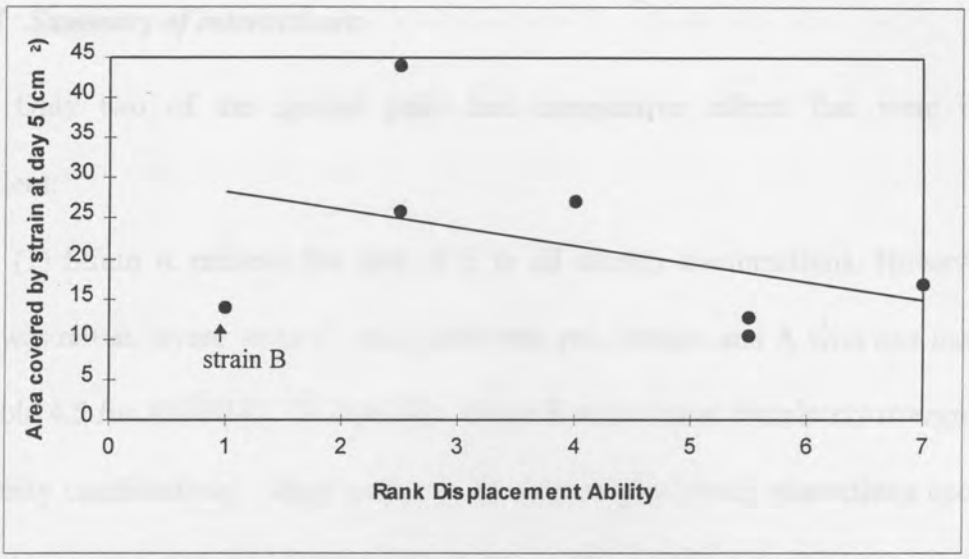

Figure 4.9: Correlation between the displacement ability of the strains and the growth rate of each of the strains (average area covered by the strains after five days of growth) r= -382, p=0.398

4.3.3 Pairwise interactions at different densities:

There were three density treatments of each pairwise interaction; (1) two inocula of strain *i* with one inoculum of strain *j* (*iij*), (2) one inoculum of strain *i* with two inocula of strain *j* (*ijj*), (3) one inoculum of strain *i* with one inoculum of strain *j* (*ij*)

The effect per inoculum was calculated as in section 4.2.7. Table 4.5 shows the results of one-way ANOVA tests for differences in competitive effects at the different densities. All data were homoscedastic. All interactions were barrage type interactions where there was no clear zone between the colonies and no intermingling of hyphae.

4.3.3.1 Summary of interactions:

Only two of the species pairs had competitive effects that were density dependent:

(1) Strain A reduced the area of E in all density combinations. However, the effect was not as severe when E was plated with two inocula and A with one inoculum (see table 4.5 for ANOVA). In this case, where E was almost completely overgrown in all density combinations, there seems to be some higher order interactions occurring where A cannot completely overgrow E. In the AAE case, E was overgrown except for a very small area. This holding onto territory is not consistent with the effect that A has on E in the AEE or AE cases. To calculate additivity therefore the AAE case was ignored. After removing this case, the effects in the other two treatments were tested. The data were heteroscedastic and no transformation could help. An independent samples two-tailed t-test for unequal variances showed that the two effects were the same ($p=0.91$, $t=-0.14$, $df=1$). The interaction type was category 3 where there was no displacement of E until there was no uncolonised space left, then A displaced E.

(2) The interaction between B and C was a function of density. Where B was outnumbered (1:2), it was slightly reduced by C, but when B had two inocula against C's one inoculum, it was able to completely overgrow C. When initial densities were the same, B overgrew C but not completely.

In cases where a strain completely overgrew the target strain, the magnitude of the effect could not be calculated. In these cases a "+" is shown. Sometimes where the stronger competitor was plated at a lower density (2:1) the target strain was not completely overgrown. In these cases a value could be given for the effect. In cases E with B, F with C and D with G the first strain was outcompeted by the second strain in

all density combination except where it had two inocula against one inoculum of the second strain (2:1). In several other cases (C with B, F with C, E with G, F with G) the first strain was only overgrown in the 1:2 treatment, so values could be determined for the other densities. In these cases only two values for competitive effect were compared (one-way ANOVA).

Table 4.5: Table of competitive effects. Effects were calculated as in section 4.2.7. + = strain completely overgrew the other strain, - = strain was completely overgrown.

Target strain	Competitor	Effect at density 1:2	Effect at density 1:1	Effect at density 2:1	df	F	p
A	B	12.7±0.01	14.9±3.3	13.2±3.4	2,3	0.18	0.844
A	C	1.5±1.0	0.6±2.8	6.0±1.1	2,3	2.5	0.230
A	D	13.6±2.1	18.4±4.6	16.7±7.8	2,3	0.21	0.822
A	E	14.3±1.2	24.0±0.01	24.3±2.2	2,3	15.84	0.025
A	F	14.8±0.7	20.2±2.3	24.9±2.5	2,3	6.32	0.084
A	G	11.0±4.7	14.4±4.8	11.1±3.1	2,3	0.21	0.821
B	A	-13.2±3.4	-14.9±3.3	-12.7±0.01	2,3	0.18	0.844
B	C	+	18.4±1.7	-3.8±0.3	1,2	159.71	0.006
B	D	+	+	+			
B	E	+	+	20.4±1.1			
B	F	+	+	+			
B	G	+	+	+			
C	A	-6.0±1.1	-0.6±2.8	-1.5±1.0	2,3	2.5	0.230
C	B	3.8±0.3	-18.4±1.7	-	1,2	159.71	0.006
C	D	+	+	+			
C	E	+	20.3±1.1	21.8±3.6	1,2	0.163	0.725
C	F	+	+	31.4±0.9			
C	G	-0.6±0.9	-6.0±2.6	-4.2±2.6	2,3	1.59	0.339
D	A	-16.7±7.8	-18.4±4.6	-13.6±2.1	2,3	0.21	0.822
D	B	-	-	-			
D	C	-	-	-			
D	E	-6.3±6.8	-0.9±5.1	-1.0±5.7	2,3	0.27	0.779
D	F	0.1±1.6	2.6±6.1	0.1±6.8	2,3	0.07	0.932
D	G	-29.1±1.2	-	-			
E	A	-24.3±2.2	-24.0±0.01	-14.3±1.2	2,3	15.84	0.025
E	B	-20.4±1.1	-	-			
E	C	-21.8±3.6	-20.3±1.1	-	1,2	0.163	0.725
E	D	1.0±5.7	0.9±5.1	6.3±6.8	2,3	0.27	0.779
E	F	14.1±0.5	17.7±0.5	16.0±0.9	2,3	7.64	0.066
E	G	-23.4±2.2	-20.6±3.5	-	1,2	0.49	0.558
F	A	-24.9±2.5	-20.2±2.3	-14.8±0.7	2,3	6.32	0.084
F	B	-	-	-			
F	C	-31.4±0.9	-	-			
F	D	-0.1±6.8	-2.6±6.1	-0.1±1.6	2,3	0.07	0.932
F	E	-16.0±0.9	-17.7±0.5	-14.1±0.5	2,3	7.64	0.066
F	G	-22.2±1.1	-20.9±0.8	-	1,2	0.87	0.449
G	A	-11.1±3.1	-14.4±4.8	-11.0±4.7	2,3	0.21	0.821
G	B	-	-	-			
G	C	4.2±2.6	6.0±2.6	0.6±0.9	2,3	1.59	0.339
G	D	+	+	29.1±1.2			
G	E	+	20.6±3.5	23.4±2.2	1,2	0.49	0.558
G	F	+	20.9±0.8	22.2±1.1	1,2	0.87	0.449

4.3.3.2 Type of interactions:

Most interactions between pairs of strains were the same in the different densities, but with the following exceptions:

Interactions between A and C: In AC inoculation density there was no displacement between A and C. Also with two inocula of C and one of A, there was no displacement. Whereas on the plates with two inocula of A and one of C, A slightly displaced C once there was no space left on the plate. This slight difference did not however show up in the tests for density dependent effects above (section 4.3.3.1).

Interactions between B and C: In both the BC inoculation density and the BBC density, there was no displacement until there was no space left on the plate. B then displaced C. However, in the BCC inoculation density, B did not displace C at all.

4.3.4 three-way interactions:

The three-way interactions could be placed into five basic categories:

(1) Additive interactions

The effect of strains i and j on k was the sum of the pairwise interaction effects of i and j on k. The calculated value for additivity was not significantly different from the real values in the three-way interaction (one-way ANOVA).

(2) Indirect effect

The effect of strains i and j on k was non-additive. The direction of the non-additive effect could be explained by an indirect effect mediated via a change in the size of either strain i or j.

(3) Higher order interaction - the result of the three-way is not additive and the deviation is in the opposite direction to any predictable indirect effect.

(4) The three-way combinations which included B and C could not be categorised with the others, because there was a higher order interaction in the two-way plates. These are therefore discussed separately.

(5) Undecided - The additivity of the three-way interactions could not be tested because one or more of the strains was overgrown by another of the strains in every pairwise density treatment.

For each three-species combination a graphical representation of the interactions is shown. The response variable in all cases is the competitive effect as calculated in section 4.2.7. From left to right, the first three categories show the interactions between pairs of species, the fourth category shows the calculated effect that two species would have on the target species in three-species interaction if the interactions were all

additive. The last category shows competitive effect of two species on the target species in three-way competition.

4.3.4.1 Explanation of graphs for three-way competition:

Figure 4.10 shows an example of the graphs showing the results of pairwise and three-way competition for a group of three strains. The competitive effects are as calculated in section 4.2.7. The dotted line shows the value at which a strain is completely overgrown (33.3) (ie 1/3 of the plate). The expected values for three-strain-interactions were calculated as in section 4.2.8, by adding the individual effects of the two strains on the target strain. For example, in figure 4.10, the expected effect of strains C and D on E was -20.2 (±1.0) + 2.7 (±5.9) = -17.5 (±6.9) and the observed effect was -25.3 (±1.5)). The expected and observed effects of two strains on a target strain were compared using one-way ANOVA. These results are displayed with the graphs (eg see table 4.6).

In cases where one strain was overgrown by another strain in every pairwise combination of the two strains (eg C overgrew D, figure 4.10), the value of the competitive effect could not be calculated. Therefore, the expected effect of these two strains on each other when with another strain also could not be calculated. Therefore, in these cases, the effect (eg effect of D on C) was given a value of 33.3. This value was used to calculate the expected value of three-strain interactions involving this pair of strains (eg the effect of D and E on C was 33.3 + 20.2 = 53.5). However, when one strain was overgrown, an exact value for the competitive effect could be given. That is, the effect of D on C was 33.3 or more. The predicted effect of D and E on C was therefore 53.5 or more. In cases where this occurs, the 'or more' factor is represented in

the graph as an arrow (↓ , ↑) indicating the direction of the potential effect (eg see figure 4.10).

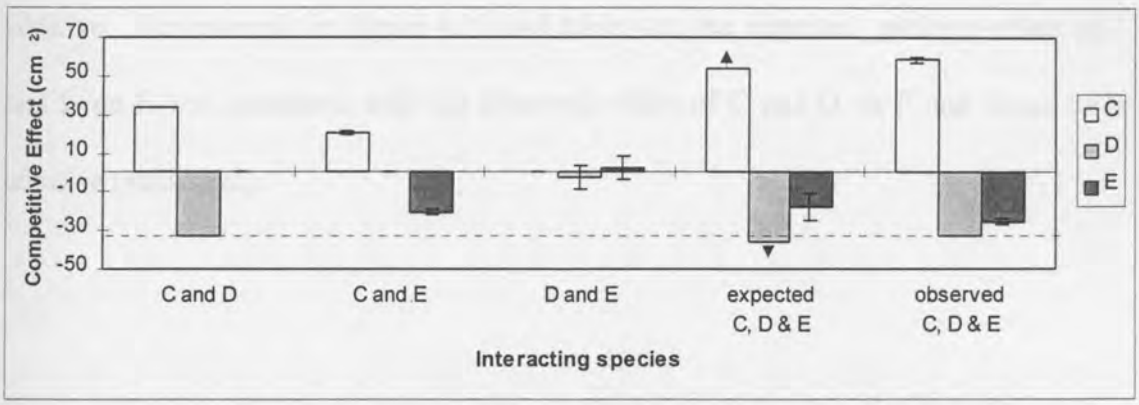

Figure 4.10: Example of graphs showing the competitive effects between three strains (in this case, strains C, D and E). error bars =±1SEM. dotted line (.....) represents the value at which a strain is completely overgrown. Arrows (↓ ,↑) indicate the direction of the predicted effect.

Table 4.6: Example of table showing comparison of the expected (additive) effect of two strains on the target strain with the observed effect in three species competition using either logical or statistical methods

	test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and D on E	logical	-	-	additive
Effect of strains C and D on E	logical	-	-	additive
Effect of strains C and D on E	statistical	1.21	0.385	additive

4.3.4.2 Statistical and logical demonstrations of additive and non-additive effects:

In cases where one strain was overgrown by another, and an exact value could not be calculated for the expected effect in three-strain interactions, the additivity or non-additivity of the interaction was usually obvious. For example, in figure 4.16, the exact value for the predicted effect of D and E on C could not be calculated, but it was 53.5 or more. The observed effect of D and E on C was 58.7±1.5. Therefore the observed effect was within the range of the expected, additive effect. The interaction was labelled additive due to logical reasons (see table 4.6).

In other cases where there was no overgrowth, an expected, additive effect was calculated and compared with the observed effect of the three-strain interaction using ANOVA. These tests of additivity are termed statistical additive or statistical non-additive. For example in figure 4.10 and table 4.6, the expected, additive effect of C and D on E was compared with the observed effect of C and D on E and found to be additive (statistical).

4.3.4.3 Additive, statistical

Seventeen of the 35 three-strain combinations did not contain any non-additive interactions. Nine of these combinations (ABC, ADE, ADF, ADG, CBE, CEG, DEF, DEG, DFG) were found to be additive using ANOVA. The pairwise interactions within these three-strain combinations are summarised in figure 4.11. In six of the combinations, two of the three strains were equal competitors. There were two three-strain combinations which contained a partial intransitivity (ABC, ADG). The other seven combinations were transitive. The pairwise interactions involved displacement while growing (d1), displacement only after there was no space left on the plate (d2), and no displacement (nd). Figures 4.12-4.20 and tables 4.7-4.15 show the three-way interactions for each of the combinations of strains as well as the test comparing the expected additive and observed competitive effects of three-way interactions. The nature of the interactions between pairs of species mostly remained the same in three-way and pairwise interactions. Two exceptions were: (1) between strains D, E and G; G displaced D as it grew in three-way interactions, whereas in pairwise interactions G didn't displace D until there was no space left on the plate; (2) between, strains A, D and G; in three-way interactions A displaced D slightly when there was no space left on the plate, whereas in pairwise treatments, there was no displacement between these two strains.

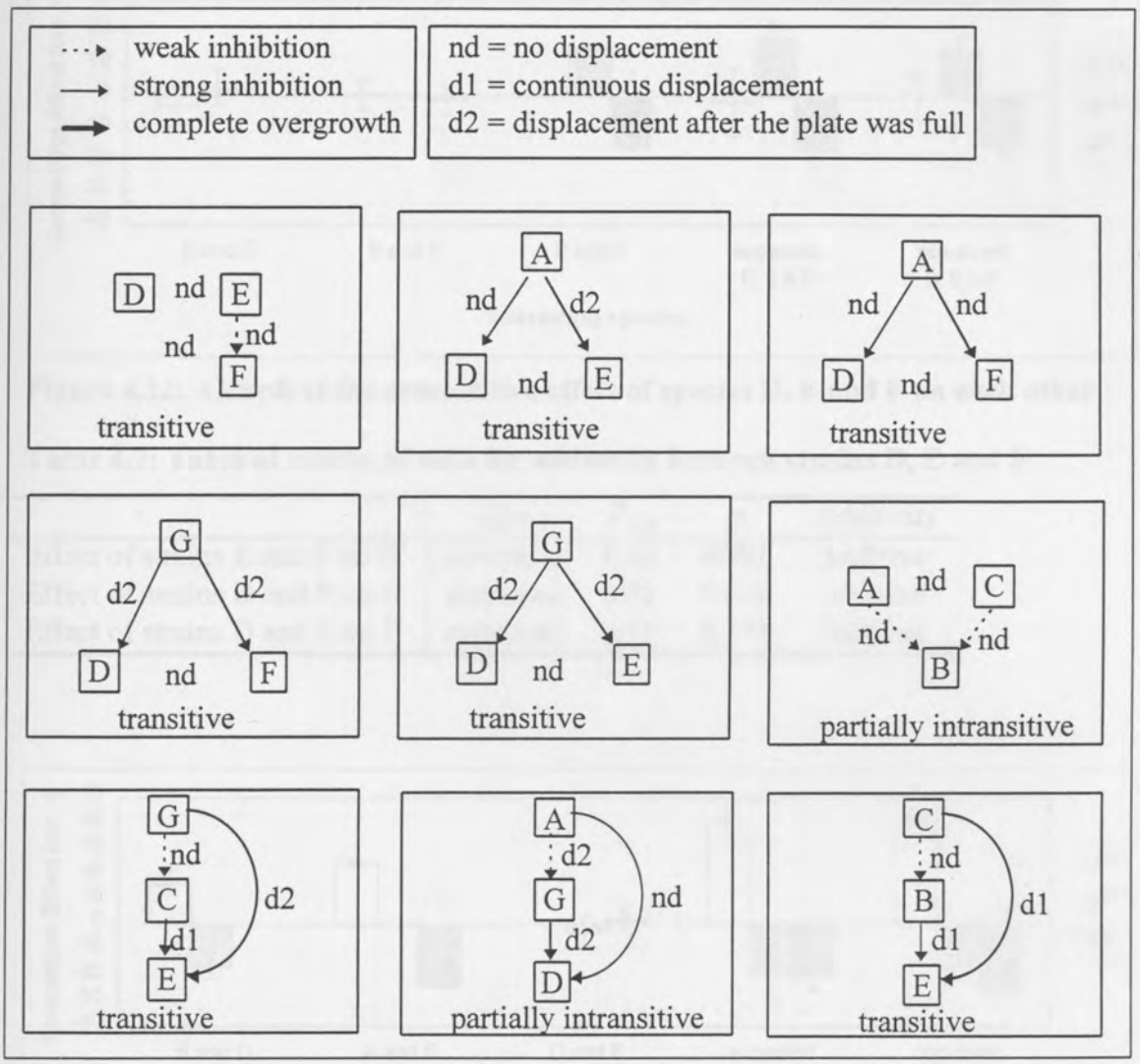

Figure 4.11: Diagrams summarising the pairwise interactions between three-strain combinations which contained no non-additive interactions (statistical)

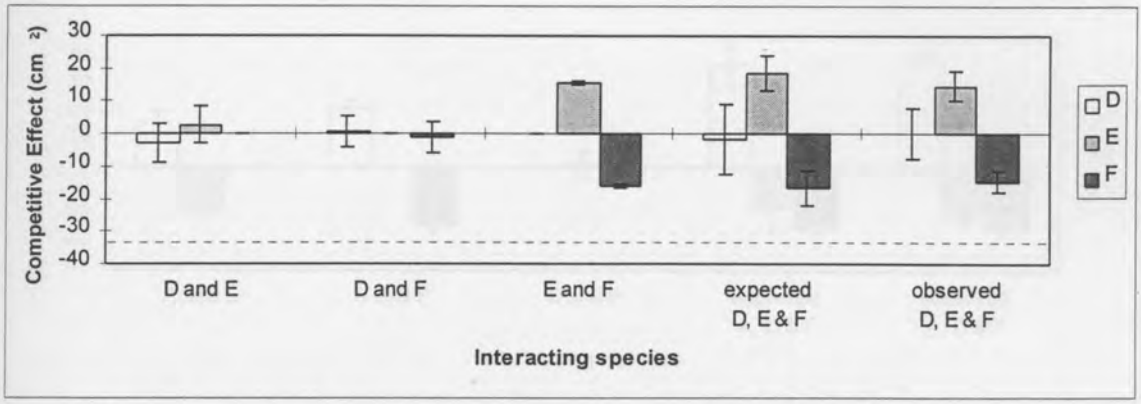

Figure 4.12: Graph of the competitive effect of species D, E and F on each other

Table 4.7: Table of results of tests for additivity between strains D, E and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and F on D	statistical	0.02	0.901	additive
Effect of strains D and F on E	statistical	0.32	0.628	additive
Effect of strains D and E on F	statistical	0.11	0.774	additive

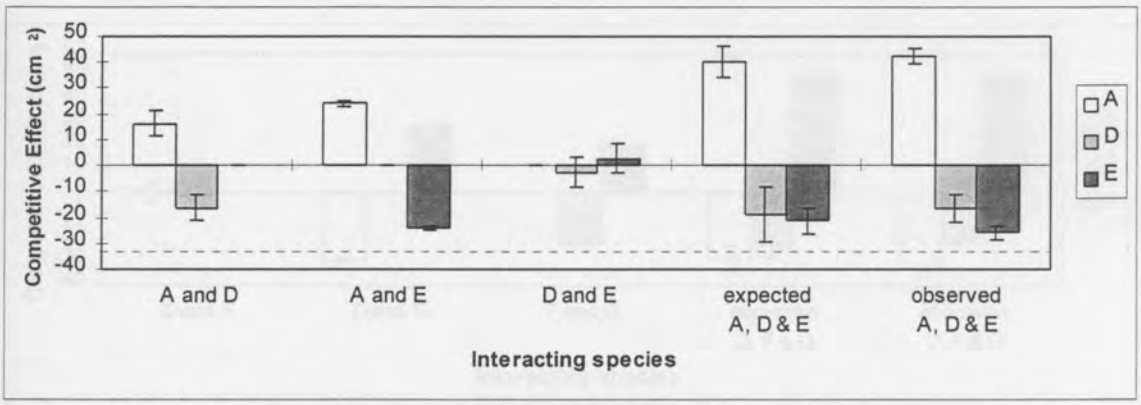

Figure 4.13: Graph of the competitive effect of species A, D and E on each other

Table 4.8: Table of results of tests for additivity between strains A, D and E

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and E on A	statistical	0.09	0.787	additive
Effect of strains A and E on D	statistical	0.04	0.855	additive
Effect of strains A and D on E	statistical	0.70	0.491	additive

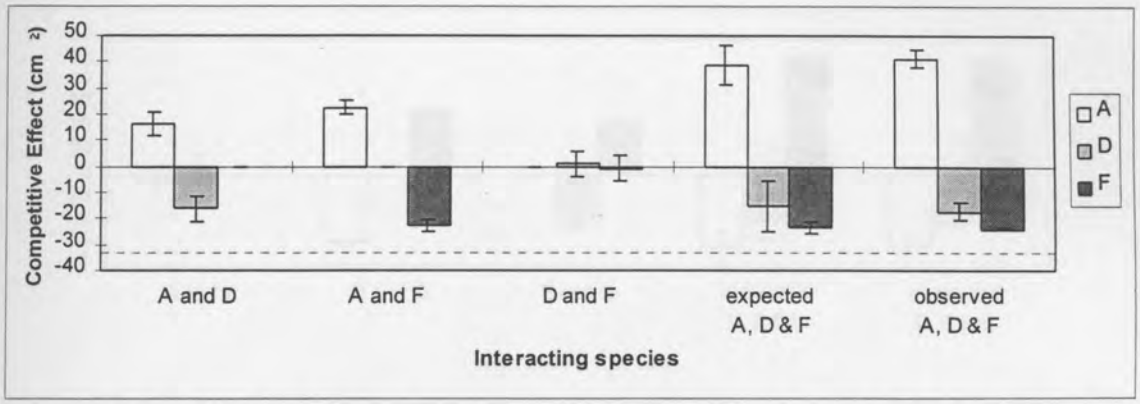

Figure 4.14: Graph of the competitive effect of species A, D and F on each other

Table 4.9: Table of results of tests for additivity between strains A, D and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and F on A	statistical	0.11	0.774	additive
Effect of strains F and A on D	statistical	0.04	0.862	additive
Effect of strains A and D on F	statistical	0.05	0.842	additive

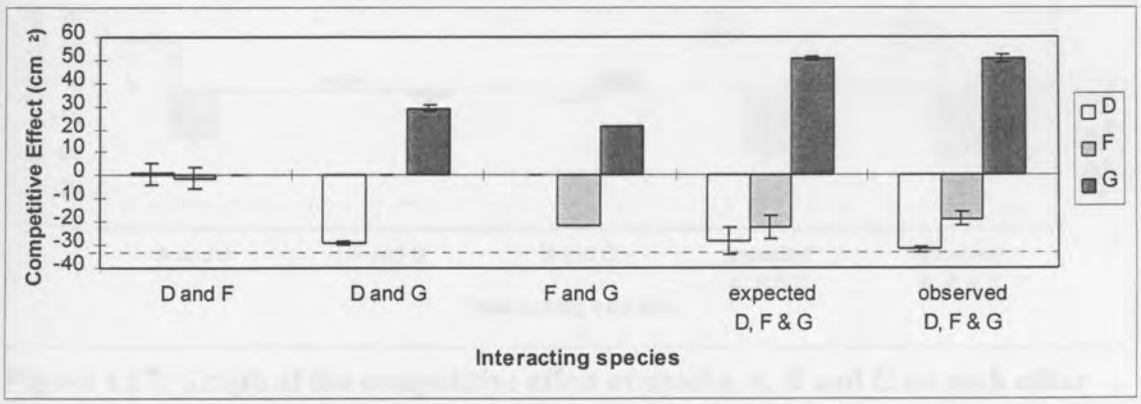

Figure 4.15: Graph of the competitive effect of species D, F and G on each other

Table 4.10: Table of results of tests for additivity between strains D, F and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains F and G on D	statistical	0.38	0.599	additive
Effect of strains D and G on F	statistical	0.43	0.578	additive
Effect of strains D and F on G	statistical	0.23	0.680	additive

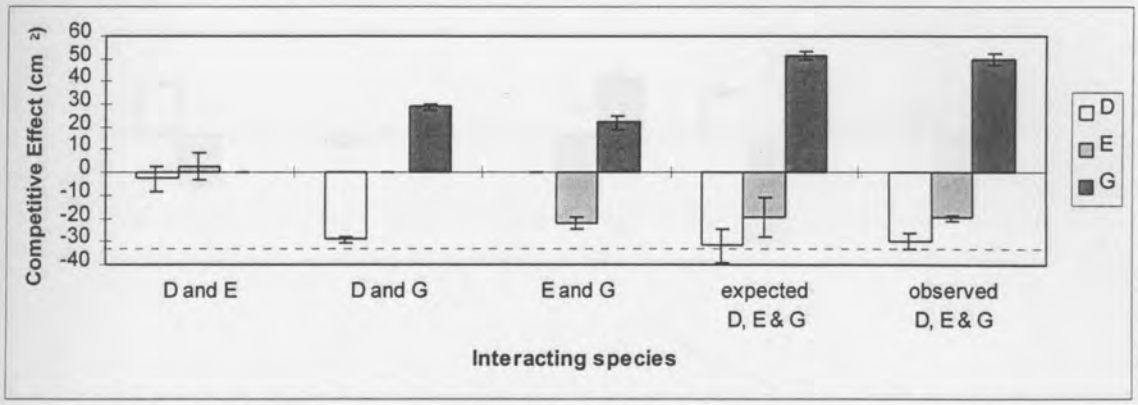

Figure 4.16: Graph of the competitive effect of species D, E and G on each other

Table 4.11: Table of results of tests for additivity between strains D, E and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and G on D	statistical	0.06	0.826	additive
Effect of strains D and G on E	statistical	0.001	0.954	additive
Effect of strains E and D on G	statistical	0.001	0.997	additive

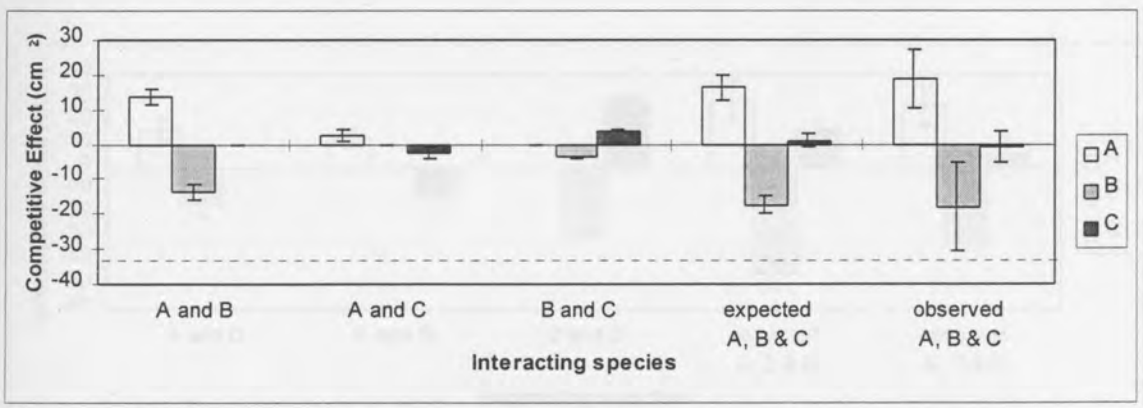

Figure 4.17: Graph of the competitive effect of species A, B and C on each other

Table 4.12: Table of results of tests for additivity between strains A, B and C

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains B and C on A	statistical	0.07	0.811	additive
Effect of strains A and C on B	statistical	4.12	0.179	additive
Effect of strains A and B on C	statistical	0.16	0.729	additive

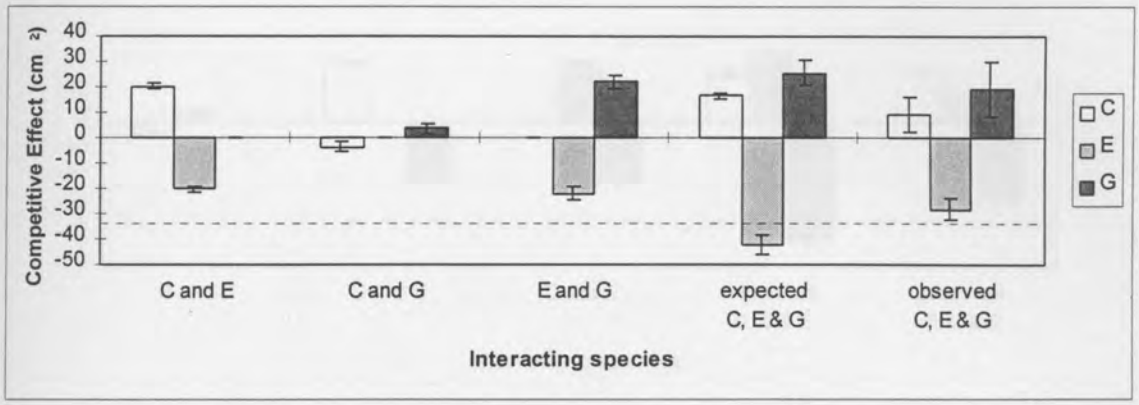

Figure 4.18 Graph of the competitive effect of species C, E and G on each other

Table 4.13: Table of results of tests for additivity between strains C, E and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and G on C	statistical	1.22	0.384	additive
Effect of strains C and G on E	statistical	6.48	0.126	additive
Effect of strains C and E on G	statistical	0.29	0.645	additive

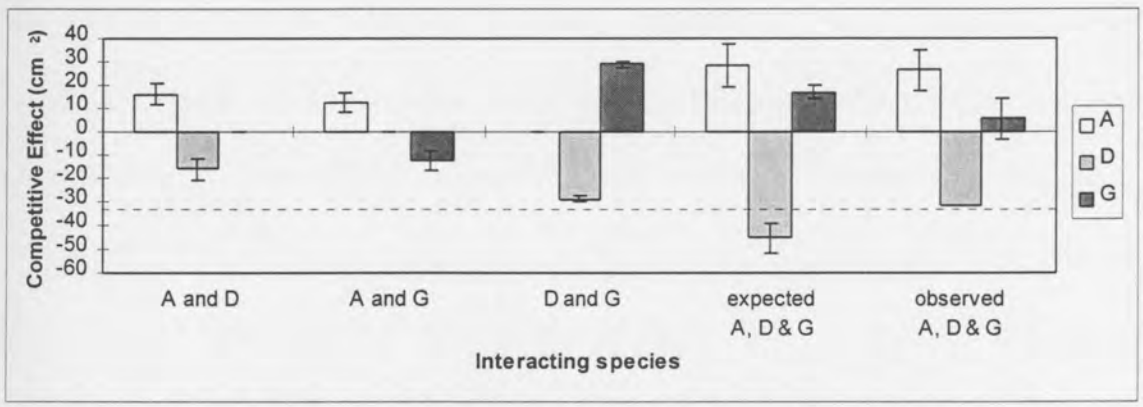

Figure 4.19: Graph of the competitive effect of species A, D and G on each other

Table 4.14: Table of results of tests for additivity between strains A, D and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and G on A	statistical	0.03	0.884	additive
Effect of strains A and G on D	statistical	5.02	0.154	additive
Effect of strains A and D on G	statistical	1.45	0.352	additive

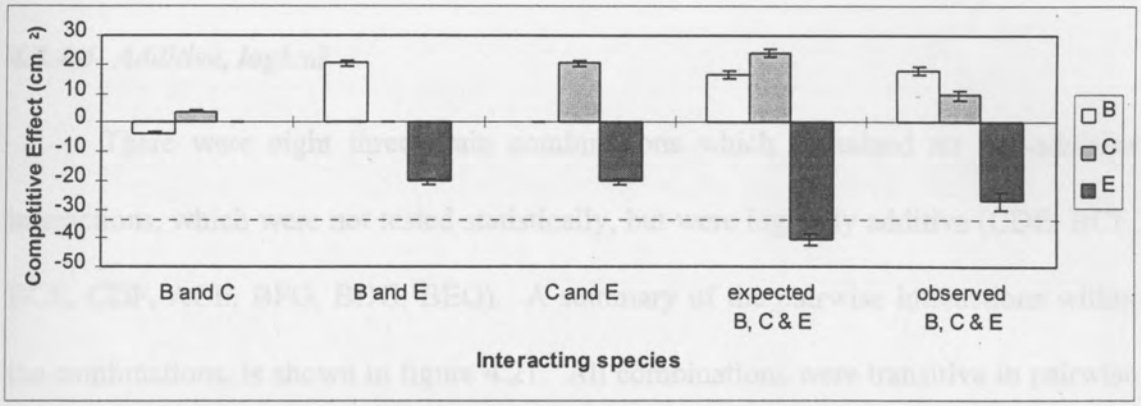

Figure 4.20: Graph of the competitive effect of species B, C and E on each other

Table 4.15: Table of results of tests for additivity between strains B, C and E

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and E on B	statistical	16.16	0.057	additive
Effect of strains B and E on C	statistical	3.55	0.200	additive
Effect of strains B and C on E	statistical	12.51	0.071	additive

4.3.4.4 Additive, logical

There were eight three-strain combinations which contained no non-additive interactions, which were not tested statistically, but were logically additive (CDE, BCF, BCE, CDF, ACE, BFG, BDG, BEG). A summary of the pairwise interactions within the combinations, is shown in figure 4.21. All combinations were transitive in pairwise interactions. In five of the three-strain combinations, a pair of the strains were equal competitors. Figures 4.22-4.29 show the competitive effects of these three-strain combinations in pairwise and three-way treatments. Tables 4.16-4.23 show the additivity of the interactions. The nature of the interactions remained the same in four of the three-strain combinations (CDE, BDF, BDE, ACE). In the CDF combination, F displaced D as it grew in three-way competition, whereas in pairwise interactions, there was no displacement between these two strains. Also, C displaced F as it grew, whereas in pairwise interactions, C did not displace F until there was no space left on the plates. In the BDG, BEG and BFG combinations, when there was no space left, in the pairwise interactions, G displaced D, E and F, whereas in the three-way plates, there was no displacement between G and D, E or F, respectively.

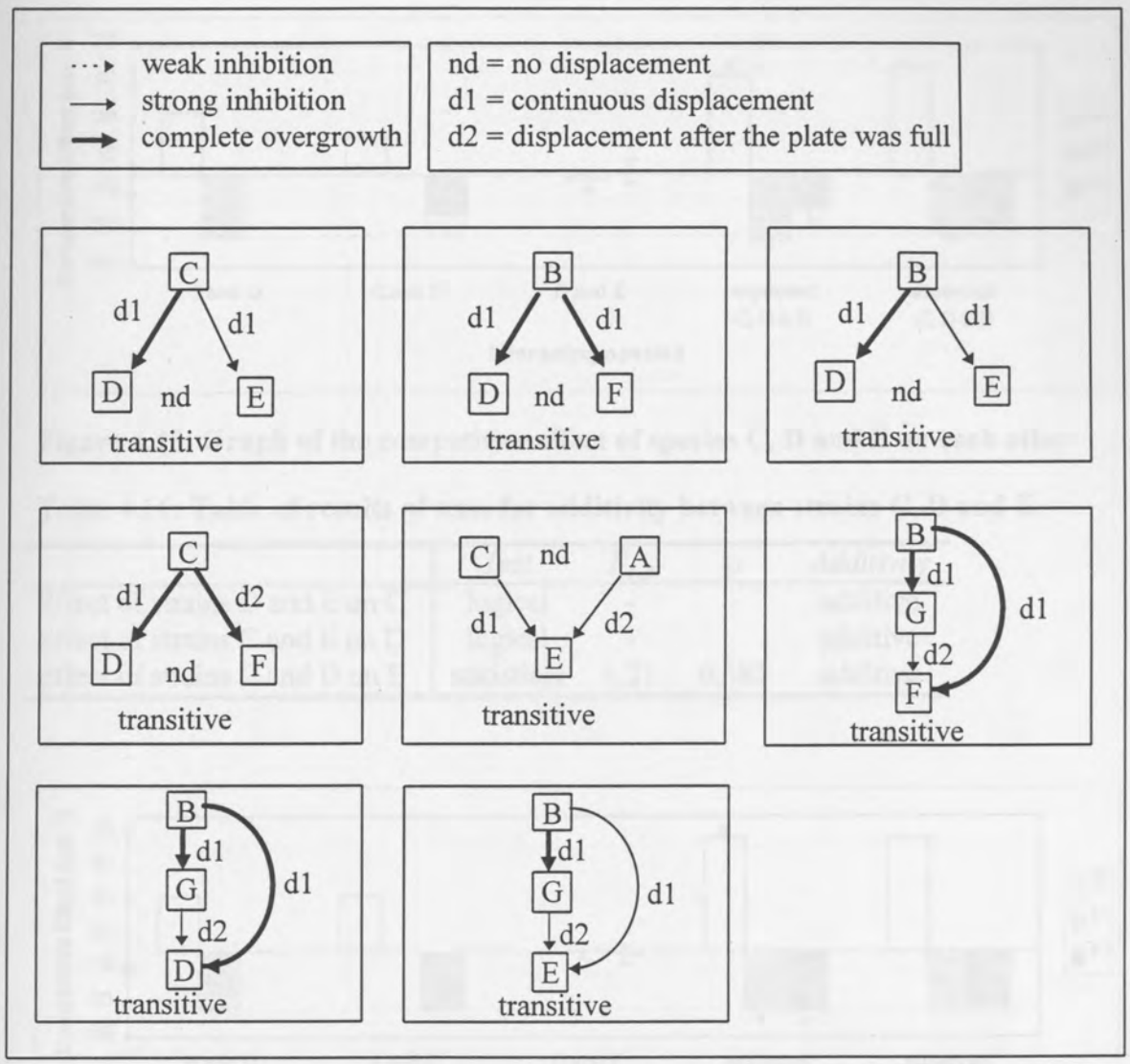

Figure 4.21: Diagrams summarising the pairwise interactions between three-strain combinations which contained no non-additive interactions (logical)

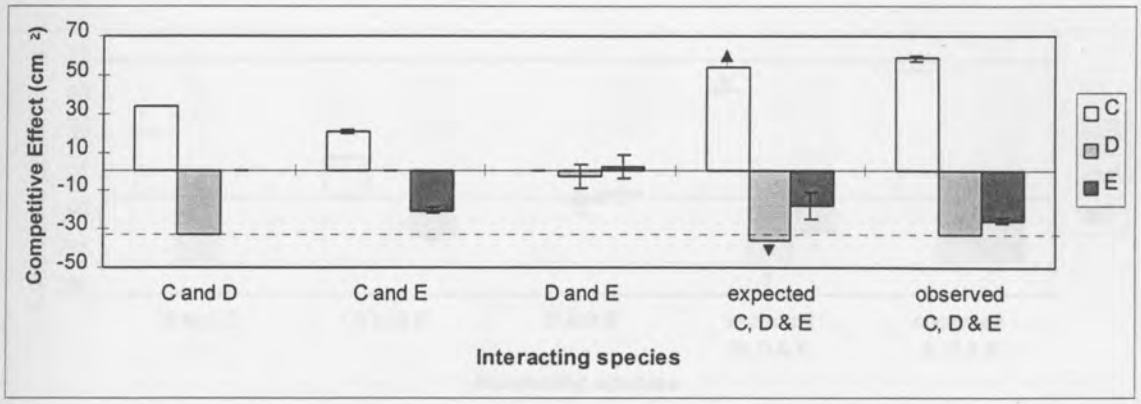

Figure 4.22: Graph of the competitive effect of species C, D and E on each other

Table 4.16: Table of results of tests for additivity between strains C, D and E.

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and E on C	logical	-	-	additive
Effect of strains C and E on D	logical	-	-	additive
Effect of strains C and D on E	statistical	1.21	0.385	additive

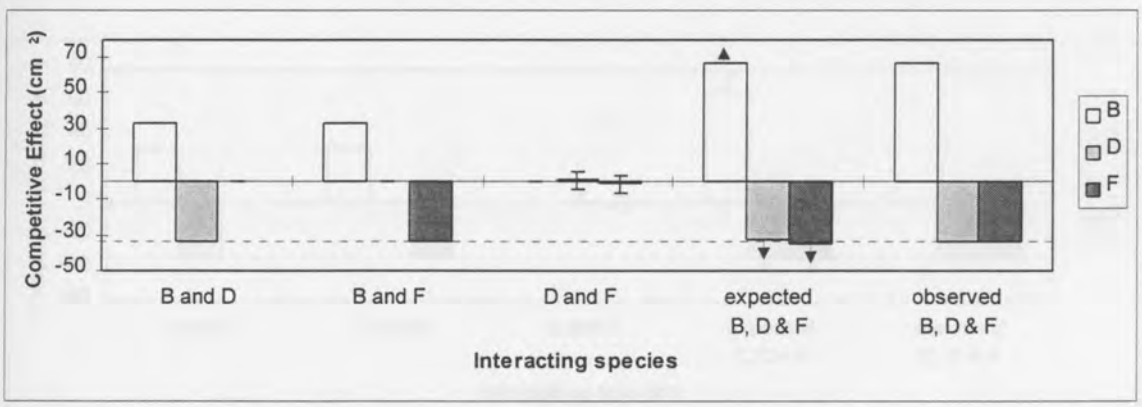

Figure 4.23: Graph of the competitive effect of species B, D and F on each other

Table 4.17: Table of results of tests for additivity between strains B, D and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and F on B	logical	-	-	additive
Effect of strains B and F on D	logical	-	-	additive
Effect of strains B and D on F	logical	-	-	additive

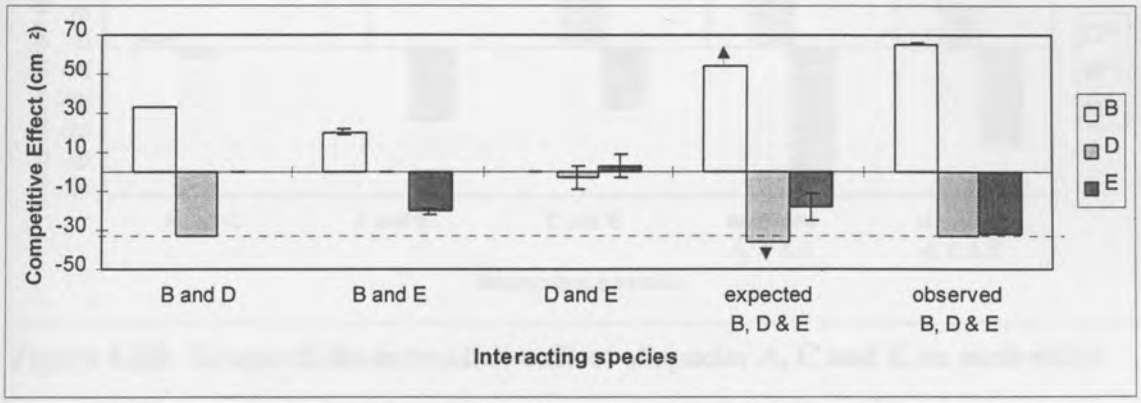

Figure 4.24: Graph of the competitive effect of species B, D and E on each other

Table 4.18: Table of results of tests for additivity between strains B, D and E

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and E on B	logical	-	-	additive
Effect of strains B and E on D	logical	-	-	additive
Effect of strains B and D on E	statistical	4.3	0.174	additive

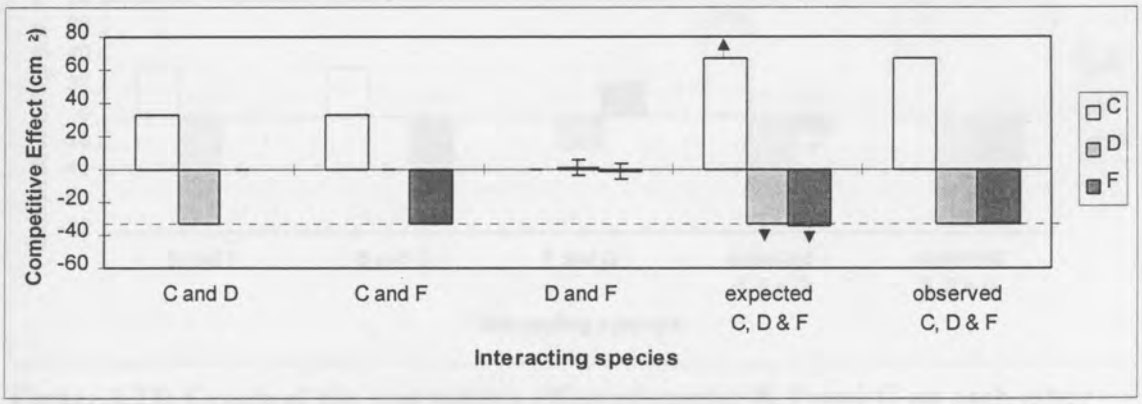

Figure 4.25: Graph of the competitive effect of species C, D and F on each other

Table 4.19: Table of results of tests for additivity between strains C, D and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and F on C	logical	-	-	additive
Effect of strains C and F on D	logical	-	-	additive
Effect of strains C and D on F	logical	-	-	additive

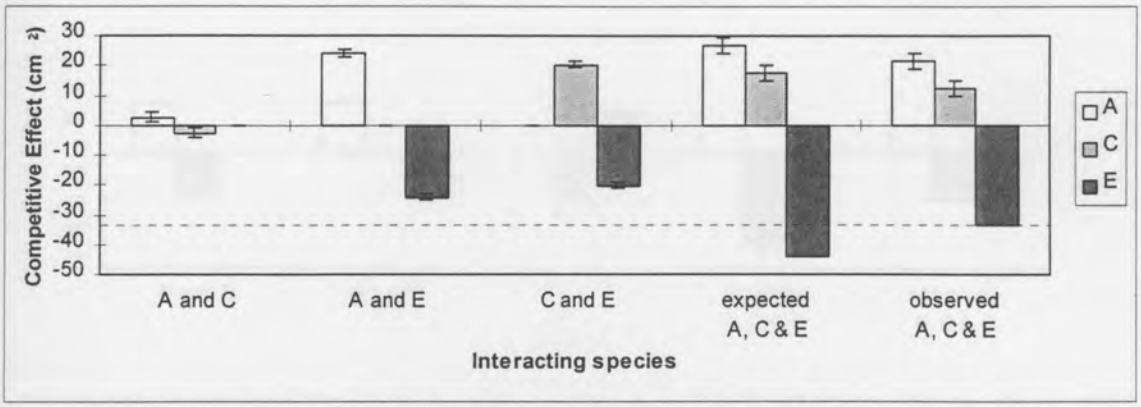

Figure 4.26: Graph of the competitive effect of species A, C and E on each other

Table 4.20: Table of results of tests for additivity between strains A, C and E.

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and E on A	statistical	2.21	0.275	additive
Effect of strains A and E on C	statistical	2.15	0.280	additive
Effect of strains A and C on E	logical	-	-	additive

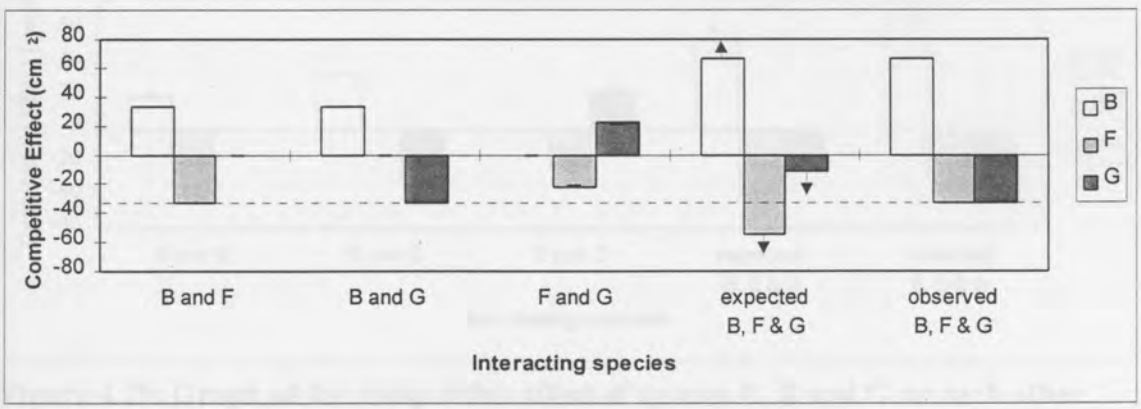

Figure 4.27: Graph of the competitive effect of species B, F and G on each other

Table 4.21: Table of results of tests for additivity between strains B, F and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains F and G on B	logical	-	-	additive
Effect of strains B and G on F	logical	-	-	additive
Effect of strains B and F on G	logical	-	-	additive

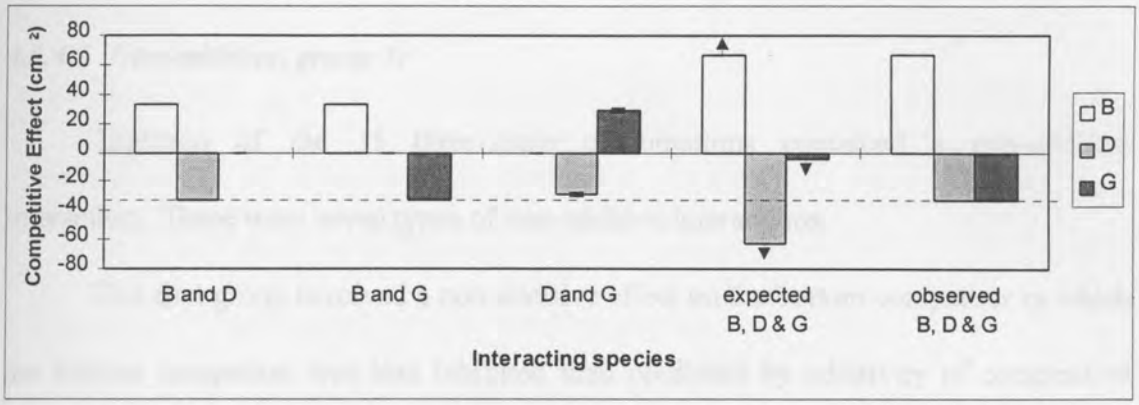

Figure 4.28: Graph of the competitive effect of species B, D and G on each other

Table 4.22: Table of results of tests for additivity between strains B, D and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and G on B	logical	-	-	additive
Effect of strains B and G on D	logical	-	-	additive
Effect of strains B and D on G	logical	-	-	additive

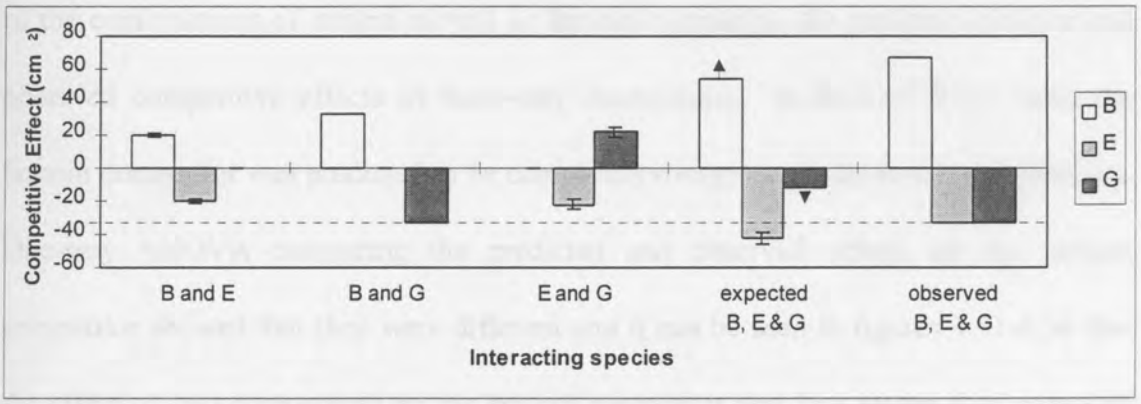

Figure 4.29: Graph of the competitive effect of species B, E and G on each other

Table 4.23: Table of results of tests for additivity between strains B, E and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and G on B	logical	-	-	additive
Effect of strains B and G on E	logical	-	-	additive
Effect of strains B and E on G	logical	-	-	additive

4.3.4.5 Non-additive, group 1:

Eighteen of the 35 three-strain combinations contained a non-additive interaction. There were seven types of non-additive interactions.

The first group involved a non-additive effect on the bottom competitor in which the bottom competitor was less inhibited than predicted by additivity of competitive effects from pairwise interactions. Four three-strain combinations fell into this category (EFG, ABE, AEG and AFG). The pairwise interactions within these three-strain combinations are summarised in figure 4.30. All interactions were transitive and there were no cases of complete overgrowth.

Figures 4.31-4.34 and tables 4.24-4.27 show the three-way interactions for each of the combinations of strains as well as the test comparing the expected additive and observed competitive effects of three-way interactions. In each of these cases the bottom competitor was predicted to be completely overgrown in three-way competition. One-way ANOVA comparing the predicted and observed effects on the bottom competitor showed that they were different and it can be seen in figures 4.31-4.34 that the effect of two competitors on the bottom competitor was less severe than complete overgrowth. These non-additive interactions are likely to be indirect effects of the top competitor on the bottom competitor via the middle competitor. For example, in figure 4.31, strain A had a direct negative effect on strain E, it also had a direct negative effect on strain B. It is possible that by A reducing the area of B in the presence of E, B had less effect on strain E thereby reducing the overall effect of strains A and B on E. The nature of some of the interactions changed. In combinations AFG and EFG, G displaced F in pairwise interactions when there was no space left on the plate, whereas

in three-way competition G did not displace F at all. In three-way interactions between strains A, B and E, A slightly displaced E as it grew, whereas in pairwise interactions A did not displace E until there was no space left.

In each of these cases the chance of the three-strains coexisting would have been increased by the presence of the non-additive effect.

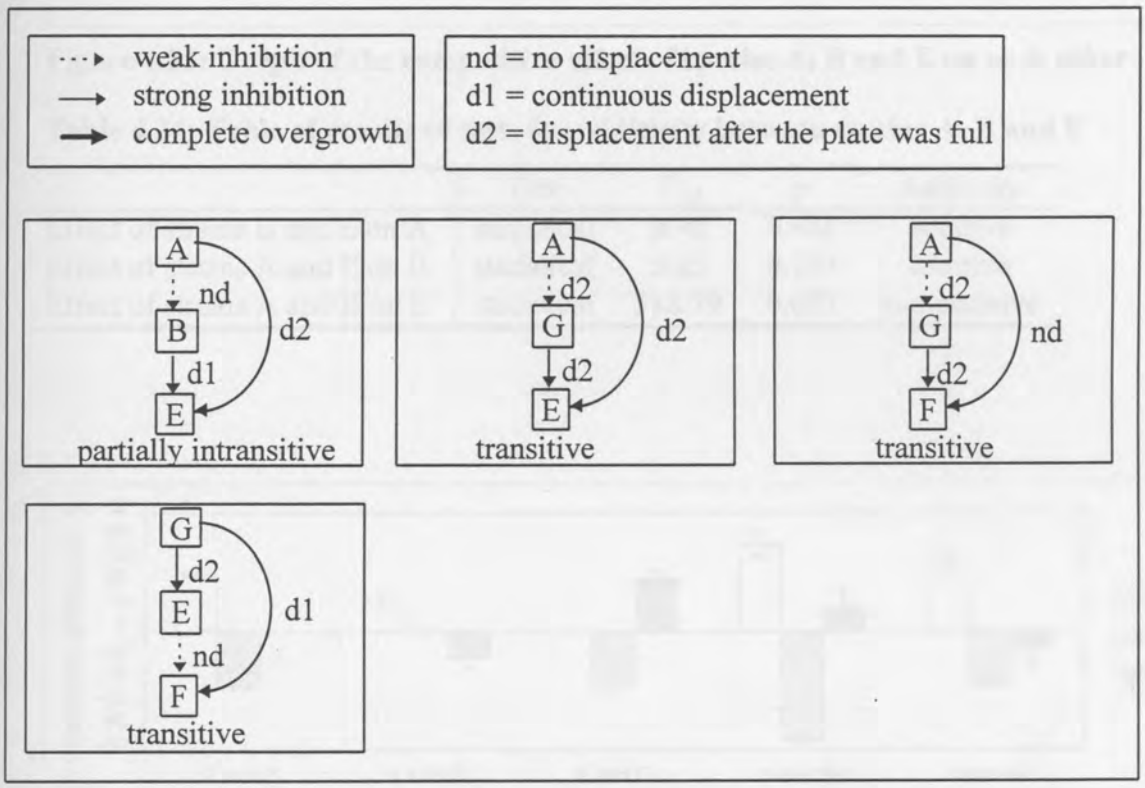

Figure 4.30: Diagrams summarising the pairwise interactions between three-strain combinations which contained non-additive interactions (group 1)

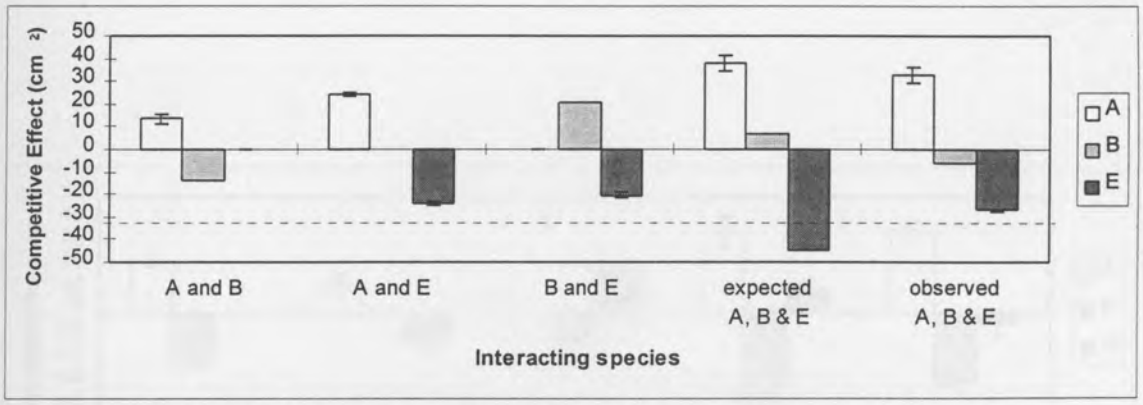

Figure 4.31: Graph of the competitive effect of species A, B and E on each other

Table 4.24: Table of results of tests for additivity between strains A, B and E

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains B and E on A	statistical	0.96	0.431	additive
Effect of strains A and E on B	statistical	5.05	0.154	additive
Effect of strains A and B on E	statistical	588.79	0.002	non-additive

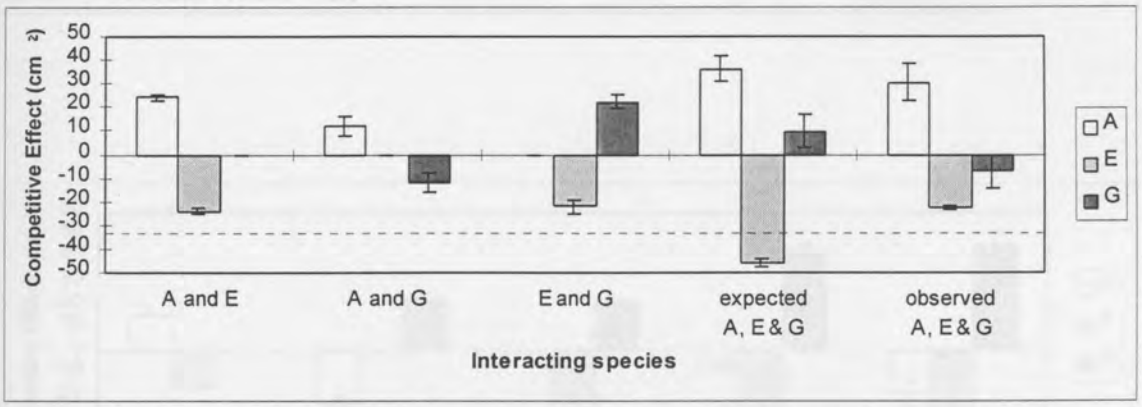

Figure 4.32: Graph of the competitive effect of species A, E and G on each other

Table 4.25: Table of results of tests for additivity between strains A, E and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and G on A	logical	-	-	additive
Effect of strains A and G on E	statistical	133.57	0.007	non-additive
Effect of strains A and E on G	statistical	3.06	0.223	additive

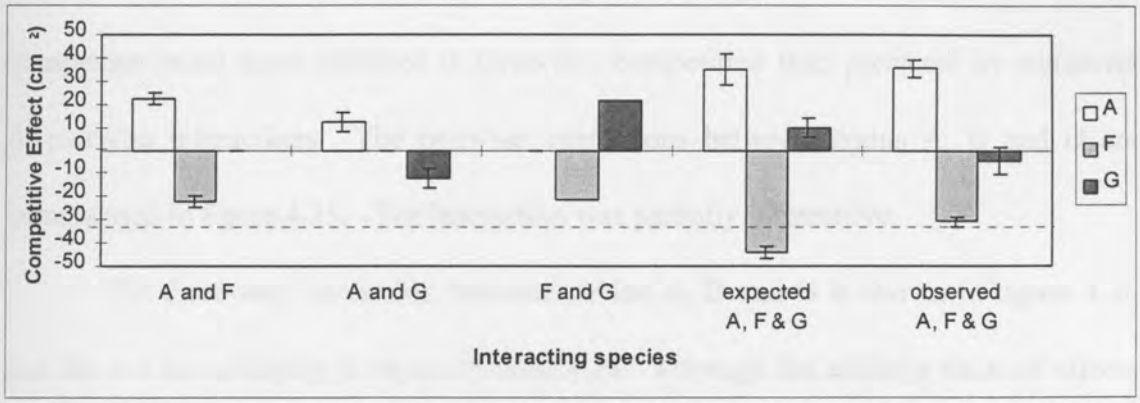

Figure 4.33: Graph of the competitive effect of species A, F and G on each other

Table 4.26: Table of results of tests for additivity between strains A, F and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains F and G on A	statistical	0.01	0.930	additive
Effect of strains A and G on F	statistical	19.41	0.048	non-additive
Effect of strains A and F on G	statistical	3.82	0.190	additive

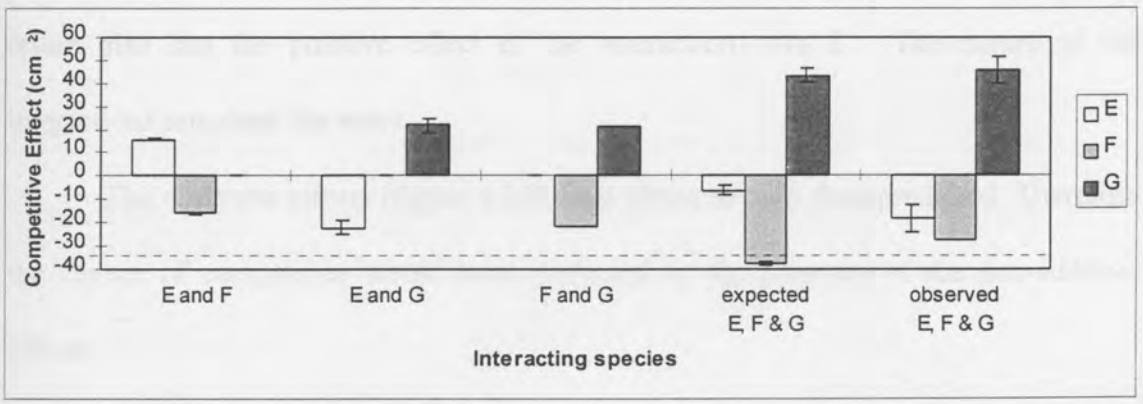

Figure 4.34: Graph of the competitive effect of species E, F and G on each other

Table 4.27: Table of results of tests for additivity between strains E, F and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains F and G on E	statistical	3.96	0.185	additive
Effect of strains E and G on F	statistical	161.60	0.006	non-additive
Effect of strains E and F on G	statistical	0.11	0.769	additive

4.3.4.6 Non-additive, group 2

Group 2 consisted of a three-strain combination (ABG) in which there were non-additive effects resulting in the bottom competitor being less inhibited and the middle competitor being more inhibited in three-way competition than predicted by additivity of pairwise interactions. The pairwise interactions between strains A, B and G are summarised in figure 4.35. The interaction was partially intransitive.

The three-way interaction between strains A, B and G is shown in figure 4.36 and the test for additivity is shown in table 4.28. Although the additive value of effects could not be calculated for B and G because B overgrew G in all pairwise combinations, the effect on strain B could be predicted to be +19.7 or more and the effect on strain G was predicted to be complete overgrowth. The observed effect on strain B was -6.5±5.0 and the effect on strain G was -13.3±4.6. The effects on strains B and G were therefore logically non-additive. The non-additive interactions were likely to be due to indirect effects whereby A reduced G, which meant that G had less effect on B and in return also lost the positive effect of the interaction with B. The nature of the interactions remained the same.

The observed effects (figure 4.36) were closer to zero than predicted. Therefore the chance of coexistence would have increased by the presence of the non-additive effects.

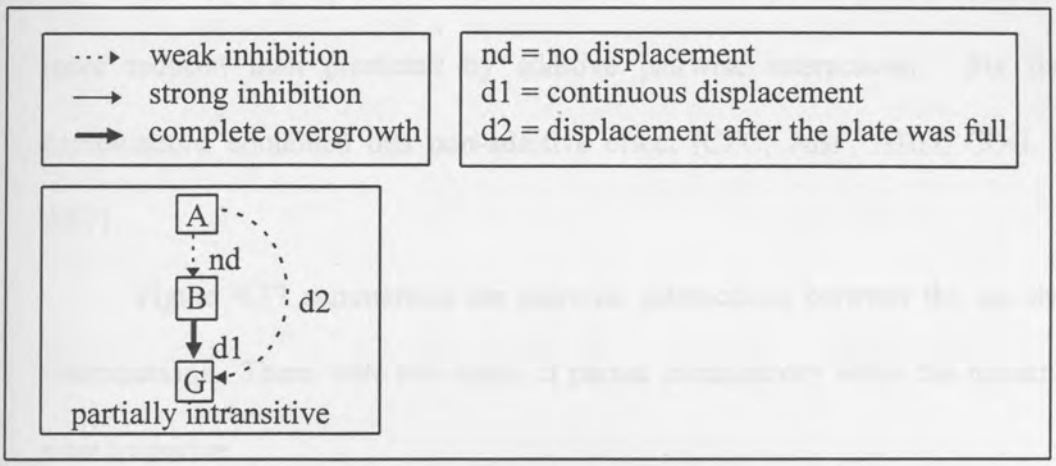

Figure 4.35: Diagram summarising the pairwise interactions between three-strain combinations which contained a non-additive interaction (group 2)

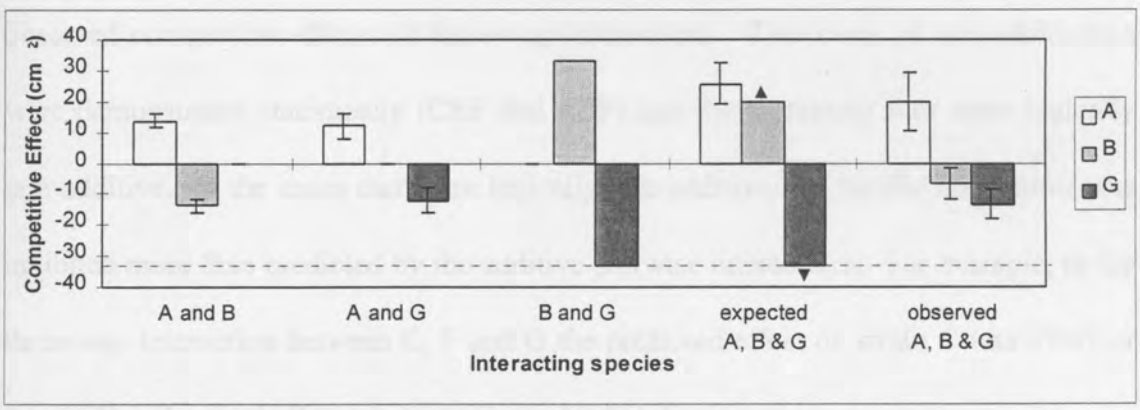

Figure 4.36: Graph of the competitive effect of species A, B and G on each other

Table 4.28: Table of results of tests for additivity between strains A, B and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains B and G on A	statistical	0.27	0.656	additive
Effect of strains A and G on B	logical	-	-	non-additive
Effect of strains A and B on G	logical	-	-	non-additive

4.3.4.7 non-additive, group 3

The third group of non-additive effects resulted in the middle competitor being more reduced than predicted by additive pairwise interactions. Six three-strain combinations contained this non-additive effect (CFG, ABF, ABD, CDG, CEF and AEF).

Figure 4.37 summarises the pairwise interactions between the six three-strain combinations. There were two cases of partial intransitivity while the remaining cases were transitive.

Figures 4.38-4.43 and tables 4.29-4.34 show the three-way interactions for each of the combinations of strains as well as the test comparing the expected additive and observed competitive effects of three-way interactions. Two cases of non-additivities were demonstrated statistically (CEF and AEF) and the remaining four were logically non-additive. In the cases that were logically non-additive, the middle competitor was inhibited more than predicted by the additive pairwise interactions. For example, in the three-way interaction between C, F and G the predicted effect on strain C was 29.67 or more. The observed effect on strain C was 12.9±1.4.

The non-additive effects in the six three-strain combinations could have been due to an indirect effect of the top competitor on the middle competitor via a change in the bottom competitor. For example, between C, F and G, it is possible that by G reducing the area of F, C did not gain the positive effect from F that it received in pairwise interactions, thereby reducing the overall positive effect on C.

The nature of the interactions remained the same except that in the combination CEF, C displaced F as it grew in three-way competition, whereas in pairwise interactions there was no displacement.

In four of the cases (CDG, CFG, ABD and ABF), the observed effects were closer to zero than the predicted effects and therefore the chance of coexistence would have increased. In the other two cases (CEF and AEF), the observed effects were more negative than the predicted (negative) effects, therefore the chance of coexistence would have decreased.

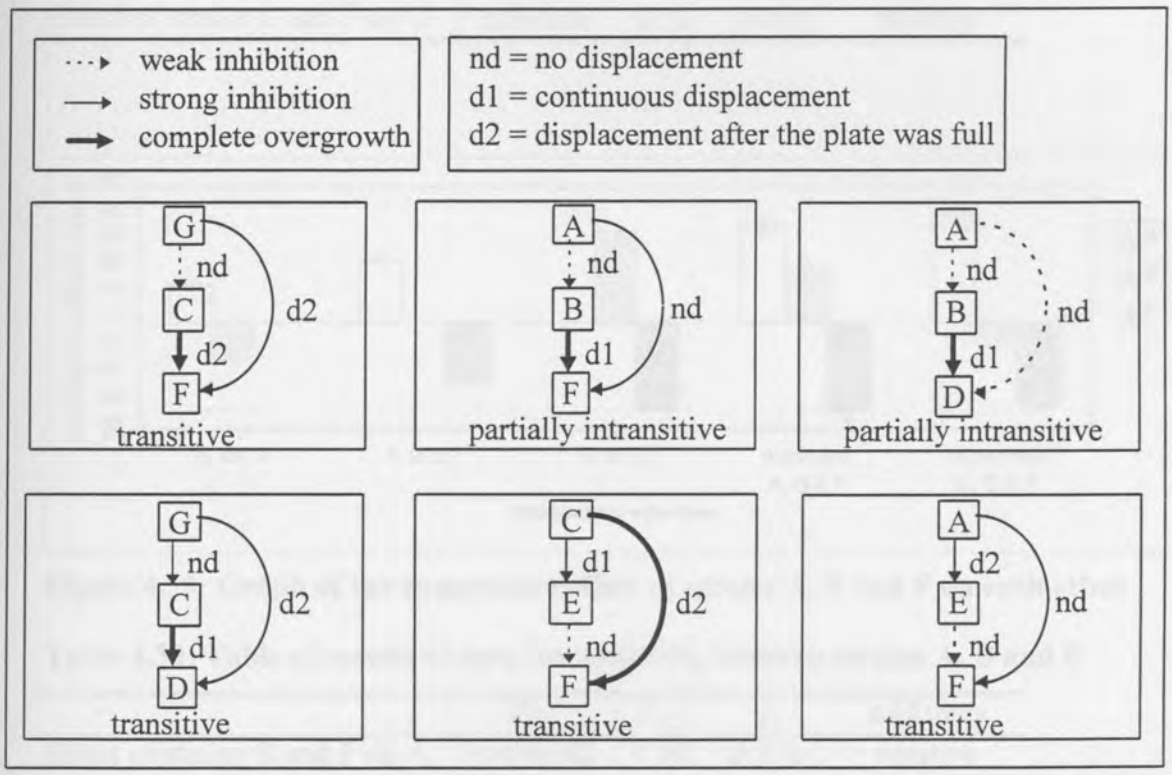

Figure 4.37: Diagrams summarising the pairwise interactions between three-strain combinations which contained non-additive interactions (group 3)

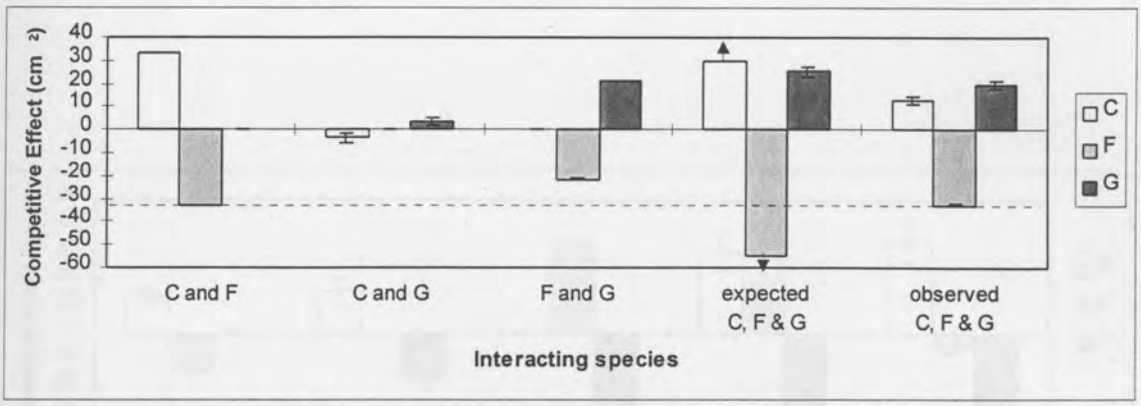

Figure 4.38: Graph of the competitive effect of species C, F and G on each other

Table 4.29: Table of results of tests for additivity between strains C, F and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains F and G on C	logical	-	-	non-additive
Effect of strains C and G on F	logical	-	-	additive
Effect of strains C and F on G	statistical	3.72	0.194	additive

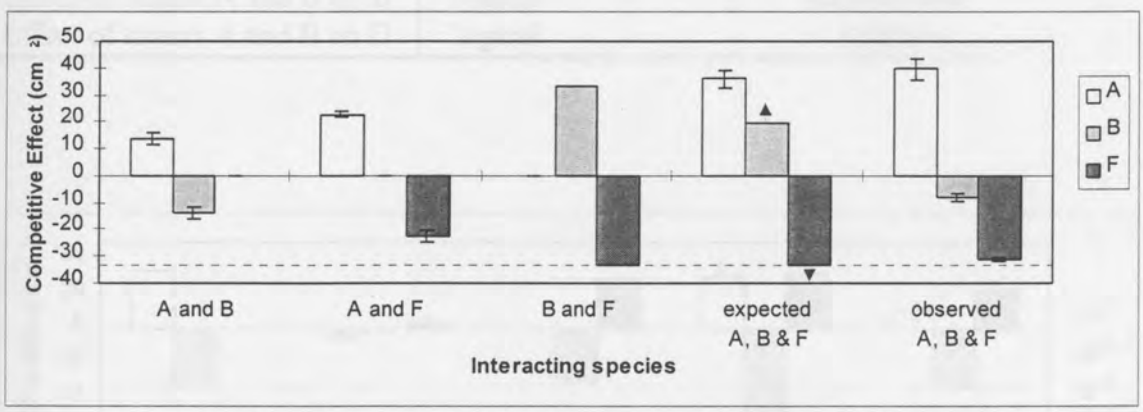

Figure 4.39: Graph of the competitive effect of species A, B and F on each other

Table 4.30: Table of results of tests for additivity between strains A, B and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains B and F on A	statistical	0.56	0.531	additive
Effect of strains A and F on B	logical	-	-	non-additive
Effect of strains B and F on A	logical	-	-	additive

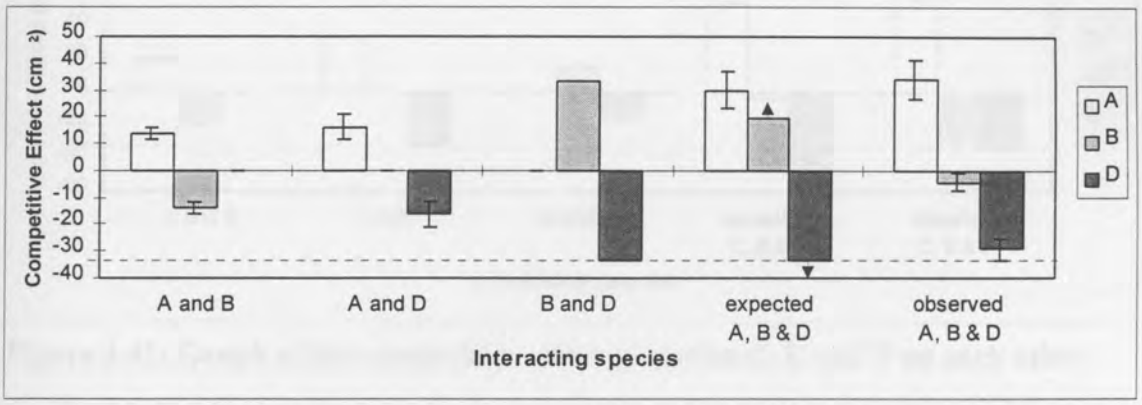

Figure 4.40: Graph of the competitive effect of species A, B and D on each other

Table 4.31: Table of results of tests for additivity between strains A, B and D

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains B and D on A	statistical	0.16	0.729	additive
Effect of strains A and D on B	logical	-	-	non-additive
Effect of strains A and B on D	logical	-	-	additive

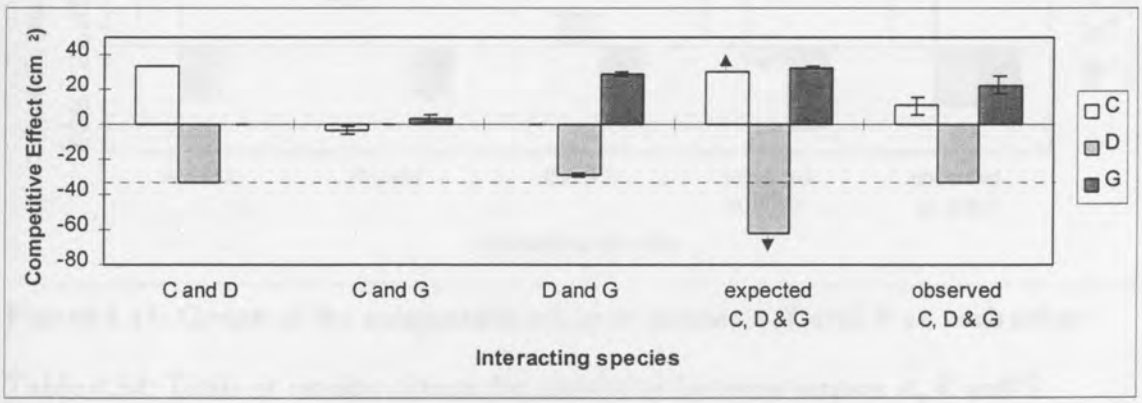

Figure 4.41: Graph of the competitive effect of species C, D and G on each other

Table 4.32: Table of results of tests for additivity between species C, D and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains D and G on C	logical	-	-	non-additive
Effect of strains C and G on D	logical	-	-	additive
Effect of strains C and D on G	statistical	4.32	0.173	additive

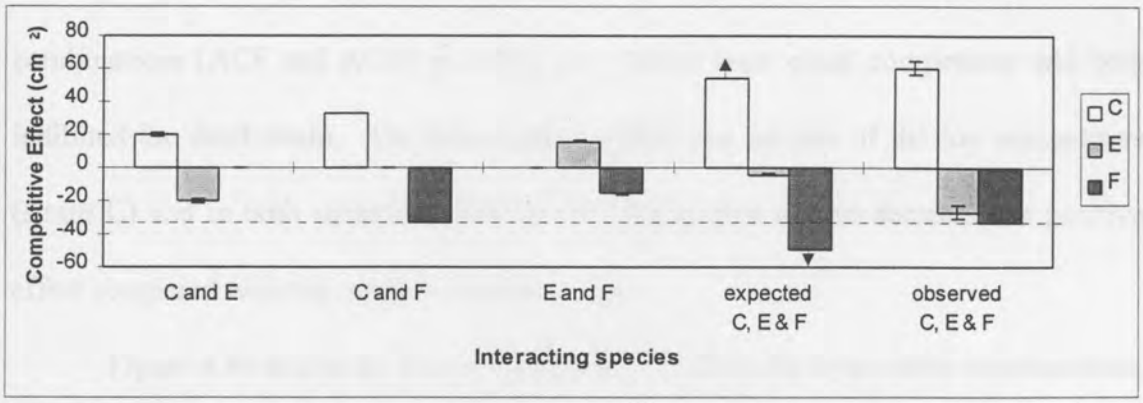

Figure 4.42: Graph of the competitive effect of species C, E and F on each other

Table 4.33: Table of results of tests for additivity between strains C, E and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and F on C	logical	-	-	additive
Effect of strains C and F on E	statistical	27.53	0.034	non-additive
Effect of strains C and E on F	logical	-	-	additive

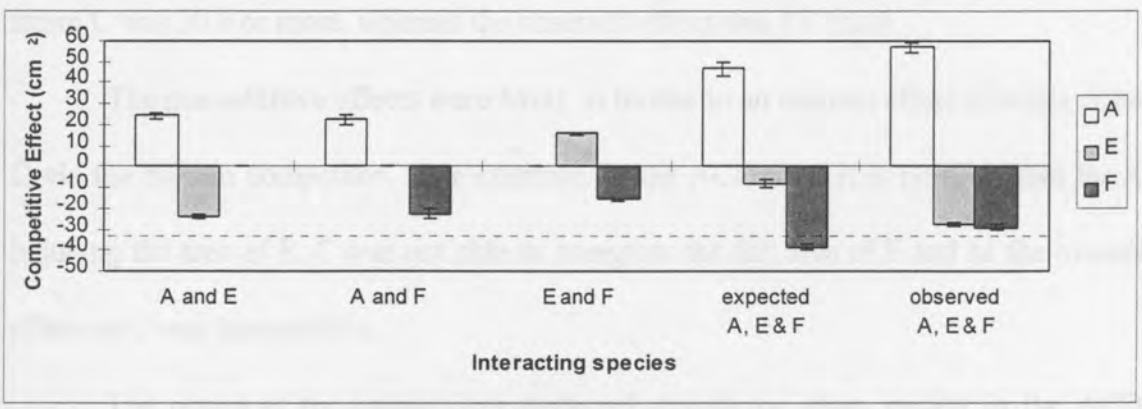

Figure 4.43: Graph of the competitive effect of species A, E and F on each other

Table 4.34: Table of results of tests for additivity between strains A, E and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and F on A	statistical	5.44	0.145	additive
Effect of strains A and F on E	statistical	77.08	0.013	non-additive
Effect of strains A and E on F	statistical	18.18	0.051	additive

4.3.4.8 Non-additive, group 4

The fourth group of non-additive interactions includes two three-strain combinations (ACF and ACD) in which two strains were equal competitors and both inhibited the third strain. The non-additive effect was on one of the top competitors (strain C) and in both cases the effect on the competitor was to decrease the positive effect compared with the predicted additive effect.

Figure 4.44 shows the pairwise interactions within the three-strain combinations. Both were partially intransitive.

Figures 4.45-4.46 and tables 4.35-4.36 show the three-way interactions for each of the combinations of strains as well as the test comparing the expected additive and observed competitive effects of three-way interactions. The effect on strain C in both cases was logically non-additive. For example, in the ACF case, the predicted effect on strain C was 30.6 or more, whereas the observed effect was 17.2±2.0.

The non-additive effects were likely to be due to an indirect effect of strain A on C via the bottom competitor. For example, in the ACF case, it is possible that by A reducing the area of F, C was not able to overgrow the full area of F and so the overall effect on C was less positive.

The nature of the interactions remained almost the same, except in the ACD case, in three-way competition, C displaced D only after there was no space left, whereas in pairwise interactions, C displaced D as it grew.

The chance of coexistence would have increased in both cases as the observed effect on C was closer to zero than predicted.

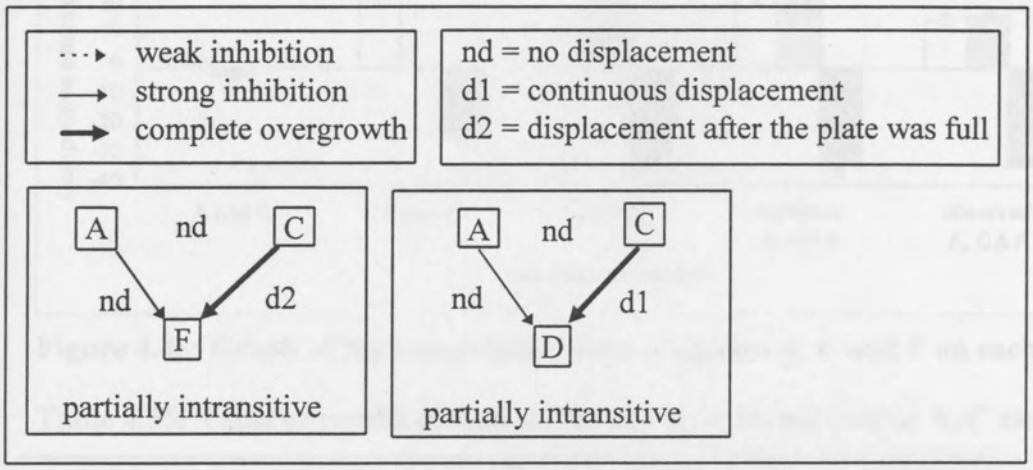

Figure 4.44: Diagrams summarising the pairwise interactions between three-strain combinations which contained non-additive interactions (group 4)

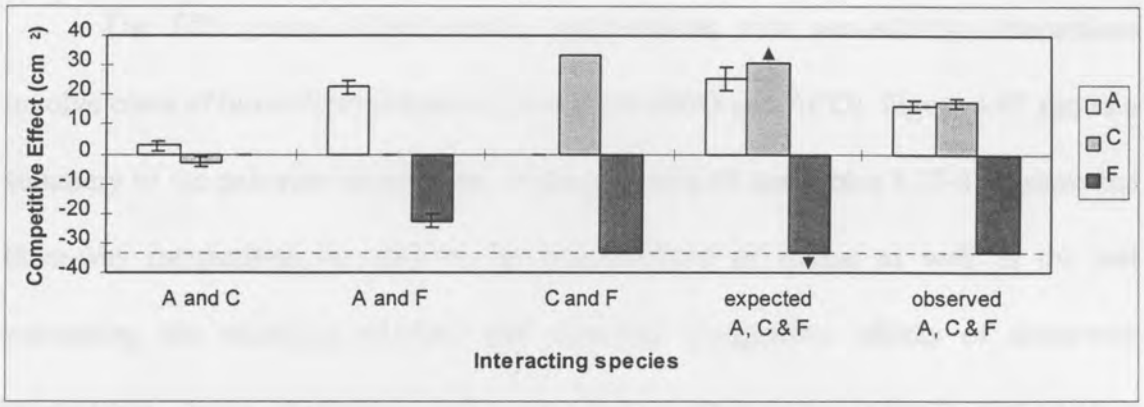

Figure 4.45: Graph of the competitive effect of species A, C and F on each other

Table 4.35: Table of results of tests for additivity between strains A, C and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and F on A	statistical	4.14	0.179	additive
Effect of strains A and F on C	logical	-	-	non-additive
Effect of strains A and C on F	logical	-	-	additive

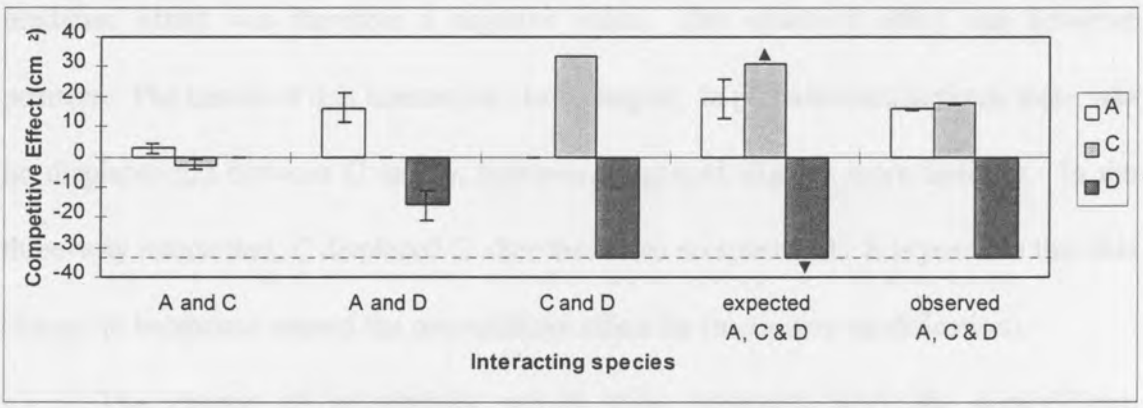

Figure 4.46: Graph of the competitive effect of species A, C and D on each other

Table 4.36: Table of results of tests for additivity between strains A, C and D

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and D on A	statistical	0.27	0.657	additive
Effect of strains A and D on C	logical	-	-	non-additive
Effect of strains A and C on D	logical	-	-	additive

4.3.4.9 Non-additive, group 5

The fifth group of three-strain combinations with non-additive interactions involve cases of intransitive pairwise interactions (BCG and ACG) Figure 4.47 shows a summary of the pairwise interactions. Figures 4.48-4.49 and tables 4.37-4.38 show the three-way interactions for each of the combinations of strains as well as the test comparing the expected additive and observed competitive effects of three-way interactions. In the combination BCG, the effect on strain G was logically non-additive because the predicted effect on G was -29.7 or more and the observed value was -16.1±4.1. The nature of the interactions did not change.

The non-additive effect (statistical) on C in the combination ACG is not easily explained. The direct effects on C were negative from G and neutral from A. The predicted effect was therefore a negative value. The observed effect was however positive. The nature of this interaction also changed. In pairwise interactions, there was no displacement between C and G, however, G gained slightly more territory. In the three-way interaction, C displaced G after there was no space left. It is possible that this change in behaviour caused the non-additive effect (ie interaction modification).

The chance of coexistence would have increased with the non-additive interaction in the BCG combination whereas in the ACG combination, the chance of coexistence would have remained the same because the predicted and observed effects were approximately the same distance from zero (-6.4±0.4 cf 7.0±2.8).

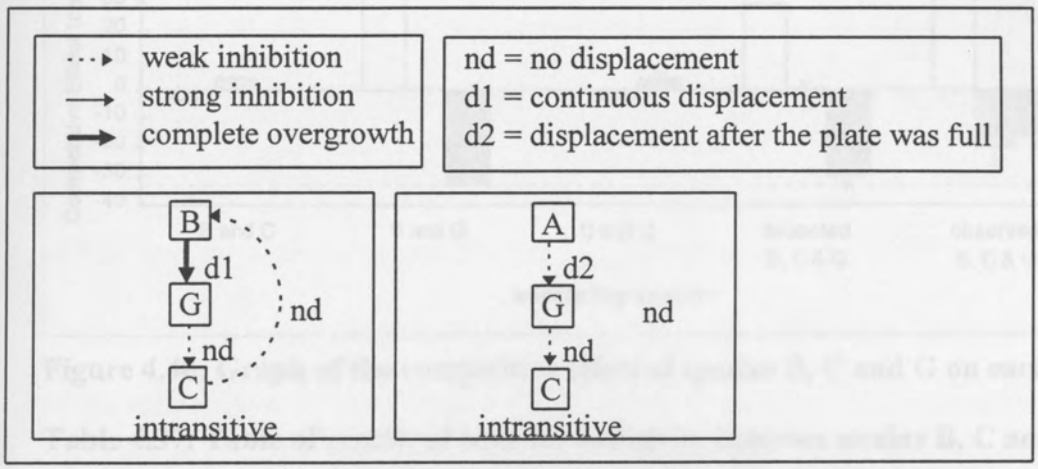

Figure 4.47: Diagrams summarising the pairwise interactions between three-strain combinations which contained non-additive interactions (group 5)

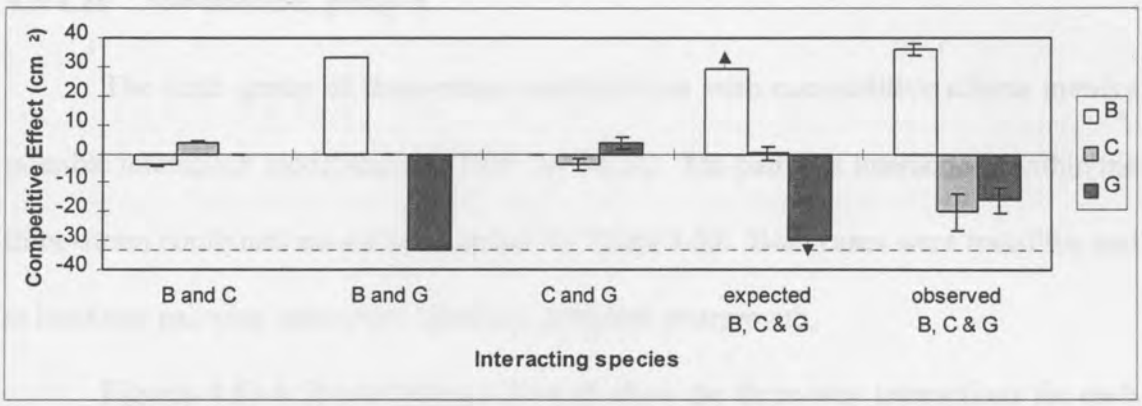

Figure 4.48: Graph of the competitive effect of species B, C and G on each other

Table 4.37: Table of results of tests for additivity between strains B, C and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and G on B	logical	-	-	additive
Effect of strains B and G on C	statistical	1.80	0.312	additive
Effect of strains B and C on G	logical	-	-	non-additive

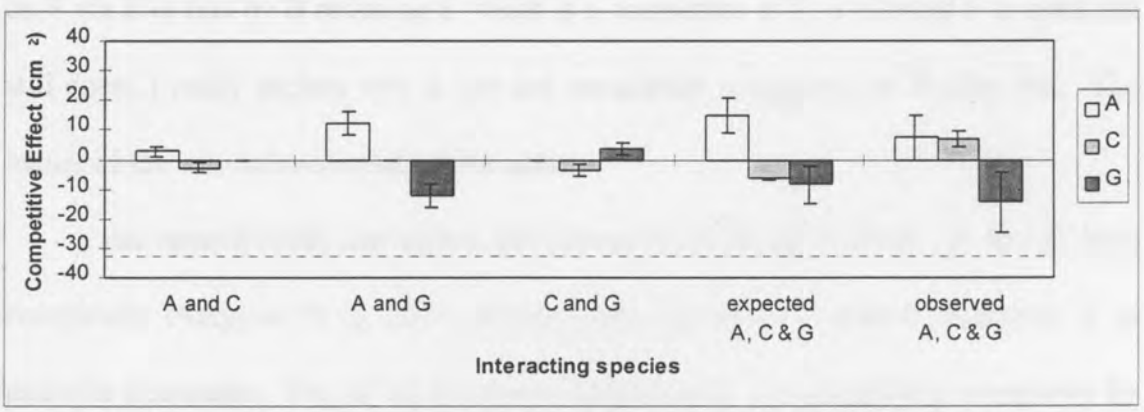

Figure 4.49: Graph of the competitive effect of species A, C and G on each other

Table 4.38: Table of results of tests for additivity between strains A, C and G

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and G on A	statistical	0.61	0.516	additive
Effect of strains A and G on C	statistical	22.46	0.042	non-additive
Effect of strains A and C on G	statistical	0.24	0.671	additive

4.3.4.10 Non-additive, group 6

The sixth group of three-strain combinations with non-additive effects involve possible interaction modifications (BEF and BCD). The pairwise interactions within the three-strain combinations are summarised in figure 4.50. Both cases were transitive and at least one pairwise interaction involved complete overgrowth.

Figures 4.51-4.52 and tables 4.39-4.40 show the three-way interactions for each of the combinations of strains as well as the test comparing the expected additive and observed competitive effects of three-way interactions. The effect on E in the BEF case was counter-intuitive. B did not completely overgrow E in the two-way interactions and the effect of F on B was positive. Conversely, F was completely overgrown in the pairwise interactions but not in the three-way. It is possible that B had an indirect effect on F via E in that by B reducing E which is a competitor of F, it allowed F to exist but still doesn't really explain why it was not completely overgrown by B after that. The nature of the interactions remained the same.

An unpredictable interaction also occurred in the BCD case. B and C both completely overgrew D in pairwise treatments, however C weakly inhibited B in pairwise treatments. Yet, in the three-way interaction C was completely overgrown by B. The presence of D has therefore indirectly reduced the area of C. This interaction is likely to be tied to the non-linear competitive interaction between B and C. In this case, B and C interacted as they did on the BC plates. The nature of the interactions remained the same except that B displaced C as in the BC pairwise interaction rather than no displacement as in the BCC interaction.

The chance of coexistence was decreased in the BCD case because the predicted result was that both B and C retained territory, whereas the observed result was that only B existed on the plate. In the BEF case, the chance of coexistence would have increased with the non-additive effect on F, but decreased with the non-additive effect on E.

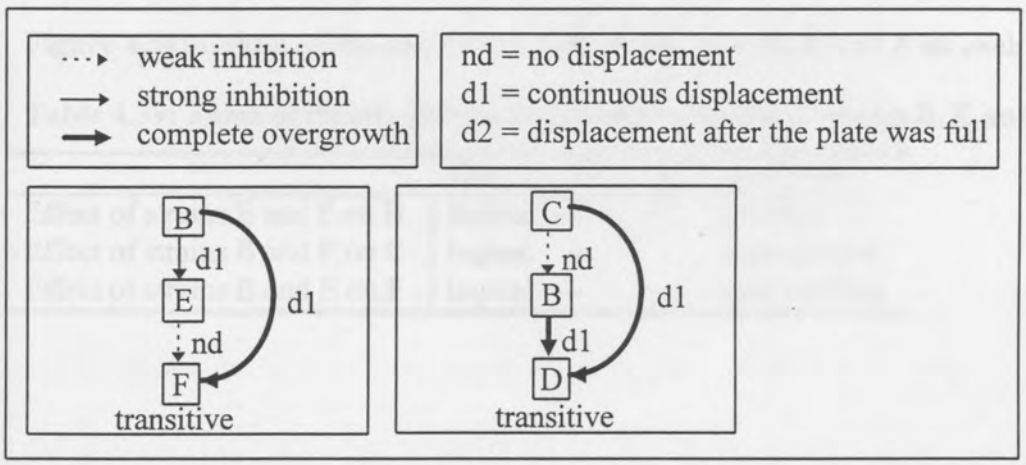

Figure 4.50: Diagrams summarising the pairwise interactions between three-strain combinations which contained non-additive interactions (group 6)

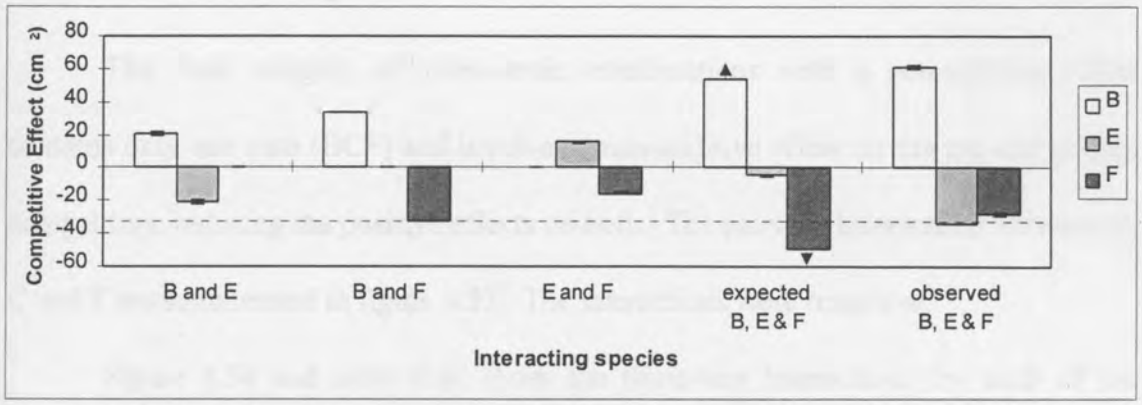

Figure 4.51: Graph of the competitive effect of species B, E and F on each other

Table 4.39: Table of results of tests for additivity between strains B, E and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains E and F on B	logical	-	-	additive
Effect of strains B and F on E	logical	-	-	non-additive
Effect of strains B and E on F	logical	-	-	non-additive

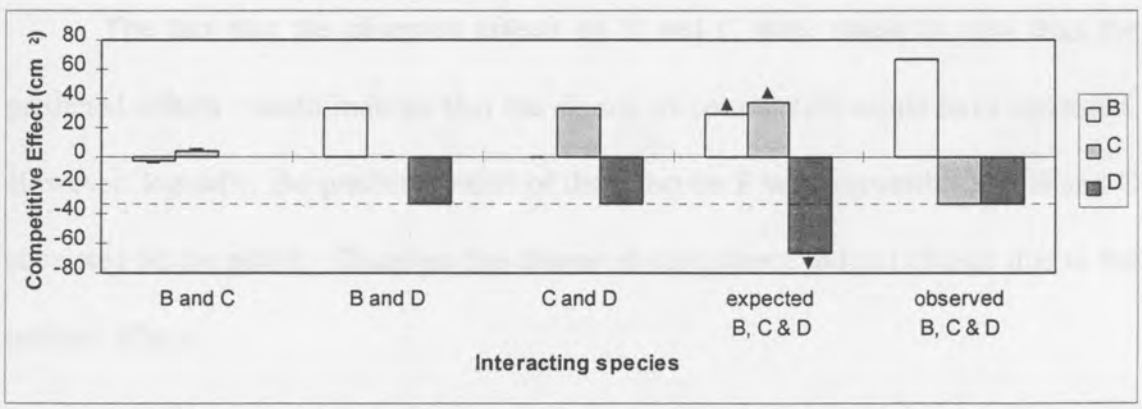

Figure 4.52: Graph of the competitive effect of species B, C and D on each other

Table 4.40: Table of results of tests for additivity between strains B, C and D

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and D on B	logical	-	-	additive
Effect of strains B and D on C	logical	-	-	non-additive
Effect of strains B and C on D	logical	-	-	additive

4.3.4.11 Non-additive, group 7

The final category of three-strain combinations with a non-additive effect contains only one case (BCF) and involves a non-additive effect on the top and middle competitors, reducing the positive effects on both. The pairwise interactions between B, C and F are summarised in figure 4.53. The interactions were transitive.

Figure 4.54 and table 4.41 show the three-way interactions for each of the combinations of strains as well as the test comparing the expected additive and observed competitive effects of three-way interactions. The observed effect on B and C were below the predicted values, and were therefore logically non-additive. It is likely that these non-additive effects were due to indirect effects of these two strains on each other via F. That is, by both of these strains reducing the area of F, each denied the other the full positive effect of F. The nature of the interactions remained the same.

The fact that the observed effects on B and C were closer to zero than the predicted effects would indicate that the chance of coexistence would have increased. However, logically, the predicted value of the effect on F was impossible and B and C coexisted on the plates. Therefore the chance of coexistence did not change due to the indirect effects.

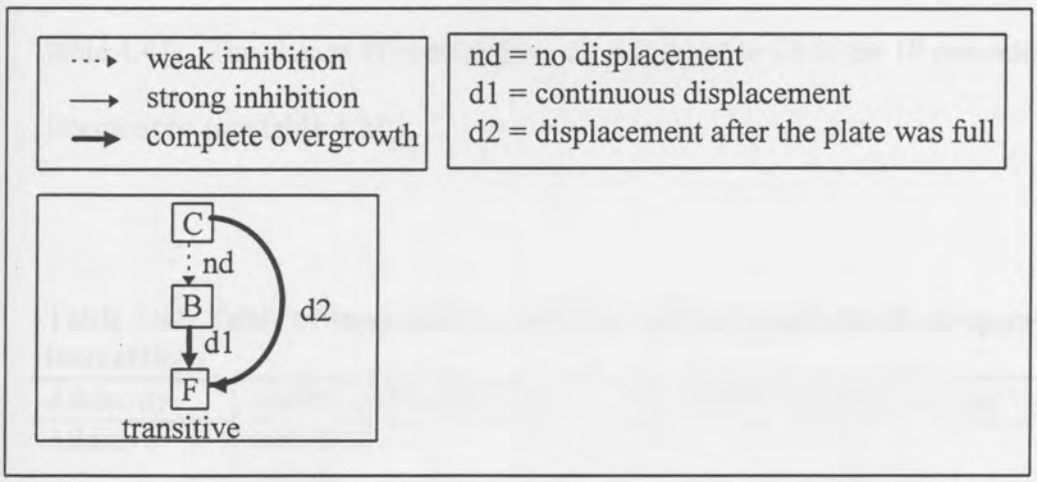

Figure 4.53: Diagrams summarising the pairwise interactions between three-strain combinations which contained non-additive interactions (group 7)

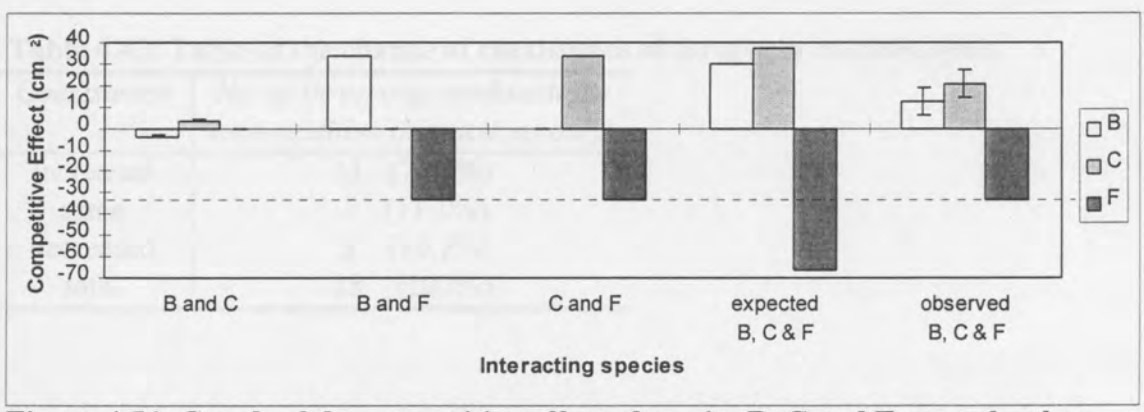

Figure 4.54: Graph of the competitive effect of species B, C and F on each other

Table 4.41: Table of results of tests for additivity between strains B, C and F

	Test	$F_{1,2}$	*p*	*Additivity*
Effect of strains C and F on B	logical	-	-	non-additive
Effect of strains B and F on C	logical	-	-	non-additive
Effect of strains B and C on F	logical	-	-	additive

4.3.4.12 Summary of three-way interactions:

Of the 35 three-way combinations, 18 contained non-additive competition (see table 4.42). The chance of coexistence was increased in 13 of the 18 non-additive interactions (see table 4.43).

Table 4.42: Table of frequencies of additive and non-additive three-species interactions

Additivity	*Method of identification*	*No. of three-species combinations*
Additive	statistical	9
	logical	8
total		17 (48.6%)
Non-additive	statistical	7
	logical	11
total		18 (51.4%)
Total		35

Table 4.43: Table of the chance of coexistence of three-way combinations

Coexistence	*No. of three-way combinations (non-additive interactions only)*
increased	13 (72.2%)
same	2 (11.1%)
decreased	3 (16.7%)
total	18 (100%)

4.4 Discussion:

4.4.1 Non-additive competition amongst WDBs

This study has demonstrated the occurrence of non-additive competitive effects between WDB's. Of the 35 three-species combinations, 18 included a non-additive competitive interaction. The number of non-additive interactions reported here are likely to be an undestimate because with only two replicates, the statistical power in the ANOVA tests was very low.

One of the hypotheses proposed for this study was that indirect effects could increase the chance of species coexisting. There is some evidence from this study to support this hypothesis. Of the 18 three-species combinations with non-additive interactions, 13 had an increased chance of coexistence. Although the magnitude of the indirect effects that enhance coexistence were not great, it must be remembered that this was only one step up in complexity from pairwise interactions. If we were to add a fourth species we would expect to see more indirect effects (Menge, 1995). By adding one more species to a pairwise system, approximately half of the interactions became non-additive. With each additional species, we would expect a rise in the probability of there being indirect effects or interaction modifications between the species (Menge, 1995).

It is interesting to note that in many of the three-species combinations which did not contain a non-additive interaction, there were a pair of species which had no effect on each other (relative to intra-specific competition). This finding could be predicted. If strain I has no direct effect on strain J, then strain I cannot have an indirect effect on strain K via strain J. An interaction modification could however still occur in this case.

4.4.2 Separating indirect effects from interaction modifications

With each reported case of non-additivity, a hypothesis was proposed as to the possible mechanism. In most cases, the simplest hypothesis was the occurrence of one indirect effect. However, in two cases there was no logical chain of interactions which could explain the observed effect. These were therefore proposed as interaction modifications. In order to test these hypotheses the three-species compintations now need to be studied in finer detail.

4.4.3 Pairwise interactions:

Pairwise interactions mostly involved asymmetric competition (where one strain gained more territory than the other). That the competitive abilities of these strains formed a hierarchy was expected based on previous studies (eg Owens *et al*, 1994; Pearce, 1990; Coates and Rayner, 1985c; Carruthers and Rayner, 1979; Boddy and Rayner, 1983; Rayner and Hedges, 1982; Thompson and Boddy, 1983; Boddy, Bardsley and Gibbon, 1987). However, I have not been able to find any previous studies reporting intransitive competitive loops or partial intransitivities in a fungal community. Within plant (Keddy and Shipley, 1989) and sessile marine communities (Rubin, 1982; Buss and Jackson, 1979; Turner and Todd, 1994; Russ, 1982; Quinn, 1982) the importance of intransitivities is debated. That intransitive loops occur in these communities is not doubted. However, the frequency and influence that they have is controversial. Karlson and Jackson, (1981) show that intransitivities can increase coexistence between competing species, whilst Keddy and Shipley (1989) argue that they are not that common and not that important in plant communities. This discovery

of two intransitive loops in a competition matrix of seven fungal strains raises questions about their frequency and importance in fungal communities. This is investigated further in chapter 6. The correlation between growth rate, competitive ability and displacement ability is discussed in chapter 6.

4.4.4 Size-dependent competition:

Most fungal competition studies have been *in vitro*, pairwise interactions where inocula of competing species are placed on the agar at the same time and at the same density. There is therefore not a lot of information on the consistency of competitive abilities at different inoculum densities. One exception to this was a study by Holmer and Stenlid (1993) who found that original inoculum size influenced the competitive ability of a strain in all four species tested. In contrast, in this study the only interaction which was size-dependent was the interaction between strains B and C. In the 1:1 and 2:1 inoculum ratios strain B displaced strain C. However, when there were two inocula of strain C and only one inoculum of strain B, there was no displacement.

4.4.5 Conclusion:

Studies which address the issue of complexity within communities have repeatedly found that indirect effects and interaction modifications are extremely important in community dynamics (Yodzis, 1988; Abrams *et al,* 1995; Menge, 1997). The finding that non-additive competitive effects occur between WDBs suggests that we need to take a whole-community perspective in future studies. That these non-additive effects could increase the diversity of WDB communities is of profound importance. It

is unknown to what extent these non-additive effects influence natural communities of WDBs, however, the present study indicates that it is an issue that is worth addressing.

5. Relative competitive ability of homokaryons and heterokaryons:

Summary:

The relative competitive abilities of mycelial homokaryons and heterokaryons of four species of wood decay fungi (*Peniophora* sp.1, *Peniophora* sp.2, *Pereniporia medulla-panis*, *Aleurodiscus lividocoeruleus*) were assessed. There was no simple relationship between nuclear status and competitive ability. The homokaryon of *Peniophora* sp.2 was competitively superior to it's heterokaryon, whereas the homokaryon of *Peniophora* sp.1 was inferior to it's heterokaryon. A hierarchy of competitive abilities of each strain revealed that *P. medulla-panis* homokaryon = *P. medulla-panis* heterokaryon > *Peniophora* sp.1 heterokaryon > *Peniophora* sp.2 homokaryon > *Peniophora* sp.2 heterokaryon > *A. lividocoeruleus* heterokaryon = *A. lividocoeruleus* homokaryon. This experiment showed that homokaryons as well as heterokaryons have the potential to influence community structure through competitive effects.

5.1 Introduction

It is a commonly held belief that homokaryons are inferior to heterokaryons of the same species (Simchen, 1966). However, there seems to be very little evidence to support this view because they have largely been neglected in ecological studies of fungi (Coates and Rayner, 1985a).

The elusive nature of homokaryons may have contributed to this belief. The fruiting bodies of heterokaryons are easily observed in the field and are often used to observe the distribution and abundance of WDB's in an area (eg Renvall 1995; Niemelä *et al*, 1995), whereas heterothallic homokaryons do not produce sexual fruiting structures (Alexopoulos *et al*, 1996). In addition, the mycelium of many heterokaryons have clamp connections (see sections 1.1) which make them easily distinguishable as basidiomycetes. Homokaryons do not have these structures.

Despite the relative difficulty in finding homokaryons, there have been several records of homokaryons in the field (Boddy and Rayner, 1984; Coates and Rayner, 1985a, 1985b, 1985c). In an extensive study by Coates and Rayner (1985a,b,c) homokaryons were found to be prevalent in early stages of decay development. The heterokaryons then gradually became more abundant as the homokaryons found compatible mates. The ability of unmated homokaryons to hold a territory against competing homokaryons and heterokaryons of other species has not been tested.

For the colonisation and maintenance of a territory, aspects such as degradative ability, hyphal extension and competitive ability are likely to be important. How then do homokaryons and heterokaryons differ in these aspects?

The relative decay capacity of homokaryons and heterokaryons varies between isolates and species. While heterokaryons of *Lenzites trabea* (Pers. ex Fries) usually have a greater decay capacity than their component homokaryons (Amburgey 1970), the reverse is true for *Serpula lacrimans* (Elliot *et al,* 1979). Similarly, studies of hyphal extension of homokaryons and heterokaryons have shown great variability even within the one species (eg *Schizophyllum commune*, Simchen 1966).

The relative competitive abilities of homokaryons and heterokaryons has not been assessed. As with competing heterokaryons, if homokaryons are able to withhold territory from a heterokaryon, then this will influence community structure. The purpose of this experiment was to observe the rate of growth and relative interspecific competitive abilities of homokaryotic and heterokaryotic mycelia of four wood decay basidiomycetes.

Four species of WDBs had been successfully crossed to form heterokaryons (see chapter 3) These were therefore used in this experiment. The species were *Peniophora* sp1 (KQ14B2), *Peniophora* sp2 (KQ14B4), *Aleurodiscus lividocoeruleus* (CQ16B12), and *Pereniporia medulla-panis* (KS1).

A single heterokaryon and one of its' parent homokaryons of each species was used in this experiment. When a heterokaryon and one of it's parent homokaryons are paired on a plate, they can fuse (Boddy and Rayner, 1982; Coates, Rayner and Boddy, 1985). Therefore the intraspecific competitive abilities of homokaryons and heterokaryons could not be recorded. However, by observing the competitive abilities of the homokaryons and heterokaryons when paired against other strains, the relative interspecies competitive abilities of homokaryons and heterokaryons of the same species could be assessed.

5.2 Methods:

5.2.1 Experimental design and inoculation:

For brevity the species used in this experiment (*Peniophora* sp1, *Peniophora* sp2, *Pereniporia medulla-panis*, *Aleurodiscus lividocoeruleus*), will be referred to as A, B, C and D, respectively. Homokaryotic strains will be referred to as A-hom, B-hom, C-hom and D-hom, while heterokaryotic strains will be referred to as A-het, B-het, C-het and D-het. One of the parent homokaryons used to synthesise the heterokaryon was randomly chosen to be used as a representative of a homokaryon of that species in this experiment. Homokaryons and heterokaryons of A, B, C and D were inoculated onto agar plates in pairs, 1cm apart in the centre of the plate. Control plates were inoculated twice with the same strain. Three replicates of each pairwise interaction were inoculated and the experiment was repeated (two blocks). Plates were incubated at room temperature (21°C) for eight weeks (two weeks after there was no further change in the interactions). The area covered by each strain on each plate was measured on days 4, 5, 6, 7, 14, 21, 28, 35, 42, 49 and 56 after inoculation.

5.2.2 Media

This experiment was performed on MEA plates which were allowed to set and air dry in a lamina flow for one day to reduce the excess moisture from the plates. This was found to successfully reduce the incidence of fungal contamination. The plates were then kept in sealed polythene bags (10 plates per bag) to stop additional moisture loss. See appendix B for details of MEA.

5.2.3 Measurement of area:

The outline of each colony was drawn onto a plastic sheet and this was scanned into a computer using a video camera. The area and perimeter of each colony was measured using an image analysis program (Optimus 5.1).

5.2.4 Checking the accuracy of area measurements:

At the end of the eight weeks, the final area covered by each strain was recorded. To ensure that the area recorded was accurate, 5x5 mm blocks of agar and mycelium were taken from areas on each plate where there could be some uncertainty. On all plates, blocks were cut from either side of an interaction zone to check for hyphae that may be growing under the interaction zone. In some cases, where a distinct barrage was not observed, many core samples were taken to ascertain where each strain grew. A wet mount was prepared of each block and examined under a light microscope at 400x. Samples taken from the plates were compared to a set of reference slides of each of the cultures.

5.2.5 Growth rate:

Single inocula were also plated to determine the growth rate of each strain. The radius of the mycelium was recorded every day until the colony had reached the edge of the plate. Only the radius at day seven was analysed as this was the last day before the first of the strains reached the edge of the plate. Radius was measured by taking two perpendicular measurements of diameter, averaging these measurements, then halving the value.

5.2.6 Data Analysis:

5.2.6.1 Growth rate:

The analysis of the growth rate data required a mixed model (model III) three-way ANOVA, with radius of the colony after seven days as the dependent variable, species and replicate experiments as independent, random factors and nuclear status as an independent fixed factor.

5.2.6.2 Relative competitive ability:

The competitive ability data could not be analysed in the same way as the growth rate because when complete overgrowth of one strain consistently occurred, the values for final area were all zero for that strain, which resulted in zero variance between replicates. The replicates within each replicate experiment were pooled (arithmetic mean) and two-way model III ANOVA were performed with final area (dependent variable) by competitor strain (random, independent factor) by nuclear status (fixed, independent factor). There were no cases where both blocks (replicate experiments) contained all zero values for all replicates, so when the mean of the replicates was used (mean of each block), there was some variance within these cells. Homogeneity of variance tests showed that the data were homoscedastic.

5.2.6.3 Interpretation of ANOVA:

In the interpretation of the three-way ANOVA table the three-way interaction was read first. If this was not significant ($p>0.05$) then the two-way interactions were

read. If these were not significant then the independent factors were studied. The factors which accounted for the most variation (highest mean square value) in the analysis were considered most important.

Likewise, the two-way ANOVA tables were interpreted from the interaction through to the independent factors.

5.2.6.4 Competitive hierarchy:

The strains were given a score for their competitive ability scoring +1 for each strain that it was able to suppress and -1 for each strain that it was suppressed by (significant effect in model II two-way ANOVA, $P<0.05$). The sum of these scores gave an overall value of the competitive ability of the strain. The two-way ANOVA compared the area covered by a strain when it was paired with itself and when it was paired with another strain, with replicate experiment and competitor strain (itself or one other) as the independent random factors and area covered as the dependent variable. In some cases the data were heteroscedastic and no transformations were able to rectify this. As there are no two-way non-parametric tests, Mann-Whitney U-tests were used, pooling the data from replicate experiments. To compensate for increased overall type I error due to conducting many individual tests, the rejection region was reduced to 1% in each test.

5.2.6.5 Interaction descriptions:

Because the area covered by each strain in each pairwise treatment was recorded regularly (see section 5.2.1), the dynamics of each interaction could be observed in

addition to the final area. The ability of one strain to displace another was recorded and each strain was given a score for its' displacement ability. For each other strain that a strain could displace it was given a score of 1 and for each strain that it was displaced by it was given a score of -1. The total gave the displacement ability of a strain.

5.2.6.6 Competitive ability, displacement ability and growth rate correlations

The strains were ranked according to their competitive abilities (1=highest, 8=lowest). The ranks were then plotted against the radius of a strain at day five when in monoculture. Spearman correlation was used to test the relationship between them. The strains were also ranked according to displacement ability using interaction descriptions and Spearman correlation was used to test the relationship between displacement ability and the radius of a strain at day five in monoculture.

5.3 Results:

5.3.1 Growth rate:

For the growth rate (radius of a colony after seven days of growth) there was an interaction between nuclear status and species (p=0.002)(see table 5.1). The direction of the difference between the growth rates of homokaryon and heterokaryon depended on the individual species. This can be seen in figure 5.1 where the hyphal extension of A and C heterokaryons was greater than that of the respective homokaryons, whereas the B homokaryon was faster growing than it's heterokaryon and D was equal. There was no difference between the replicate experiments.

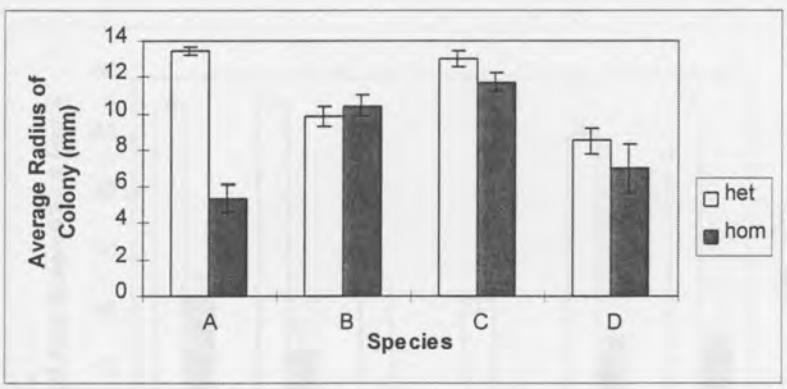

Figure 5.1: Graph of the average radius of homokaryotic and heterokaryotic mycelia of each species after seven days of growth on MEA. Error bars=±1SEM.

Table 5.1: Source of variation in radius (mm) at day seven due to nuclear status, replicate experiments and species.

Sources of variation	*df*	*MS*	*F*	*p*
within + residual	176	48.48		
Species	3	197.31	4.07	0.008
Nuclear status	1	307.55	6.34	0.013
Replicate experiments	1	4.08	0.08	0.772
Species x replicate experiments	3	21.65	0.45	0.720
Species x nuclear status	3	251.74	5.19	0.002
Nuclear status x replicate experiment	1	44.08	0.91	0.342
Species x nuclear status x replicate experiment	3	10.88	0.22	0.879

5.3.2 Competitive ability:

5.3.2.1 Species A:

For species A the heterokaryon consistently had a greater final area than the homokaryon in competition with the six other strains (see figure 5.2). However, the difference between the area covered by the homokaryon and heterokaryon of A varied with the competing strain (p=0.033)(see table 5.2). For example, the homokaryon of A was almost always completely overgrown by C heterokaryon, but fared better against C homokaryon, whereas A heterokaryon fared slightly worse against C homokaryon than C heterokaryon. The most variation in area (highest MS) was accounted for by nuclear status of species A.

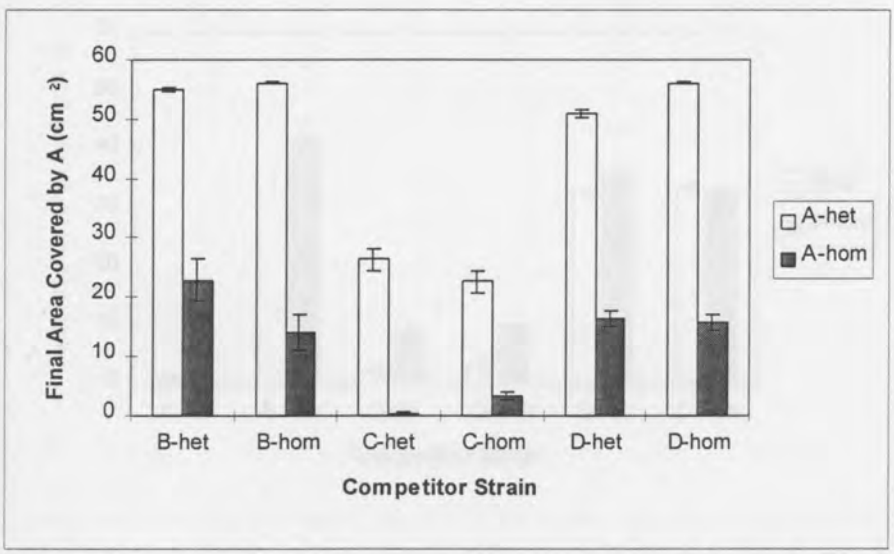

Figure 5.2: Graph of the area (cm^2) covered by A homokaryon and heterokaryon in competition. Error bars=±1SEM.

Table 5.2: Source of variation of final area covered by A due to nuclear status, competing strain and their interaction

Source of variation	*df*	*MS*	*F*	*p*
within + residual	12	19.87		
Nuclear status	1	6192.09	311.57	0.000
Competitor strain	5	554.15	27.88	0.000
Nuclear status x competitor strain	5	70.84	3.56	0.033

5.3.2.2 Species B:

The final area of B was influenced by competitor strain (p=0.000) and perhaps by nuclear status (p=0.047) (although nuclear status was only marginally significant) (see table 5.3). There was no interaction between these two factors (p=0.580). The competitor strain caused the greatest variance (highest MS). The homokaryotic strain held a greater area than the heterokaryon against most competitor strains, although the probability value shows that this is marginally significant (p=0.047). This is probably due to the area covered with D-hom and A-het. It is clear that the homokaryon had a greater final area than the heterokaryon with A-hom, C-het, C-hom and D-het (see figure 5.3).

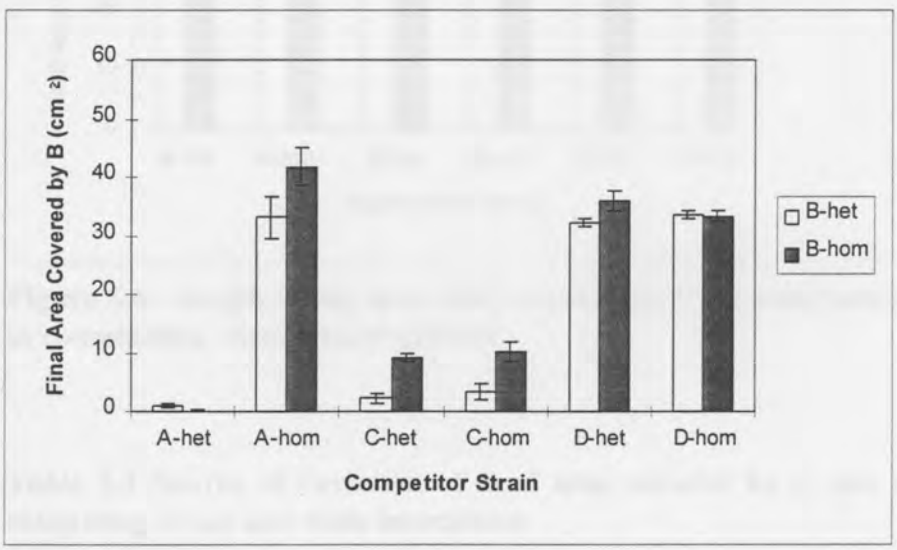

Figure 5.3: Graph of the area (cm^2) covered by B homokaryon and heterokaryon in competition. Error bars=±1SEM

Table 5.3: Source of variation of final area covered by B due to nuclear status, competing strain and their interaction

Source of variation	*df*	*MS*	*F*	*p*
within + residual	12	21.35		
Nuclear status	1	104.79	4.91	0.047
Competitor strain	5	1153.88	54.06	0.000
Nuclear status x Competitor strain	5	16.75	0.78	0.580

5.3.2.3 Species C:

The only strong influence on the final area of C was the competitor strain ($p=0.000$) (see table 5.4). This can be seen in figure 5.4 where A-het reduced the area considerably whereas A-hom, B-het, D-het and D-hom were unable to reduce the area of C. B-hom was able to reduce the area of C slightly. The homokaryon and heterokaryon of species C had the same competitive ability.

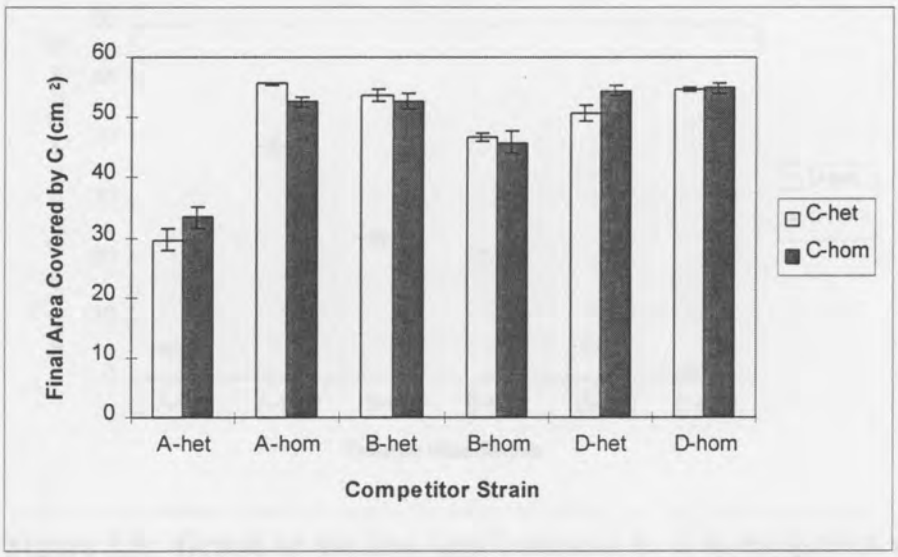

Figure 5.4: Graph of the area (cm^2) covered by C homokaryon and heterokaryon in competition. Error bars=±1SEM.

Table 5.4 Source of variation of final area covered by C due to nuclear status, competing strain and their interaction

Source of variation	*df*	*MS*	*F*	*p*
within + residual	12	2.69		
Nuclear status	1	1.35	0.50	0.492
Competitor strain	5	320.95	119.26	0.000
Nuclear status x Competitor strain	5	6.89	2.56	0.085

5.3.2.4 Species D:

The competitor strain strongly altered the final area covered by species D (p=0.000) (see table 5.5). Figure 5.5 shows that the area of both strains of D were strongly reduced when paired with A-het, C-het and C-hom, whereas D was able to hold nearly half the plate when paired against A-hom, B-het and B-hom. The homokaryon and heterokaryon of species D had the same competitive ability.

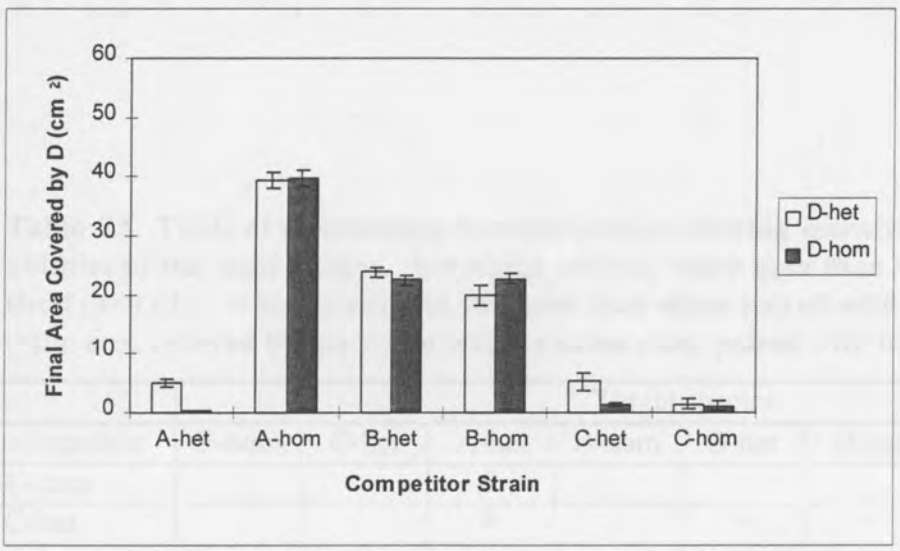

Figure 5.5: Graph of the area (cm^2) covered by D homokaryon and heterokaryon in competition. Error bars=±1SEM.

Table 5.5: Source of variation of final area covered by D due to nuclear status, competing strain and their interaction

Source of variation	*df*	*MS*	*F*	*p*
within + residual	12	3.48		
Nuclear status	1	9.19	2.64	0.13
Competitor strain	5	950.29	273.29	0.000
Nuclear status x competitor strain	5	7.99	2.30	0.110

5.3.3 Hierarchy of competitive abilities:

The eight strains were placed into a hierarchy of competitive ability (see table 5.6) based on the number of strains which a strain replaced and was replaced by. The main point to see here is that one of the highest competitor was a homokaryon (C-hom). Figures 5.6 and 5.7 show the interactions between C-hom/B-het and C-hom/B-hom, respectively.

Table 5.6: Table of interactions between strains showing hierarchy of competitive abilities of the eight strains. + = strain covered more area than when paired with itself ($p<0.01$). - = strain covered less area than when paired with itself ($p<0.01$). 0 = the area covered by the strain was the same when paired with itself ($p>0.01$).

	Target species							
competitor	C-hom	C-het	A-het	B-hom	B-het	D-het	D-hom	A-hom
C-hom			0	-	-	-	-	-
C-het			0	-	-	-	-	-
A-het	+	0		-	-	-	-	
B-hom	+	+	+			-	-	-
B-het	+	+	+			-	-	0
D-het	+	+	+	+	+			-
D-hom	+	+	+	+	+			-
A-hom	+	+		+	0	+	+	
Effect	5	5	4	3	2	1	1	0
Response	0	0	0	-3	-3	-5	-5	-5
Score	5	5	4	0	-1	-4	-4	-5

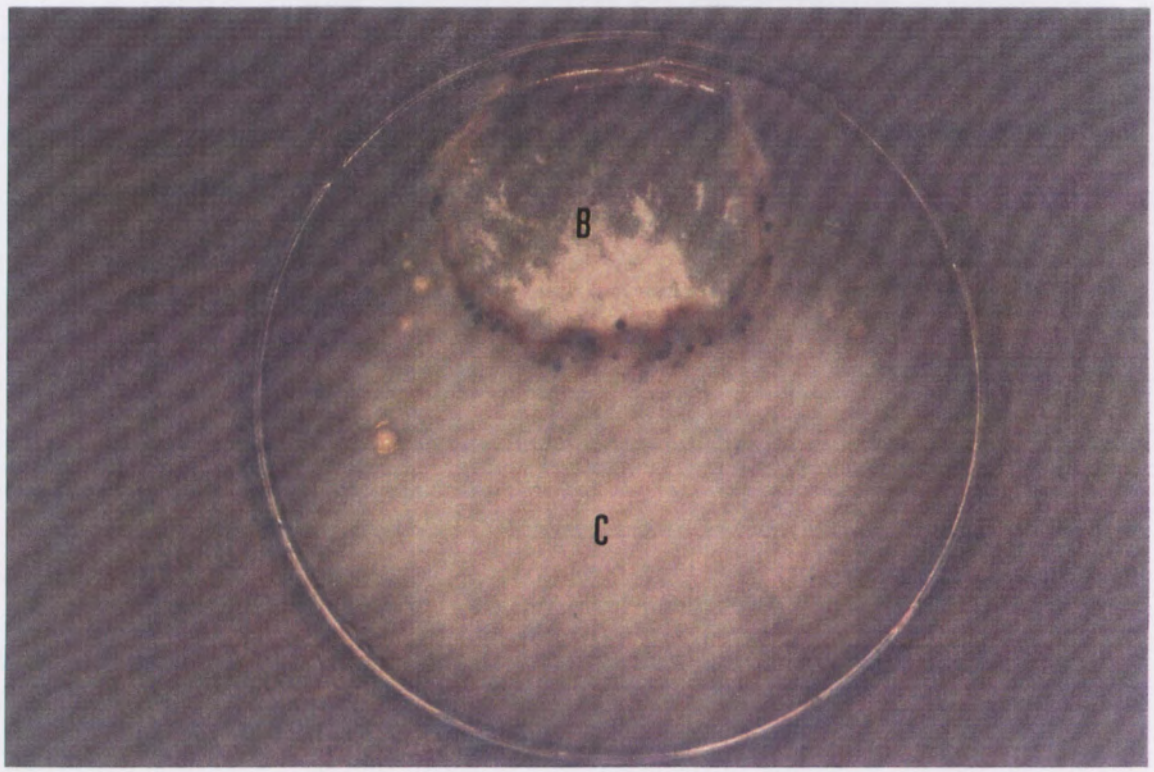

Figure 5.6: Photograph of interaction between C-hom and B-het

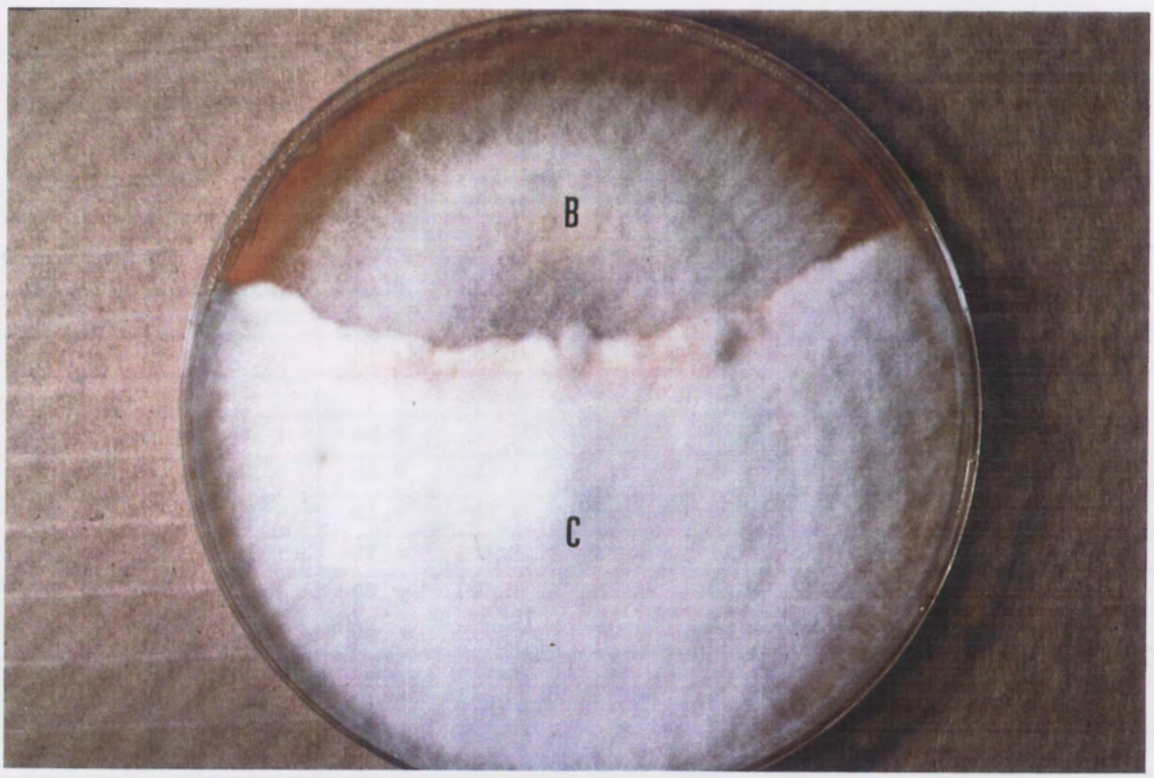

Figure 5.7: Photograph of interaction between C-hom and B-hom

5.3.4 Interaction descriptions:

Interspecies interactions could be placed into three categories:

(nd) there was no displacement between the strains. If one of the strains grew faster than the other, then it gained more territory than the other strain.

(d1) One strain grew faster than the other and the faster growing strain displaced the other while still growing across the plate.

(d2) Initially there was no displacement, but after the two colonies had filled the plate, one strain displaced the other.

Table 5.7 shows the type of interaction between each pair of strains. Categories d1 and d2 involve displacement. The displacement ability of each strains is shown. There were no consistent patterns in interaction types with regard to homokaryons and heterokaryons.

Table 5.7: Table of interaction types between each pair of strains and their displacement abilities. +, - and 0 signs are the same as in table 5.6, showing that the strain covered more, less or the same area respectively as when it was paired with itself ($p<0.05$). Numbers 1, 2 and 3 indicate the type of interaction between the pair of strains as described in section 5.3.4.

	Target species							
competitor	C-hom	C-het	A-het	B-hom	B-het	D-het	D-hom	A-hom
C-hom			nd 0	d2 -	d2 -	d1 -	d1 -	d2 -
C-het			nd 0	d2 -	d2 -	d1 -	d1 -	d2 -
A-het	nd +	nd 0		d1 -	d1 -	d1 -	d1 -	
B-hom	d2 +	d2 +	d1 +			nd -	nd -	nd -
B-het	d2 +	d2 +	d1 +			d2 -	d2 -	nd 0
D-het	d1 +	d1 +	d1 +	nd +	d2 +			nd -
D-hom	d1 +	d1 +	d1 +	nd +	d2 +			nd -
A-hom	d2 +	d2 +		nd +	nd 0	nd +	nd +	
displaced	5	5	4	0	2	0	0	0
displaced by	0	0	0	-3	-3	-4	-4	-2
Score	5	5	4	-3	-1	-4	-4	-2

5.3.5 Competitive ability, displacement ability and growth rate correlations:

The radius of each strain after five days of growth in monoculture was negatively correlated with the rank competitive ability of the strains (r= -0.916, p=0.001) (see figure 5.8). That is, the more superior competitors also had higher growth rates. The rank displacement ability was also correlated with the radius of each strain after five days of growth in monoculture (r= -0.723, p=0.043) (see figure 5.9).

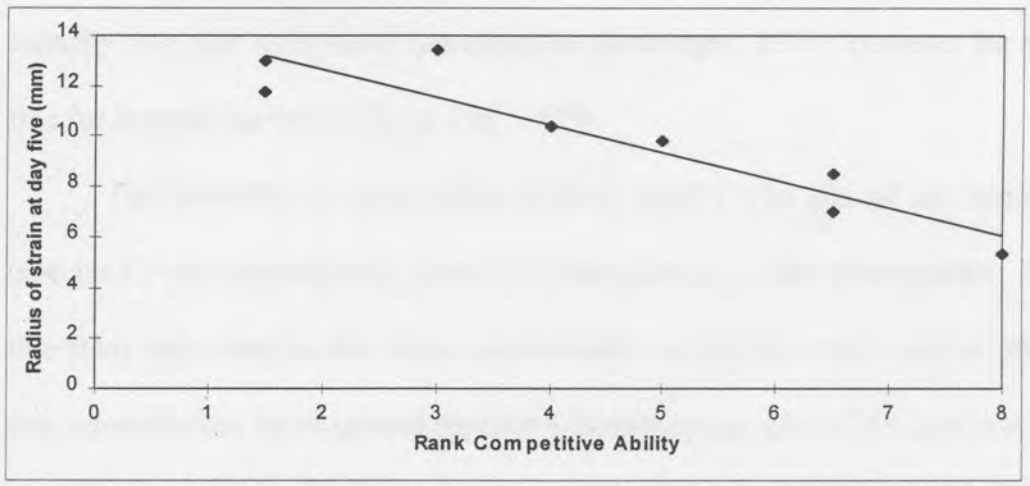

Figure 5.8: Graph of the rank competitive ability of each strain with the radius of each strain after five days of growth in monoculture (mm). r= -0.916, p=0.001. Line shows linear regression.

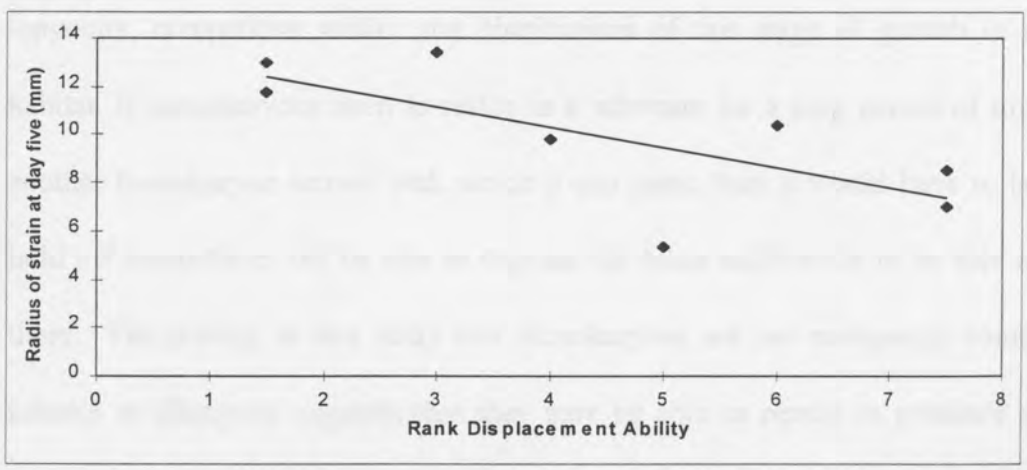

Figure 5.9: Graph of the rank displacement ability of each strain with the radius of each strain after five days of growth in monoculture (mm). r= -0.723, p=0.043. Line shows linear regression.

5.4 Discussion:

This experiment has demonstrated that both the relative competitive abilities and growth rates of homokaryons and heterokaryons of the same species varies between the different species. While the heterokaryon of species A was superior to the homokaryon of species A, the reverse was true for species C. This finding is consistent with previous studies on the degradative abilities of homokaryons and heterokaryons. For example, the heterokaryons of *Lenzites trabea* (Pers. ex Fries) usually have a greater decay capacity than their component homokaryons (Amburgey, 1970). However the reverse is true for *Serpula lacrimans* (Elliot *et al,* 1979).

The hierarchy of competitive abilities showed that one of the homokaryons (species C) was competitively superior to heterokaryons of the other species. Although this study only investigated single representative strains from four species, the notion that heterokaryons are in general superior to homokaryons should be questioned.

While this study has shown that homokaryons are capable of influencing the community dynamics of wood decay fungi, no inferences can be made to the field situation. However, these results do suggests that it could be important to investigate the longevity, competitive ability and distributions of this stage of growth in a natural habitat. If homokaryons need to reside in a substrate for a long period of time before another homokaryon arrives with which it can mate, then it would have to be able to hold off competitors and be able to degrade the wood sufficiently to be able to remain there. The finding in this study that monokaryons are not necessarily competitively inferior to dikaryons suggests that they may be able to persist in presence of strong competitition in the field.

The hyphal extension was found to be correlated with both displacement and competitive abilities of the strains. This finding is discussed in chapter 6.

5.4.1 Future directions:

The role of basidiomycete homokaryons in wood decay community dynamics needs to be examined. A quantitative survey of mycelia from fallen branches in natural habitats would be necessary. Kirby *et al* (1990) have developed a particle plate method for non-selectively isolating fungi from wood. This technique along with developing methods for identifying homokaryotic basidiomycetes in culture could be used to non-selectively quantify the distribution and abundance of homokaryons and heterokaryons.

6. The transitivity of a competitive hierarchy of species in a wood decay basidiomycete community.

Summary:

Intransitive competitive loops (A>B, B>C but C>A) are a mechanism that allow species to coexist and have been found in many communities. The purpose of this study was to determine if intransitivities occur in WDB communities. Nineteen species of WDB strains were paired in every possible combination on MEA plates. The area covered by each strain after 10 weeks was recorded and used as a measure of competitive ability. The 19 species could be placed into a transitive hierarchy of competitive abilities. However, there were two intransitive loops embedded within the overall hierarchy. In addition, there were many inconsistencies found in competitive abilities within the hierarchy which were termed partial intransitivities. This is where A>B>C, but the effect of B on C was greater than the effect of A on C. Possibly, these inconsistencies could increase the chance of the coexistence of species of WDB.

6.1 Introduction:

This study examined a competition matrix of 19 WDBs for intransitive loops. Complete transitivity exists where species A outcompetes species B, B outcompetes C and therefore A also outcompetes C (A>B>C). Any deviation from this pattern means that the competitive abilities are intransitive (eg A>B, B>C but C>A) (Taylor and Aarssen, 1990).

Intransitive competition has been proposed as a mechanism for the coexistence of species (Gilpin, 1975; May and Leonard, 1975). A computer simulation of competition on marine hard substrata provided support for this hypothesis (Karlson and Jackson, 1981). It was found that complex networks with many intransitive loops had a higher species richness than simple networks. Simple networks also had a higher species richness than a transitive hierarchy.

Intransitivities have commonly been found in marine benthic communities (Rubin, 1982; Buss and Jackson, 1979; Turner and Todd, 1994; Russ, 1982; Quinn, 1982) and there is some evidence that they occur in plant communities (Keddy and Shipley, 1989). Despite the potential importance and frequency of intransitivities within competition hierarchies, studies on fungal communities have overlooked them (Owens *et al,* 1994; Carruthers and Rayner, 1979; Dowson, Rayner and Boddy, 1988; Wicklow and Hirschfield, 1979). Owens *et al* (1994) studied a competition hierarchy of 16 fungal species. They focussed on overall trends and therefore did not report the presence of an intransitive loop within their data (*Neolentinus lepideus* replaced *Gloephyllum trabeum*, *G. trabeum* replaced *Schizophyllum commune* (ii), but *N. lepideus* was replaced by *S. commune*). However, Chapela, Boddy and Rayner (1988) reported an intransitive loop

within a competition matrix in which *Psathyrella hydrophilum* replaced *Phallus impudicus*, *P. impudicus* replaced *Tricholomopsis platyphylla* and *T. platyphylla* replaced *P. hydrophilum*.

Fungal communities are likely to contain intransitive loops because of the various mechanisms of competition used by fungi (see section 1.3). For example, species A may be able to inhibit species B using a diffusible substance, B may be able to outcompete C by direct hyphal interference, but this does not necessarily mean that A will be able to inhibit C. If C is tolerant of the diffusible substance produced by A then C may be able to overgrow A.

6.1.1 Objectives:

Previously (see chapter 4) one intransitive loop was found in a seven-species competition hierarchy. The purpose of this experiment was to estimate the frequency of intransitive loops within a larger competitive hierarchy (19 strains) and to test the hypothesis that fungal communities form competition hierarchies.

Data from chapters 4 and 5 demonstrated a relationship between competitive ability, displacement ability and growth rate. One further purpose of this chapter was to test this correlation on a larger data set (19 strains vs 7 or 8 strains).

6.2 Methods:

6.2.1 Cultures and inoculation:

The methods used to culture the strains used in this experiment are outlined in chapter 3. For convenience they were assigned a number in chapter 3 and will be referred to by that number in this and subsequent chapters. The strains were: 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, and 24.

These strains were paired in every combination on MEA plates. Cubes, 5x5 mm of agar and mycelium were cut from actively growing colonies. These cubes were then placed 1cm apart in the centre of a plate. Controls had two inocula of the same strain. Duplicates of each combination were prepared and allowed to grow for 10 weeks at room temperature. The experiment was then repeated.

As in chapters 4 and 5 the areas covered by each strain were checked by taking hyphal samples (see section 4.2.5). The final area covered by each strain on each plate was measured as in section 4.2.4.

6.2.2 Data analysis:

The duplicate plates that were prepared at the same time were pooled (mean) and analyses were conducted on these values (n=2). A one-way ANOVA was performed for each strain comparing the final areas covered when paired with itself and with each of the other strains. Contrast tests compared the final area covered by a strain co-inoculated with a competitor and with itself (when it occupied half of the area of the plate).

6.2.3 Competitive hierarchy:

The strains were given a score for their competitive ability by giving a score of 1 for each strain that it was able to suppress (ANOVA, $P<0.05$) and -1 for each strain that it was suppressed by (ANOVA, $P<0.05$). The sum of these scores gave an overall value of the competitive ability of the strain.

In addition, for a particular strain, the competitors were ranked according to the mean area covered by the tester strain. This was done for each test strain and a Friedman test was used to test for differences in the rankings.

6.2.4 Displacement ability

A strain was given a score of one (+1) for each other strain that it could displace and minus one (-1) for each strain that could displace that strain. The scores were added and the strains were ranked according to their displacement ability.

6.2.5 Competitive ability and displacement ability vs growth rate

Spearman correlation was used to measure the relationship between rank competitive ability, rank displacement ability and growth rate of monoculture (area covered on day 5 by a the mycelium of a strain growing from a single inoculum with no competitors).

6.3 Results:

6.3.1 Competitive ability:

The strains tested could be placed into a general hierarchy of competitive ability based on the area covered by each species when paired with a competitor (see table 6.1). To test for general transitivity the strains were ranked for the effect that they had on each target strain (ie the strains were ranked 18 times according to the effect on each target strain). The rank series were then compared using a Friedman test and found to be the same (χ^2_{19} =8.18, P>0.5) demonstrating a general transitive hierarchy of competitive ability.

Although the hierarchy was mostly transitive, there were two intransitive loops within the hierarchy. Strains 5, 17 and 22 formed one intransitive loop (5>17, 17>22 and 22>5) while 12 and 14 formed less obvious intransitivities with 6, 20, 7 and 24 (14>12, 6>14, but 6=12; 14>12, 7>14, but 7=12; 14>12, 20>14, but 20=12; 14>12, 24>14, but 24=12).

Table 6.2 shows the mean area covered (±SEM) by each strain when paired with each other strain. The strains were ranked in the same order as in table 6.1. The mean area covered by each strain over all competitors generally agrees with the rankings. The mean area decreased with rank. However there were a few exceptions. Strain 17 was ranked below 21, 11, 5 and 22 but had a higher mean area than all of these. There were similar inconsistencies between 11 and 21, 9 and 15, 2 and 1, 13 and 12. These could be accounted for by sampling variance.

There were a number of cases of partial intransitivities. For example, strain 5 was ranked below 18,11 and 21, but it faired better against 8 than 18, 21 or 11. Using contrast tests ($p<0.01$) within the original one-way ANOVA's, 114 of these partial intransitivities were found out of a possible 969 cases.

6.3.2 Displacement ability:

Interactions between strains could be placed into three categories:

(nd) there was no displacement between the strains. Usually one strain grew faster than the other and therefore ended up with more territory,

(d1) one strain displaced the other as soon as they made contact. ie as they grew, one displaced the other,

(d2) initially there was no displacement between the strains, until there was no space left on the plate, then one strain displaced the other.

Table 6.3 shows the categories for each pairwise interaction. The strains are ordered as in tables 6.1 and 6.2. The strains were ranked according to their displacement ability.

Table 6.1: Results of interspecific competition (ANOVA, $p<0.05$). + means that the strain had a greater final area when paired with the competitor than when paired with itself, - means that the strain had a smaller final area when paired with the competitor than when paired with itself, = means that the strain covered the same amount of area whether paired with the competitor or itself. Effect = the number of strains that were suppressed by the target strain. Response = the number of strains that were able to suppress the target strain. Score = effect + response.

	Target strain																		
	19	8	18	21	11	5	22	17	15	9	1	2	7	20	6	24	12	13	14
Competitor																			
19		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	+	+		=	=	-	=	-	-	-	-	-	-	-	-	-	-	-	-
21	+	+	=		=	=	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	=	=		=	=	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+		+	-	-	=	-	-	-	-	-	-	-	-	-
22	+	+	+	=	=	-		+	=	-	-	-	-	-	-	-	-	-	-
17	+	+	+	+	+	+	-		-	-	-	-	-	-	-	-	-	-	-
15	+	+	+	+	+	+	=	+		=	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	=	=	+	=		=	-	-	-	-	-	-	-	-
1	+	+	+	+	+	+	+	+	+	=		=	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	=		-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+		=	-	=	=	-	-
20	+	+	+	+	+	+	+	+	+	+	+	=	=		=	-	=	-	-
6	+	+	+	+	+	+	+	+	+	+	=	+	+	=		=	=	-	-
24	+	+	+	+	+	+	+	+	+	+	+	+	=	+	=		=	=	-
12	+	+	+	+	+	+	+	+	+	+	+	+	=	=	=	=		=	+
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	=	=	=		=
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	=	
Effect	18	17	13	13	12	11	11	12	9	8	7	7	3	3	2	1	0	0	1
Response	0	-1	-2	-2	-2	-4	-4	-6	-7	-7	-9	-10	-12	-12	-13	-13	-13	-15	-16
score	18	16	11	11	10	7	7	6	2	1	-2	-3	-9	-9	-11	-12	-13	-15	-15
Rank	1	2	3.5	3.5	5	6.5	6.5	8	9	10	11	12	13.5	13.5	15	16	17	18.5	18.5

Table 6.2: Table of % mean area covered (±SEM) by a target strain when paired with a competitor

	Target strain									
	strain 19	strain 8	strain 18	strain 21	strain 11	strain 5	strain 22	strain 17	strain 15	strain 9
competitor										
19	50±0.1	25.4±4.0	6.3±2.4	3.0±1.4	5.9±1.6	1.7±0.4	0±0	8.5±2.6	0±0	3.2±0.7
8	74.6±4.0	50±0.1	0±0	0.7±0.7	1.9±0.3	17.4±2.4	4.6±0.9	4.7±0.2	1.5±0.4	0±0
18	93.7±2.3	100±0.4	50±0.2	56.5±3.7	56.5±0.9	40.6±5.6	31.8±2.3	35.0±1.6	10.1±0.2	32.7±1.9
21	97.0±1.5	99.3±0.7	43.5±3.8	50±0.1	48.6±5.7	37.3±1.5	51.8±2.8	28.4±4.8	23.2±0.2	28.1±0.9
11	94.1±1.9	98.1±0.6	43.5±0.7	51.4±5.8	50±0.1	40.3±1.3	55.1±0.1	33.3±1.7	16.3±5.4	28.7±0.9
5	98.3±0.5	82.6±2.9	59.4±6.0	62.7±1.1	59.7±1.3	50±0.3	62.0±9.6	39.0±0.4	16.9±3.0	46.7±3.3
22	100±0.5	95.4±0.6	68.2±3.5	48.2±2.4	44.9±0.1	38.0±10.6	50±0.2	100±0	35.5±22.9	40.7±1.6
17	91.5±2.4	95.3±1.2	65±2.0	71.6±4.4	66.7±1.5	61.0±0.7	0±0	50±0.1	0±0	38.8±2.6
15	100±0.7	98.5±0.4	89.9±0.2	76.8±0.2	83.7±5.2	83.1±2.2	64.5±24.4	100±0.1	50±0.1	50.4±2.5
9	96.8±0.2	100±0.1	67.3±1.8	71.9±1.0	71.3±0.8	53.3±3.3	59.0±9.3	61.2±2.9	49.6±2.6	50±0.1
1	100±0.3	100±0.5	100±0.4	100±0.2	100±0.1	100±0.1	100±0.3	100±0.4	75.3±9.2	57.5±6.7
2	100±0.1	100±0.1	96.4±1.2	100±0.1	96.8±2.6	96.6±3.4	100±0.1	100±0	82.1±2.7	74.0±5.9
7	100±0.1	100±0.4	84.9±0.1	81.9±6.7	76.7±0.3	100±0.1	100±0.1	100±0.1	100±0.1	66.7±2.3
20	98.7±1.4	100±0.2	100±0.1	100±0.1	100±0	100±0.2	100±0.7	100±0.2	89.0±0.1	79±2.2
6	100±0.7	100±0.1	88.6±1.5	87.5±5.2	84.8±3.9	100±0.1	100±0.8	100±0.5	100±0.5	77.1±2.5
24	100±0.1	100±0.1	94.6±4.2	92.5±4.7	93.0±2.6	91.9±0.6	100±0	100±0.1	76.7±5.9	80.5±6.9
12	100±0.5	100±0.7	100±0.1	100±0.1	100±0.4	100±0.7	73.3±0.5	100±0.1	100±0.1	100±0.1
13	100±0.2	100±1.0	100±0.4	100±0.4	100±0.8	100±1.0	100±0	100±1.0	100±0.8	98.3±1.6
14	100±0.1	100±0.4	100±0.1	100±0.1	100±1.0	100±0.4	100±0.1	100±0.1	100±0.1	100±0.1
Mean of mean area	94.4	91.3	71.5	69.7	70.3	68.7	67.7	71.1	54.0	55.4

Table 6.2(continued): Table of % mean area covered (±SEM) by a target strain when paired with a competitor

	Target strain								
	strain 1	strain 2	strain 7	strain 20	strain 6	strain 24	strain 12	strain 13	strain 14
Competitor									
19	0±0	0±0	0±0	1.4±1.4	0±0	0±0	0±0	0±0	0±0
8	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
18	0±0	3.6±1.1	15.1±0	0±0	11.4±1.6	5.4±4.3	0±0	0±0	0±0
21	0±0	0±0	18.1±6.8	0±0	12.5±5.6	7.5±4.0	0±0	0±0	0±0
11	0±0	3.2±1.6	23.3±0	0±0	15.2±4.0	7.0±2.7	0±0	0±0	0±0
5	0±0	3.4±2.6	0±0	0±0	0±0	8.1±0.5	0±0	0±0	0±0
22	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
17	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
15	24.7±8.2	17.9±2.6	0±0	11.0±0.9	0±0	23.3±6.9	0±0	0±0	0±0
9	42.5±6.5	26.0±2.4	33.3±2.2	21.0±2.2	22.9±2.4	19.5±7.9	0±0	1.7±1.7	0±0
1	50±0.1	54.0±10.2	29.8±4.0	24.0±5.7	33.7±2.3	24.3±7.9	13.6±5.7	16.5±7.2	5.0±0.1
2	46.0±9.7	50±0.2	28.8±7.1	33.4±8.4	28.6±3.9	16.6±10.7	10.9±2.5	19.6±0.5	9.2±7.0
7	70.2±4.0	71.2±7.2	50±0.1	45.8±9.3	0±0	45.3±8.7	44.5±2.6	31.8±2.5	10.8±0.5
20	76.0±5.2	66.6±8.6	54.2±9.4	50±0.1	57.6±11.0	28.6±3.8	35.9±4.5	31±1.9	2.7±0.1
6	66.3±2.1	71.4±3.5	100±0.1	42.4±9.9	50±0.1	46.7±7.4	42.1±4.5	34.3±4.6	14.2±6.7
24	75.7±7.2	83.4±10.5	54.7±9.2	71.4±2.7	53.3±6.4	50±0.1	36.5±6.3	52.2±3.4	24.7±2.3
12	86.4±5.8	89.1±2.9	55.5±2.2	64.1±4.4	57.9±4.3	63.5±9.8	50±0.1	53.1±4.0	65.5±3.7
13	83.5±5.3	80.4±0.4	68.2±2.7	69.0±1.7	65.7±4.5	47.8±3.5	46.9±2.7	50±0.1	44.2±0.6
14	95.0±0.5	90.8±6.5	89.2±0.2	97.3±0.1	85.8±6.5	75.3±1.9	34.5±3.8	54.8 ±0.2	50±0.2
Mean of mean area	37.5	37.2	32.5	27.8	25.9	25.7	16.4	17.9	11.9

Table 6.3: Table of displacement abilities. nd = no displacement, d1= displacement as it grew, d2= displacement only after there was no space left on the plate. Above the shaded box the target strain was displaced, below the box, the competitor was displaced. * = the opposite. Effect = the number of strains that were displaced by the target strain. Response = the number of strains that were able to displace the target strain. Score = effect + response.

	Target strain																		
	19	8	18	21	11	5	22	17	15	9	1	2	7	20	6	24	12	13	14
Competitor																			
19		nd	d2*	d2*	d2*	nd	nd	d2*	nd	nd	nd	nd	d2	d2*	nd	d2	d1	d2*	d2
8	nd		nd	nd	nd	nd	nd	d2*	nd	d2*	d1	d1	d2	nd	d2	d2*	nd	nd	nd
18	d2*	nd		nd	nd	d2	d2	nd	d2	nd	d2	d2	d2	d2	d2	d2	d2	d2	d1
21	d2*	nd	nd		nd	nd	nd	nd	nd	nd	d2	d2	nd	d1	d2	nd	d2	d1	d1
11	d2*	nd	nd	nd		nd	nd	nd	d2	nd	d1	d2	nd	d1	d2	nd	d2	d2	d1
5	nd	nd	nd	nd	nd		d2	d2	d2	nd	d1	d1	d2	d2	d2	nd	d2	d2	d2
22	nd	nd	d2	nd	nd	d2		d2*	d2	nd	d1	d1	d1	d1	d1	d1	d1	d1	d1
17	d2*	d2*	nd	nd	nd	d2	d2*		d1	d1	d1	d1	d1	d1	d1	d1	d1	d1	d1
15	nd	nd	d2	nd	d2	d2	d2	d1		nd	nd	d2	d2	d2	d2	d2	d1	d1	d1
9	nd	d2*	nd	nd	nd	nd	nd	d1	nd		nd	nd	nd	d1	nd	nd	d2	d2	d1
1	nd	d1	d2	d2	d1	d1	d1	d1	nd	nd		nd	nd	nd	nd	nd	nd	nd	d1
2	nd	d1	d2	d2	d2	d1	d1	d1	d2	nd	nd		nd	nd	nd	d2	nd	nd	d1
7	d2	d2	d2	nd	nd	d2	d1	d1	d2	nd	nd	nd		d1	nd	nd	d2	d2	d1
20	d2*	nd	d2	d1	d1	d2	d1	d1	d2	d1	nd	nd	d1		nd	nd	nd	nd	d2
6	nd	d2	d2	d2	d2	d2	d1	d1	d2	nd	nd	nd	nd	nd		nd	nd	nd	nd
24	d2	d2*	d2	nd	nd	nd	d1	d1	d2	nd	nd	d2	nd	nd	nd		nd	nd	d1
12	d1	nd	d2	d2	d2	d2	d1	d1	d1	d2	nd	nd	d2	nd	nd	nd		nd	nd
13	d2*	nd	d2	d1	d2	d2	d1	d1	d1	d2	nd	nd	d2	nd	nd	nd	nd		nd
14	d2	nd	d1	d1	d1	d2	d1	d1	d1	d1	d1	d1	d1	d2	nd	d1	nd	nd	
Effect	4	4	12	8	9	11	12	14	8	5	1	2	4	2	0	2	0	1	0
Response	-6	-3	0	0	0	-1	-1	-1	-5	-1	-7	-8	-7	-9	-8	-6	-10	-9	-14
score	-2	1	12	8	9	10	11	13	3	4	-6	-6	-3	-7	-8	-4	-10	-8	-14
Rank	10	9	2	6	5	4	3	1	8	7	13.5	13.5	11	15	16.5	12	18	16.5	19

6.3.3 Competitive ability and displacement ability vs growth rate:

Competitive ability and displacement ability were both negatively correlated with growth rate (r= -0.961, p=0.000 and r= -0.635, p=0.005). That is, the fastest growing strains were also the most competitive. However, the correlation between competitive ability and growth rate was much stronger than between displacement ability and growth rate.

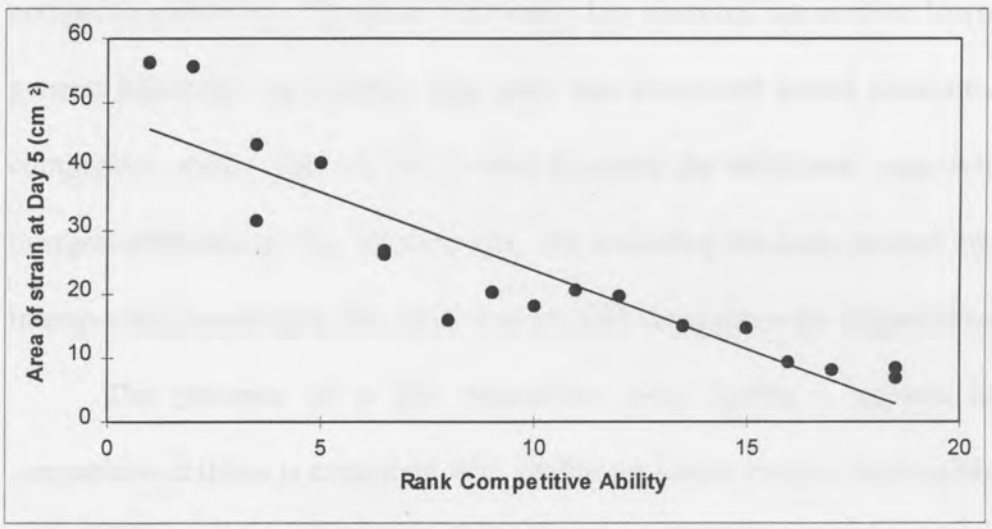

Figure 6.1: Graph of competitive ability with the area covered by monoculture at day 5 (r= -0.961, p=0.000). Line represents linear regression.

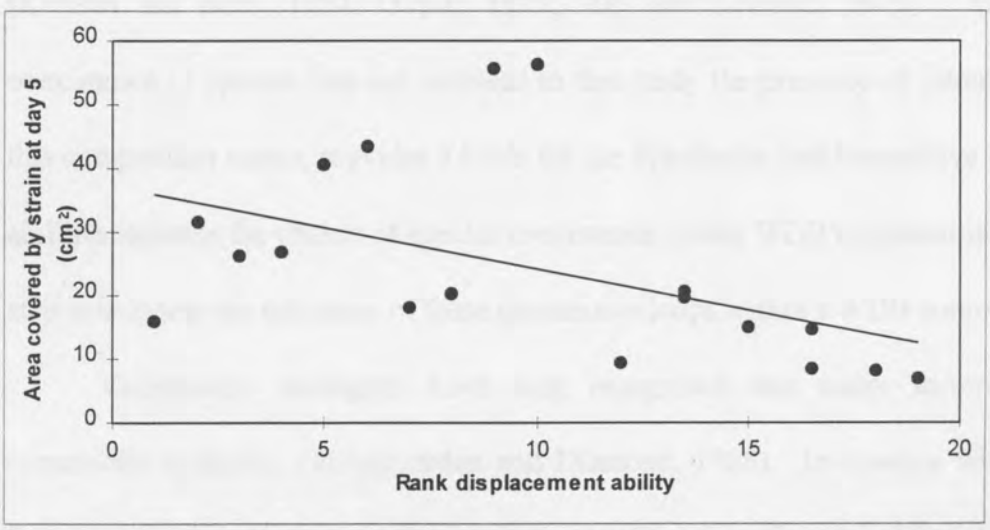

Figure 6.2: Graph of rank displacement ability with the area covered by monoculture at day 5 (r= -0.635, p=0.005). Line represents linear regression

6.4 Discussion:

6.4.1 Intransitivities:

This study is consistent with previous studies (Dowson, Rayner and Boddy, 1988; Wicklow and Hirschfield, 1979; Owens *et al* 1994; Carruthers and Rayner 1979; Robinson, Dighton and Frankland, 1993) in that the 19 strains formed a hierarchy of competitive abilities. However, this study has observed intransitive loops within the general hierarchy. In addition, this study has uncovered partial intransitivities in the competition matrix that are not evident by using the traditional approach of scoring overgrowth/deadlock (see section 1.3). By recording the area covered by a strain in interspecific competition, this study has revealed more about the degree of competition.

The presence of a few intransitive loops within a general hierarchy of competitive abilities is consistent with studies on sessile marine communities (Karlson, 1985; Rubin, 1982; Buss, 1980; Buss and Jackson, 1979; Quinn, 1982; Russ, 1982) and has been proposed as a mechanism for species coexistence in these communities (Karlson and Buss, 1984; Gilpin, 1975; May and Leonard, 1975). Although the coexistence of species was not assessed in this study the presence of intransitivities in this competition matrix provides a basis for the hypothesis that intransitive competitive abilities increase the chance of species coexistence within WDB communities. The next step is to assess the influence of these intransitive loops within a WDB community.

Community ecologists have long recognised that many factors influence community dynamics (Roughgarden and Diamond, 1986). In systems where no one factor can explain the community structure a combination of many subtle factors may be determining the structure (eg Wilbur, 1987; Minchinton and Scheibling, 1993). The

existence of two intransitivities within a 19-species competition matrix may seem trivial. However, in the context of the whole community, they may be one of these subtle factors which increase the chance of coexistence without singly being able to allow the coexistence of species.

6.4.2 Competitive ability, displacement ability and growth rate:

Data from chapters 4 and 5 and also this chapter have demonstrated a relationship between the competitive ability of a strain and its growth rate. Generally strains with faster growth rates also gained more territory. By observing the dynamics of the interactions it could be seen in a lot of cases that there was no displacement of hyphae. The strain which covered the territory the fastest, would then be able to hold that territory and hence be competitively superior. In addition, in cases where there was displacement, the faster growing species usually displaced the slower growing species. However, there appeared to be no advantage gained in the acquiring of resource faster for the displacement of the other strain. That is, a strain did not displace other strains more if it gained more resource. In chapter 4 where the strains were paired in different densities, the competitive effects per inoculum remained the same. If there were to be any advantage in having resource to displace the other strain then the competitive effects would have changed in the different densities. The only exception was strain B in chapter 4 which overgrew strain C in 1:1 and 2:1 treatments.

This correlation between competitive ability and growth rate is not consistent with R-, C- and S- selection. If there were a trade-off between ruderal and combative strategies then the faster growing strains would be less combative. This could be due to

several things. Firstly, it could mean that R-, C- and S-selection theory does not operate within this subcommunity of wood decay fungi. However, Rayner and Boddy (1988) classified most WDB as C-selected. Therefore within this subcommunity, perhaps the R-, C- and S- selection theory does not hold up. Secondly, in this study, only two life-history traits have been measured. Perhaps dispersal rates make up the difference. Thirdly, this finding could be an artefact of agar plate competition. It may be that the slower growing species may displace the faster growing species in wood, however, on agar where the nutrients might be quickly utilised the hyphae detect that there are no resources left in that area where the competitor strain grew and therefore do not replace that strain. In wood, where it takes considerably more time to deplete nutrients, the slower growing strain may displace the faster growing strain. The relationship between competitive ability and growth rate therefore needs to be tested in wood.

7. General discussion and conclusions:

7.1 Main conclusions:

The major results of this thesis are: (1) that the distribution and abundance of WDB species is influenced by many unknown, different factors (chapter 2); (2) that indirect effects and potentially interaction modifications occur between individuals of wood decay basidiomycetes; (3) that these non-additive interactions generally increase the chance of coexistence of species; (4) that intransitive loops exist within a competition matrix of wood decay basidiomycetes; (5) that heterokaryons are not necessarily always competitively superior to homokaryons; and (6) the growth rate of an individual fungus on agar is correlated with it's competitive ability on agar. In addition, this study has given valuable information on the identity of WDBs and their abundance patterns in two prominent conservation parks in South Australia.

7.2 Coexistence of competing species:

The coexistence of WDBs may be due to one or a combination of many different factors such as variable environment, patchiness of the landscape, non-equilibrium theories (eg disturbance), interactions with other trophic levels and neighbourhood models, intransitive competition or indirect effects/HOIs. Two hypotheses on coexistence were tested in this thesis: (a) that intransitive competition and (b) non-additive interactions increase the chance of species coexistence.

7.2.1 Intransitive competition:

This thesis revealed the presence of intransitive loops within a WDB community. Although the influence of intransitive competition on diversity was not tested, Karlson and Buss (1984) have previously demonstrated that intransitive competition can increase species diversity. However, they point out that intransitive competition may only be a factor in habitats with low or no disturbance. In habitats with high levels of disturbance, intransitive competition would be inconsequential to the coexistence of species. Disturbance can be a strong factor in increasing species diversity (Rogers, 1993; Sommer, 1993; Sommer *et al*, 1993; Karlson and Hurd, 1993; Duncan, 1993; Lavorel *et al*, 1994; Tanner, Hughes and Connell, 1994; McGrady-Steed and Morin, 1996) and in habitats with strong disturbance this factor overrides the influence of intransitive competition (Karlson and Buss, 1984). The influence of intransitive competition therefore needs to be assessed in a natural WDB community. In addition, competitive abilities are likely to fluctuate with different environmental conditions. Boddy (1983) found that the growth rate of different species had different optimum temperatures and humidity conditions. The association between growth rate and competitive ability of a fungus demonstrated in this thesis suggests that different fungi may be competitively superior at different temperatures and moisture contents. The experiments in this thesis were all conducted on the same media, under very similar environmental conditions. I would expect a greater incidence of swapping of competitive abilities in fluctuating environmental conditions. The possibility of condition specific competitive abilities between WDBs needs investigation.

7.2.2 Non-additive competition:

This thesis reports for the first time the existence of indirect interactions and possible interaction modifications between fungi. In addition it reports the role that these non-additive interactions may play in influencing species diversity (coexistence). In a WDB community with many competitors and therefore many interaction links indirect effects are likely to greatly influence community dynamics. This, along with intransitive loops and other mechanisms of coexistence, may help to explain the coexistence of many competing WDBs in the same habitat.

In other communities (Menge, 1995) the number of indirect effects increases with the number of species interacting. If WDBs follow this same trend then this study has underestimated the frequency of indirect effects in this community. It is unknown whether these extra indirect effects with more species would also enhance coexistence. These are issues which require further investigation.

Although this study has demonstrated that non-additive interactions occur between fungi on agar plates, their effect on a natural community of WDBs is unknown. To test their role in the field, it would be necessary to inoculate a series of sterile wood blocks with one, two or three species and place the blocks in the field, then collect them at a later stage and observe the resultant three-dimensional colonisation patterns. This experiment would yield information about the role of both direct and indirect competition on colonisation of wood by naturally occurring wood decay fungi.

There has been an increasing amount of interest in indirect effects and interactions modifications in the last 10 years with ecologists realising the interconnectedness of species. However, there are many questions yet to be answered about the role of non-additive interactions, such as: how do these interactions interplay

with disturbance regimes, abiotic fluctuations and habitat heterogeneity? The answer is likely to be complex and well worth investigating.

7.2.3 Conclusion:

It is therefore concluded that indirect effects and intransitive loops may act as mechanisms for coexistence between competing WDBs. However, both mechanisms need to be tested in the field along with other mechanisms.

7.3 Field based ecological studies on WDBs:

More information is needed about: (1) how many species interact within a single branch; (2) for how long; (3) are they homokaryons or heterokaryons? There are a series of studies which give us information about the development of WDB communities. Although there have been a number of studies (Rayner, 1977; Rayner et al, 1981; Rayner and Todd, 1977; Coates and Rayner, 1985a, 1985b, 1985c) which have looked at mycelial distributions of WDB, they have generally been from artificial situations. There have been a number of other studies (Boddy et al, 1987; Boddy and Rayner, 1982; Boddy and Rayner, 1983a, 1983b; Boddy and Rayner, 1984; Boddy and Swift, 1984) which have looked at a relatively natural situation but these studies have been on branches that have been still attached to the tree. The community of fungi in branches before and after branch fall may be considerably different (Rayner and Boddy, 1988). A thorough survey of the mycelia inhabiting naturally fallen branches within natural habitats is required. This survey could assess the frequency of homokaryons vs heterokaryons, the number of species interactions in a branch, etc. Surveys of fruiting

bodies only (such as in chapter 2) are likely to underestimate the number of mycelia existing within each branch because most fungi do not have fruiting bodies all of the time.

This thesis raises questions about the role or influence of homokaryons on the WDB community. In particular: how long do they persist before they either mate or are overgrown? In a community where there are many conspecific propagules arriving at a branch, homokaryons may not have a long life span before they mate. However, in a highly diverse community, the chances of a spore of the same species arriving at the same branch would be much lower. Therefore homokaryons may be more common. Although the study by Coates and Rayner (1985a, 1985b, 1985c) was ground breaking in that it demonstrated the influence of interactions between WDBs *in vivo*, it would have underestimated the importance of the homokaryotic stage. Homokaryons were found to be prominent in the first 12 months only. After this time, they had mostly mated and the heterokaryotic stage became more prominent. Their study could have underestimated the duration of the homokaryotic stage because it was conducted within a plantation forest which notoriously have many individuals of just a few species. In addition, they cut stumps for colonisation, therefore increasing the surface area available for colonisation by aerial spores. These two factors would tend to mean that there were more spores of the same species present. Therefore, the homokaryons would mate very quickly and therefore not be found after 12 months. To truly assess the frequency and importance of the homokaryotic stage we need to conduct a survey in a more natural situation.

7.4 Non-equilibrium theories:

There has been a strong tendency within mycology to consider only equilibrium theories (Strong, 1992). The information that is now available on non-equilibrium conditions suggests that many communities are influenced strongly by non-equilibrium conditions generated by factors such as disturbance. In particular, within Australia, non-equilibrium conditions may be particularly relevant with the random occurrence of bushfires. Cooke and Rayner (1984) point out that the community structure within harvested forests are considerably different to natural vegetation.

7.5 Measures of competition:

Measuring the final area covered by mycelia in interspecific competition in this thesis was crucial to observing the intricacies of these interactions. Image analysis software made the measurement of area very simple, although still time consuming. Further improvements in hardware and software may make this even easier in the future.

7.6 Populations vs individuals:

A feature of fungal studies is that we usually observe interactions between only individuals rather than populations. This approach was justified in section 1.8. However, there are some problems with dealing with individuals rather than populations. As pointed out in section 1.8 we must make assumptions when extrapolating from interactions between individuals to interactions between populations. Intraspecific variation in growth rates and or competitive ability, dispersal etc could

profoundly influence the outcome of interactions between real populations. Apart from a study by Simchen (1966) on the degrading ability of WDBs of the same species, intraspecific variation has not been extensively studied. A related problem is that interactions between individual mycelia on agar plates study only one portion of the life-cycle of WDBs. It is possible that WDBs interact strongly in another part of the life cycle. For example, one species may be able to fruit better if it has a fruiting body of another particular species on which to put its fruiting structure(s). Neimella *et al* (1995) observed *Skeletocutis* sp.1 WR fruiting on top of fruiting bodies of *Phellinus ferrugineofuscus* (P. Karst.) Bourdot WR. In these cases, *Skeletocutis* sp.1 may be facilitate to produce more propagules. This is an issue that requires further investigation.

7.7 Use of WDB communities to study ecological theories:

This system has the potential to be of great benefit to ecological theory. The wood decay basidiomycete community has the advantage of being cheaply repeatable and storable. Experiments on agar plates take only weeks. If we are to gain an overall picture of the natural world then communities from all Kingdoms require consideration. In this case, in an ecologically under-investigated system, this aim can easily be achieved.

7.8 Future directions:

Throughout this discussion, a number of areas have been highlighted which require further study. This thesis has enhanced our knowledge of the complexity of competition between WDBs and yet there are still many unanswered questions. There are still wide gaps in our knowledge of basic factors affecting WDBs such as: (1) the influence of direct competition in a natural community; (2) the number of individuals which inhabit each branch; (3) the frequency and longevity of homokaryotic and heterokaryotic stages; and many others.

To follow on from my research on indirect effects/interaction modification between WDBs, future studies need to look for non-additivities between more than three species using wood as the resource unit rather than agar plates. This would answer several questions: (1) do non-additivities occur within the natural substrate; (2) does the number of non-additivities increase with the number of species; (3) do non-additivities via more than one other species also increase the coexistence of species?

To assess the role of non-additivities in the field requires a lot more work. We will always have the problem that each generation of WDBs takes 15-200 years (see section 1.5). This means that we cannot feasibly test the influence of competition (direct or indirect) on the whole community dynamics over several generations. An experiment as suggested in section 7.2.2 would test the influence of direct and indirect competition on colonisation patterns in one generation.

The conclusions from this thesis all point to the fact that we need extensive, long term surveys and field experiments of WDB mycelia in natural habitats. However, the use of laboratory experiments such as those conducted in this thesis is essential for

testing the possibility of ecological phenomena such as intransitive loops, indirect effects and interactions modifications.

A. Appendix A

Table A.1: Table of site descriptions, locations and date of sampling at Kyeema

Site	Quadrat	Date	Location	habitat
Kyeema	1	9 may 1994	S35°16.310' E138°41.464'	medium *Eucalyptus baxteri.* thick, knee high cover
Kyeema	2	11 may 1994	S35°16.001' E138°40.556'	medium *E. obliqua* sparce ground cover
Kyeema	3	16 may 1994	S35°15.810' E138°40.960'	dense patch *E. obliqua* med ground cover
Kyeema	4	23 may 1994	S35°16.451' E138°40.543'	medium patch *E. obliqua* sparce to medium ground cover
Kyeema	8	28 june 1994	S35°15.838' E138°40.495'	north facing hill *E. obliqua* medium to dense med to dense ground cover
Kyeema	9	2 july 1994	S35°15.903' E138°40.480'	medium *E. obliqua* patch *Xanthorrhea* medium to thick thick ground cover
Kyeema	10	4 july 1994	S35°15.947' E138°40.523'	dense patch of young *E. obiliqua* medium to sparce ground cover *Xanthorrhea* three plants
Kyeema	11	12 july 1994	S35°16.147' E138°40.562'	Many *Hakea* sp. plants very wet medium to sparce ground cover, evenly spread *E. obliqua* ie not in patches - sparce
Kyeema	12	25 july 1994	S 35°16.909' E138°40.112	medium E.obliqua sparce ground cover
Kyeema	14	5 april 1995	S 35°16.112' E138°41.736'	dense *E. obliqua* medium to dense, knee high ground cover
Kyeema	15	28 april 95	S35°15.878' E138°41.644'	sparce *E. obliqua* medium ground cover few shrubs

Table A.2: Table of site descriptions, locations and date of sampling at Cox Scrub

Site	Quadrat	Date	Location	habitat
Cox	4	7 june 94	S35°20.002' E138°44.873'	top of hill *E. baxteri* patch medium density *Hakea* thick ground cover
Cox	5	8 june 94	S35°19.875' E138°44.889'	*E. baxteri* stand medium ground cover
Cox	6	9 june 94	S 35°20.018' E138°44.022'	*E. baxteri* stand knee to waist heathland dense ground cover *Xanthorrhea* sp
Cox	7	9 june 94	S35°20.268' E138°43.885	*E. baxteri* patch in lower lying area with sedges medium - low ground cover
Cox	8	10 june 94	S35°20.170' E138°44.515'	*E. baxteri* stand medium ground cover
Cox	15	21 july 94	S35°20.181' E138°43.032'	young *E. baxteri* patch little ground cover thigh high shrubs
Cox	16	21 july 94	S 35°20.338' E138°44.192'	*E. baxteri* stand sparce ground cover
Cox	17	12 Sept 94	S35°20.499' E138°43.899'	*E. baxteri* stand medium to thich ground cover
Cox	18	23 march 95	S35°19.857' E138°44.591	medium to thick *E.baxteri* patch medium ground cover
Cox	20	30 apr 95	S35°19.977' E138°42.445'	*E. baxteri* patch very few fallen branches medium ground cover
Cox	21	1 jun 95	S35°19.878' E138°43.975'	medium *E. baxteri* patch sparce ground cover

B. Appendix B:

B.1 Stains

B.1.1 Cotton blue

1 mg	Cotton blue
100 mL	Distilled water

B.1.2 Sulphovanillin

2.0 g	Vanillin
6.0 mL	Distilled water
16.0 mL	Conc. sulphuric acid

B.1.3 Melzers Reagent

20 gm	Chloral hydrate
1 g	Potassium Iodide
0.3 g	Iodine
10 mL	Distilled water

B.1.4 KOH

1 g	KOH
9 mL	Distilled water.

B.2 Media

B.2.1 Malt agar

Oxoid Malt Extract Agar CM59

Usual formula:

Malt extract = 30g/L

Mycological peptone = 5 g/L

Agar = 15 g/L

pH=5.4±0.2

B.2.2 Wood/guaiacol agar (WG agar)

To test for lignin degrading ability (Loneran *et al,* 1993).

1g sawdust from a mixture of wood from *Eucalyptus baxteri* and *Eucalyptus obliqua* from Kyeema and Cox Scrub (fits through a 1mm mesh seive)

6g bacteriological agar (Oxoid agar no. 1, code L11)

made up to 500mL with distilled water

Autoclaved at 121°C for 20 minutes

allowed mixture to cool to 65°C.

Added 16.2 µL of guaiacol (0.01% w/v)

pH=4.5

B.2.3 Potato Dextose Agar (PDA)

Oxoid CM139

usual formula

Potato extract = 4 g/L

Glucose = 20 g/L

Agar = 15 g/L

pH=5.6±0.2

B.3 Maintenance and permantent storage of cultures

B.3.1 Slopes

Malt agar slopes were used for short term storage of cultures. Malt agar was prepared as above. While the media was still molten, 10mL was poured into each of 50mL plastic storage containers. The lids were then loosely placed on the containers, and autoclaved. Immediately after taking the containers out of the autoclave the lids were screwed on tight and the containers were put at a 45° angle and allowed to set.

Fungal cultures were inoculated onto these slopes and allowed to grow ~1cm diameter. Approximately 5mL of sterile parafin oil was then poured into the containers to cover the culture. The containers were then stored at 4°C.

C. Appendix C

Table C.1: Table of locations and date of collection of WDB specimens which were cultured. * = specimen was collected as part of the field survey (chapter 2). Locations Kyeema and Cox Scrub are as described in chapter 2. Location Kuitpo Forest is a mixed *Eucaplytus* plantation (S S35° 14' E 138° 40').

Culture code	Species	Specimen code	location	date
	From Fruit bodies			
25	*Xylobolus illudens*	34	Kuitpo forest	17 Aug 1993
38	*Ceriporia purpurea*	48	Cox Scrub	1 Sept 1993
kq14b3*	*Peniophora* sp. 2	kq14b3*	Kyeema	5 April 1995
8	*Pycnoporus australiensis*	14	Kuitpo forest	20 July 1993
kq2b1*	*Peniophora* sp. 1	kq2b1*	Kyeema	11 May 1994
kq12b7*	*Hymenochaete innexa*	kq12b7*	Kyeema	25 July 1994
43	unidentified corticioid 1	53	Cox Scrub	1 Sept 1993
15	*Stereum* sp.1	25A	Kyeema	10 Aug 1993
17.2	*Stereum* sp.2	27	Kyeema	10 Aug 1993
Cq16b12*	*Aleurodiscus lividocoeruleus*	cq16b12*	Cox Scrub	21 July 1994
Kyeema 1	unidentified hydnum	Kyeema 1	Kyeema	12 May 1995
ks1	*Pereniporia medulla-panis*	ks1	Kyeema	15 June 1995
k1 stalked	unidentified agaricoid	k1 stalked	Kyeema	12 May 1995
kq14b1*	*Phanerochaete filamentosa*	kq14b1*	Kyeema	5 April 1995
C1	too old to identify - hydnum	C1	Cox Scrub	12 May 1995
kq12b5*	*Aleurodiscus lividocoeruleus*	kq12b5*	Kyeema	25 July 1994
	From wood			
KQ14B2*	unknown	KQ14B2*	Kyeema	5 April 1995
KQ14B3*	unknown	KQ14B3*	Kyeema	5 April 1995
KQ12B6*	unknown	KQ12B6*	Kyeema	25 July 1994
KQ14B7*	unknown	KQ14B7*	Kyeema	5 April 1995

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