THE EFFECTS OF NITROGENOUS COMPOUNDS ON DECAY OF WOOD BY SOFT-ROT FUNGI.

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Summary

The amino acids in the sapwood of *Fagus sylvatica* L., *Pinus sylvestris* L. and *Tilia europaea* L. were investigated. The amino acids were identified and quantified in three sections; those present as free amino acids, those bound in soluble proteins and those bound in insoluble protein. The amino acid content of the soft-rot organism, *Chaetomium globosum* Kunze ex Fr., was also determined and found to be similar in amino acid range and ratio, but lower in magnitude than the timbers.

The *in vitro* ability of three soft-rot fungi and one Basidiomycete to grow and produce cellulase when amino acids were supplied as a nitrogen source was investigated. The fungi were found, in general, to produce greatest growth and cellulase production in the presence of aspartic acid and glutamic acid, the two amino acids found to be most abundant in the sapwood of timbers investigated.

A humidity chamber technique was employed to study soft-rot decay of wood. This enabled pure fungal cultures to be used to inoculate the wood and may be considered to simulate conditions which result in soft-rot decay of wood not in ground contact. Fungal spore suspensions were used to inoculate wood blocks which had received additional levels of inorganic or organic nitrogen. The criteria used to assess decay were weight loss and loss of bending strength. Bending strength was measured on a tensometer with specially designed three-point loading jaws. Strength loss and weight loss were closely correlated in decay tests with *Chaetomium globosum* but in experiments with *Trichoderma viride* Pers. ex Fr. and *Alternaria tenuissima* (Fr.) Wiltsh. correlation was apparent only at the highest weight losses.

The addition of organic nitrogen to wood, as aspartic or glutamic acid, was found to increase decay by *Chaetomium globosum* to a greater extent than the addition of inorganic nitrogen.

Amino acids/soft-rot/strength loss/weight loss/timber.

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CONTENTS

CHAPTER 1

INTRODUCTION.

1.1 Fungal decay and its economic importance

Wood has been used for structural purposes for many centuries. In recent years, with the wider use of metals, concrete and plastics, the role of wood as a building material has been questioned but the high strength-to-weight ratio inherent in wood and its flexural rigidity (modulus of elasticity x moment of inertia), ensure its continued use. The working life of wood structures is often shortened by biological decay caused by insects and fungi. Insects are the main agents of wood deterioration in the tropics, while fungi are more important in temperate regions (Dhanarajan, 1974). In situations where the biological factor is precluded wooden structures may have extremely long working-life spans (Borgin, 1971).

An overall estimation of economic losses caused by timber decay is difficult since the monetary value of a commodity depends, in part, on its relative abundance or scarcity. The risk of decay varies greatly from one type of wood product to another, and from one kind of timber to another, and so specific accounts of economic loss are usually cited. Realisation that timber decay was a matter of national importance came early in the nineteenth century, when the wooden warships of the British Fleet suffered from a disastrous attack of fungal decay (Cartwright and Findlay, 1958). House timbers also suffer from fungal attack and Findlay (1967) estimated that the cost of repairing houses, damaged by dry rot (Serpula lacrimans) in the United Kingdom, was £1 million per annum before 1939 and £10 million per annum after 1945. Tyrer (1961) found that 27% of

13,000 dwellings surveyed during 1960 had wet rot (Coniophora puteana) and 17% revealed dry rot. He estimated the expenditure necessitated by decay to be above £10 million per year.

Another well documented subject is the fungal damage to wooden structures in water cooling towers. Findlay and Savory (1950) established that the deterioration observed in towers was caused by fungi and not, as previously thought, by chemical means. Fungal damage to a redwood water cooling tower over 34 months of operation cost approximately £4,000 in repairs 18 years ago (Baechler, Blew and Duncan, 1961).

For many years, the United Kingdom has imported very large quantities of timbers from overseas (about 85% of consumption) and is unlikely to reach total self-sufficiency in this commodity. The imported timber should therefore be used as efficiently and economically as possible and wastage caused by biodeterioration should be minimised. With this end in mind, a great deal of research has been undertaken to investigate fungal decay of timbers.

1.2 Wood-attacking fungi.

In the first half of this century the economic loss of wood caused by fungal colonisation was divided into two groups;

a) that caused by Basidiomycete decay of wood which destroys the cell wall constituents and so reduces the strength of wood.

b) that caused by fungal staining of wood in which the fungi utilise material from the cell cavities for their nutrition and produce pigments which spoil the appearance of the wood.

Of these two groups the former is considered to be the more economically important.

The description of a different type of wood decay, generally localised to the surface of wood and termed soft-rot decay, was made by Findlay and Savory (1950). The term soft-rot is now considered to include wood decay caused by fungi belonging to the Ascomycetes and Fungi Imperfecti. King (1972) suggested that many of the microfungi which were considered to colonise wood passively possessed a potential wood decay ability. This was later confirmed by Nilsson (1973) who showed that the majority of 169 commonly found wood-colonising microfungi produced soft-rot decay in wood under laboratory conditions.

The nomenclature used to classify wood-colonising microfungi became confused with the general acceptance of the term "soft-rot". Fungal species concerned in passive colonisation of wood had been termed "staining" or "mould" fungi, while those which produced soft-rot decay were termed "soft-rot" fungi. With continued research on soft-rot decay, it became increasingly obvious that many of the wood-colonising microfungi could be classified in more than one group depending upon the conditions in which they were growing. The term "micromyces" has been suggested as an alternative to "staining", "mould" and"soft-rot" fungi and

covers all microfungi colonising wood (Gorshin and Krapivina, 1969). However, this term is not yet in general use and the anomolies of nomenclature of non-Basidiomycete colonisation of wood still exist.

The succession of organisms which appear in wood during its decay has been studied by a number of workers (Butcher, 1968; Banerjee and Levy, 1971; Kaarik, 1975). Much of the work has been concerned with studies on wood in soil contact and has shown a succession of organisms from an initial phase of bacteria, Actinomycetes, moulds, stain and soft-rot fungi to the take-over by Basidiomycete decay fungi. Kaarik (1975) proposed three interacting hypotheses to explain the succession;

a) ability of the fungi to reach and decay wood e.g. periodicity of spore production and magnitude of spore release is generally greater in the microfungi than in the Basidiomycetes.

b) competitive or synergistic interactions among fungi.

c) changes in the wood substrate with time of exposure e.g. moisture content and acidity.

Microfungi appear early in the succession and may remain in the wood throughout the decay process. It may be postulated that the chemical and physical changes in wood, brought about by colonising microfungi, increases the susceptibility of wood to the Basidiomycetes. The investigation of soft-rot fungi is still relatively recent

and the importance of their role in the succession of decay organisms has yet to be elucidated.

1.3 Antagonistic interactions of wood colonisers.

The antagonistic action of some bacteria and Actinomycetes against wood decay Basidiomycetes was observed by Jacquiot (1968) and Greaves (1970). In laboratory tests the Fungi Imperfecti, *Trichoderma viride*, *Gliocladium* spp. and *Penicillium* spp., were also shown to be effective in the inhibition of growth of wood decay organisms (Kerner-Gang, 1970). Some Fungi Imperfecti behaved similarly in tests using wood as a growth substrate (Shields and Atwell, 1963; Toole, 1971b; Ricard, 1976). Hulme and Shields (1970) explained the ability of *T. viride* to retard colonisation by other organisms by its removal of non-structural simple nutrients or by the production of antibiotics.

The possibility exists that some early colonisers of wood may behave in a protective capacity against Basidiomycete decay. However, the majority of work on fungal interactions has been carried out in laboratories and has utilised pure cultures. Thus, care must be taken in extrapolating these results to cover natural situations. The activities of microorganisms and their interactions may be affected by conditions in their natural habitat (Harley, 1971).

1.4 Effects of preservatives on wood colonisation.

The amount of decay caused by Basidiomycetes in

large dimensional timbers is much greater than that which can be produced by microfungi (Savory, 1954a). The Basidiomycetes are generally considered to be the most economically important agents of decay and the toxicity trials of preservatives are geared towards them. Chou, Preston and Levi (1974) have shown that Basidiomycete decay fungi vary widely in their tolerance to wood preservatives. Other recent work has shown that wood treated with a wide range of preservatives, although resistant to Basidiomycete colonisation, was susceptible to colonisation and decay by microfungi (Gorshin and Krapivina, 1969). The colonisation and decay may occur rapidly (Butcher, 1971) and may even occur in wood treated with copper-chrome-arsenic solutions (Greaves, 1972).

The degradation of wood preservatives by fungi *in vitro* has been demonstrated by Madhosingh (1961), Unligil (1968) and Duncan and Deverall (1964) and the fungi most efficient at degrading preservatives were the microfungi. Experiments carried out by Duncan and Deverall (1964) involved exposure of preservative treated wood blocks to an Ascomycete or Fungi Imperfecti, followed by exposure to Basidiomycete decay fungi. Control preservative treated blocks remained untouched by the Basidiomycetes but blocks which were exposed to Ascomycetes or Fungi Imperfecti, prior to exposure to Basidiomycetes, were decayed by the Basidiomycetes. Hence, it appears that wood-colonising microfungi play some part in the failure of timber treated with preservatives, although the amount of wood decay produced directly by them may only be small.

Teyegaga (1976) thought it probable that preservative tolerant fungi adopted shunt-metabolic pathways in order to evade the toxic action of the chemicals. This, he postulated, would result in the production of large amounts of organic acids which would react with the preservatives to detoxify them. An alternative or contributory explanation may lie with the distribution of preservatives in wood. The investigation of micro-distribution of copper-chrome-arsenic in two hardwoods revealed a build up of preservative at the lumen/cell wall interface and little penetration into the cell walls (Dickinson, 1974). The pattern of attack by wood-decaying Basidiomycetes and soft-rot fungi was clearly demonstrated in scanning electron microscope studies by Bravery (1971); the Basidiomycetes caused decay at the lumen/cell wall interface while the soft-rot organism, Chaetomium globosum, formed cavities within the middle (S_2) layer of the cell wall. It is therefore possible for soft-rot organisms to decay the bulk of vessel or fibre walls within the S_2 layer of preservative treated wood, without approaching the heavily treated lumen/cell wall interface.

Until recently, much of the work documented on soft-rot decay of wood has been concerned with timber in soil contact. However, the decay of timber joinery such as window frames and door jambs, which are out of soil contact, is now becoming increasingly important. The presence of soft-rot organisms has been recorded in these situations (Savory, Carey and Stribbling, 1977; Carey,

pers. comm.). In such decayed joinery, the nutrient levels in the timber may be the dominant factor in decay susceptibility since soil nutrients are not available.

It is evident that soft-rot fungi play a significant role in the economic losses caused by fungal decay of wood, and any further information on this decay process will help to understand the problems associated with timber utilisation.

1.5 Factors affecting initial colonisation of wood.

The factors which influence the initial colonisation of wood are numerous, particularly when wood is in soil contact, but those of greatest importance for wood in all situations are moisture content, acidity and nutrients of wood.

1.5.1 Moisture content

Wood-destroying Basidiomycetes were found to influence the moisture content of wood by the production of water during the decay process (Ammer, 1964). The lower limit of wood moisture content for the initiation of active decay by these fungi was found to be approximately 30% of the dry weight. Soft-rot organisms have occasionally been found in wood at moisture contents of 20% and below but it was not clear if the organisms were actively decaying wood at these moisture levels (Morton and Eggins, 1976). Becker and Kaune (1966) determined the lower limit of moisture content for obtaining soft-rot in pine and beech to be in the order of 30-35%. The upper limits for soft-rot decay were

determined at 60-80% for pine and 60-120% for beech. Moisture content of wood tended to increase with decay and so made the determination of optimum levels difficult.

The wide range of substrate moisture contents at which fungal decay occurs makes it extremely difficult to maintain wood at moisture contents low enough to prevent decay.

1.5.2 pH

The importance of pH in wood decay was stressed by Edgecombe (1941) who found that acidic levels favoured Basidiomycete growth. Henningsson (1967) found that the optimal growth of Basidiomycetes occurred at pH 5-6 with a general tolerance over the pH range of 1.5-8.0. The data available for the pH tolerances of soft-rot fungi are inconsistent. Duncan (1960) found the optima of 32 isolates to be pH6 with little growth occurring below pH3 and above pH9, while in a comparative study of test methods there was some indication that neutral conditions were most favourable for soft-rot organisms (Savory and Bravery, 1970). Sharp and Eggins (1970) however, observed little change in rate of decay in an unsterile soil over a pH range of 3.7 - 8.6. Further experiments showed that some soft-rot fungi were favoured by acidic conditions and others by alkaline conditions.

Although the optimal pH for wood decay may vary from species to species, a pH approaching neutrality is generally employed in soil burial tests. For experiments on wood decay, in which wood is not surrounded by a burial substrate, the natural pH of the wood should not be altered.

1.5.3 Nutrients.

No living organism can survive and multiply without an external source of nutrients and so the presence or absence of nutrients in timber surfaces is important in the consideration of wood colonisation. A depletion of nutrients in fungal-colonised wooden fenceposts, after five months of exposure, was considered to be the causal factor in the curtailment of the activity of existing fungi (Banerjee and Levy, 1971). At the same time the wood surface was rendered uninhabitable for newly colonising organisms.

Nutrients, and nitrogen in particular, play a major role in the susceptibility of different timbers to microbial attack. Nitrogen is scarce in wood and rarely exceeds 0.3% of the dry weight (Merrill and Cowling, 1966). The deficiency of nitrogen in wood is considered to be the major limiting factor of wood decay (Findlay, 1934; Schmitz and Kaufert, 1936; Cowling and Merrill, 1966; Levi and Cowling, 1969). Not only does the nitrogen content vary between different species of tree but it also varies within a species, depending on the time of year felling takes place. This yearly variation in nitrogen affects the susceptibility of wood to decay e.g. it is more susceptible to decay if felled in the summer rather than winter because of the higher nitrogen content at that time (Levi and Cowling, 1968; Bletchly, 1969).

The importance of nitrogen in the decay process was emphasised in the work of King, Oxley and Long (1974) in which the redistribution of soluble nitrogen during

drying of wood was observed. Nitrogen analyses carried out on plank surfaces and on the centres of planks showed a higher nitrogen concentration in the plank surfaces in both commercial and laboratory material. Both air-drying and oven-drying of wood caused the migration of nitrogen in sapwood. However, nitrogen accumulation did not occur at the surfaces of heartwood where there is very little soluble nitrogen. The possibility of redistribution of other nutrients was suggested and a similar movement of soluble carbohydrates was later demonstrated by Long (1978).

The migration of nitrogen was considered to be important in the susceptibility of wood to decay but the chemical forms in which the nitrogen exists in timber and the ability of decay fungi to utilise them has not been extensively studied. It was the purpose of this study to investigate the nature of nitrogenous substances present in wood, and to examine their effects as nutrient sources on the growth, cellulase production and decay ability of a selection of soft-rot organisms.

CHAPTER 2

THE AMINO ACID CONTENTS OF TIMBERS AND OF THE SOFT-ROT FUNGUS, *CHAETOMIUM GLOBOSUM*.

2.1 Introduction

There are a vast number of organic compounds which contain nitrogen; of these, the ones of interest in biodeterioration are those occurring in substrates which support microbial growth. In practice, this means amino acids, proteins and the products of protein hydrolysis.

Much work has been carried out on the amino acid contents of living plants, which includes some work on trees and woody plants. Engard (1939) pointed out that nitrate was the principal form of nitrogen translocated in the xylem of raspberry plants but that the conversion of nitrates to amino acids and protein was not restricted to leaves but occurred in all living cells of the plant. The amino acids were found to constitute 0.5% of the dry weight of phloem exudate in summer and considerably more in autumn, prior to leaf fall. Analyses of the amino acids in exudates from plants have shown glutamine and valine to be predominant in Yucca flaccida sap (Tammes and van Die, 1964) and glutamine to predominate in young tea plants (Selvendran and Selvendran, 1973). Glutamine was also shown to be the most abundant amino acid in the xylem sap of loblolly pine (Pinus taeda) and tracer studies revealed glutamic acid to be a precursor of the glutamine (Barnes, 1962). Glutamic acid and aspartic acid appeared as the predominant amino acids in the sap of Quercus borealis, Q. robur, Q. petraea and Acer platanoides (Ziegler, 1956).

The most documented sap constituents must be those of the tracheal sap of apple trees (Malus spp.).

Oland and Yemm (1956) analysed ethanol and water extracts of apple wood and reported arginine and asparagine to be the chief nitrogenous reserves, with arginine accounting for more than 60% of the soluble nitrogen. Bollard (1957 a, b) could detect no nitrate in apple tracheal sap and revealed that asparagine and glutamine accounted for over 50% of the nitrogen in sap. This discrepancy of results was due to the different extracts analysed. Tromp and Ovaa (1976) clarified the position when they showed arginine to be the main form of storage nitrogen in apple, while newly absorbed nitrogen was translocated as asparagine. Asparagine, glutamine, aspartic acid, glutamic acid and arginine accounted for 90-95% of the amino-nitrogen fraction of apple root tissue (Tromp and Ovaa, 1976).

The vast array of information available on amino acid content of plants was concerned, in particular, with growing organisms and on the amino acids transported in sap. There are few data available which record the free amino acids,or amino acids in proteins, of converted timber. One of the few quantified, comprehensive examinations of the chemical nature of nitrogenous substances in timber was carried out on pine (Laidlaw and Smith, 1965; Baker, Laidlaw and Smith, 1970).

Lack of detailed information on nitrogen sources in timber, which could be used in the nutrition of decay fungi, limits our understanding of the decay process. Hence, the organic nitrogen contents of a number of timbers, and that of the soft-rot organism, Chaetomium

globosum, were determined.

2.2 Materials and methods.

2.2.1 Preparation of timber samples.

Samples of air-dried sapwood of Scots pine (Pinus sylvestris L.), beech (Fagus sylvatica L.) and lime (Tilia europaea L.) were supplied by the Princes Risborough Laboratories (P.R.L.), Aylesbury, Buckinghamshire. A further sample of lime (oven-dried at 50° C) was supplied by Dr. B. King, Dundee College of Technology. The samples of wood were cut into small cubes on a band-saw and milled in a Culatti micro-hammer mill to pass a number 20 mesh sieve (0.84mm. internal diameter).

2.2.2 Extraction of free amino acids and soluble protein from timbers.

Samples of air-dried wood (15g) in Whatman extraction thimbles, were placed in separate extractors of a Soxhlet leaching apparatus. The samples were extracted with 1:2 alcohol/benzene for 24 hours, 60% aqueous ethanol for 17 hours and water for 24 hours. The combined extracts were evaporated to dryness, under reduced pressure at a temperature of 60° C, on a rotary evaporator. The residue was extracted with 70% aqueous ethanol and this solution evaporated to a small volume. Hydrochloric acid (HCl) was added to the solution until the pH reached 2.0. The solution was then centrifuged and the precipitate

which appeared contained the soluble protein. This precipitate was removed and hydrolysed as described below. The supernatant contained the free amino acids and was stored in a deep freeze until analysed.

Empty extraction thimbles were subjected to the leaching procedure and the leachate analysed as a control.

2.2.3 Protein hydrolysis

Extracted wood was oven-dried to constant weight and samples (3g) were placed in flasks with 200ml 6N HCl. The solution was boiled under reflux for 24 hours to hydrolyse the protein. Soluble protein obtained from the solvent extraction was also hydrolysed in this manner. After cooling, the hydrolysed mixture was filtered through Whatman No. 54 filter paper, the residue washed several times with water and the combined filtrate and washings evaporated to dryness on a rotary evaporator. Repeated addition and evaporation of water removed residual HCl. The solution was evaporated to a small volume and stored in a deep freeze until analysed.

2.2.4 Preparation of Chaetomium globosum samples

A spore suspension of C. globosum Kunze ex Fr. was prepared from a culture (No. S70P) obtained from the Princes Risborough Laboratories and grown on 2% malt agar. A medium was prepared, as described in B.S. 838 (1961) for soft-rot organisms and poured into Petri plates. Sterile filter paper was placed on the surface of the set agar and

inoculated with spores. Petri plates which contained 2% malt agar were also prepared and inoculated with spores. Both types of plate were incubated at 25°C for one week. Growth of C. globosum on the filter paper had remained vegetative while growth on the malt agar was predominantly reproductive. The mycelia and perithecia were harvested separately from the two types of plate and each was oven-dried to constant weight.

2.2.5 Hydrolysis of Chaetomium globosum

The mycelial and perithecial fractions of the fungus were hydrolysed in 6N HCl as described in the section for protein hydrolysis in timber (section 2.2.3). The samples were stored in a deep freeze until analysed.

2.2.6 Amino acid determination

Amino acid analyses were carried out on a Locarte amino acid analyser. Sulphosalicylic acid was added to each sample prior to analysis, in order to precipitate any remaining protein which may block the analyser column. With each set of analyses, a standard and a blank were also run. The blank contained sulphosalicylic acid only and gave a base line trace which could be subtracted from the sample traces. The standard contained 25 nannomoles of a range of amino acids commonly found in plants and gave the basis for identification and quantification of the amino acids present in the samples.

2.2.7 Total nitrogen determination

Samples of milled sapwood and of harvested fungus were oven-dried to constant weight. Dry samples of 100mg were weighed and placed in microkjeldahl flasks. A small amount of the following catalyst was added to each flask:-

K_2SO_4	8.0g
$CuSO_4 \cdot 5H_2O$	2.0g
$Na_2 SeO_4 \cdot 10H_2O$	0.05g

Concentrated H_2SO_4 (1.5ml) was pipetted into each flask and the flasks then placed on heated Kjeldahl digestion racks and left for 6-7 hours. Nitrogen analysis was carried out on the digested samples by the method of Humphries (1956) using the following indicator:-

Equal volumes of 0.2% Methyl red in ethanol
0.1% Methylene blue in ethanol
Purple in acidic conditions and green in alkaline conditions.

Control flasks which contained catalyst and H_2SO_4 only were treated in the same manner.

2.3 Results

Extraction of wood yielded a mixture of amino acids and a polymeric material which has been designated soluble protein by Baker et al. (1970). The amino acid composition of samples derived from free amino acid leachates, soluble protein hydrolysis and insoluble protein hydrolysis are given in Tables 1,2 and 3.

Table 1: Free amino acids of timber (arranged in the order of appearance on analyser traces).

μmoles amino acids per 100g wood

	PINE	BEECH	P.R.L. LIME	DUNDEE LIME
Aspartic acid	1.2	1.1	10.9	21.5
Threonine	0.7	)	)	
		) 1.2	) 9.7	9.2
Serine	1.8	)	)	
Glutamic acid	0.7	0.3	3.6	2.4
Proline	0.2	0.2	151.3	0.6
Glycine	0.1	0.7	4.0	4.7
Alanine	1.6	0.7	22.2	10.4
Cysteine	-	-	-	-
Valine	0.7	0.2	3.6	1.3
Methionine	trace	trace	trace	trace
Isoleucine	0.4	0.2	1.5	2.2
Leucine	0.4	0.1	1.3	4.5
Tyrosine	0.3	0.1	0.9	1.0
Phenylalanine	0.2	0.1	0.5	0.6
Histidine	0.2	0.2	3.0	3.0
Ornithine	*	*	*	*
Lysine	0.1	0.1	0.8	1.5
Arginine	0.2	-	33.3	26.0
ɣ amino-butyric acid	-	trace	14.3	12.3
Total	8.8	5.2	260.9	101.2

* Ornithine present in these extracts at a measurable level but not included in the standard.

Table 2: Amino acid constituents of the soluble protein of timber.

μmoles amino acids per 100g wood

	PINE	BEECH	P.R.L. LIME	DUNDEE LIME
Aspartic acid	2.8	4.0	9.9	10.9
Threonine	1.1	1.5	2.3	1.7
Serine	1.9	2.7	2.1	2.7
Glutamic acid	3.6	3.6	6.5	8.4
Proline	1.0	1.9	0.9	-
Glycine	3.3	4.8	5.0	4.5
Alanine	2.1	2.2	5.2	4.5
Cysteine	-	3.7	-	-
Valine	1.2	2.9	2.8	3.4
Methionine	trace	0.5	trace	trace
Isoleucine	1.4	3.0	1.3	1.8
Leucine	1.4	2.2	2.1	1.8
Tyrosine	0.3	0.5	0.3	-
Phenylalanine	0.8	1.2	-	-
Histidine	0.4	0.8	0.2	-
Ornithine	trace	trace	trace	trace
Lysine	0.5	0.7	0.9	1.4
Arginine	0.3	-	7.4	6.2
γ amino-butyric acid	0.8	-	5.5	5.5
Total	22.9	36.2	52.4	52.8

Table 3: Amino acid constituents of the insoluble protein of timber.

μmoles amino acid per g wood

	PINE	BEECH	P.R.L. LIME	DUNDEE LIME
Aspartic acid	1.09	2.47	5.16	4.44
Threonine	0.92	1.68	3.04	2.23
Serine	0.98	1.83	3.76	2.77
Glutamic acid	1.10	2.01	3.87	3.45
Proline	0.86	1.57	2.88	2.54
Glycine	1.19	2.20	4.92	3.14
Alanine	1.23	2.13	3.97	3.06
Cysteine	-	-	-	-
Valine	0.98	1.65	3.02	2.73
Methionine	trace	1.04	0.95	1.03
Isoleucine	0.73	1.23	2.43	1.87
Leucine	0.88	1.70	3.64	3.03
Tyrosine	0.08	0.45	0.86	0.78
Phenylalanine	0.46	1.02	1.96	1.73
Histidine	0.19	0.34	0.75	0.52
Ornithine	trace	trace	trace	-
Lysine	0.43	0.92	1.91	1.14
Arginine	0.17	0.22	1.12	1.08
γ amino-butyric acid	-	-	-	-
Total	11.29	22.46	44.24	34.54

Table 4 records the amino acid composition of the hydrolysed vegetative and reproductive fractions of the soft-rot fungus.

The Tables exclude some amino acid compounds which appeared on the analyser traces. These peaks were too small to be quantified and no attempt was made to identify them.

The figures recorded in Tables 1-4 are the means of two analyses and are calculated on a dry, un-extracted, original material basis.

The greatest quantities of amino acids in wood were found in the insoluble proteins and were, in total, approximately a hundred-fold greater than those found in the soluble protein. The free amino acid fraction yielded the lowest total amino acids in beech and pine but the two lime woods examined showed a greater quantity of amino acids in this fraction than in the soluble protein. In general, pine sapwood contained lower quantities of amino acids than beech (with the exception of the free amino acid analysis) while lime sapwood revealed a greater amount of total amino acids than the other two woods in all three fractions.

Table 4 shows the much higher nitrogen content of fungi compared with that of the timbers. The range of amino acids identified was similar to that in the woods but the amino acids were present in higher quantities in both the vegetative and reproductive fractions.

Table 5 includes the total nitrogen contents of

Table 4: Amino acids of Chaetomium globosum.

	µmoles amino acid/g fungus	
	VEGETATIVE FRACTION	REPRODUCTIVE FRACTION
Aspartic acid	87.1	67.5
Threonine	53.3	42.2
Serine	54.9	45.3
Glutamic acid	135.7	86.6
Proline	41.3	30.5
Glycine	78.5	67.6
Alanine	77.9	62.3
Cysteine	7.2	9.1
Valine	52.7	29.4
Methionine	8.2	6.4
Isoleucine	57.7	126.0
Leucine	74.8	127.0
Tyrosine	20.4	18.2
Phenylalanine	25.7	21.4
Histidine	14.8	15.8
Ornithine	*	*
Lysine	28.0	30.5
Arginine	41.3	32.4
ɣ amino-butyric acid	3.1	2.8
Total	862.6	821.0

* Present in extract at a measurable level but not included in the standard.

Table 5: Total nitrogen and amino acid nitrogen of timber and of Chaetomium globosum.

	% Nitrogen		
	Kjeldahl analysis	Amino acid analysis	% nitrogen recovered as amino acids
Pine	0.030	0.021	70
Beech	0.054	0.035	65
P.R.L. Lime	0.135	0.093	69
Dundee Lime	0.110	0.080	72
C. globosum:			
Vegetative	1.64	1.43	87
Reproductive	1.70	1.35	79

timbers and C. globosum as determined by the Kjeldahl analyses. Each figure is the mean of 10 analyses. Data from Tables 1-4 were converted to percentage nitrogen and also entered in Table 5 as the total percentage nitrogen identified as amino acids. These data could then be compared with the percentage nitrogen determined by Kjeldahl analysis to give an estimate of the percentage nitrogen recovered as amino acids.

2.4 Discussion

2.4.1 Percentage nitrogen recovered by amino acid analysis

The percentage recovery of nitrogen by amino acid analysis was based on the assumed total recovery of nitrogen by Kjeldahl analysis. Amino acid analyses accounted for 65-87% of the total nitrogen contents of the wood and fungus. The majority of these losses could probably be accounted for in the number of unidentified amino acids which were revealed on the analyser traces. These amino acids only occurred in low quantities and even if identification had been accomplished, their levels could not have been quantified.

Small quantities of all the amino acids were probably also lost in the preparation of the samples for analysis as the samples were transferred to different containers on several occasions (e.g. from flask to rotary evaporator, from evaporator to centrifuge, from centrifuge to storage phial) and so the final calculation of amino acids/unit weight of wood can be regarded as lower than

the true level.

Quantities of inorganic nitrogen present in the wood would not be accounted for in the amino acid analyses. This would lead to a lower percentage of nitrogen recovery by this method when compared with the total nitrogen content revealed by Kjeldahl analyses.

Amino acid recovery was greater in the analyses of C. globosum than wood. The fungal samples were merely hydrolysed prior to analysis and so the amino acid results came from only one treatment. The wood samples, however, were subjected to an extraction procedure and two sets of hydrolysis, one for soluble protein and one for insoluble protein. The three amino acid mixtures from the procedures were then reduced separately in the rotary evaporator and so losses could be expected to be higher than those in the fungal analyses.

Consideration of these losses reveals the percentage nitrogen recovery in amino acid analyses to be at a reasonable level. The levels of amino acids/unit weight of wood or fungus, recorded in Tables 1-4, can thus be regarded as minimum quantities.

2.4.2 Comparison of timber amino acids

One of the first things to be noticed from the amino acids in each wood (Tables 1-3) was that there was very little difference in the types and ratios of amino acids in each of the three fractions examined. The amino acids which most frequently predominated were aspartic acid, glutamic acid, glycine, alanine, threonine and

serine. Glutamine and asparagine were not revealed because, on hydrolysis, they revert to glutamic and aspartic acids and so become incorporated into the quantities recorded for these two acids. Threonine and serine were also sometimes calculated together because they were not always resolved on the traces of free amino acid analyses.

The amino acids found in greatest quantities in the present analyses were also found to predominate in the sapwood of pine (Laidlaw and Smith, 1965; Baker et al, 1970) and of cherry (Lee, Bada and Peterson, 1976). They were also identified qualitatively in aspen and white pine (Merrill and Cowling, 1966).

The amounts of amino acids, present as free amino acids or in soluble and insoluble protein in pine, differed only slightly from the results of Baker et al. (1970). The quantities of total amino acids found in each fraction were a little lower in the present work but the range of amino acids was similar. Pine, generally contained the lowest quantities of amino acids of the woods examined but the quantity of free amino acids in pine was higher than that found in beech.

The two lime sapwoods which were examined showed a consistency in the total quantity of amino acids in each fraction. The level of amino acids in the free amino acid fraction was higher than that in the soluble protein fraction and lime differed in this way from the other two woods. Within the free amino acid fraction the two lime woods differed in composition. Dundee lime showed a pre-

dominance of aspartic acid and arginine but P.R.L. lime contained a predominance of proline. The proline level in P.R.L. lime was approximately 250 times greater than that in the Dundee lime. This rather dramatic increase in one particular amino acid is unlikely to be explained by variations in the time of felling of the trees since variations in the season of cutting would probably lead only to a reduction or increase in the quantities of all amino acids. However, it is possible that the quantity of proline may be increased during certain physiological changes in the wood, such as budor flower production and at leaf fall or, it may accumulate at wound sites.

The arginine levels in the free amino acid fraction of both lime woods were high in comparison with other amino acids present and with the analyses of the other woods. Measurement of this particular amino acid was difficult, since the peak corresponding to arginine appeared at the precise location of a base-line aberration revealed on the blank trace. This may have resulted in an over-estimation of this amino acid and it would not be advisable to conclude too much from these apparent high levels.

A further point of interest from the analyses of free amino acids in lime was the appearance of relatively high levels of γ amino-butyric acid. It was also found in the soluble protein fraction of lime but otherwise only occurred as a trace in the free amino acids of beech and in small quantities in the soluble protein of pine.

γ amino-butyric acid is formed as a product of decarboxylation of glutamic acid and was the only amino acid identified in the present analyses which is not considered to be a protein amino acid. Butyric acid is a volatile, saturated fatty acid and northern hardwoods are not generally noted for containing volatile oils. Lime, however, has previously been reported to contain an oil consisting of butyric and higher fatty acids (Mutton, 1962). The presence of volatiles is also discussed in Appendix II in connection with the inhibition of spore germination on lime sapwood.

2.4.3 Amino acids of *Chaetomium globosum*

The total quantities of amino acids, revealed by the analysis of vegetative mycelia and the reproductive structures (Table 4), showed a similar amount of amino acid/g fungus in the two fractions. The total nitrogen contents determined by Kjeldahl analysis also showed a similar level in the two fractions (Table 5). The difference between the nitrogen content of the vegetative and reproductive fractions was revealed in their respective amino acid compositions. Glutamic acid predominated in the vegetative fraction with aspartic acid as the next most abundant amino acid. In the reproductive fraction, the level of glutamic acid was greatly reduced along with a reduction in the quantities of many of the other amino acids. The levels of isoleucine and leucine, however, were increased to approximately twice their vegetative level in the re-

productive fraction. It is possible that this difference in amino acid composition was due to the different growth media used to provide vegetative and reproductive growth. It may also be postulated that this increase in leucine and isoleucine was brought about by, or responsible for, the physiological changes which occur on sporulation of the fungus.

The composition of the nitrogen fraction of the fungus is, in general terms, similar in ratio, though not in the order of magnitude, to the timbers. Aspartic acid, glutamic acid, glycine and alanine were amongst the most abundant amino acids of the fungus, as they were in the timbers.

2.4.4 Significance of wood and fungal amino acid composition

The ability of decay fungi to grow on wood as the sole source of nutrients indicates their apparent efficiency in utilising nitrogen from the wood. As much of the nitrogen in wood occurs as amino acids, either free or bound in proteins and peptides, it is important for the wood attacking fungi to be able to utilise amino acid nitrogen. The amino acids of wood may be taken up by the decay fungi and be incorporated directly into their own proteins or, alternatively, the amino acid nitrogen may be converted to ammonium-nitrogen prior to re-synthesis into fungal protein. The similarity between the major amino acids of the timbers and the soft-rot organism make it

possible for a direct uptake of many of the amino acids from wood and incorporation into fungal proteins. This would not be possible if the amino acids predominant in the timbers were not those which predominated in the fungus. A breakdown of amino-nitrogen to ammonium-nitrogen would then have to take place, prior to fungal utilisation.

The direct assimilation of organic nitrogen was hinted at by Foster (1949) and the more rapid growth of Basidiomycete wood decay fungi on organic nitrogen than on inorganic nitrogen was discussed by Cowling and Merrill (1966). Henningsson (1968) found that a number of Basidiomycete fungi, capable of attacking birch and aspen pulpwood, were able to utilise the amino acids found in aspen wood by Merrill and Cowling (1966). The growth of these decay fungi was greater on some of these amino acids than on inorganic nitrogen sources. These reports appear to support the possibility of direct amino acid assimilation by the fungi. If this ability to directly utilise organic nitrogen sources is widespread amongst decay organisms, then it also means that the lysis of hyphae of soft-rot fungi (and other early wood colonisers) will release nitrogen compounds which can readily be utilised by succeeding organisms in the timber. Thus, the nitrogen compounds may be recycled several times during the decomposition process.

CHAPTER 3

STUDIES ON THE AMINO ACID NUTRITION OF WOOD DECAY FUNGI.

3.1 Introduction

Nitrogen is present in wood as amino acids, nitrates, peptides and proteins. The free amino acids and the amino acid constituents of wood proteins were identified in the previous chapter and the fairly close correspondence with the amino acid content of a typical soft-rot organism was discussed.

Basidiomycete fungi which are able to cause wood decay are known to be able to utilise amino acid nitrogen (Henningsson, 1968) but little is known about the utilisation of amino acids by soft-rot fungi. The lack of information in this particular field prompted the execution of experiments reported in this chapter on the amino acid nutrition of a selection of wood decay fungi.

One of the most important physiological factors to be considered when dealing with wood decay fungi is that of cellulolytic activity. The availability of different nitrogen sources may affect the cellulase production of fungi and so alter their decay abilities. This aspect of fungal physiology is also investigated in the present chapter.

3.2 Test organisms

Cultures of the Fungi Imperfecti, Trichoderma viride Pers. ex Fr. and Alternaria tenuissima (Fr.) Wiltsh. were obtained from the culture collection at the Biodeterioration Information Centre, University of Aston in Birmingham.

Cultures of the Basidiomycete, Coriolus versicolor (L. ex Fr.) Quél. and the Ascomycete, Chaetomium globosum Kunze ex Fr. were provided by the Princes Risborough Laboratories, Aylesbury, Buckinghamshire.

C. globosum, A. tenuissima and T. viride are fungi capable of causing soft-rot decay of timber, while Coriolus versicolor causes white rot of timber.

Princes Risborough Laboratories supplied 10 isolates of Chaetomium globosum and a further isolate was obtained from the culture collection of the Biological Sciences Department, Aston University. The growth rates and relative cellulolytic activities of the isolates were determined by inoculation on to a cellulose medium in Petri dishes and test tubes. These were incubated at 25°C and, after 6 days, the colony diameters were measured from the Petri plates and, after 14 days, the depth of cellulose clearing was measured in the test tubes.

A wide variation in growth and cellulolytic activity was revealed (Table 6) and so a single isolate (S70P), which closely approximated the mean in colony diameter and cellulose clearing, was selected to represent C. globosum in decay tests. Throughout the present investigation, isolate No. S70P was used when C. globosum was required.

Fungal cultures were maintained on 2% malt agar at 25°C unless otherwise stated.

3.3 Amino acids as sole nitrogen source

Table 6: A comparison of C. globosum isolates.

Identification Code	Colony diameter (cm)	Depth of cellulose agar cleared (mm)
2/2 SCNc	6.7	3.2
3/1 SCNa	6.4	3.8
S70E	7.4	1.6
51/5 SCNb	6.6	4.3
S70G	4.1	1.3
S70L	8.0	2.0
16/5MAa	6.4	4.7
S70D	7.6	3.7
S70P *	6.5	3.3
S70F	5.8	1.7
2g	7.3	4.3
Average	6.6	3.1

Results are the means of 5 replicates.

* S70P was the isolate used in subsequent experiments.

3.3.1 Materials and methods

A basal cellulose agar medium, without a substantial nitrogen source, was prepared as proposed in Bravery's (1968) modification of Eggins and Pugh's (1962) cellulose agar. The nitrogen inherent in agar and that contained in thiamin hydrochloride was considered to be negligible when compared to the nitrogen levels subsequently added.

Basal medium:

KH_2PO_4	1.0g
KCl	0.5g
$MgSO_4.7H_2O$	0.2g
$Ca\ Cl_2$	0.1g
Thiamin hydrochloride	0.001g
Agar	12.0g
Cellulose	10.0g
Distilled water	1000 ml

Whatman cellulose powder was ballmilled for 72 hours to reduce particle size and then used as the cellulose source. It was prepared as a 4% suspension in distilled water and incorporated into the medium as 250ml/litre, which gave 1% cellulose in the final medium.

The nitrogen sources investigated included the amino acids identified from the sapwoods of pine, beech and lime, and two inorganic sources: $(NH_4)_2\ SO_4$ and $NH_4\ NO_3$. The percentage nitrogen content of each nitrogen source was calculated (Appendix I). The agar medium was divided into 100ml aliquots and nitrogen sources added to

give 10mg, 15mg and 20mg of nitrogen per aliquot.

Soft-rot fungi have been shown to grow within a wide pH range (Sharp and Eggins, 1970) but to simulate decay conditions a pH similar to that of timber was required. Samples of lime, beech and pine were milled in a Culatti micro-hammer mill and 10ml of distilled water added to 2g of milled wood. Samples were mechanically shaken for 15 minutes before measuring the pH. The results are summarised below as the means of 10 samples per timber:

Lime	pH 4.6
Beech	pH 5.2
Pine	pH 4.8

The aliquots of medium were subsequently buffered to pH 5.0 with sodium acetate and autoclaved at 15 p.s.i.* (120^{o}C) for 20 minutes. Little or no pH change occurred in the medium when autoclaved.

When cool, the agar was poured into labelled Petri dishes in quantities of 10ml/dish and allowed to set. This procedure resulted in plates which contained nitrogen levels of 1.0mg, 1.5mg and 2.0mg for each nitrogen source. The carbon to nitrogen (C:N) ratios of these plates were approximately 40:1, 30:1 and 20:1 respectively.

One week old cultures of the test fungi were used to inoculate the agar plates. The plates were incubated at 25^{o}C and two colony diameters, at right angles to one another, measured on each plate on alternate days of incubation.

* 15 p.s.i. = 1.0 x 10^{5} Pascals

For each fungus, nitrogen source and nitrogen concentration, three replicate plates were incubated.

3.3.2 Results

The results are summarised as histograms in Figures 1-4. Colony diameters of C. globosum, A. tenuissima and Coriolus versicolor were recorded after six days of incubation, while colony diameters of the rapid growing T. viride were recorded after only two days of incubation.

The fungi revealed a number of responses to increased nitrogen concentration and each of the following patterns was found:

a) increased colony diameter with increased nitrogen.

b) decreased colony diameter with increased nitrogen.

c) similar colony diameter with each nitrogen concentration.

d) maximum colony diameter with intermediate nitrogen concentrations.

e) minimum colony diameter with intermediate nitrogen concentrations.

Results were subjected to an analysis of variance for each nitrogen source at three concentrations and the statistical probabilities recorded in Appendix I. Confidence limits were calculated at the 95% level for each

Figure 1: Growth of Chaetomium globosum after six days incubation.

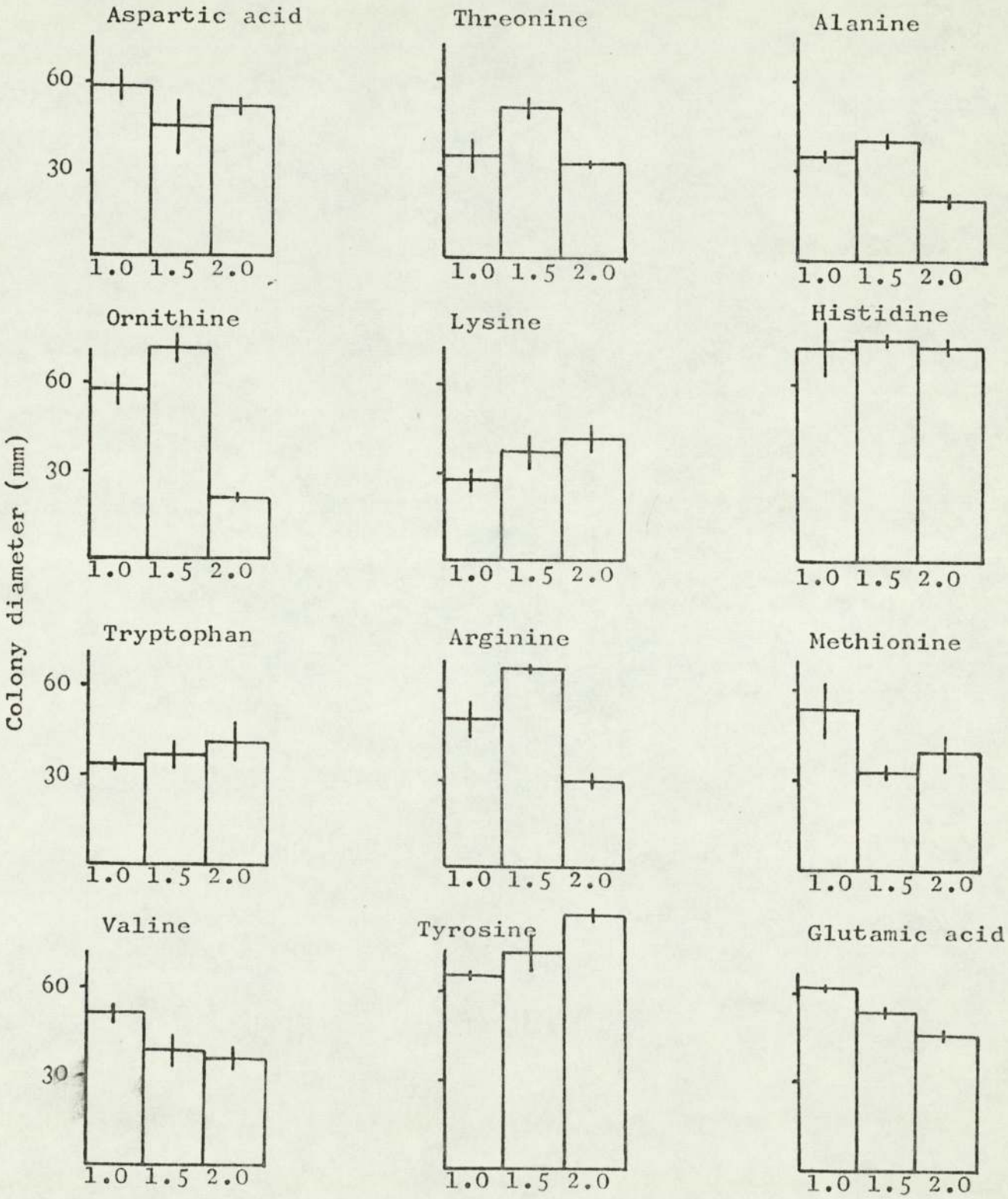


Figure 1 (continued):

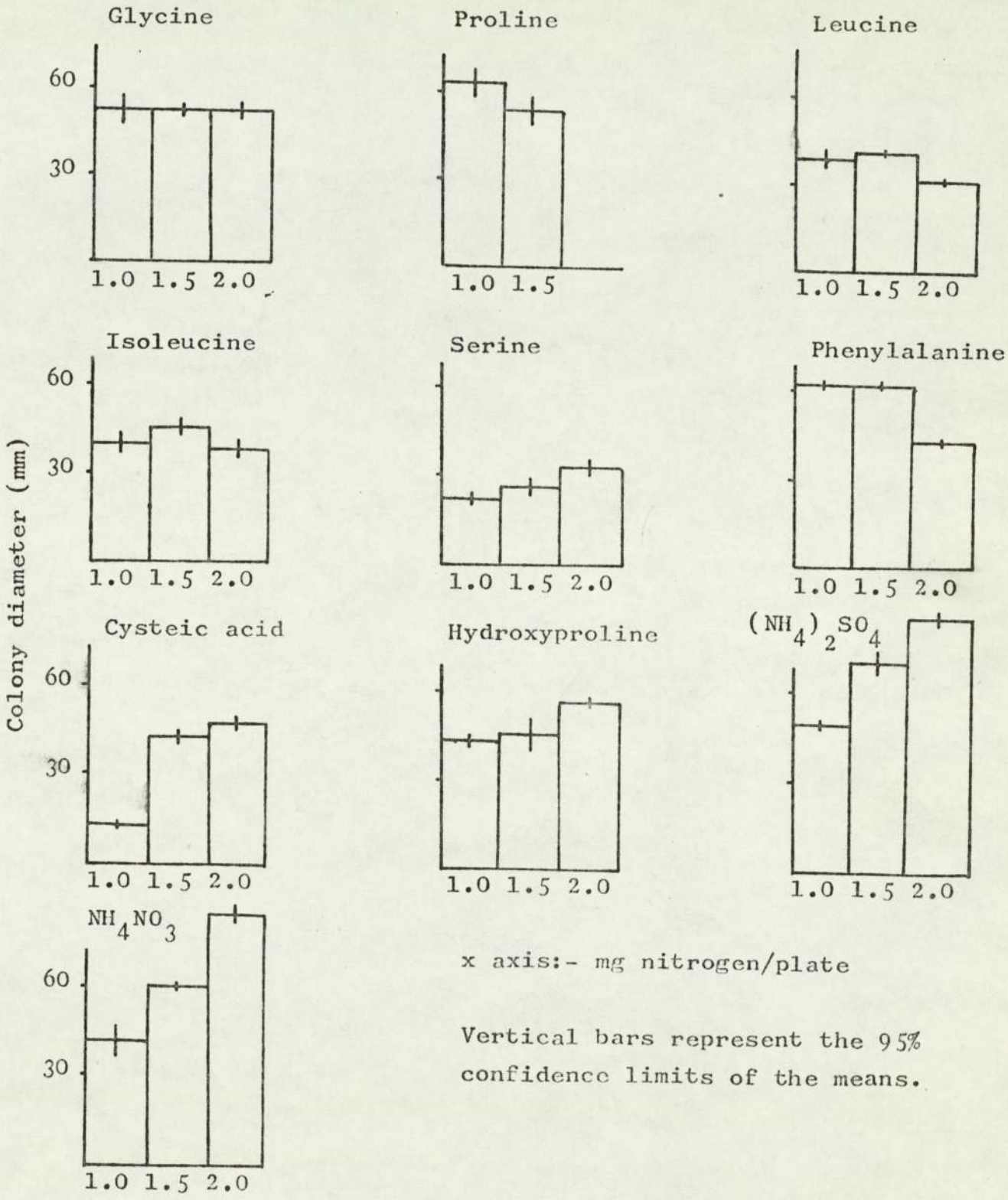


Figure 2: Growth of Alternaria tenuissima after six days incubation.

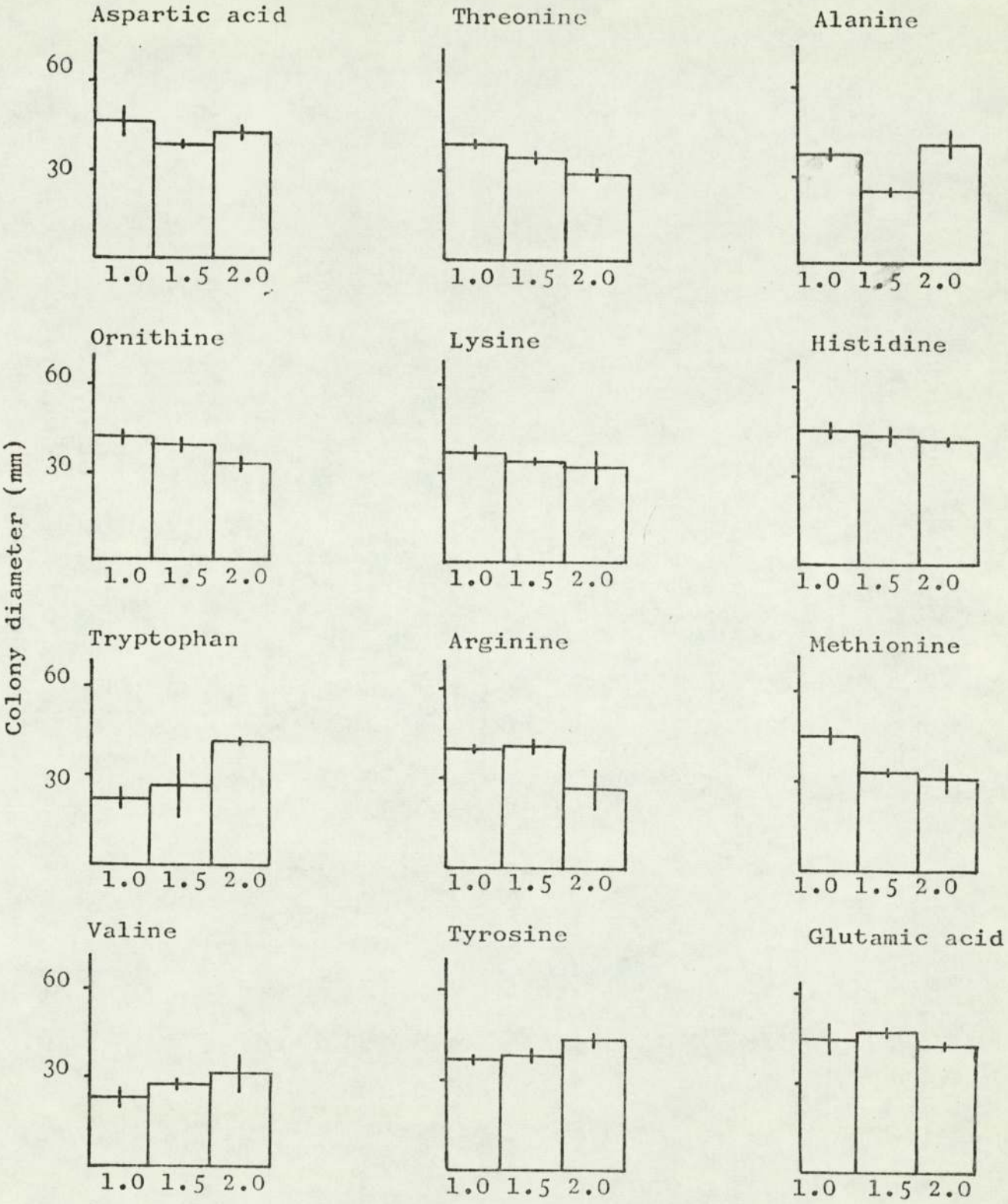


Figure 2 (continued):

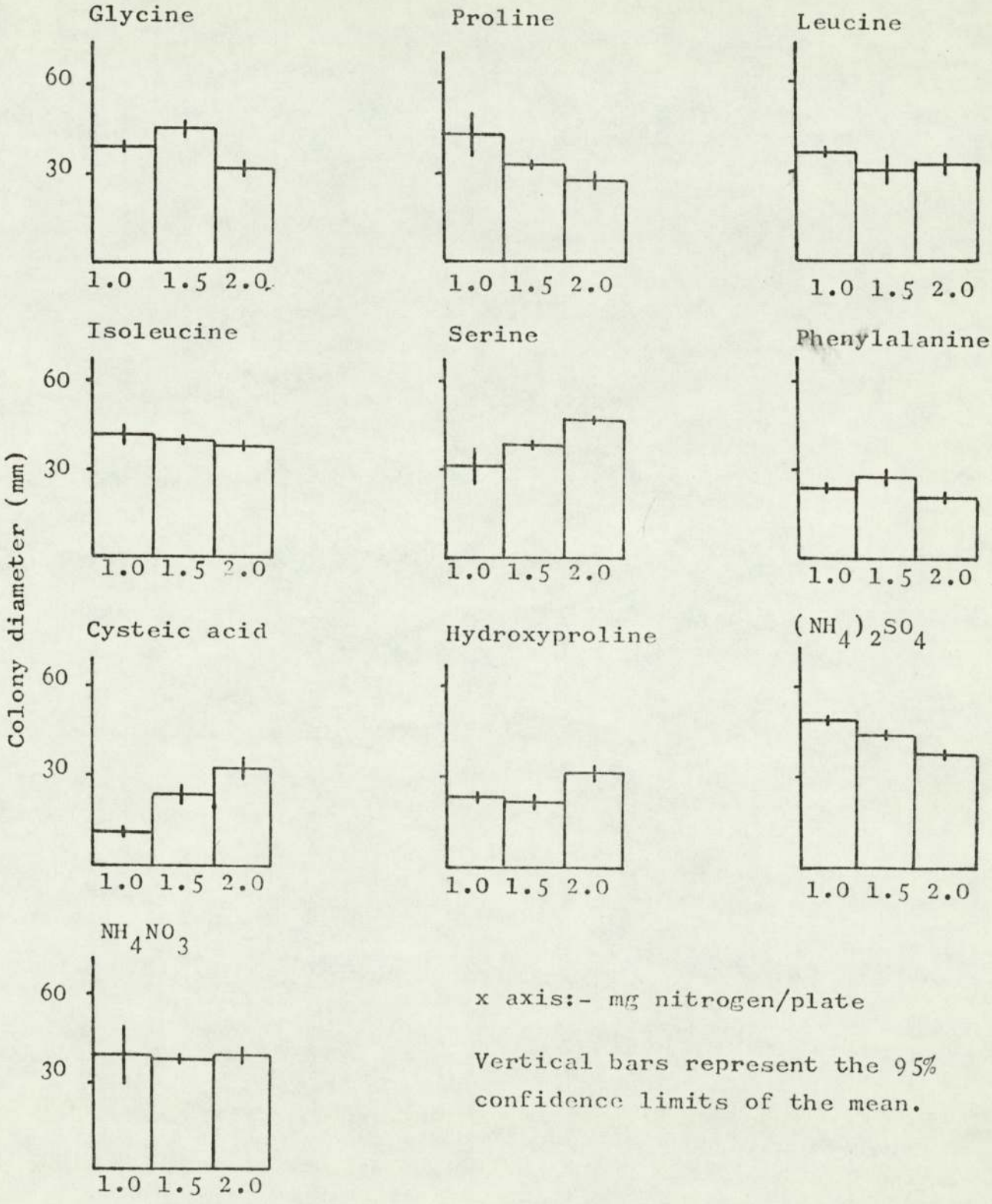


Figure 3: Growth of Trichoderma viride after two days incubation.

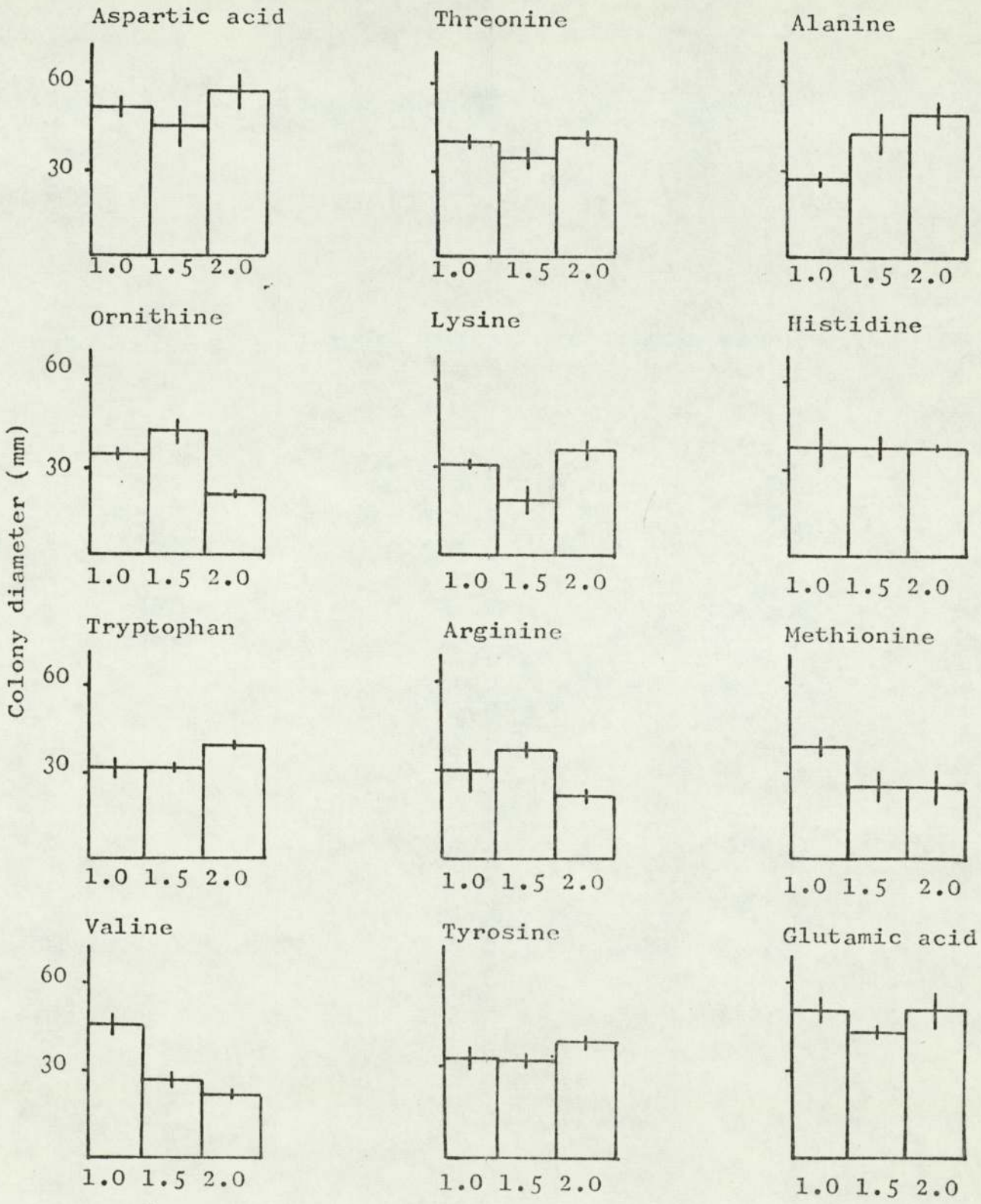


Figure 3(continued):

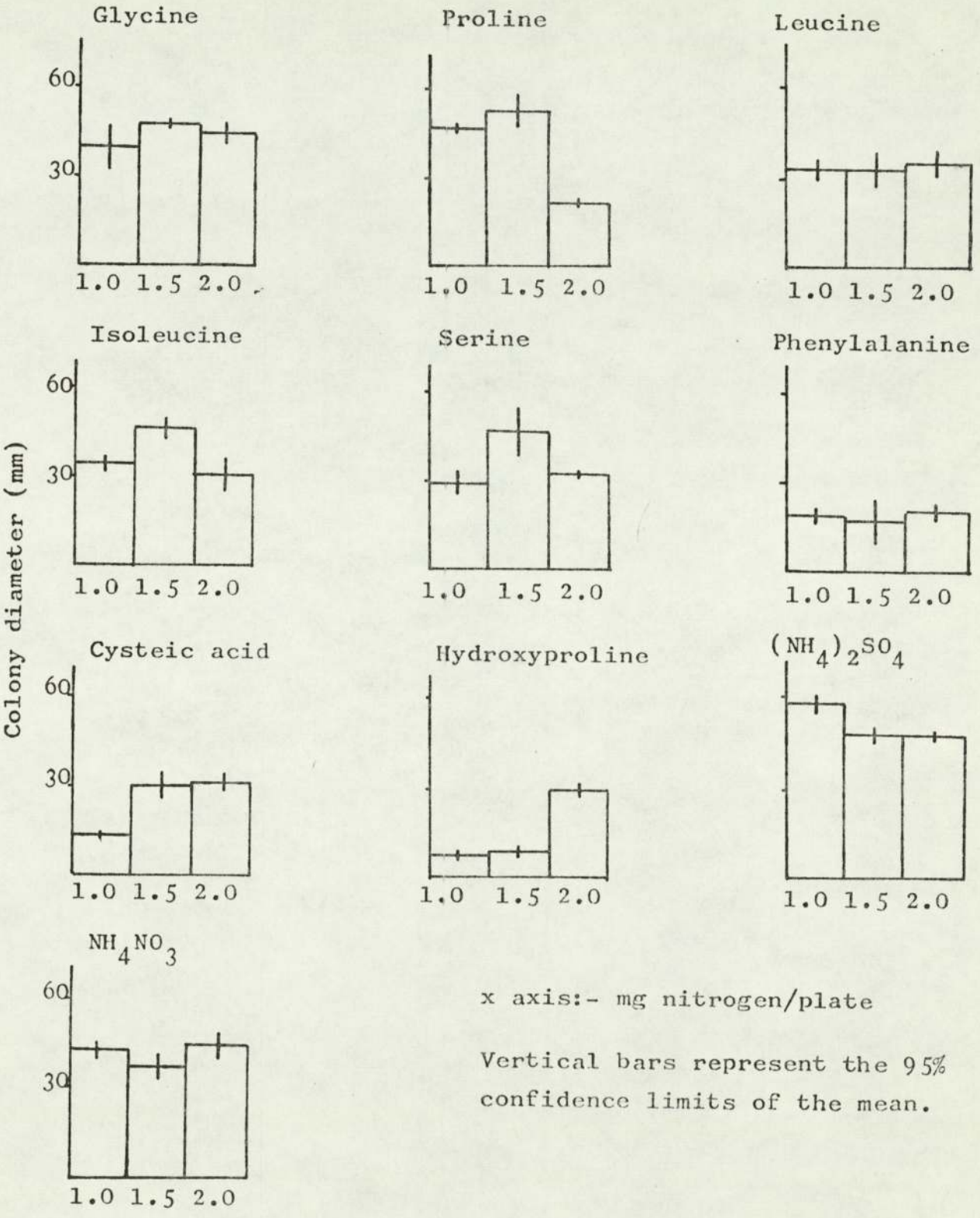


Figure 4: Growth of Coriolus versicolor after six days incubation.

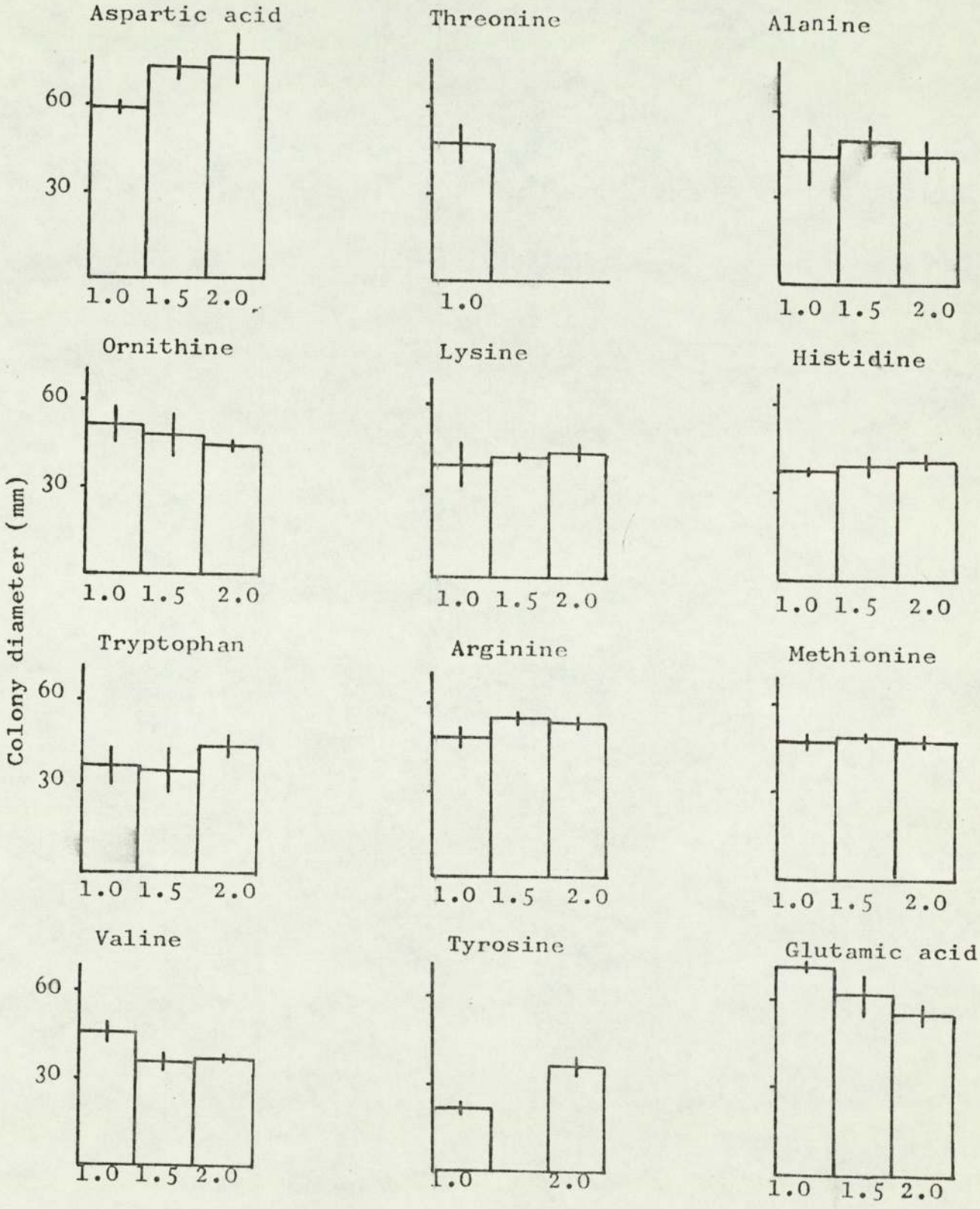
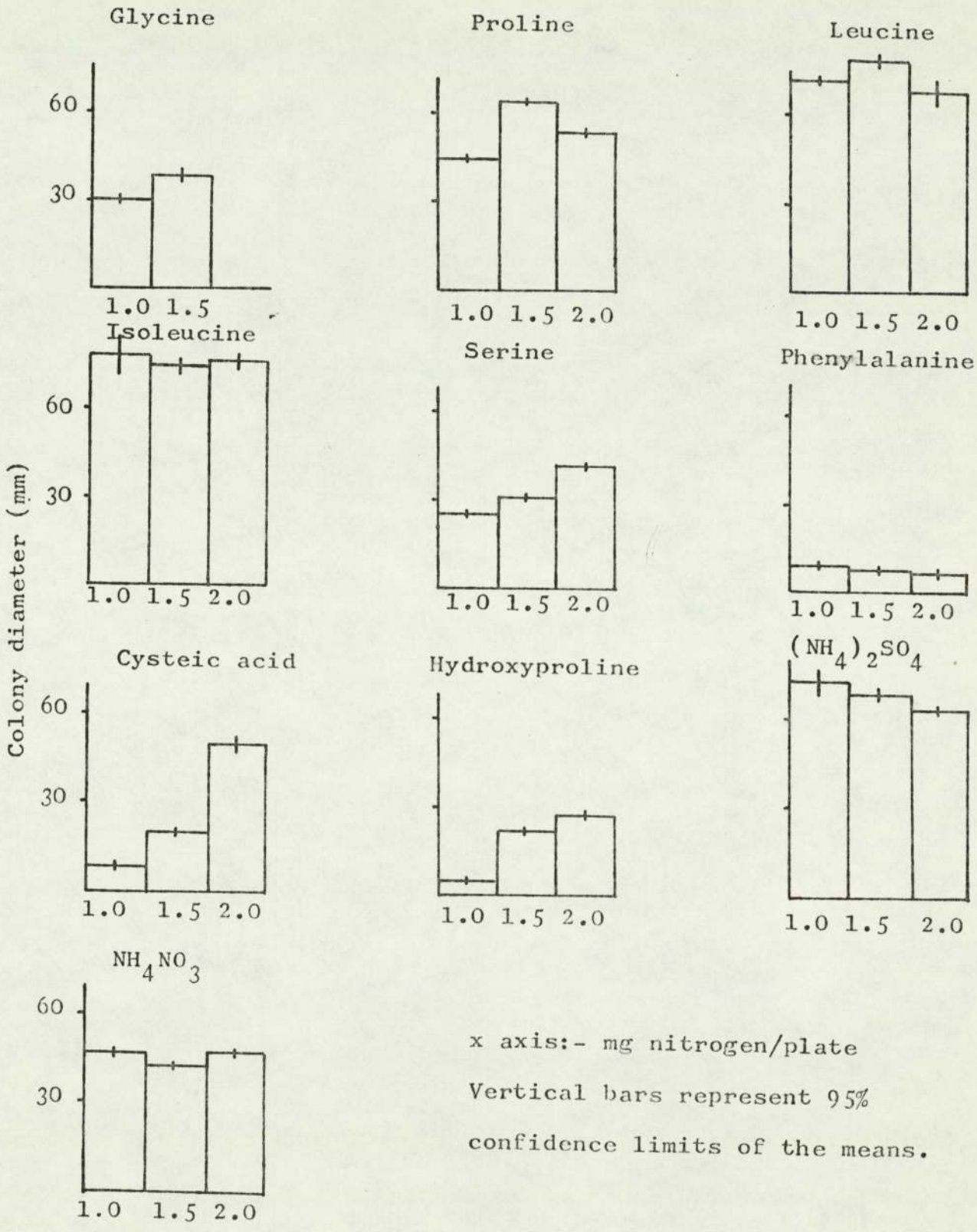


Figure 4 (continued):



set of results and recorded on individual histograms (Figs. 1-4).

The fungi showed the same response on only three nitrogen sources. All four fungi increased their colony diameters with increased concentrations of tyrosine, cysteic acid and hydroxyproline. Coriolus versicolor was the most insensitive fungus to nitrogen concentration increases and showed no response to increased concentrations of nine of the nitrogen sources. The soft-rot organisms showed no response to increased nitrogen on four nitrogen sources in the case of T. viride and A. tenuissima and five nitrogen sources in the case of Chaetomium globosum.

Figures 5-8 show the relative growth of the fungi on the different nitrogen sources. Growth on $(NH_4)_2SO_4$ was given the value of 100%. Each relative value was based on the mean of 27 observations (three concentrations, three incubation periods and three replicates). The fungi showed some similarities in preference of nitrogen source:- glutamic and aspartic acid were good growth supporters while cysteic acid, phenylalanine and hydroxyproline were, in general, poor growth supporters. C. globosum was able to exploit the latter two amino acids to a greater extent than the other fungi. The soft-rot organisms were able to utilise both inorganic nitrogen sources well, but achieved their best growth on $(NH_4)_2SO_4$. Coriolus versicolor differed in having relatively poor growth on NH_4NO_3 and its growth on leucine, isoleucine and aspartic acid was better than that on $(NH_4)_2SO_4$.

Figure 5: Relative growth of Chaetomium globosum on different nitrogen sources.

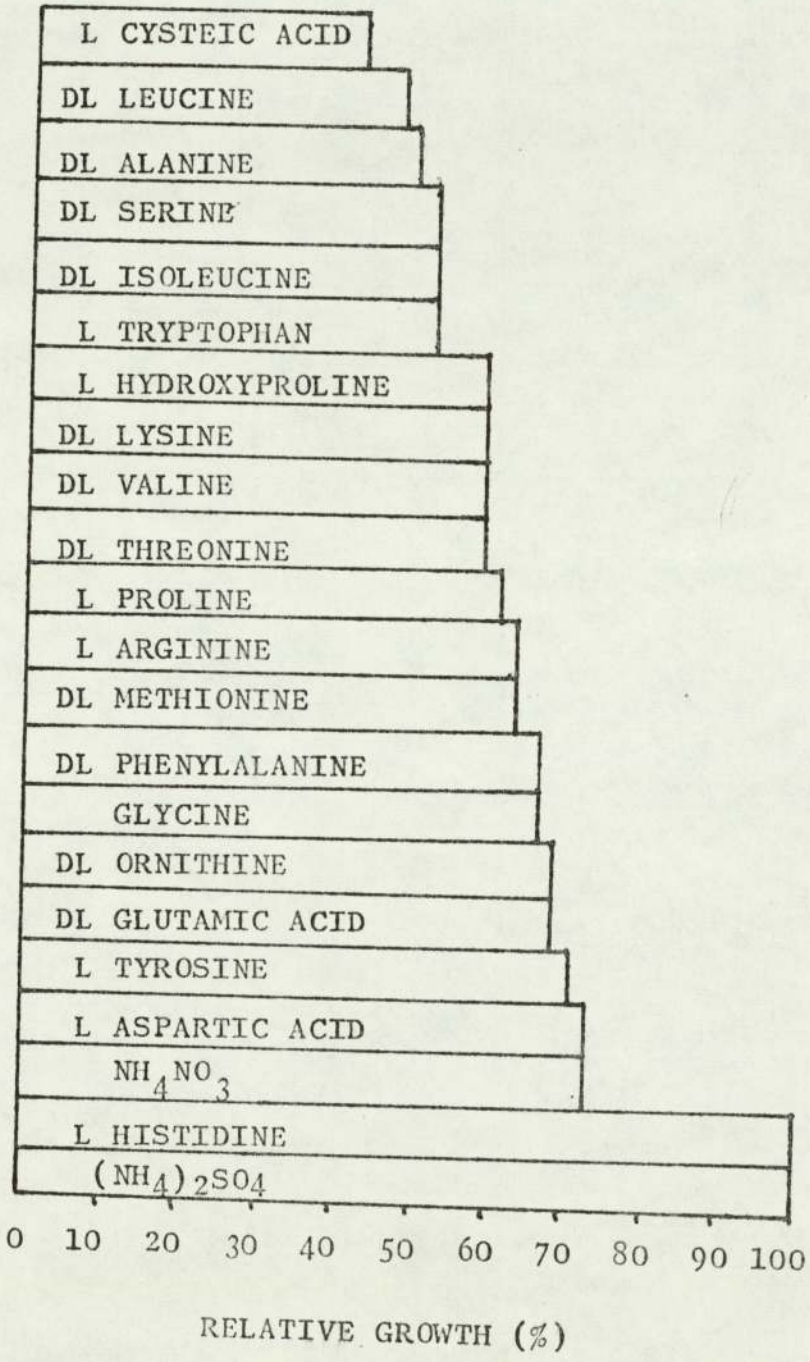


Figure 6: Relative growth of Alternaria tenuissima on different nitrogen sources.

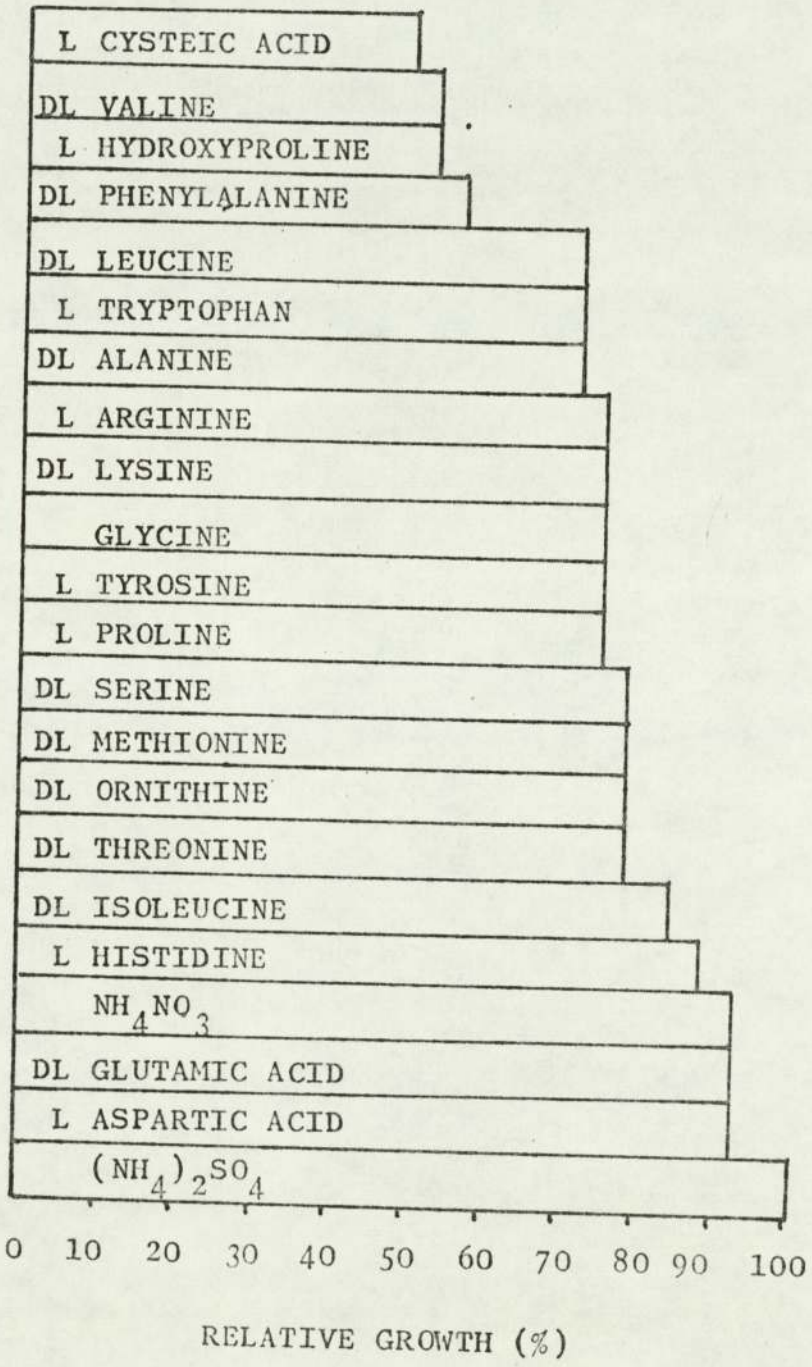


Figure 7: Relative growth of Trichoderma viride on different nitrogen sources.

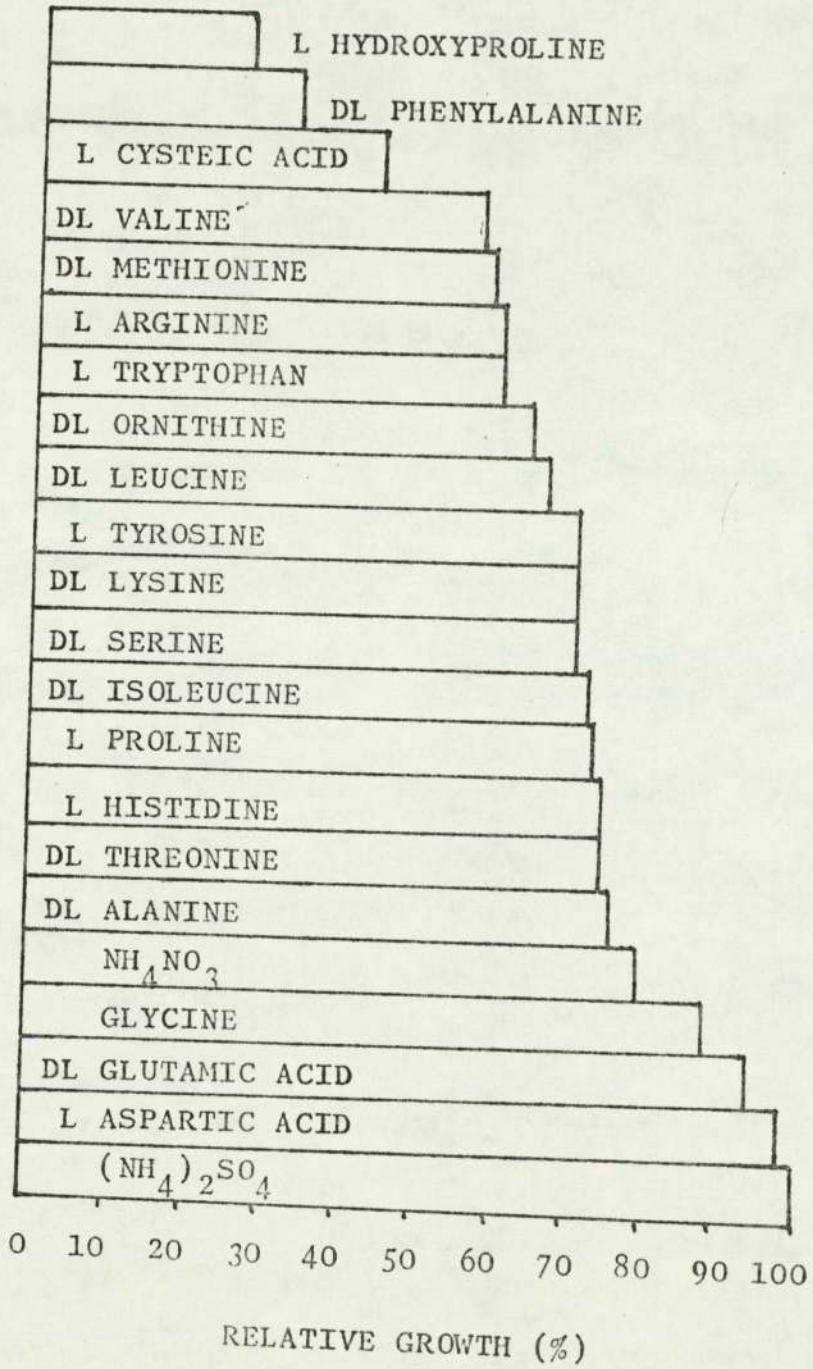
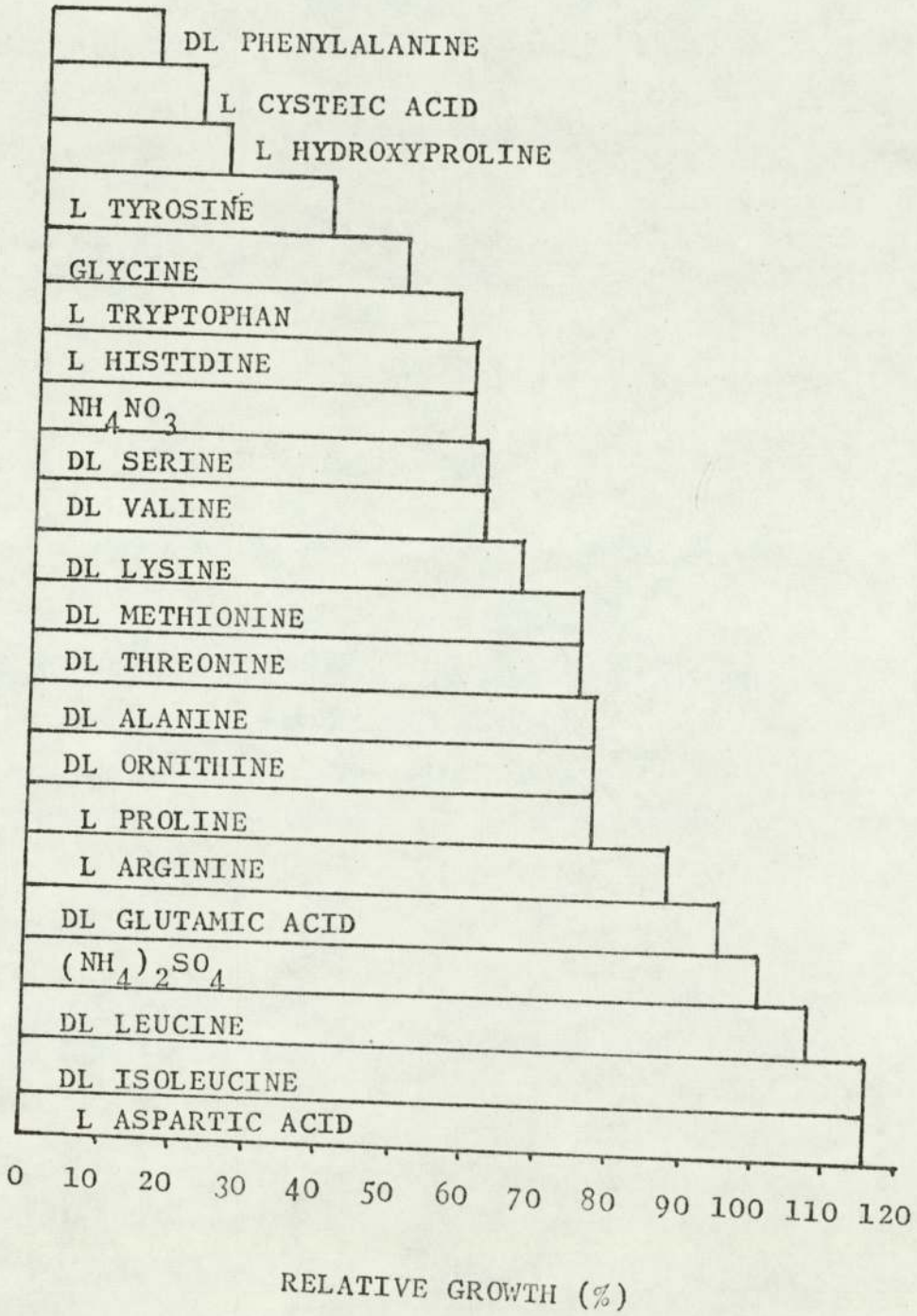


Figure 8: Relative growth of Coriolus versicolor on different nitrogen sources.



3.3.3 Discussion

The amino acids utilised in these laboratory experiments were either L-isomers or racemic mixtures. In living organisms, amino acids are generally found as the L-isomer. It may be debated that the use of different isomeric configurations of pure amino acids in nutritional experiments does not allow comparison of amino acid utilisation. However, it has been shown that fungi belonging to the Ascomycetes and Fungi Imperfecti are capable of similar growth on either D- or L-isomers of amino acids. The fungi contained D-amino acid oxidases which were thought to be capable of breaking down the D-isomer and allowing the resynthesis as the L-isomer (Horowitz, 1944; Emerson, Puziss and Knight, 1950).

The presence of D-amino acid oxidases in Basidiomycetes has not been detected and Jennison and Perritt (1960) found that a number of wood decay Basidiomycetes were not able to utilise D-isomers of two particular amino acids. When supplied with racemic mixtures of these amino acids the fungi were able to grow but only produced half the growth of that produced on similar levels of pure L-isomers. This apparent inability to utilise the D-isomer of amino acids may also be true of Coriolus versicolor and hence affect the results of the present experiments.

Results from the present experiments which involved C. versicolor showed that the growth on racemic mixtures was generally higher than that on amino acids supplied solely as the L-isomer. If C. versicolor was unable to utilise the

D-isomers then the growth on the racemic mixtures should be doubled in order to compare them with growth on amino acids supplied as L-isomers. This would give very high results on these amino acids and the relative growth would be much higher than on $(NH_4)_2SO_4$. It appears likely, then, if C. versicolor cannot utilise D-isomers, that the fungus had not exhausted the supply of L-isomers in the amino acids during the present experiment.

Merrill, Levi and Cowling (1966) and Levi, Merrill and Cowling (1968) carried out experiments which showed that C. versicolor rapidly utilised various nitrogenous compounds which occurred naturally in wood or became available from autolysis of mycelium during decay. Their experiments indicated that amino acids, proteins and peptides supported more rapid growth than nucleic acids, nucleotides and cell wall materials.

In the present investigation, Chaetomium globosum, A. tenuissima, T. viride and Coriolus versicolor utilised a variety of amino acids as nitrogen sources, and aspartic and glutamic acids generally supported the greatest amount of growth. Much of the earliest work concerned with the effects of amino acids as nitrogen sources on fungal growth was carried out by Leonian and Lilly (1938, 1940) and Steinberg (1942) in liquid cultures. Studies of this type have been repeated by Pelletier and Keitt (1954), Jennison, Newcomb and Henderson (1955), Merrill and Cowling (1968), Henningsson (1968) and Ibbotson (1974) with a variety of fungi. Most investigations have shown aspartic and glutamic acids to be the amino acids most likely to support

good fungal growth. The relative utilisation of amino acids shown in Figures 5-8 followed a pattern broadly based on the chemical nature of the amino acids supplied. The monoamino dicarboxylic acids, glutamic and aspartic, were consistently amongst the supporters of good growth. The amino acids which supported slightly poorer growth included glycine, alanine, serine, threonine, valine, leucine and isoleucine i.e. the monoamino monocarboxylic acids. Those amino acids which contain sulphur (cysteic acid and methionine) and those with a cyclic structure (proline, tyrosine, phenylalanine, hydroxyproline, tryptophan, lysine, arginine and histidine) did not tend to support such high fungal growth.

Results on the influence of nitrogen source concentration on the radial growth of Chaetomium globosum, A. tenuissima, T. viride and Coriolus versicolor showed individual preferences between the fungi. A number of growth response patterns to nitrogen concentration were seen which did not conform to the widely held idea of increased nitrogen leading to increased growth. The fungi did not always respond to increases in nitrogen concentration and in some instances growth was lower on higher nitrogen concentrations. Similar experiments have been carried out by Pelletier and Keitt (1954) in which Venturia inaequalis was grown in liquid cultures containing different amino acids as nitrogen source. They, too, found that the fungus responded in a diverse fashion to the amino acids and that growth responses varied with both different amino acids and their concentration. Park (1976) found a number of

fungal response patterns to different nitrogen concentrations of inorganic nitrogen when various cellulolytic fungi were used. Substrate weight losses at three nitrogen concentrations were recorded and his results showed similar growth response patterns to those observed in the present investigation.

3.4 Amino acids as sole nitrogen and carbon source

3.4.1 Materials and methods

A basal medium was prepared which omitted a major carbon and nitrogen source. The constituents of the medium were:

KH_2PO_4	1.0g
KCl	0.5g
$MgSO_4.7H_2O$	0.2g
$CaCl_2$	0.1g
Thiamin hydrochloride	0.001g
Distilled water	1000ml

Aspartic acid, glutamic acid, leucine, threonine, histidine and asparagine were used as organic sources of carbon and nitrogen. $(NH_4)_2SO_4$ and NH_4NO_3 were used as inorganic nitrogen sources with glucose added to provide a carbon source.

The basal medium was divided into 450ml aliquots. Amino acids were added in quantities which gave 45mg carbon/450ml medium. C:N ratios were calculated for each amino

acid (Appendix I) and the two inorganic nitrogen sources plus glucose were added to the medium at a ratio equivalent to the average amino acid C:N ratio i.e. a 3:1 ratio. The media were buffered to pH 5.0 with sodium acetate.

50ml quantities of media were pipetted into 250ml Erlenmeyer flasks and closed with cotton wool plugs. The flasks were sterilised at 15 p.s.i. (120°C) for 20 minutes and allowed to cool.

Flasks were inoculated with mycelium from the growing edge of 7 day-old cultures of Chaetomium globosum, T. viride or Coriolus versicolor and incubated at 20°C on a shaker table. Flasks were replicated three times for a fungus on each carbon and nitrogen source.

After 21 days incubation the flasks were removed and the contents filtered through Whatman No. 1 filter papers. The filter papers and mycelial masses were oven-dried to constant weight and the mycelial dry weights recorded.

3.4.2 Results

The dry weights of mycelia produced in the culture flasks are recorded in Table 7. The fungi utilised the carbon and nitrogen from the amino acids to varied extents. Growth on glutamic and aspartic acids was similar to that on the two inorganic nitrogen sources plus glucose in all of the fungi investigated. Threonine supported relatively good growth of Chaetomium globosum and Coriolus versicolor but it supported only poor growth of T. viride. Leucine supported mediocre growth of Chaetomium globosum and

Table 7: Mycelial dry weights of fungi grown on a selection of amino acids as sole carbon and nitrogen source.

Mycelial dry weight (mg /100ml nutrient)

Carbon + nitrogen source	Chaetomium globosum	Trichoderma viride	Coriolus versicolor
NH_4NO_3 + glucose	32.5	43.0	29.3
$(NH_4)_2SO_4$ + glucose	29.1	39.5	20.6
Aspartic acid	27.5	41.3	19.0
Glutamic acid	26.8	43.8	25.8
Leucine	16.0	16.1	16.8
Threonine	22.2	15.6	25.7
Histidine	14.5	4.6	4.3
Asparagine	14.0	15.8	21.1

Results are the means of 3 replicates.

Coriolus versicolor and only poor growth of T. viride. Growth on asparagine followed a similar pattern; C. versicolor produced relatively good growth, T. viride poor growth and Chaetomium globosum mediocre growth. Coriolus versicolor and T. viride produced very little growth on histidine, although Chaetomium globosum again produced mediocre growth.

In summary, aspartic and glutamic acids proved to be good growth supporters when supplied as sole carbon and nitrogen sources. The other amino acids investigated supported lower growth than aspartic and glutamic acids, with the exception of the good growth of Coriolus versicolor on threonine and histidine.

3.4.3 Discussion

The results of these experiments revealed the ability of the test fungi to utilise amino acid carbon when amino acids were supplied as the major carbon source. Keratinophilic fungi have also been shown to have the ability to utilise carbon in amino acids (Ibbotson, 1974). The relevance of this information is concerned with the cellulolytic abilities of decay fungi. Cellulases are only produced by fungi when cellulose is present i.e. they are induced enzymes, and the presence of other more readily utilised carbon sources may reduce cellulase production. This phenomenon has been reported by Bravery (1968) and Park (1973) who found that media which contained asparagine supported less cellulolytic activity than media from which asparagine was omitted.

The effect of different concentrations of an alternative carbon source (glucose) on the cellulolytic activity of a number of mesophylic fungi was observed by Malik (1970). He reported that the relative cellulolytic activity of most of the investigated fungi tended to decrease with the increase in alternative carbon source.

It is possible, therefore, that the observed cellulolytic activities of decay fungi on laboratory media containing inorganic nitrogen sources are higher than the organisms true activity on wood.

3.5 The effect of amino acids on cellulolytic activity

3.5.1 Materials and methods

A modification of the method originally described by Rautela and Cowling (1965) was employed. The method involves the measurement of the depth of clearing of an opaque column of cellulose agar contained in a tube.

Cellulose agar was prepared as described in section 3.3.1 and nitrogen sources added to give 1mg nitrogen/10ml agar (C:N ratio of approximately 40:1). The nitrogen sources investigated were aspartic acid, glutamic acid, leucine, histidine, glycine, serine, asparagine, tyrosine, $(NH_4)_2SO_4$ and NH_4NO_3. The media were buffered to pH 5.0 with sodium acetate and 10ml aliquots pipetted into test tubes. Aluminium Oxoid caps were used to cover the test tubes which were then autoclaved at 15 p.s.i. (120°C) for 20 minutes. On removal, the test tubes were transferred to a 40°C water bath and allowed to cool. The tubes were

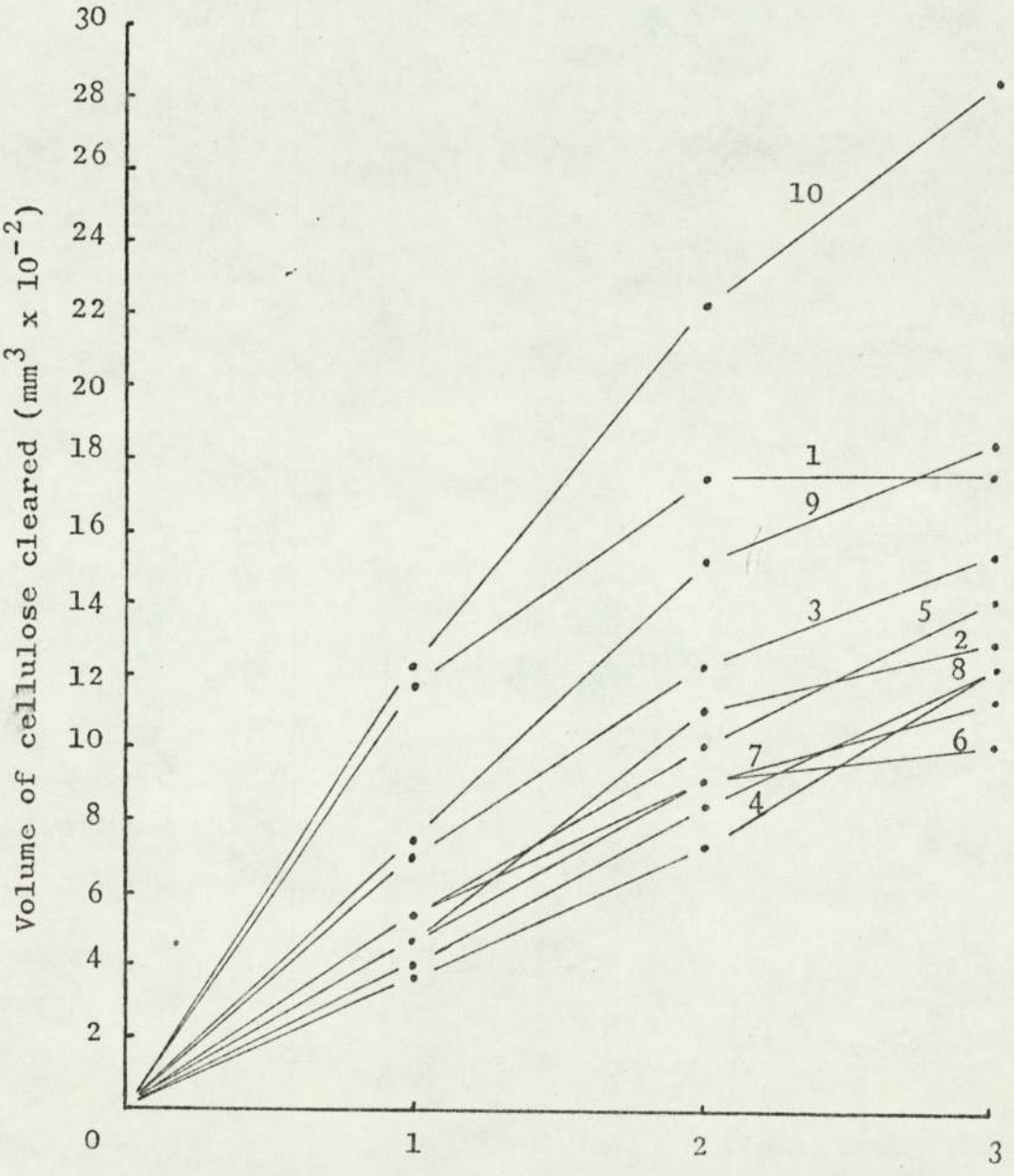
agitated on a rotary Whirlimix when cool to ensure that the cellulose particles were evenly dispersed. Freshly crushed ice was packed around the test tubes so that the agar set rapidly. This ensured that the cellulose particles did not precipitate out.

The opaque media were inoculated with mycelium of T. viride, A. tenuissima, Chaetomium globosum or Coriolus versicolor taken from the growing edge of a 7 day old culture. The tubes were then incubated at 25°C. At weekly intervals, the tubes were removed from incubation and the depth of cellulose cleared was measured in three replicates for each organism on each nitrogen source.

3.5.2 Results

The results are expressed graphically in Figures 9 and 10 as volumes of cellulose agar cleared against incubation time. Volumes were calculated to remove possible errors of comparison between test tubes of different diameters. Coriolus versicolor, Chaetomium globosum and A. tenuissima had reached maximum cellulose clearing on many of the nitrogen sources by the end of two weeks incubation. In contrast, T. viride showed increases in the volumes of cellulose cleared up to the final incubation period. Coriolus versicolor, Chaetomium globosum and A. tenuissima all produced the greatest volumes of cellulose cleared when grown on medium which contained aspartic acid as the nitrogen source. The next most effective nitrogen sources for promoting cellulose clearing by these fungi were the inorganic sources and glutamic acid. T. viride cleared

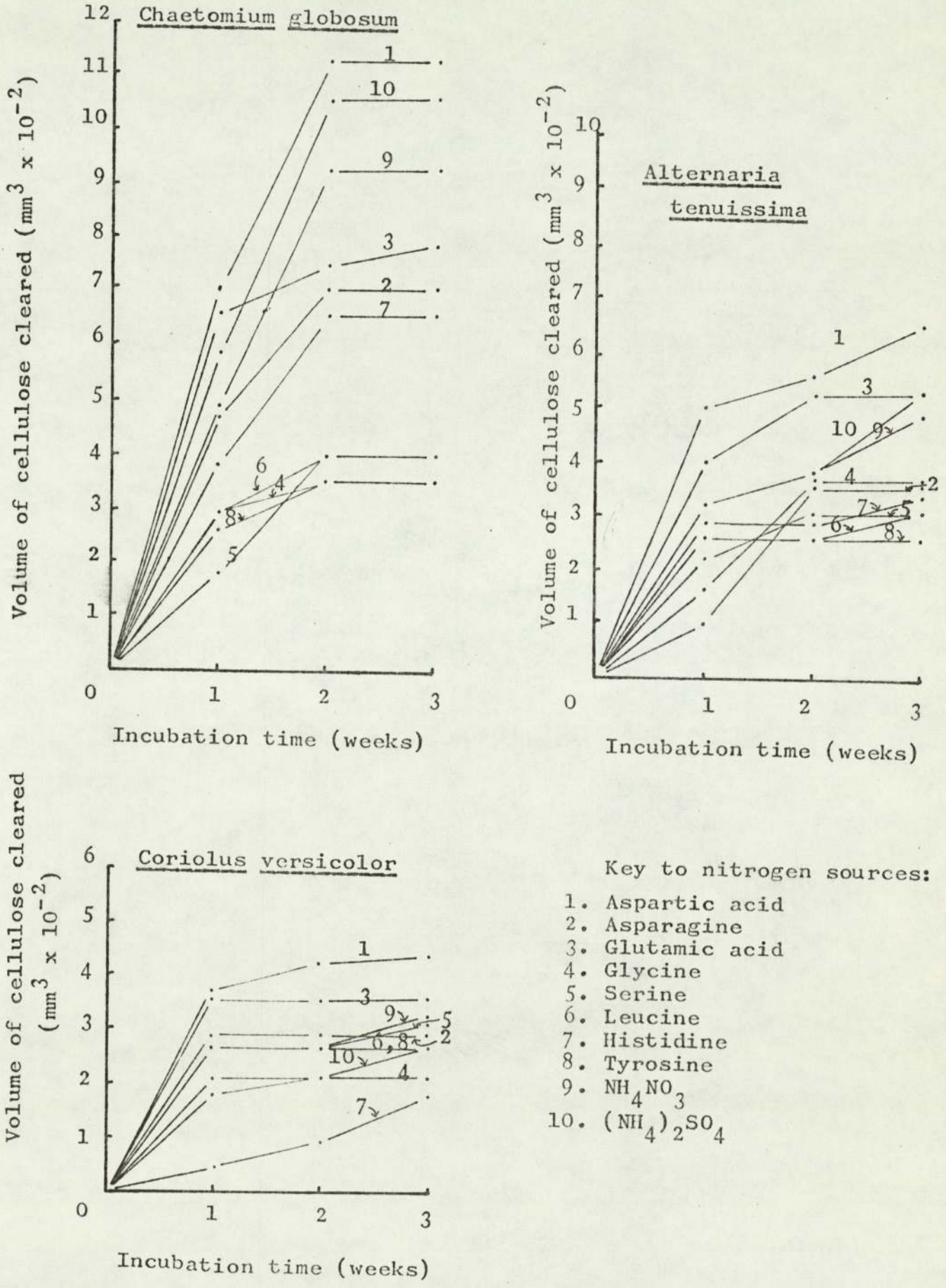
Figure 9: Cellulose clearing by Trichoderma viride.



Key to nitrogen sources:

1. Aspartic acid
2. Asparagine
3. Glutamic acid
4. Glycine
5. Serine
6. Leucine
7. Histidine
8. Tyrosine
9. NH_4NO_3
10. $(NH_4)_2SO_4$

Figure 10: Cellulose clearing by fungi.



Key to nitrogen sources:

1. Aspartic acid
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3. Glutamic acid
4. Glycine
5. Serine
6. Leucine
7. Histidine
8. Tyrosine
9. NH_4NO_3
10. $(NH_4)_2SO_4$

the greatest volumes of cellulose of the test fungi and produced the largest cellulose clearing on $(NH_4)_2\ SO_4$. Cellulose clearing by T. viride grown on aspartic acid was only slightly less than that on $(NH_4)_2SO_4$ although clearing had finished after two weeks. By the third week of incubation the amount of cellulose cleared by T. viride grown on NH_4NO_3 was greater than that on aspartic acid.

3.5.3 Discussion

Relative cellulolytic abilities of the test fungi, with amino acids as sole nitrogen source, were reported in section 3.5.2. The majority of amino acids investigated supported less cellulolytic activity than the inorganic sources. These results confirmed previous evidence of reduced cellulase production in the presence of alternative carbon sources discussed in section 3.4.3. However, glutamic and aspartic acids, in some cases, gave rise to greater cellulase activity of fungi than the inorganic nitrogen sources. A similar apparent stimulation of cellulolytic activity by amino acids was observed by Szajer (1975) in experiments on Fusarium isolates in which stimulation depended on the amino acid and the fungal strain used.

It would appear that alternative carbon sources do not always reduce the cellulase production of fungi but, where the alternative source is an amino acid, may enhance the production depending on the amino acid and fungus involved.

3.6 Chapter discussion

C:N ratios of the experimental media used in this chapter were restricted below 50:1. Maximum cellulolytic activities have been found to occur at these levels of C:N ratio (Levi and Cowling, 1966, 1968; Butcher and Drysdale, 1974).

A comparison of results from sections 3.4.2 and 3.5.2 showed a significant correlation between mycelial production and cellulase activity. A scatter diagram was drawn, relating the cellulose volume cleared (after two weeks of incubation) on media which contained aspartic acid, glutamic acid, asparagine, leucine, histidine, $(NH_4)_2SO_4$ or NH_4NO_3 as the sole nitrogen sources, and the dry weights of mycelium produced when these amino acids, or inorganic nitrogen sources plus glucose, were supplied as sole carbon and nitrogen source (Figure 11). The results were transformed into a percentage of the mean growth on all media before they were plotted on the diagram in order to compensate for the different growth rates of the fungi. The correlation coefficient was calculated and found to be significant at a probability level of $p = 0.001$. Thus, the mycelial dry weight and cellulose clearance were positively related and so the quantity of cellulose cleared was limited by the size of the fungal colony.

A similar comparison was carried out on the mycelial dry weight production, when amino acids were the sole carbon source, and the radial growth of colonies when cellulose was supplied as the carbon source (Figure 12).

Figure 11: Correlation between dry weight production and cellulose clearance on media containing different amino acids as nitrogen source.

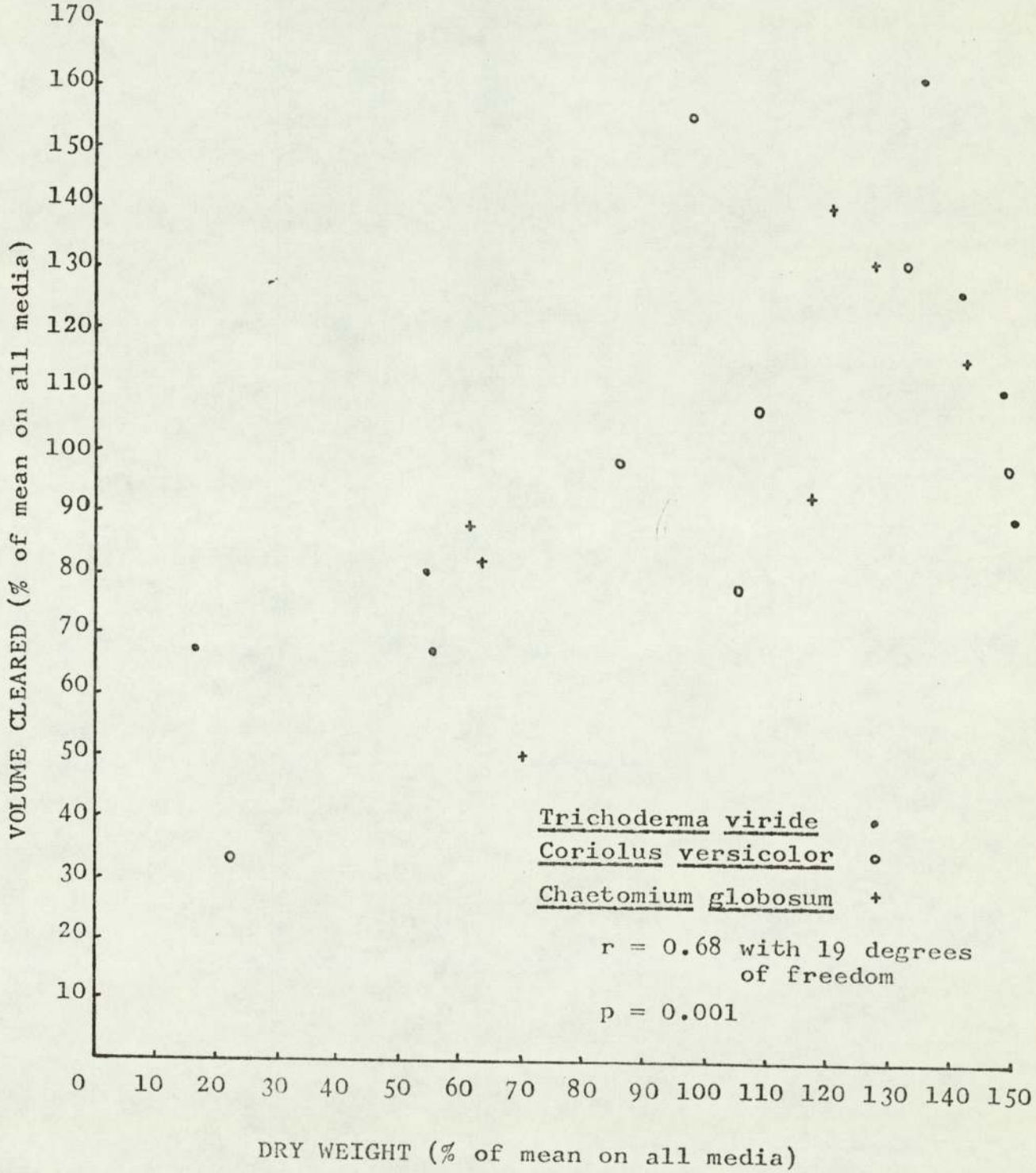
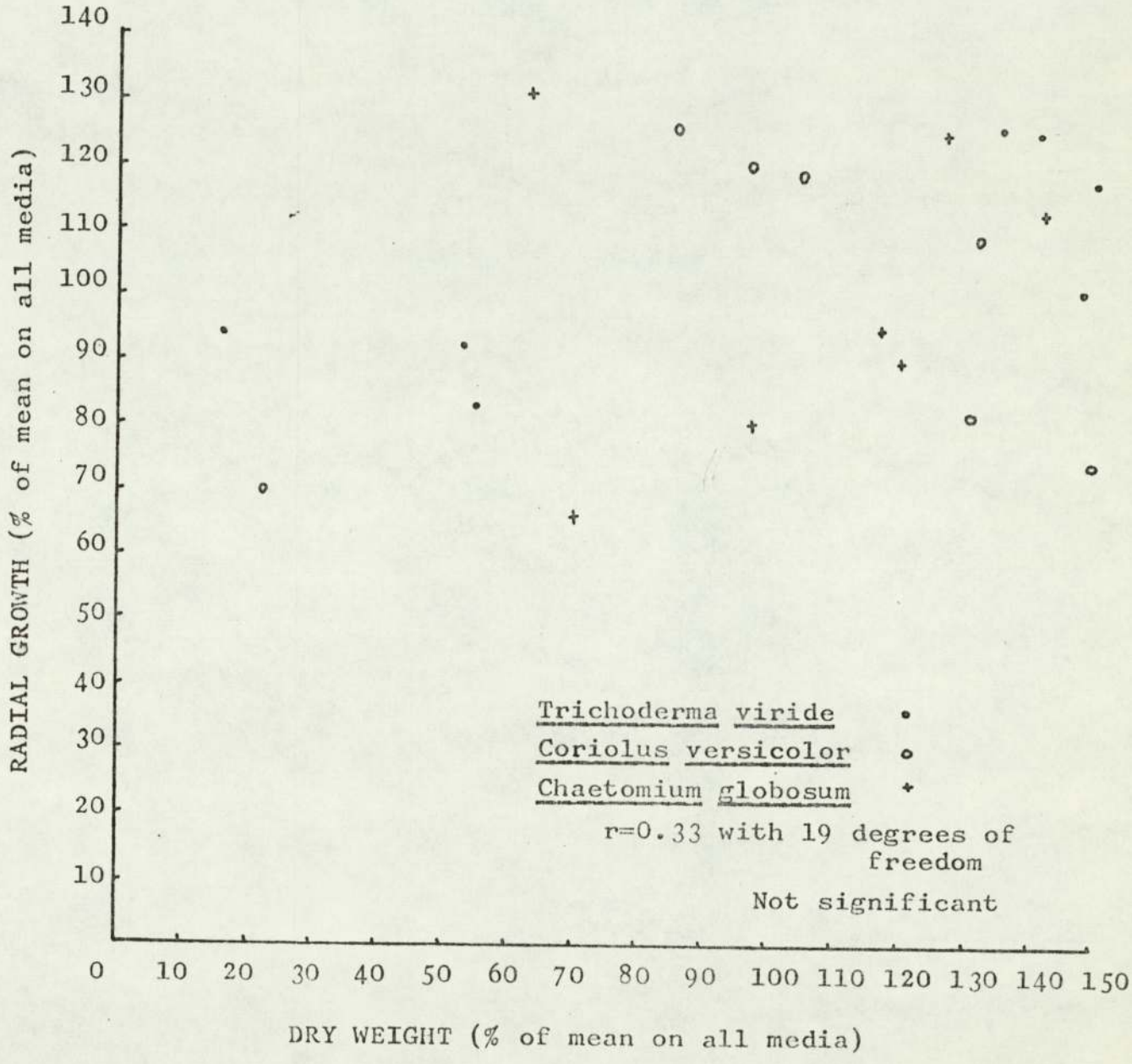


Figure 12: Correlation between dry weight production and radial growth on media containing different amino acids as nitrogen source.



The radial growth of the fungi on three different nitrogen concentrations was averaged and results were transformed into a percentage of the mean growth on all media prior to inclusion in the correlation diagram. The correlation coefficient of the collective data was 0.33 and not significant. Correlation coefficients were then calculated for the individual fungi:

Chaetomium globosum	r = 0.20	5 degrees of freedom
Coriolus versicolor	r = 0.09	
Trichoderma viride	r = 0.78	

The only coefficient to show any significance was that of T. viride at the relatively low probability level of $p = 0.05$. It can therefore be concluded that radial growth produced on solid media containing cellulose as the carbon source was not related to the dry weight of mycelium produced in liquid media with amino acids as the carbon source .

Measurement of radial growth is not an accurate method of determining the fungal response to different nutrients because it does not take the density of the fungal colony into consideration. This, in itself, may explain the lack of correlation seen between the two sets of results but an alternative explanation lies in the difference in carbon source in the two experiments. The efficiency of the fungi in utilising different nitrogen sources will alter with changes in the available carbon source. The nature of the carbon source was reported to

have an effect on the ability of fungi to utilise a particular nitrogen source by Hawker (1950) and, more recently, by Ibbotson (1974). Further experiments would be necessary to determine the role of carbon source in the efficiency of nitrogen uptake by fungi.

CHAPTER 4

BURIAL TRIALS

4.1 Introduction

Fungal decay of timber has usually been found where timber is in contact with soil. The soil is a source of inoculum containing a wide range of microflora, some of which are capable of cellulose degradation. In order to stimulate fungal decay in the laboratory burial tests are often used in which wood samples are buried in soil and removed at intervals to study the colonisation process. The test can be modified to study pure cultures of decay organisms in sterile soil and, more recently, substitute soils have been tested (Kaune, 1970; Takahashi and Nishimoto, 1973; Baines, Dickinson and Levy, 1977).

Soil generally contains sufficient nutrient salts to promote active decay (Theden, 1961; Becker and Kaune, 1966) but, in experimental studies, supplementary nutrients can be added if required (Savory, 1955). The moisture content of the soil is important in burial tests but maintenance at 100% water holding capacity gives good results (Duncan, 1965).

The aims of this chapter were to compare soil, vermiculite and perlite as burial substrates and to assess the value of burial tests for nutritional experiments.

4.2 Total nitrogen content of timbers

A major limiting factor to microbial breakdown of timber is considered to be the scarcity of nitrogen. It is important, therefore, to determine the nitrogen content of wood prior to decay studies.

The timbers investigated (lime, beech and pine)

were supplied by the Princes Risborough Laboratories. Sapwood samples were taken from planks $1\frac{1}{2}$ or 2 inches thick, depending on availability, which had been air-dried at the Princes Risborough Laboratories. The outer 5mm of each plank surface was discarded to remove wood which contains nutrients redistributed during drying (King, Oxley and Long, 1974; Long, 1978). Samples were cut into small cubes on a bandsaw and milled in a Culatti micro-hammer mill to pass a No. 20 mesh sieve (0.84mm apertures). The milled wood was oven-dried at 80°C until a constant dry weight was recorded. Dry samples of 100mg were weighed and subjected to Kjeldahl analyses, as described in section 2.2.7.

The results of the analyses are summarised in Table 8. Lime sapwood had the highest nitrogen content of 0.144%, while beech (0.06%) and pine (0.03%) were much lower.

4.3 Movement of nitrogen between test block and burial substrate

The burial technique involves the burial of moist wood blocks in a substrate held at 100% water holding capacity. The test blocks are usually impregnated under vacuum with a nutrient solution (Butcher and Drysdale, 1974) or with a preservative in the case of toxicity trials (Savory and Bravery, 1970; Savory, 1972), and allowed to dry partially before burial.

The moisture content of burial substrates and test blocks have not normally been matched and so, during burial, an equilibration of moisture contents must occur. This

Table 8: Nitrogen content of lime, beech and pine sapwood.

Timber	Number of samples	Mean % nitrogen content	Standard deviation	Standard error
Lime	25	0.144	0.012	0.002
Beech	30	0.057	0.016	0.003
Pine	30	0.030	0.005	0.001

equilibrium will involve the movement of water from the test block to substrate or vice versa and nitrogen may be transported with the water. Movement of soluble nitrogen in either direction could have an effect on the rate of decay.

The natural soil used in the following experiments was provided by the Princes Risborough Laboratories from the same site as soil used in pine burial experiments carried out by King (1975). Nitrogen content of this soil was investigated and shown to be 0.3% with soluble nitrogen amounting to 0.03% (King, 1975). The expanded silicates, vermiculite and perlite, were repeatedly washed in distilled water, dried and sieved through a No. 10 mesh sieve (2.0mm apertures). The perlite was then mixed thoroughly with aluminium oxide grit in the ratio 2:1 to reduce its water holding capacity. Water hdding capacities of the substrates were determined by the method outlined by Carey and Grant, (1975).

Substrates were brought up to water holding capacities by the addition of distilled water. Each substrate was thoroughly mixed to ensure even distribution of water and then dispensed into soil burial jars (575cc glass jars). These were autoclaved at 15 p.s.i. (120°C) for 60 minutes and the weight of each jar checked after autoclaving to ensure that no uptake of moisture had occurred.

Lime sapwood was chosen as the test wood to be examined because of its high nitrogen content. Test blocks were cut, 5mm x 10mm x 70mm (tangential x radial x longitudinal), and sterilised for 18 hours in an oven at

110°C. Some of these sterile blocks were buried in the substrates in a dry condition while others were vacuum impregnated with sterile distilled water prior to burial. The burial jars were maintained at 27°C for 4 weeks and then the test blocks removed. Analyses for nitrogen were carried out as in section 2.2.7. Control blocks of both dry and saturated lime were also prepared and maintained in Petri dishes for the experimental period and nitrogen contents determined.

The results of these nitrogen analyses are summarised in Table 9. Statistical analysis of the control results showed a significant loss of nitrogen when wood blocks were saturated. This may have been due to a leaching effect which could have occurred during the impregnation and removal of wood blocks from water. Analysis of the burial block results showed no significant difference in nitrogen content between blocks buried dry or saturated. The substitute soils contained no nitrogen source and, therefore, only movement from saturated wood to burial substrate was feasible.

Results from the blocks buried in soil showed a significant increase in nitrogen in both the dry and saturated blocks when compared with the control (Table 10). The soil burial blocks contained more than twice the amount of nitrogen found in the controls. The increase could not be accounted for by the relatively small soluble nitrogen content of the non-sterile soil but autoclave-sterilisation of soil causes a large increase in soluble nitrogen by mobilisation of that contained in soil organisms. Nitrogen

Table 9: Nitrogen content of lime blocks after burial.

Burial substrate	Nitrogen content (%) Dry wood	Saturated wood	Student's 't' value	Degrees of freedom	Probability
Control	0.143	0.127	2.76	28	0.01
Soil	0.286	0.312	1.68	13	N.S.
Vermiculite	0.148	0.146	0.22	13	N.S.
Perlite	0.142	0.130	0.89	13	N.S.

N.S. = not significant

Table 10: Comparison of soil burial blocks and control blocks.

Moisture status of wood blocks		Burial substrate		Student's 't' value	Degrees of freedom	Probability
		Soil	Control			
% nitrogen	Dry	0.286	0.143	21.3	18	0.001
	Saturated	0.312	0.127	605.3	18	0.001

had moved from the soil into the wood regardless of the moisture status of the wood. Nitrogen movement from moist soil to dry wood may be mainly along with a unidirectional flow of water, but the increase in nitrogen in the saturated wood implies that diffussion must also play a large part in nitrogen movement. Although King (1975) observed the movement of soil nitrogen into wood, in burial experiments which involved non-sterile soil, and explained it as a translocation of nitrogen into wood by microorganisms, test blocks plated out on agar in the present investigation did not show signs of contamination. Hence, the movement of nitrogen appeared to be of a physical nature and not biological.

4.4 Decay criteria

In any biodeterioration study it is essential to assess quantitatively the extent of substrate breakdown. The method chosen must have a high sensitivity and be capable of reproducibility. Weight loss has been used by the majority of workers as a decay criterion but it suffers the disadvantage that long incubation periods may be required to obtain significantly high levels of weight loss.

Strength loss determinations of wood have been investigated as an alternative to weight loss. Hardness, crushing, tensile and various bending and toughness tests have all been described by Hartley (1958). Tests using weight loss and loss of bending strength carried out by Wälchli (quoted in Savory and Bravery, 1970) showed the latter criterion to be the more sensitive for detecting

attack of untreated wood but that toxic limits established by the two methods were closely comparable. The measurement of bending strength introduced the need for particular care in selection of samples (Abou Heilah and Hutchinson, 1977).

The method used for measuring bending strength in this investigation has evolved from the nail-head pull-through principle of Merrill and French (1964). Similar methods of strength assessment have been used by Armstrong and Savory (1959) on beechwood, Liese and Pechmann (1959) on birchwood and Sharp (1970) on veneers.

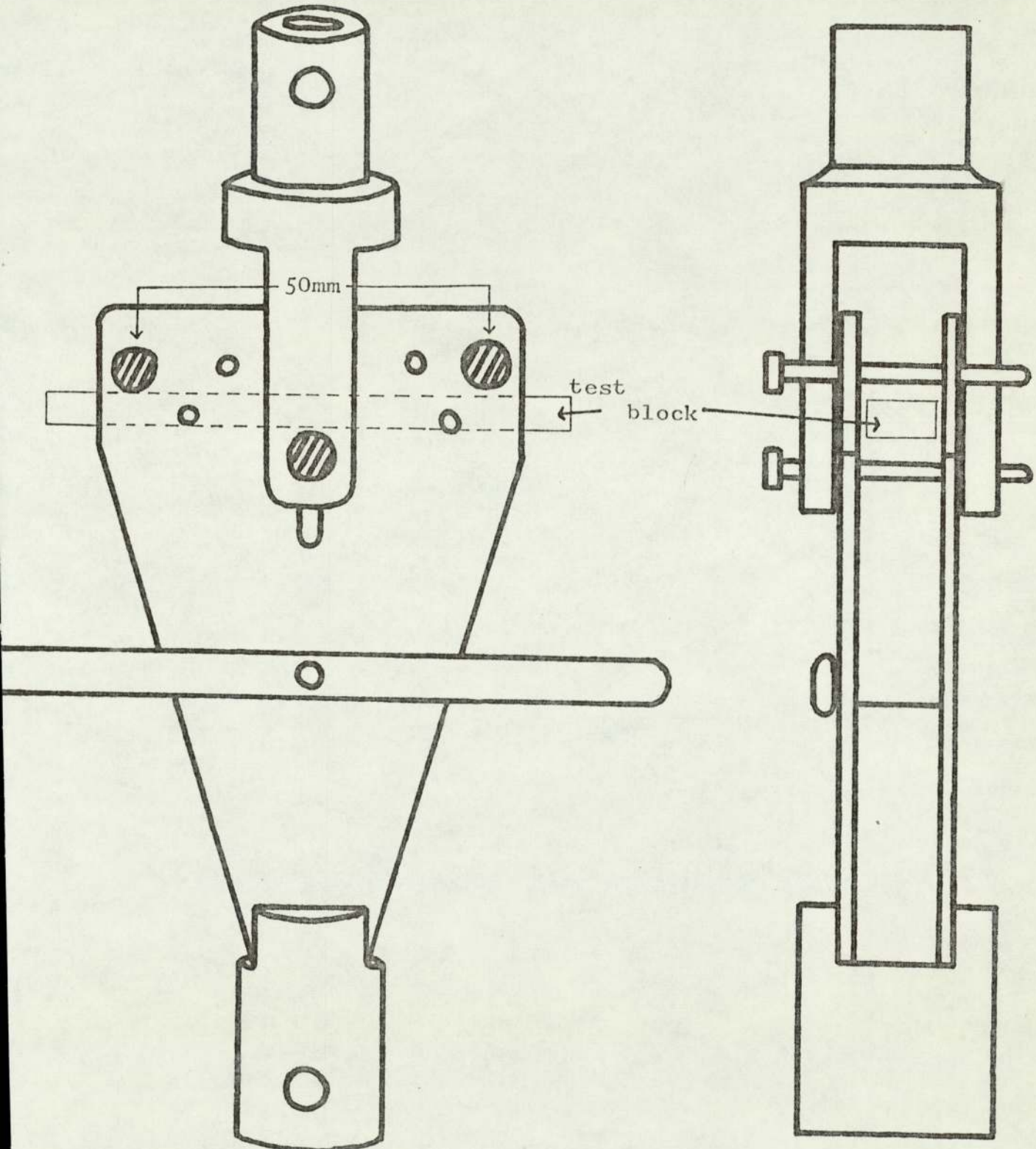
A Monsanto tensometer with specially designed 3-point loading jaws (Figure 12A, Plates I and II) was used for breaking test pieces. Bending strength was measured under an evenly increasing strain (jaw separation of 2.5cm/minute) to breaking point. The tensometer was used in a room maintained at 80% relative humidity and test blocks were saturated with water before bending to ensure standard conditions.

4.5 Timber conversion, sample size and selection

The design of the tensometer jaws imposed restrictions on the size of test samples: they could not be greater than 10mm in depth and not less than 50mm in length. The two outer pins which were to come into contact with test samples were maintained at a span of 50mm.

Air-dried timber of pine, lime and beech were provided by the Princes Risborough Laboratories and were converted into test blocks, as shown in Figure 13.

Figure 12 A: Tensometer jaws for determining bending strength.



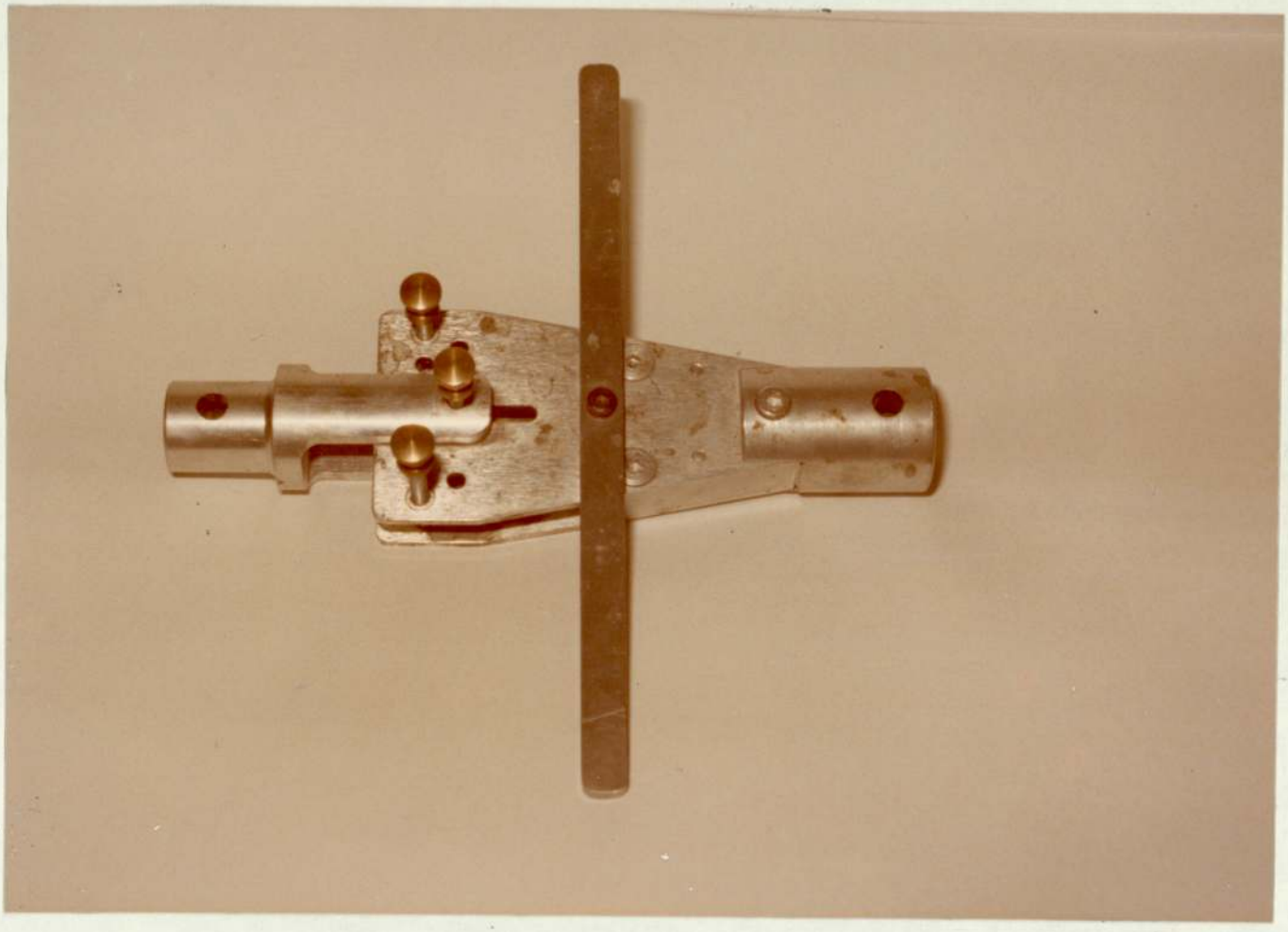


PLATE I: Bending strength jaws for use with the tensometer.

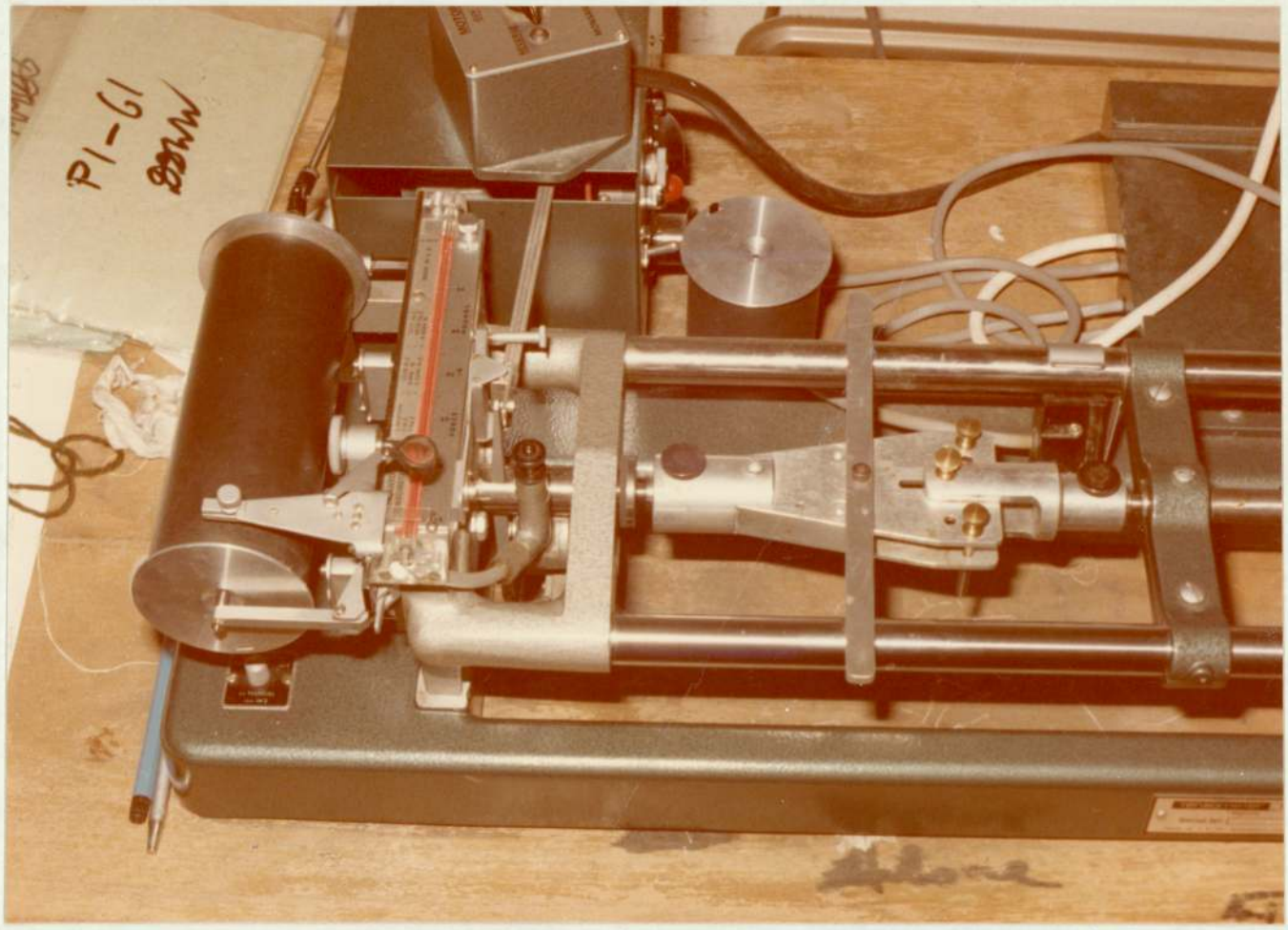


PLATE II: Bending strength jaws positioned in the tensometer.

Figure 13: Conversion of planks to test blocks

A: Plain-sawn plank, 37mm or 50mm thick, air-dried.

B: Air-dried plank converted into strips with a cross section of approximately 12mm x 7mm and with the annual rings running parallel to the 7mm edge (I). Strips with fewer than five annual rings, annual rings at grain angles greater than 10^{o} (II) and strips containing heartwood (III) were discarded.

C: Quarter-sawn strips were planed down to 10mm x 5mm (+ or - 0.1mm) and sawn into 450mm lengths for transport to the laboratory.

D: Conversion of blocks into 70mm lengths and drilled at one end to facilitate suspension in a humidity chamber.

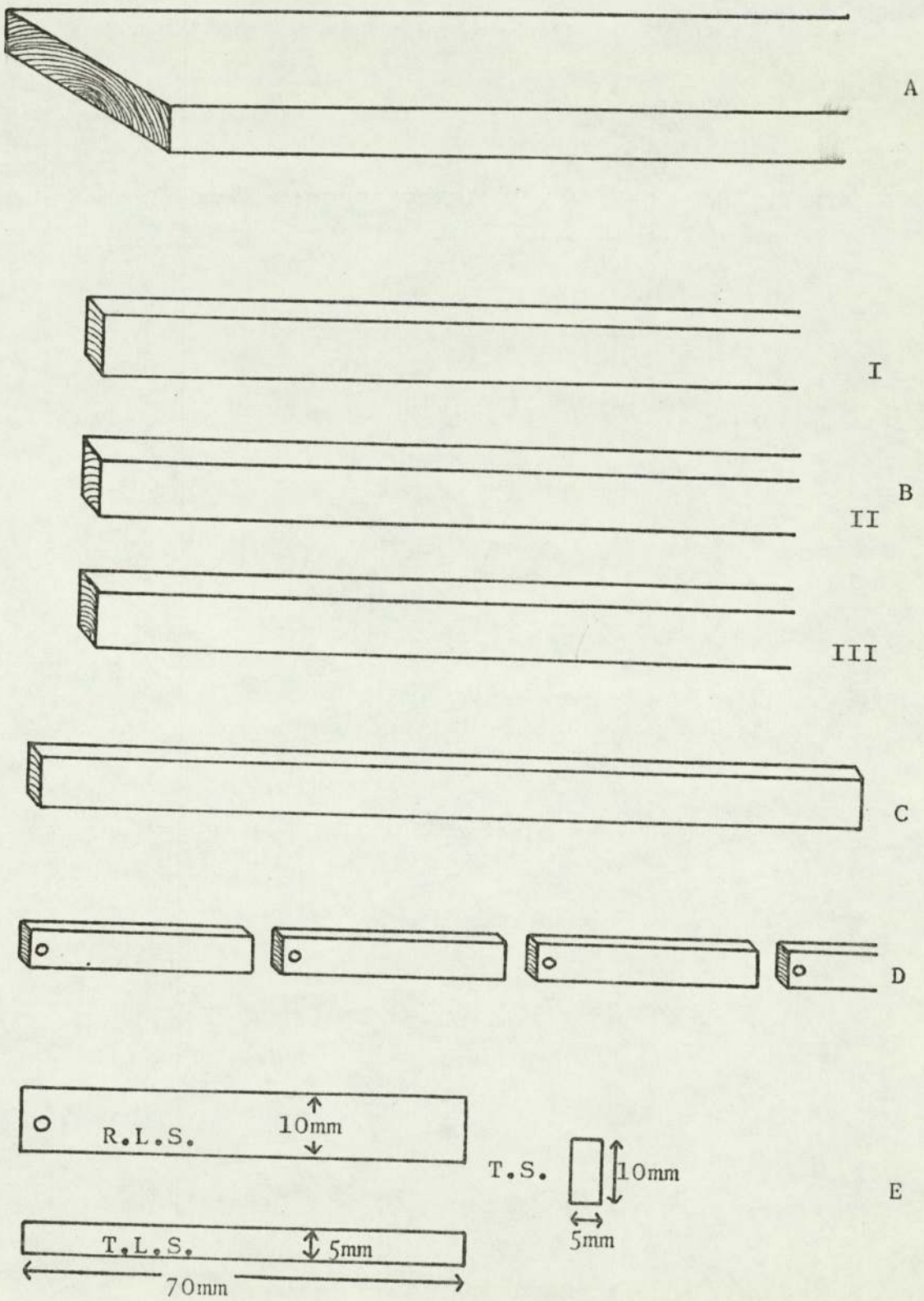
E: Exact orientation and dimensions of test blocks.

R.L.S. Radial longitudinal surface

T.L.S. Tangential longitudinal surface.

T.S. Transverse surface.

Figure 13:



Conversion of the wood was carried out in a saw mill at Princes Risborough Laboratories where the transverse sections of the samples (10mm x 5mm) were planed to an accuracy of $\pm$ 0.1mm.

Test material was selected for straightness of grain and orientated so that the annual rings ran parallel to the narrowest face. Orientation of the annual rings is important in strength testing and material with a small grain angle (the angle between the plane of the narrowest edge and that of the tangent to the sector of the annual ring) was selected by eye. Abou Heilah and Hutchinson (1977) found that selection of material with grain angles of less than 10^{o} reduced the breaking strength differences between blocks. Material in which the transverse face showed fewer than five annual rings was also discarded.

The drilling of a hole at one end of the test blocks was necessary only when experiments were conducted in a humidity chamber (Chapters 5-8). The hole was drilled well clear of the 50mm test span and was considered unlikely to have any effect on the strength testing of the specimen.

4.6 Effect of burial substrate moisture content on decay rate

As early as 1939, Leutritz emphasised the need for soil moisture control for good results in soil burial experiments. A standard method for determining water holding capacity (w.h.c.) was later incorporated into the American method for testing wood preservatives (A.S.T.M., D1413-61). This method was, however, time consuming and a shorter, more

convenient method was devised at the Princes Risborough Laboratories (Carey and Grant, 1975). This latter method of determining w.h.c. was utilised in the present experiments.

The w.h.c. of soil, vermiculite and perlite/aluminium oxide (2:1) were determined and quantities of these substrates brought to 40, 60, 80 or 100% w.h.c. by the addition of distilled water. Soil burial jars (575cc glass jars) were filled with the burial substrates and their weights recorded. The jars were autoclave-sterilised at 15 p.s.i. (120°C) for 60 minutes and the weights checked when cool to ensure that no uptake of moisture had occurred. After a two day equilibration period at 25°C in a humid room, the weights were re-checked and sterile water added if needed.

Sample blocks of lime sapwood were sterilised for 18 hours at 110°C and vacuum impregnated with sterile water.

A standard spore suspension of Chaetomium globosum was prepared from a 14 day old culture grown on 2% malt agar. A small amount of sterile water was added to the Petri plate and a sterile needle used to dislodge the spores. The suspension was filtered through sterile glass fibre cloth to remove broken hyphae and perithecial hairs. A haemacytometer was used to assess the density of spores in the suspension and, where necessary, the suspension was diluted to give approximately 10,000 spores/ml.

The surface of each lime block was inoculated with the spore suspension by using a sterile hypodermic syringe. Blocks were buried in each substrate, 15 blocks per moisture

content, and incubated at 25°C.

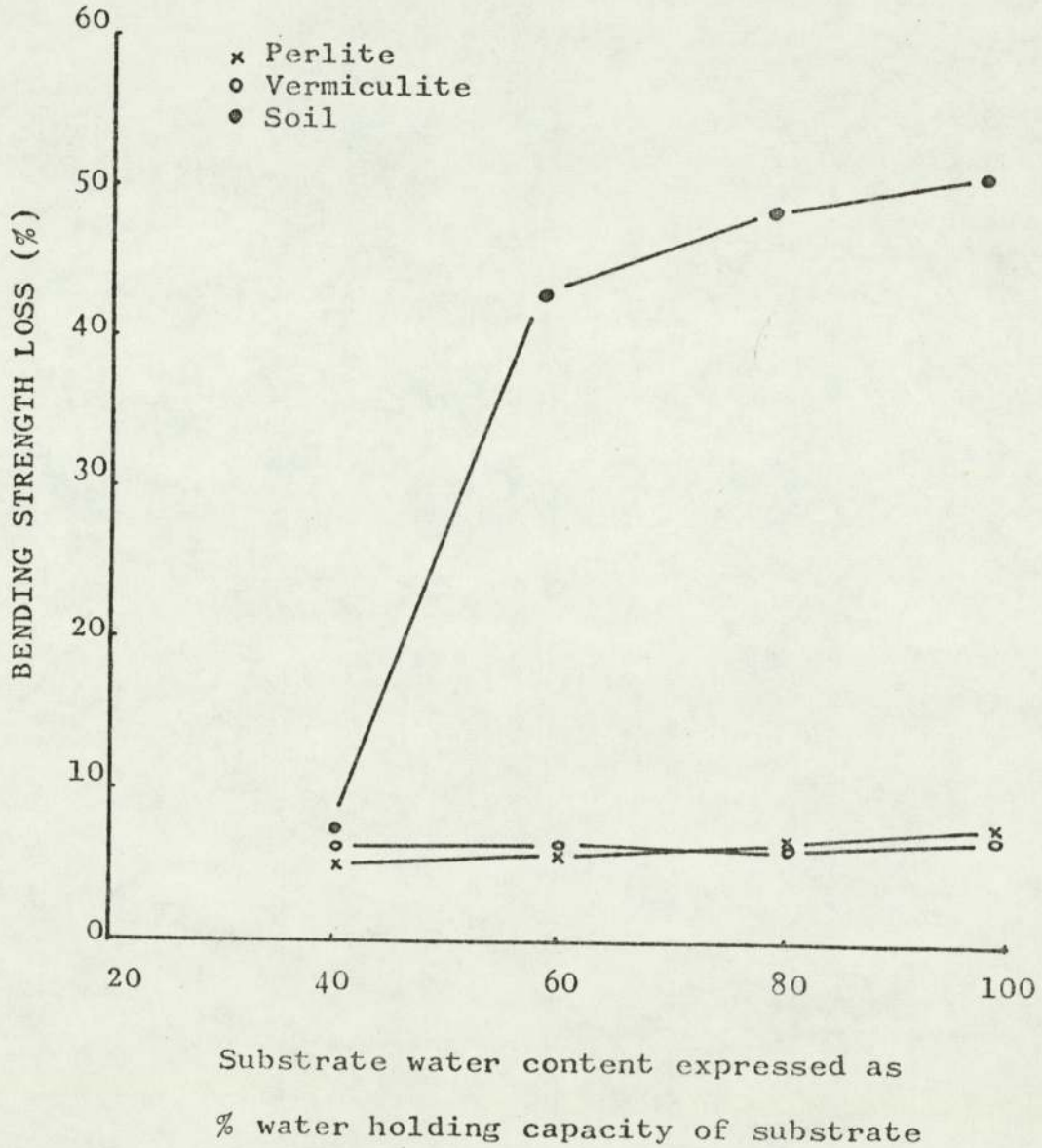
The weights of the burial jars were checked weekly and sterile water added when needed. After 4 weeks of incubation the lime blocks were removed and freed of adhering soil and hyphae. All blocks were then saturated under vacuum before the bending strengths were determined on the tensometer.

The results are presented graphically in Figure 14 and analyses of variance are given in Appendix I. It was immediately obvious that appreciable strength loss had only occurred in those blocks buried in soil. Losses of less than 10% occurred in those blocks buried in the substitute soils. Loss of strength was little affected by w.h.c. of 60% or more. At 40% w.h.c. there was a significant reduction in loss of strength.

Previous work by Carey and Grant (1975) had revealed that a wide range of moisture levels in both soil and vermiculite maintained the moisture content of buried wood blocks at levels suitable for decay. At lower moisture contents (40% w.h.c. and below) they found a tailing off of block moisture content which would reflect the low decay level seen here in soil at the lower w.h.c.

Low decay rates in blocks buried in artificial soils may be explained by insufficient nutrients. It was thought that the fungus would find sufficient nutrients in the wood to support growth. However, the level of nutrients in wood may not be sufficient when infection is from a spore inoculation and not from actively growing mycelium. The

Figure 14: Strength loss in lime inoculated with Chaetomium globosum and held in burial substrates at different moisture contents.



extra nutrients in soil, compared with the absence of nutrients in the expanded silicates, would explain the occurrence of higher decay rates in blocks buried in soil.

4.7 Discussion

The nitrogen contents of lime, beech and pine sapwood were determined in section 4.2 and lime was shown to have the highest. Lime is known to be susceptible to decay (Sharp and Levy, 1974) but it should not be assumed that timbers with high nitrogen contents will always be the most susceptible to decay. A number of factors other than nitrogen content are involved in decay susceptibility. Lewis (1975) and Rypáček (1977) suggested that variation in hemicelluloses may be important while Zainal (1976, 1977) reported that lignin content affected the rate of decay. The ratio of wood constituents may also be involved in decay susceptibility and a number of workers have shown the effect of different C:N ratios on the decay capabilities of wood decay fungi (Levi and Cowling, 1966, 1969; Butcher and Drysdale, 1974). However, the level of nitrogen in a growing tree changes with the season of year and the timber is more susceptible to decay if felled at a time of year when its nitrogen content is high (Findlay, 1931; Bletchly, 1966, 1969; Levi and Cowling, 1968). This seasonal variation of nitrogen makes it necessary to determine the nitrogen content of timber on each occasion a tree is converted for use in experimental work. Comparisons of results between workers can only be carried out if the original nitrogen content of the wood is known.

Results obtained in section 4.4 indicated that the nitrogen contents of experimental wood blocks were greatly altered during burial in soil. The complexity of soil and inadequate knowledge on the effects of autoclaving soil can lead to difficulties both in the reproducibility of experimental conditions and in the interpretation of results.

Although the level of nutrients in lime was much greater than in either of the other two timbers studied, it was not sufficient to promote decay in inoculated blocks buried in vermiculite or perlite. In order to simulate soil burial using substitute soils a complete analysis of soil nutrients would be needed and nutrients added, in the correct ratio, to the substitute soil. This would be time consuming and costly and lead to the loss of preparation simplicity inherent in these tests.

Variation of soil and the difficulties of using substitute soils led to problems which would obscure any differences in decay rate of wood with added nitrogen. It was thought advisable to devise an alternative test technique in which these difficulties would be avoided.

CHAPTER 5

THE HUMIDITY CHAMBER

5.1 Introduction

In pure culture experiments soil burial enabled the wood moisture content to be controlled during the long incubation periods required for decay investigations. An alternative method of controlling the wood moisture content was needed which would avoid the complications found with soil, i.e. gain or loss of nutrients from the substrate, as shown in the previous chapter. The assessment of nutrient requirements of fungi attacking wood demands a situation in which the wood neither gains nor loses nutrients from the medium in which it is placed. Humidity chambers have been used by a number of workers to control the moisture content of substrates (Ayerst, 1969; Fermor, 1975; Kerner-Gang, 1977). The traditional, and most convenient, means of providing standard relative humidities covering a wide range of values is the use of saturated salt solutions. Any salt solution at a given concentration and a constant temperature is in equilibrium with a fixed partial vapour pressure of water and hence defines a fixed relative humidity. Comprehensive reviews of salt solutions and the relative humidity at a given temperature have been published by Young (1967) and Winston and Bates (1960).

Snow (1949) and Ayerst (1969) have shown that germination of fungal spores was greater at higher relative humidities and that mould spores, in particular, require humidities of 60% and above. The humidity requirements for spore germination of wood decay Basidiomycetes has been investigated by Morton and French (1966) but the requirements of soft-rot organisms are not known. Decay fungi are not

only found in wood which has been in soil contact and so the infection of wood must result from spores. Thus, a knowledge of the effect of wood substrate and ambient conditions on spore germination and development would be advantageous. The humidity chamber described in this chapter was designed to investigate the germination of Chaetomium globosum spores on wood substrates and the suitability of the technique for soft-rot decay studies.

5.2 Humidity chamber description

Culture chambers were designed to hold individual wood blocks suspended above a saturated salt solution. The chambers consisted of 19mm x 150mm rimmed glass test tubes covered with 25mm Oxoid aluminium caps. Each cap was drilled to take a short length of aluminium wire with a 90^{o} bend, 5mm from the lower end, to serve as a hook. The upper end of the wire was bent back on itself and sealed to the top of the cap with "Araldite" epoxy resin (Figure 15a). The resin effectively sealed the drilled holes in the caps.

Different saturated salt solutions could then be pipetted into the test tubes to maintain the humidity at known levels.

Individual culture chambers were autoclave-sterilised at 15 p.s.i. (120^{o}C) for 20 minutes, placed in test tube racks and lowered into plastic aquarium tanks, 400mm x 200mm x 250mm. The same saturated salt solution as was used in the test tubes was also poured into the tanks to a depth of 30mm and the tanks covered with sheets

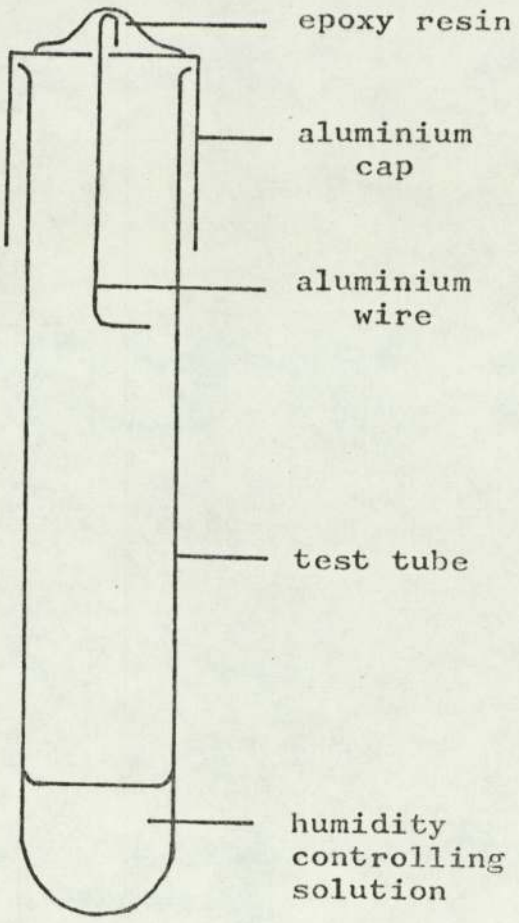


Figure 15a:

Individual culture chamber

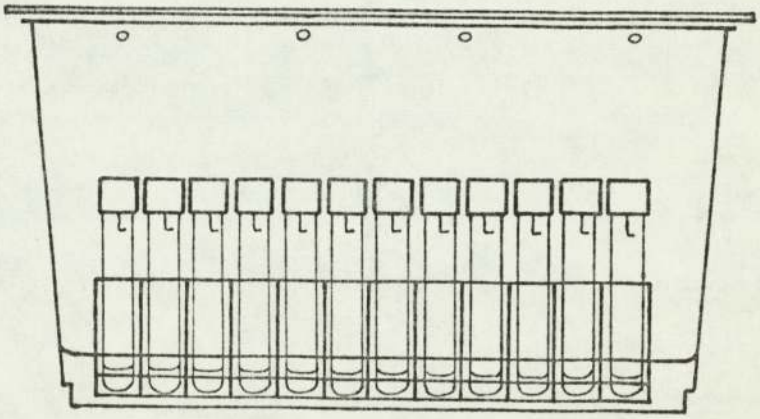


Figure 15b:

Culture chambers enclosed in a large humidity tank.

of perspex 5mm thick (Figure 15b). The tanks could then be incubated at 25°C in a waterjacketed incubator.

The disadvantage of controlled humidity chambers for long term experiments is that they frequently do not allow gaseous exchange to occur. Tight fitting lids such as those used by Ayerst (1969) lead to a build up of carbon dioxide and possible toxic effects on the test organism. However, the loosely fitting aluminium caps on rimmed test tubes, utilised in the humidity chamber described here, should not prevent gaseous exchange.

Holes were drilled in the tank sides to allow gaseous exchange but were positioned immediately below the rim of the tank to prevent air spora falling directly into the tank. Prior to use the tanks were washed in detergent, rinsed with boiling water and swabbed with alcohol to reduce the possibility of contamination of the culture chambers.

5.3 Sample preparation

The size of wood blocks had already been determined by the design of the bending strength jaws for the tensometer. This block size was also convenient for use in the culture chambers. The only modification required was to drill a hole in one end of the block (Figure 13D) to facilitate suspension from the aluminium cap of the chamber.

A major problem in using a humidity chamber was connected with the addition of nutrients to the wood blocks while maintaining sterility. Pure cultures of organisms

were to be used for decay and so wood blocks of known dry weight were oven-sterilised at 110°C for 18 hours. Nutrients could then be added by total impregnation of blocks by vacuum with solutions of known concentration. This would, however, result in water-logged blocks which would not permit fungal growth below the wood surface. Partial drying of the blocks could be carried out before inoculation to bypass the problem but would lead to difficulties in maintenance of sterility. Nutrient migration to the block surfaces would also occur (King, Oxley and Long, 1974).

A method was devised to overcome these problems, in which only partial moistening of the wood blocks was achieved. This involved the addition of a known quantity of liquid, which contained the desired amount of nutrient in solution, to bring individual wood blocks to a moisture content suitable for decay but below saturation.

Glass tubing, 15mm internal diameter, was cut into 90mm lengths and rubber bungs used to close both ends. The glass tubes were weighed and autoclave-sterilised at 15 p.s.i. (120°C) for 20 minutes. When cool, sterile wood blocks were aseptically inserted into individual tubes and quantities of sterile nutrient media added to each tube to bring the wood blocks to 80% moisture content based on the block dry weight. The narrow diameter of the glass tubing meant that the nutrient media covered a large surface area of the wood block. The tubes were placed in an incubator at 37°C to increase the rate of absorption of medium and were inverted several times to encourage even absorption.

After 48 hours, very little medium could be seen in the tubes and the wood blocks were removed. The empty tubes were reweighed and amounts of media left in the tubes calculated. The levels of media not absorbed by the wood were small (Appendix I).

Several of the moist wood blocks were cut into 1.0cm lengths and their moisture contents determined to assess the moisture distribution. It was found that moisture contents were slightly higher at the ends (Table 11) but it was considered that even the lower moisture contents would be sufficient for decay to occur. The strength test involved bending the wood blocks in the central region which would consistently be at the area of lowest moisture content and so not lead to undue error.

5.4 Preliminary experiments

Pine, beech and lime blocks of known dry weight were oven-sterilised at 110°C for 18 hours. The blocks were then brought to approximately 80% moisture content, based on the block dry weight, by addition of calculated quantities of the following autoclave-sterilised medium (see section 5.3 for method of attaining moisture content):

KH_2PO_4	1.0g
KCl	0.5g
$MgSO_4.7H_2O$	0.2g
NH_4NO_3	3.0g
Distilled water	1000 ml

Table 11: Moisture content of wood blocks after impregnation with nutrients.

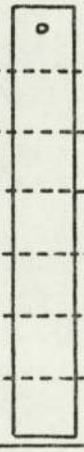
	Wood block	Moisture Content (%)						Mean	Standard deviation
		PINE		LIME		BEECH			
1-10mm		88	85	80	82	84	84	84	3
10-20mm		82	80	80	79	81	78	80	1
20-30mm		80	77	73	74	74	71	75	3
30-40mm		69	64	70	65	70	63	67	3
40-50mm		74	76	75	69	70	64	71	5
50-60mm		78	76	77	74	77	74	76	2
60-70mm		82	79	78	77	77	80	79	2
	Mean	79	77	76	74	76	73		

Table 12: Growth of C. globosum on wood at different ambient relative humidities.

Relative humidities (%)

	64	80	97.5	100
Pine	+	+	++	+++
Beech	+	++	+++	+++
Lime	-	-	-	-

\+ growth covering 0-25% surface area of block

++ growth covering 25-50% surface area of block

+++ growth covering 50-100% surface area of block

\- no growth

Controlled humidity chambers were prepared with individual culture chambers containing 5ml of either saturated $NaNO_2$, $(NH_4)_2SO_4$, K_2SO_4 or distilled water. These saturated solutions give humidities of 64%, 80%, 97.5% and 100% respectively at 25°C (Winston and Bates, 1960).

A spore suspension of C. globosum (see section 4.6) was used to inoculate the sterile wood blocks. The blocks were then suspended in the culture chambers (Plate III) within the humidity tank and incubated at 25°C.

After 14 days of incubation, mycelial growth of the fungus was apparent on the beech and pine blocks but not on the lime blocks (Table 12). A further 14 days of incubation did not reveal growth on the lime blocks at any of the humidities. The lime blocks were aseptically removed from the chambers and placed on 2% malt agar in Petri dishes in an attempt to induce spore germination and growth. However, the blocks behaved as though sterile and no growth of C. globosum occurred.

5.5. Discussion

Both the humidity chamber and method of moistening the wood blocks provided adequate conditions under which C. globosum spores could germinate and grow on beech and pine. Growth of the fungus did not occur on lime sapwood and could not be encouraged when the inoculated wood was placed on a nutrient agar. Further investigations into this phenomenon were carried out but, since they lie outside the main theme of this thesis, the work has been recorded in

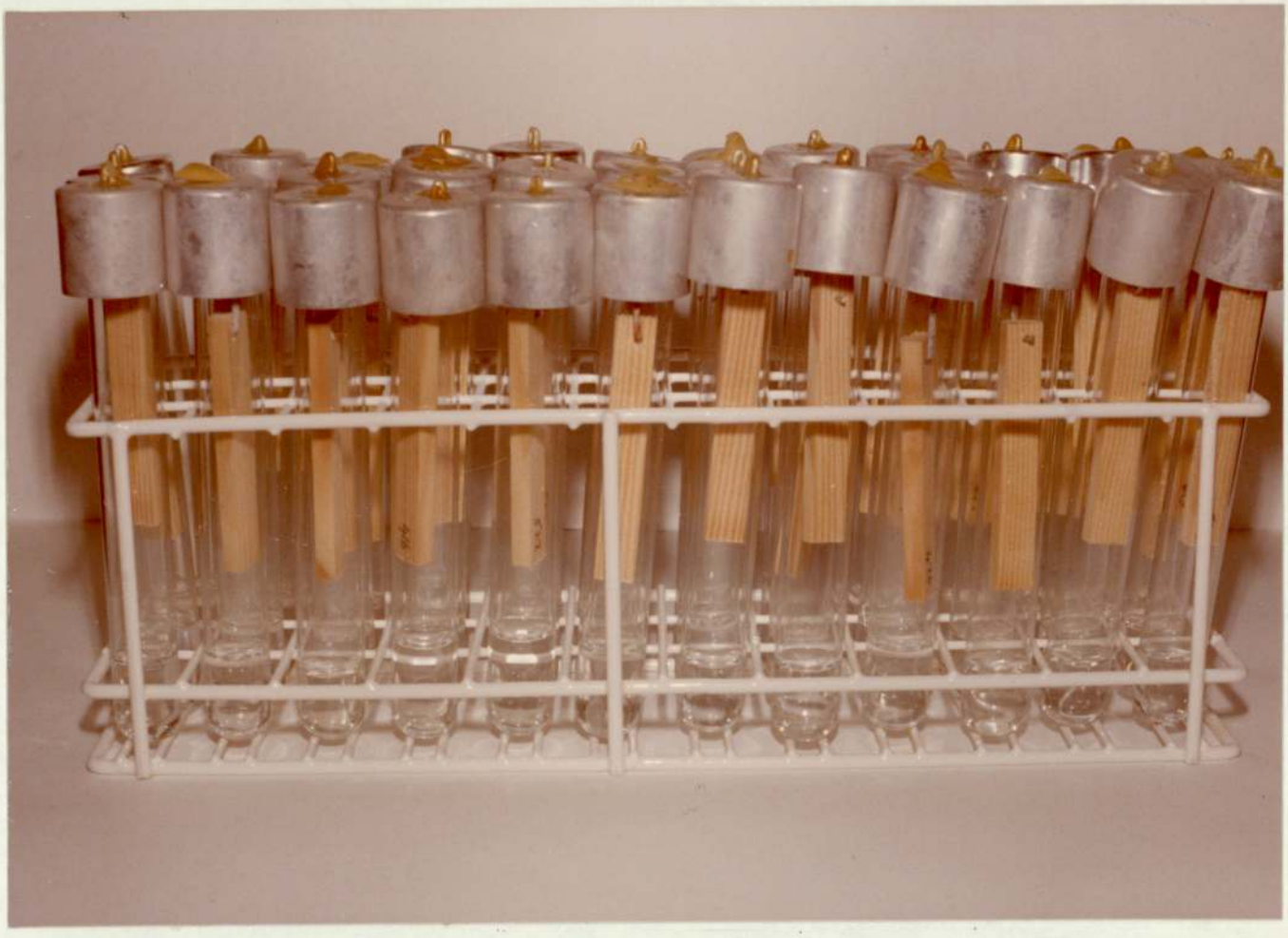


PLATE III: Individual culture chambers for growth of soft-rot fungi on sapwood test blocks.

Appendix II.

The lower moisture content of the central regions of the blocks did not deter germination and growth of the fungal spores. Growth of C. globosum on beech and pine at 100% relative humidity was greater than on lower humidities and this saturated level was used in later work.

CHAPTER 6

THE EFFECT OF INORGANIC NITROGEN ADDITION TO WOOD ON THE RATE OF DECAY BY *CHAETOMIUM GLOBOSUM*

6.1 Introduction

Experiments on the effect of nitrogen addition to wood on fungal decay were pioneered by Findlay (1934) and Schmitz and Kaufert (1936) who found that the addition of suitable nitrogen sources increased the rate of decay by up to 50%. In recent years, Lundström (1973) and Butcher (1975a) have confirmed that nitrogen additions to wood increase the rates of soft-rot decay.

Nitrogen source and concentration have been shown to have an effect on both growth and cellulolytic activity of the fungi under investigation (Chapter 3). This chapter specifically deals with the ability of *Chaetomium globosum* to cause decay in wood samples impregnated with different concentrations of an inorganic nitrogen source.

6.2 Materials and methods

Lime, beech and pine sapwood blocks were numbered, oven-dried overnight at 80°C to constant weight and the dry weights recorded. The blocks were then returned to an oven and heat-sterilised at 110°C for 18 hours. Individual sterile blocks were placed in glass tubes as described in section 5.3.

A basal medium which contained the following constituents was prepared:-

KH_2PO_4	1.0g
KCl	0.5g
$MgSO_4.7H_2O$	0.2g
Distilled water	1000ml

NH_4NO_3 was added to the medium to give nitrogen concentrations in the media of 0%, 0.035%, 0.07% and 0.11% (0.0g, 1.0g, 2.0g and 3.0g NH_4NO_3/litre). The media were autoclave-sterilised at 15 p.s.i. (120°C) for 20 minutes and allowed to cool.

Exact quantities of sterile medium were aseptically added to the sterile wood blocks to bring them to a moisture content of 80% as described in section 5.3.

A standard spore suspension of C. globosum (see section 4.6) was prepared and used to inoculate the beech and pine blocks. A sterile syringe was used to apply the spore suspension to each block surface. In view of the difficulties with lime, described in the previous chapter, the lime blocks were inoculated with mycelium taken from the growing edge of 7 day old cultures of C. globosum. Each block was inoculated half way along both radial longitudinal faces. The inoculations were carried out in a lamina flow inoculation cabinet to reduce contamination. After inoculation, each block was suspended above water in a sterile culture chamber and incubated at 25°C in a humidity chamber (see section 5.2).

Blocks were removed from incubation at intervals and the amount of decay assessed by the measurement of bending strength and weight loss.

A minimum of 8 replicates were measured for each treatment at each incubation period sampled.

6.3 Results

The percentage weight losses which occurred in the two hardwoods, beech and lime, are summarised in Figures 15 and 17. The experiment which used lime blocks was

terminated after 8 weeks of incubation but the beech experiment was continued for 12 weeks. The maximum weight loss obtained was 6.9% for beech impregnated with the highest concentration of nitrogen. The corresponding losses in bending strength are recorded in Figures 16 and 18, which show strength losses of 26% in beech blocks incubated for 12 weeks with the highest level of nitrogen addition, and 23.3% for the equivalent lime blocks incubated for 8 weeks.

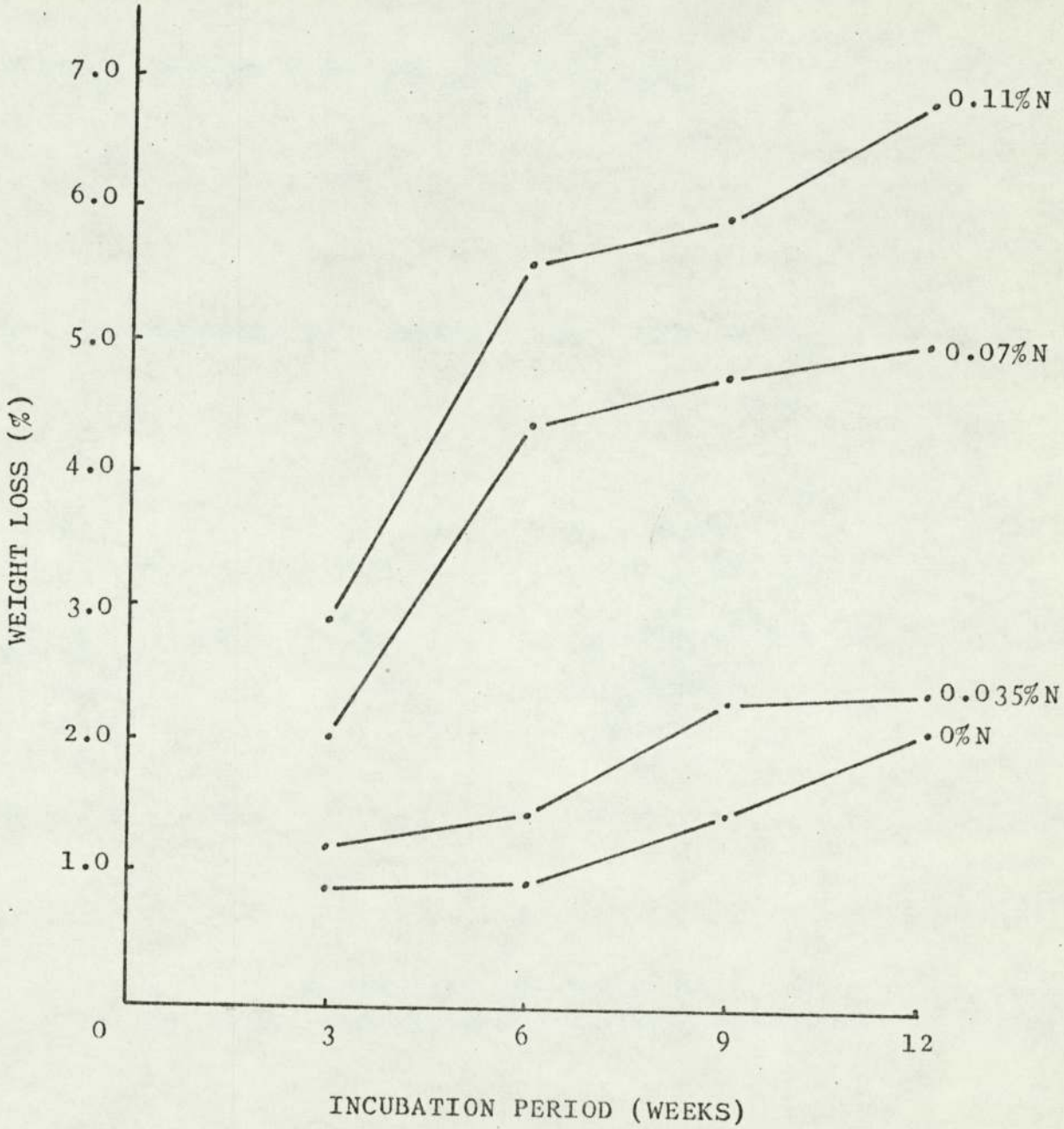
The percentage weight and strength losses found in the pine blocks are recorded in Figures 19 and 20. The vertical axes of the graphs are expanded to show a maximum weight loss of 1.55% and strength loss of 18.5% at the highest level of nitrogen addition after 16 weeks of incubation.

To determine the significance between results from different nitrogen additions, analyses of variance were carried out on results from each incubation period sampled. Results from the beech blocks showed significant differences in bending strengths and weight losses at each level of nitrogen addition from the first incubation period sampled. The lime blocks showed significant differences in the decay criteria after 4 weeks of incubation and the pine blocks showed significant differences in weight losses after 4 weeks of incubation and in strength losses after 8 weeks. Standard deviations and the results of the analyses of variance are recorded in Appendix I.

6.4 Discussion

C. globosum showed its ability to degrade pine,

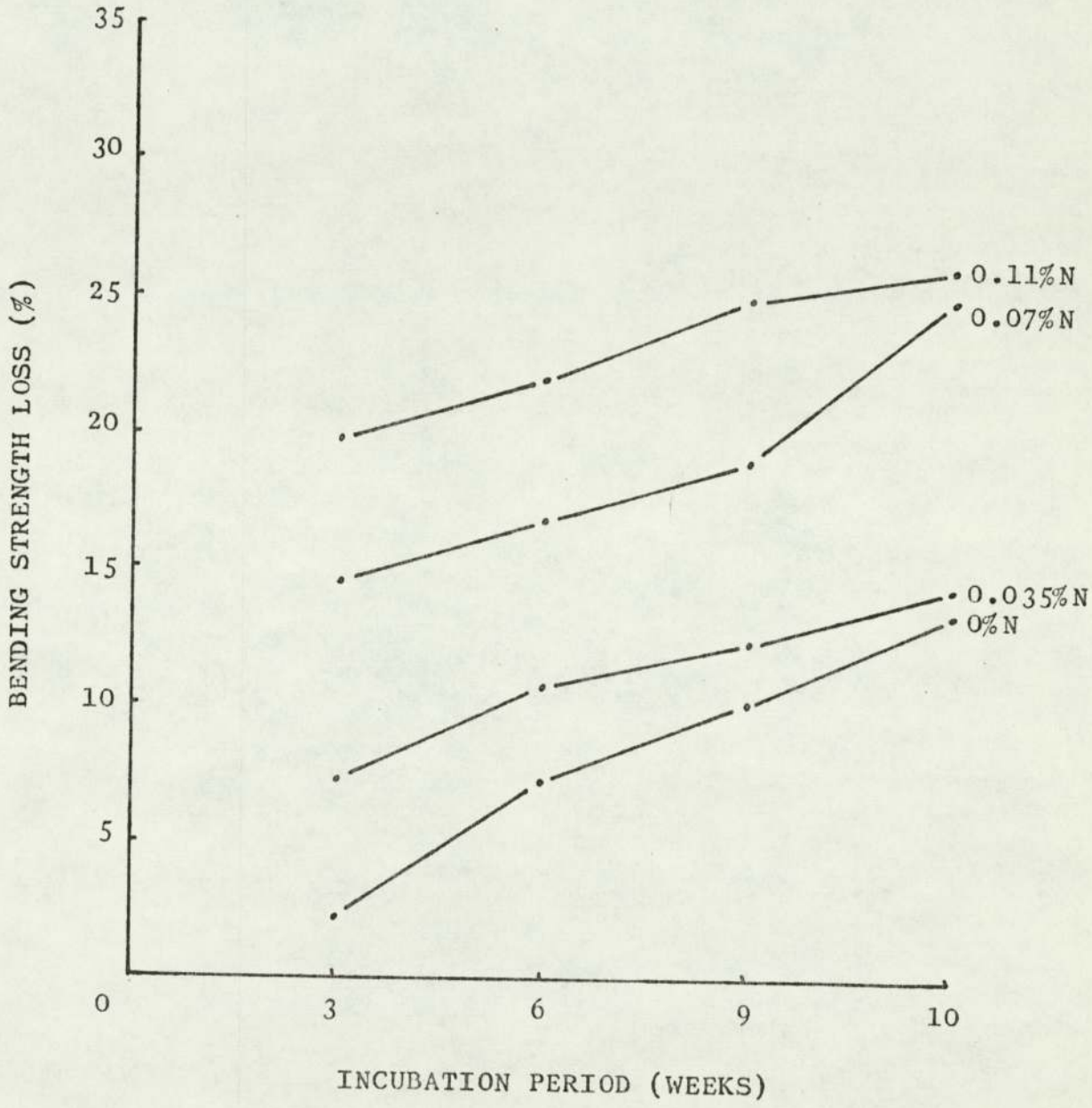
Figure 15: Weight loss in beech sapwood supplemented with inorganic nitrogen and decayed by Chaetomium globosum.



0%N = no added inorganic nitrogen

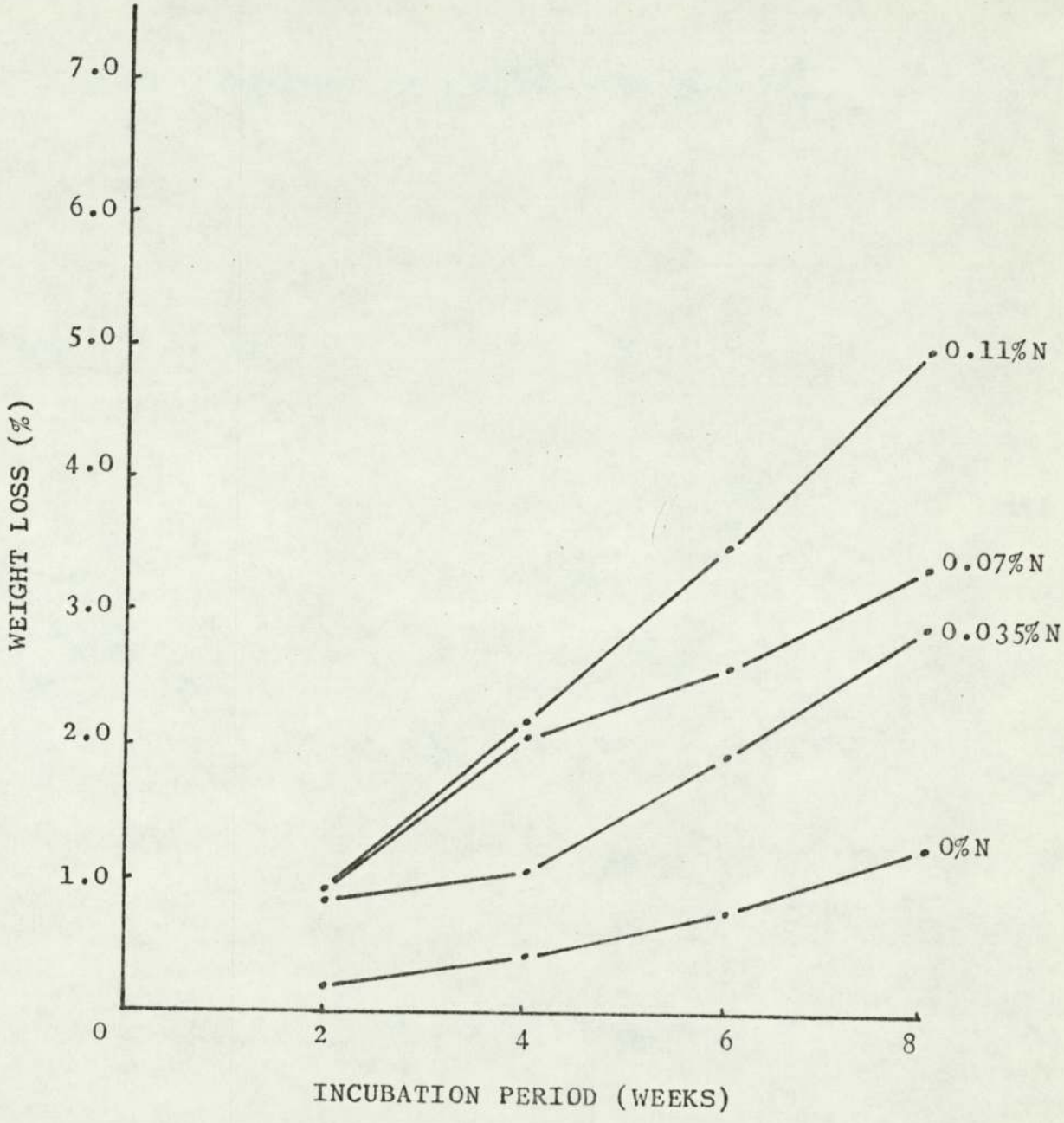
0.035%N, 0.07%N, 0.11%N = nitrogen concentrations of the media used to moisten the wood blocks

Figure 16: Strength loss in beech sapwood supplemented with inorganic nitrogen and decayed by Chaetomium globosum.



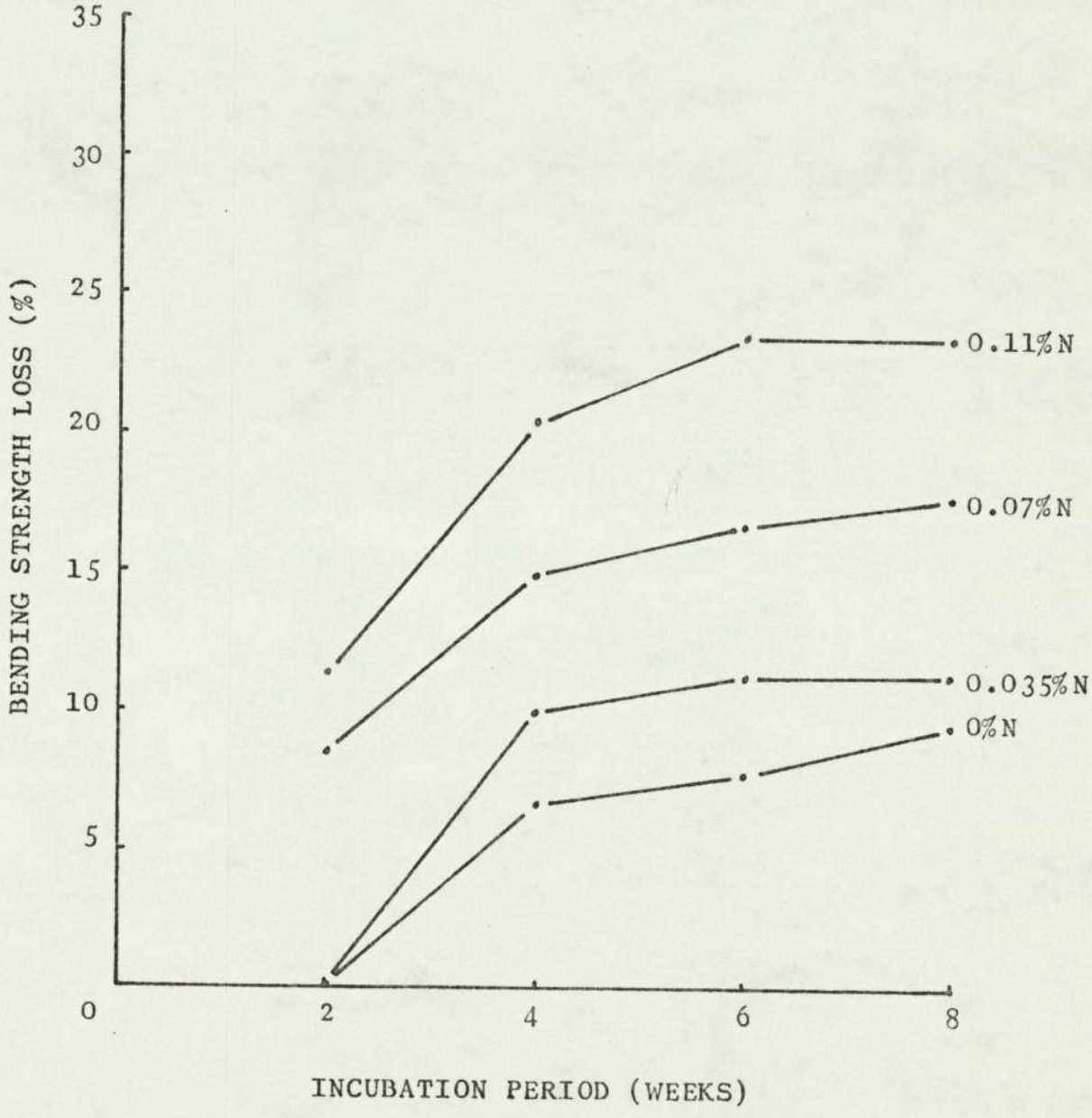
0%N = no added inorganic nitrogen

Figure 17: Weight loss in lime sapwood supplemented with inorganic nitrogen and decayed by Chaetomium globosum.



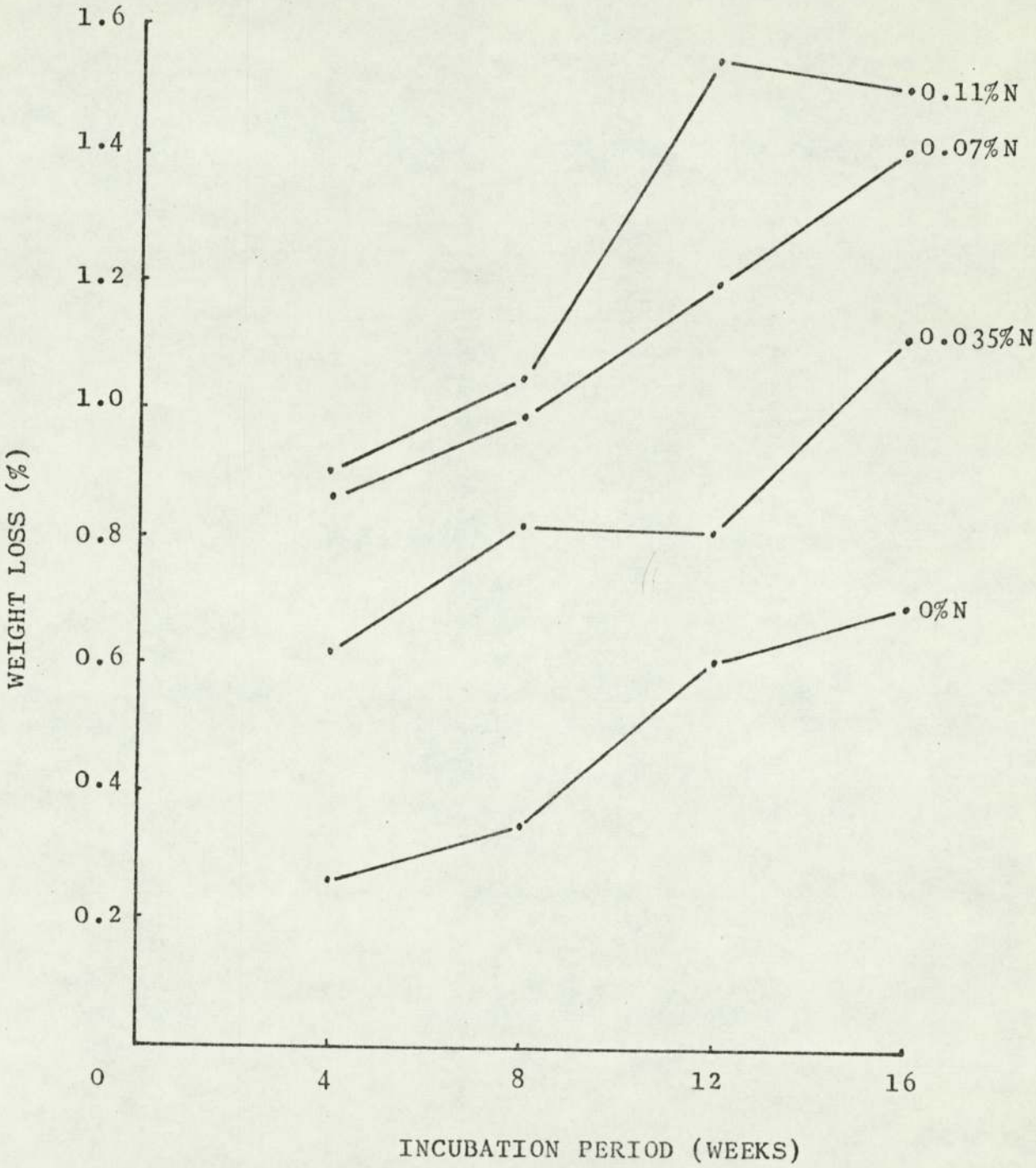
0%N = no added inorganic nitrogen

Figure 18: Strength loss in lime sapwood supplemented with inorganic nitrogen and decayed by Chaetomium globosum.



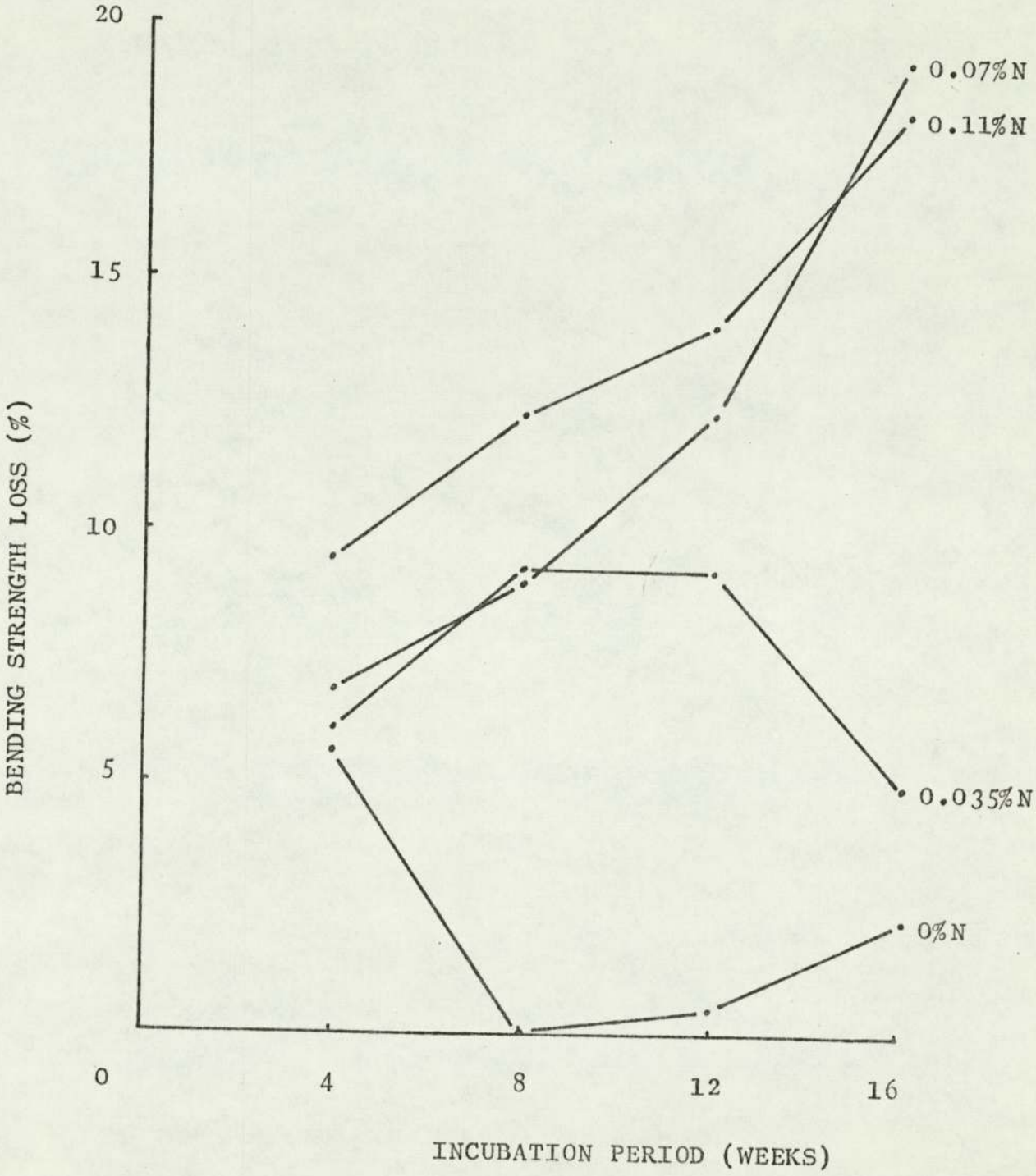
0%N = no added inorganic nitrogen

Figure 19: Weight loss in pine sapwood supplemented with inorganic nitrogen and decayed by Chaetomium globosum.



0%N = no added inorganic nitrogen

Figure 20: Strength loss in pine sapwood supplemented with inorganic nitrogen and decayed by Chaetomium globosum.



0%N = no added inorganic nitrogen

beech and lime sapwood and its decay rate was increased by the addition of an inorganic nitrogen source to the wood. NH_4NO_3 has not only been used as a nitrogen source to promote wood decay in the present investigation but also by a number of other workers. Savory (1954b) and Duncan (1965) found that decay of wood specimens was greatest when Abrams' (1948) salts were used which contained NH_4NO_3 as the nitrogen source. Greatest decay rates were promoted by NH_4NO_3 in experiments carried out by Kaune (1970) and Lundström (1973). Butcher (1975a) observed different rates of soft-rot attack in Pinus radiata buried in sand moistened with solutions of nitrogen at different concentrations. In the absence of nitrogen, hyphal development was sparse and no soft-rot cavities were formed. Addition of nitrogen at a rate of 0.25g nitrogen per Kg sand allowed good hyphal development and production of soft-rot cavities but, if the nitrogen content was doubled, hyphal development was intense but soft-rot did not develop. No evidence of a similar decrease in decay with increased concentration of added nitrogen was seen in the present experiments but the nitrogen concentrations used fell within nitrogen limits found by Lundström (1973) to promote maximum decay. Thus, the nitrogen levels used may have been too low to observe the phenomenon reported by Butcher (1975a).

The lime blocks inoculated with actively growing mycelium, and the beech blocks inoculated with a spore suspension, showed similar losses of both strength and weight after 8 weeks of incubation. The similarity in

amount of decay may indicate an equal susceptibility to attack by C. globosum in these two woods. However, the lime blocks might have shown a different rate of decay if it had been possible to inoculate them with a spore suspension but the inhibition of C. globosum spores on lime sapwood, observed in Chapter 5, prevented this. Inoculation of wood with spores could lead to an initial lag phase in the decay rate, because spore germination must occur before wood degradation can begin, while actively growing mycelium may be able to initiate wood breakdown immediately.

The pine blocks showed a much lower susceptibility to attack by C. globosum which resulted in weight losses less than 25% of those observed in beech and lime. The strength losses were relatively high in the pine blocks which had been impregnated with the two higher concentrations of nitrogen. The strength losses at the two lower nitrogen concentrations were inconsistent and little could be concluded from them. The low weight losses observed in pine, even after 16 weeks of incubation, may be too low to be considered important. However, analysis of variance on the results indicated that there were statistically significant increasing weight losses incurred with increasing nitrogen concentrations.

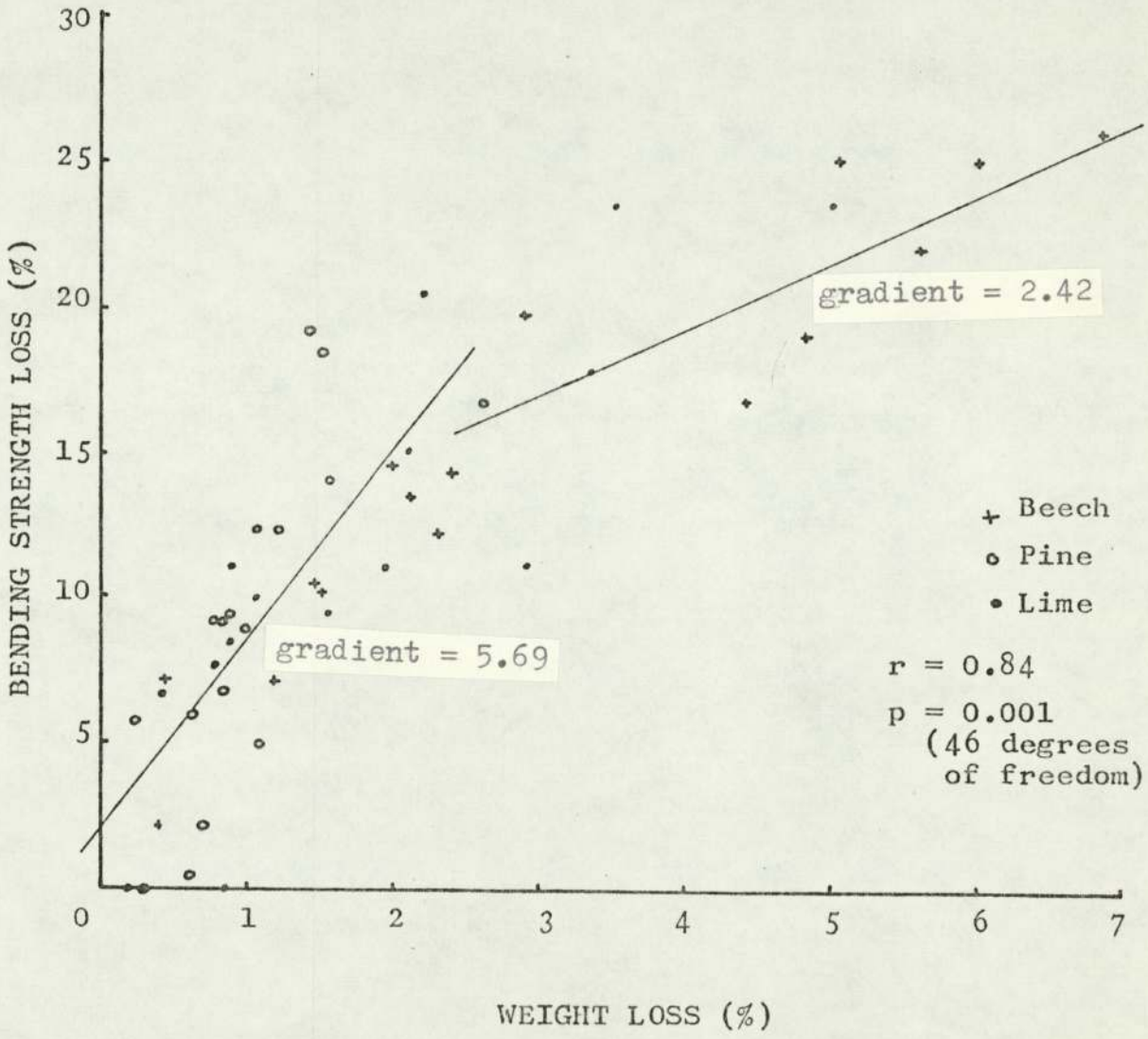
The comparison of strength and weight losses show large losses in bending strength corresponding to small weight losses. The timbers investigated showed strength losses of 5 - 10% for the first 1% weight loss. The pine blocks appeared to be particularly susceptible to loss in strength and incurred 16 - 20% strength loss when the weight

loss was 1.3 - 1.6%. Beech and lime samples showed strength losses of this order only with higher weight losses of 2.5 - 4.5%.

Measurement of bending strength seemed to be a more sensitive criterion for assessing levels of decay than weight loss at these early stages of colonisation. The standard deviations of the strength losses generally fell within an accepted 10% limit of the result mean, while those of the weight losses were often higher (Appendix I). Some anomalies in bending strength results did occur, as in the pine block experiment at the two lower nitrogen concentrations, but were probably caused by variations in wood samples and not by errors in measurement, especially in view of the very low weight losses.

The relationship between weight and strength losses can be studied by drawing a scatter diagram of results (Figure 21). All data were included and the correlation coefficient calculated to provide a measure of association. The coefficient value, $r = 0.84$, indicated a rectilinear relationship between weight loss and strength loss, in which the two variables are positively related. The significance of this value was checked on statistical tables and was significant at a level of $p = 0.001$. The effect of C. globosum on the weight and strength losses of beech has also been studied by Armstrong and Savory (1959). They, too, found that the bending strength of the wood was directly related to its loss in weight during decay and the relation between the two losses was of the same order of magnitude as that found in the present investigation.

Figure 21: Scatter diagram of percentage strength loss and percentage weight loss results.



If a rectilinear relationship between strength and weight loss continued during prolonged colonisation of sapwood, extrapolation of results indicated that a complete loss of bending strength would occur when weight losses reached 25%. It is possible that the rectilinear relationship only occurs during the early colonisation of timber and that if experiments had continued for several months, the percentage weight loss may have increased at a faster rate than the percentage strength loss. This would give rise to a curvilinear relationship. When the linear regressions and best lines of fit were calculated for the data from 0% to 2.5% and from 2.5% to 7.0% weight loss, they revealed a steeper gradient in the earlier data (Figure 21). This demonstrated the tendency toward a curvilinear relationship between weight and strength loss.

CHAPTER 7

THE EFFECT OF ASPARTIC ACID ADDITION TO WOOD ON THE SOFT-ROT DECAY RATE.

7.1 Introduction

The availability of different amino acids as nitrogen sources was shown to affect the growth rate and cellulose breakdown by test fungi when grown on laboratory media (Chapter 3). In particular, aspartic and glutamic acids were shown to promote good growth and cellulase production in comparison with that seen on inorganic nitrogen sources. The effect of aspartic acid addition to wood on the decay rate by soft-rot fungi was investigated in this chapter.

7.2 Materials and methods

Numbered pine and beech blocks were oven-dried at 80°C to constant weight and the dry weights recorded. A number of the blocks were then placed in a Soxhlet apparatus with 60% aqueous ethanol and leached for 24 hours to remove soluble materials. The leached blocks were oven-dried and the dry weights determined a second time. The loss in weight caused by leaching was calculated. The leachates from pine and beech blocks were reduced to a small volume in a rotary evaporator and analysed for amino acids.

Leached and unleached blocks were returned to an oven and heat-sterilised for 18 hours at 110°C. Individual sterile blocks were placed in sterile glass tubes closed at each end with a rubber bung.

Media were prepared as described in section 6.2 but with aspartic acid added as the nitrogen source to replace NH_4NO_3. The sterile wood blocks were moistened

to 80% moisture content by the addition of calculated quantities of media (see section 6.2) and the final quantities of added organic nitrogen/g wood were:- 0.0mg, 0.1mg and 1.0mg.

Conidial suspensions of Trichoderma viride and Alternaria tenuissima and a spore suspension of Chaetomium globosum were prepared by the method described for C. globosum in section 4.6.

Leached pine and beech blocks, with three levels of organic nitrogen, were inoculated with C. globosum spores. The unleached blocks were divided into three groups which contained equal numbers of blocks at each added nitrogen level. The three groups were inoculated with either T. viride, A. tenuissima or C. globosum.

After inoculation, each block was suspended above water, in a sterile culture chamber, and incubated at 25°C in a humidity chamber (see section 5.2).

Blocks were removed from incubation at intervals and the progress of decay assessed by the determination of weight and bending strength loss.

7.3 Results

7.3.1 The effects of leaching on the wood blocks

The mean total weight losses caused by leaching with aqueous ethanol were 0.2% in pine blocks and 2.0% in beech blocks i.e. a 1:10 ratio of total leaching losses between the two woods. Analysis of the leachates revealed the loss of a wide range of amino acids (Table 13).

Table 13: Amino acids leached from wood by extraction in alcohol/water.

	nM amino acid/g wood	
	Pine	Beech
Aspartic acid	0.85	15.23
Threonine	0.51	-
Serine	1.38	15.30
Glutamic acid	0.38	2.61
Proline	0.16	1.48
Glycine	1.28	7.05
Alanine	2.81	4.79
Cysteine	-	0.70
Valine	0.56	0.56
Methionine	-	trace
Isoleucine	0.56	1.41
Leucine	0.22	0.92
Tyrosine	0.31	0.56
Phenylalanine	0.06	0.49
Histidine	-	0.92
Ornithine	trace	trace
Lysine	0.30	0.85
Arginine	1.50	1.01
Total	10.88	53.88

Leaching caused a greater loss of amino acids/g wood in beech than in pine but only in a ratio of 5:1 i.e. less than the 10:1 ratio of total leaching losses.

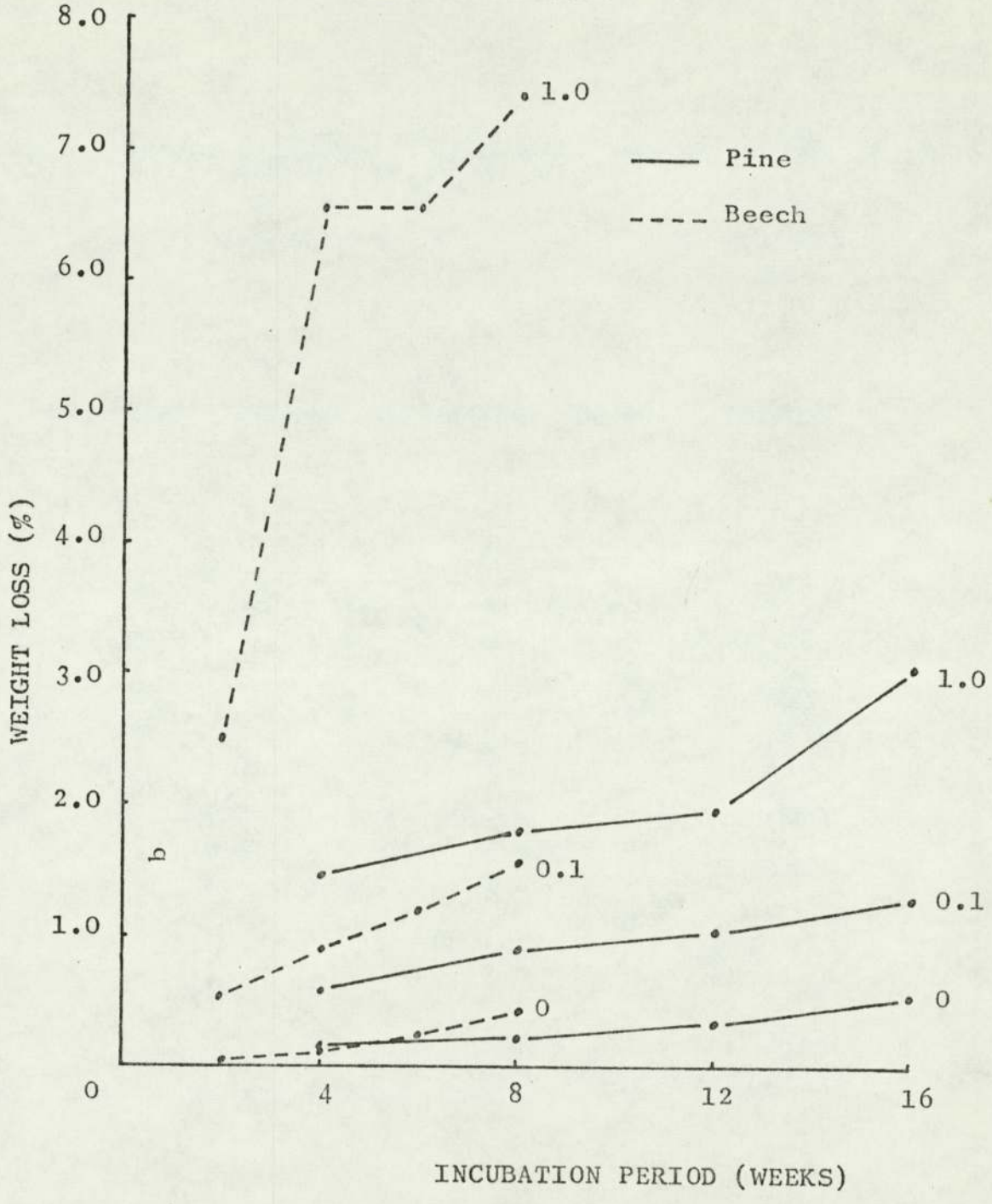
7.3.2 Soft-rot decay by Chaetomium globosum

The percentage weight and strength losses in unleached beech and pine decayed by C. globosum are shown in Figures 22 and 23. The pine blocks were incubated for twice as long as the beech blocks because of the slower decay rate of softwoods. The appearance of pine blocks inoculated with C. globosum and incubated for 16 weeks can be seen in Plate IVa. The production of perithecia on the block surfaces increased with the increase in added aspartic acid. Plate IVb demonstrates the effect of increased decay, caused by increased nitrogen addition, on the surface fibres of pine blocks decayed by C. globosum for 16 weeks. The break lines were of greater regularity in the blocks with the highest level of added aspartic acid when compared with the blocks with lower nitrogen addition.

Analyses of variance were carried out on results recorded in Figures 22 and 23. The differences between the decay criteria at each added nitrogen level were statistically significant for each incubation period sampled (Appendix I).

Incubation of leached beech and pine blocks inoculated with C. globosum was terminated after 9 weeks. Weight and strength losses are recorded in Figures 24 and 25. The physical appearance of leached beech blocks decayed by

Figure 22: Weight loss in beech and pine sapwood supplemented with aspartic acid and decayed by Chaetomium globosum.



0, 0.1 and 1.0 = levels of nitrogen (mg/g wood) added to moisten the blocks.

Figure 23: Strength loss in beech and pine sapwood supplemented with aspartic acid and decayed by Chaetomium globosum.

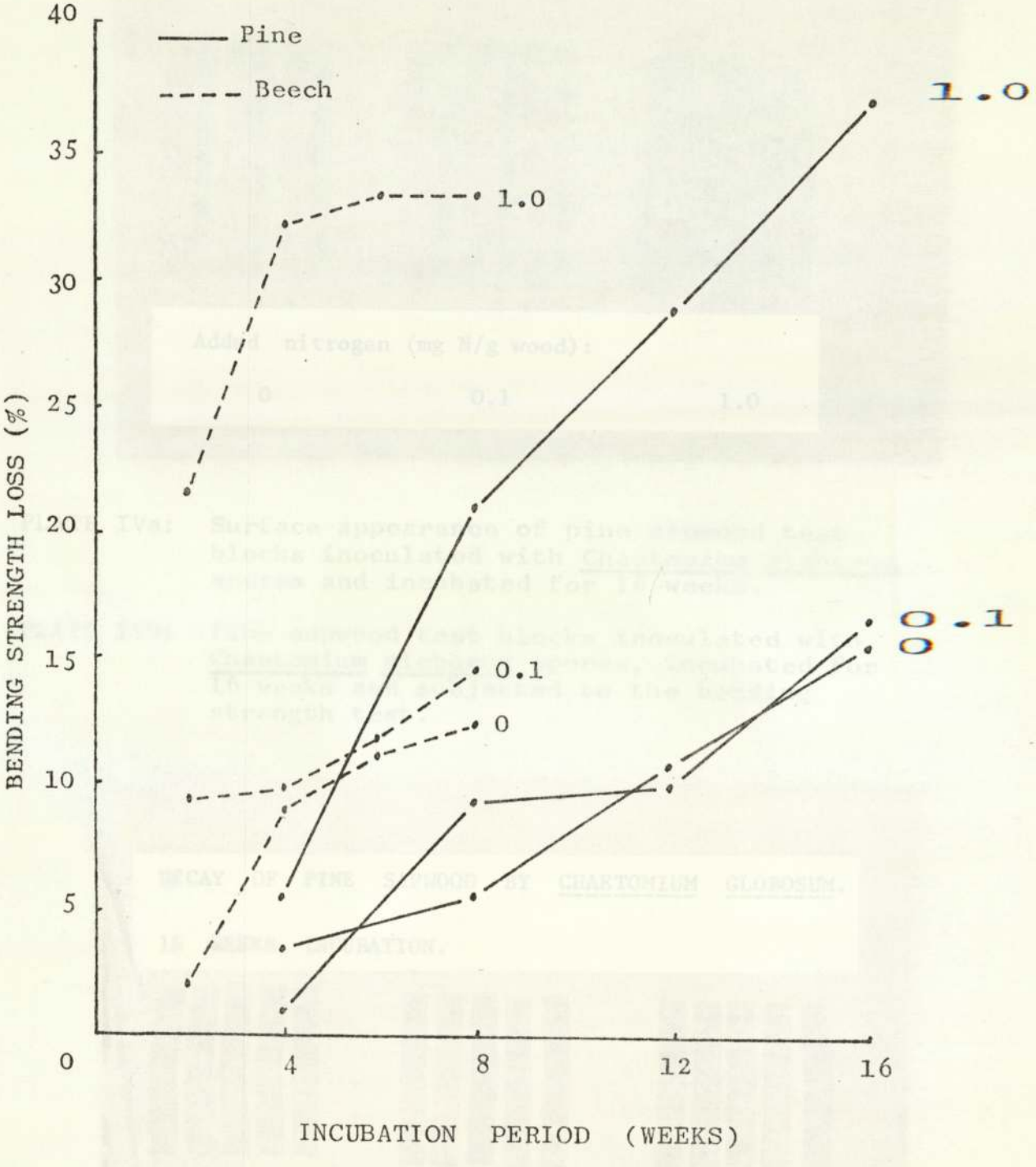


Figure 24: Weight loss in leached beech and pine sapwood supplemented with aspartic acid and decayed by Chaetomium globosum.

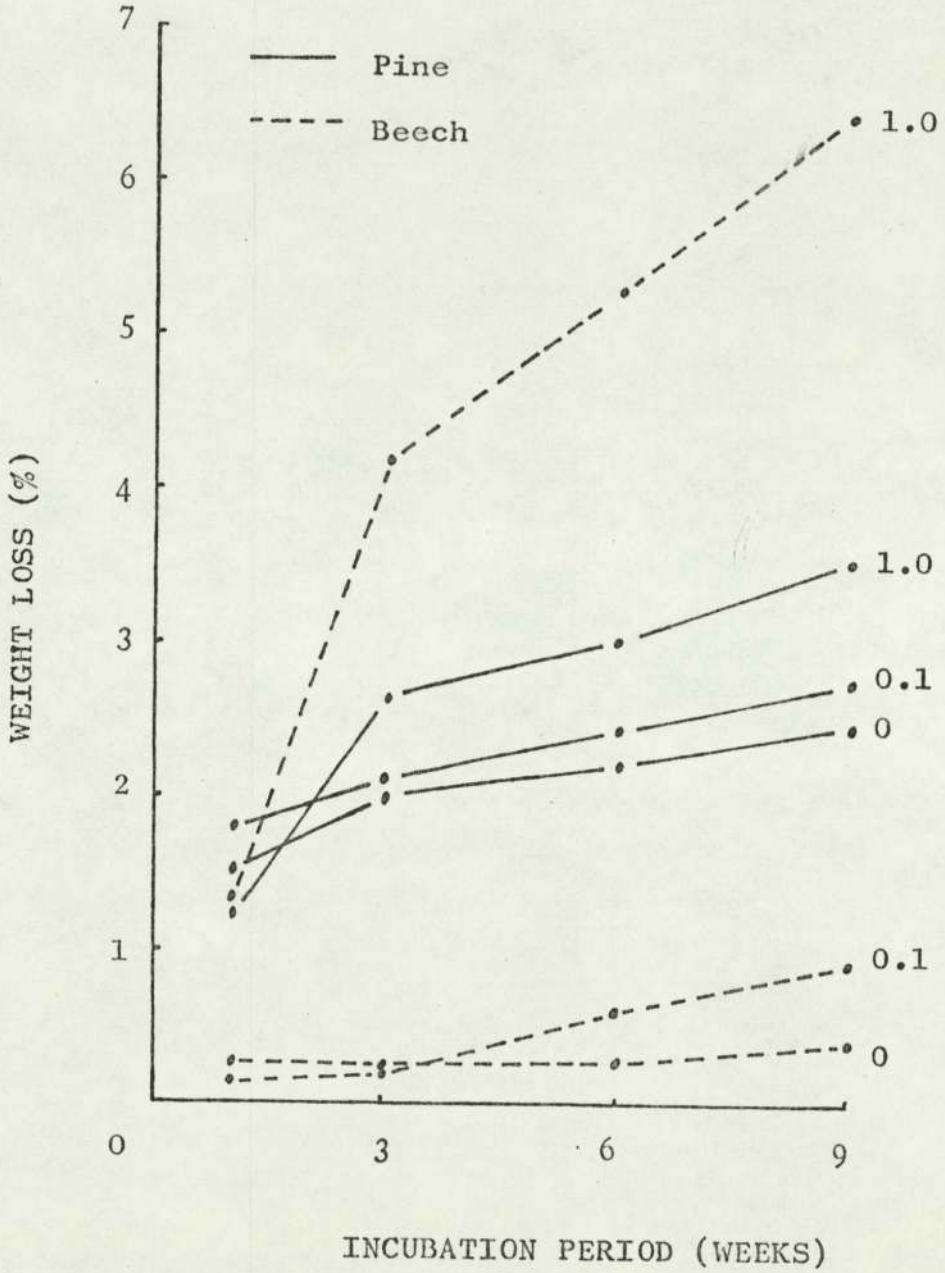


Figure 25: Strength loss in leached beech and pine sapwood supplemented with aspartic acid and decayed by Chaetomium globosum.

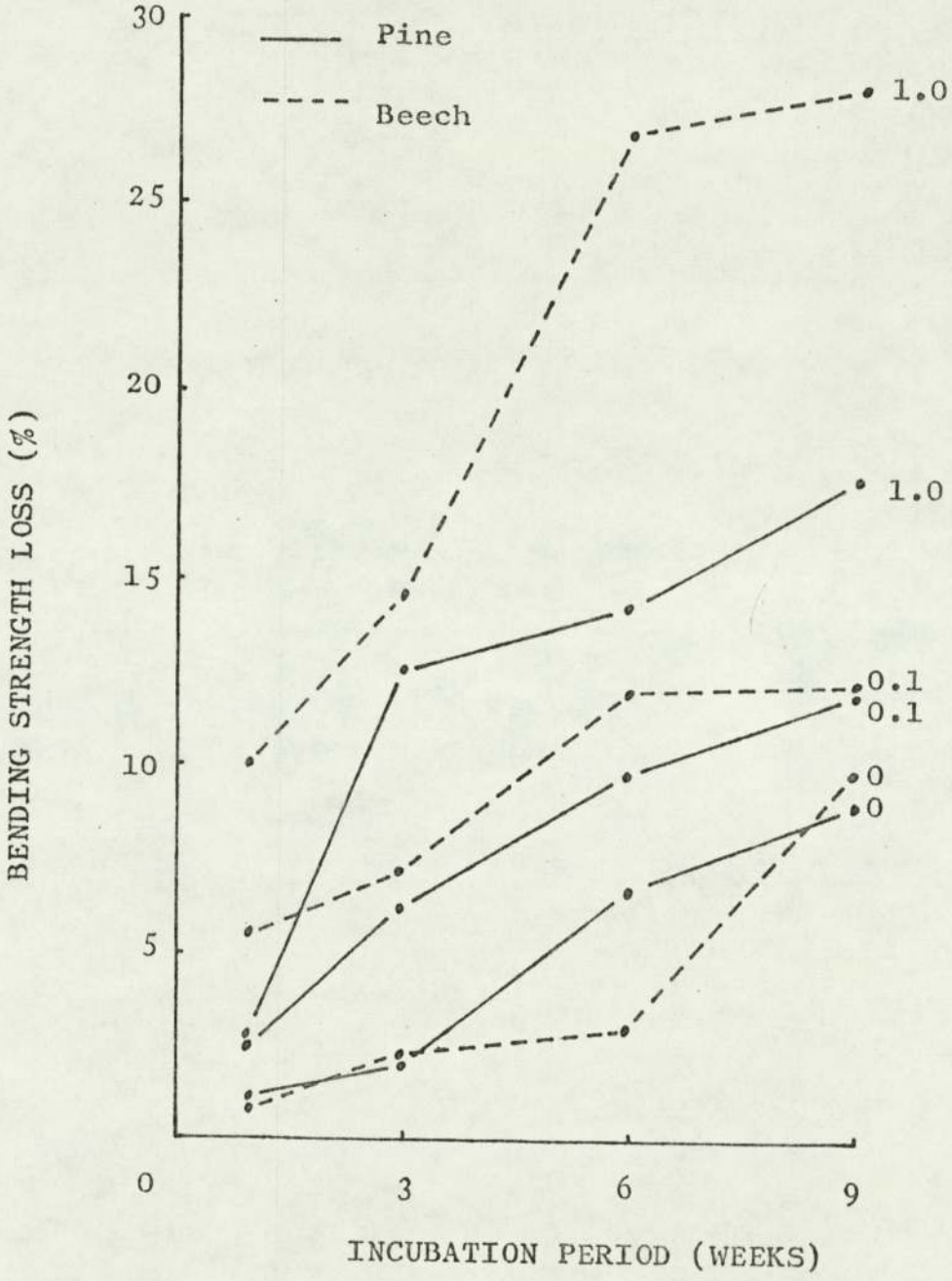
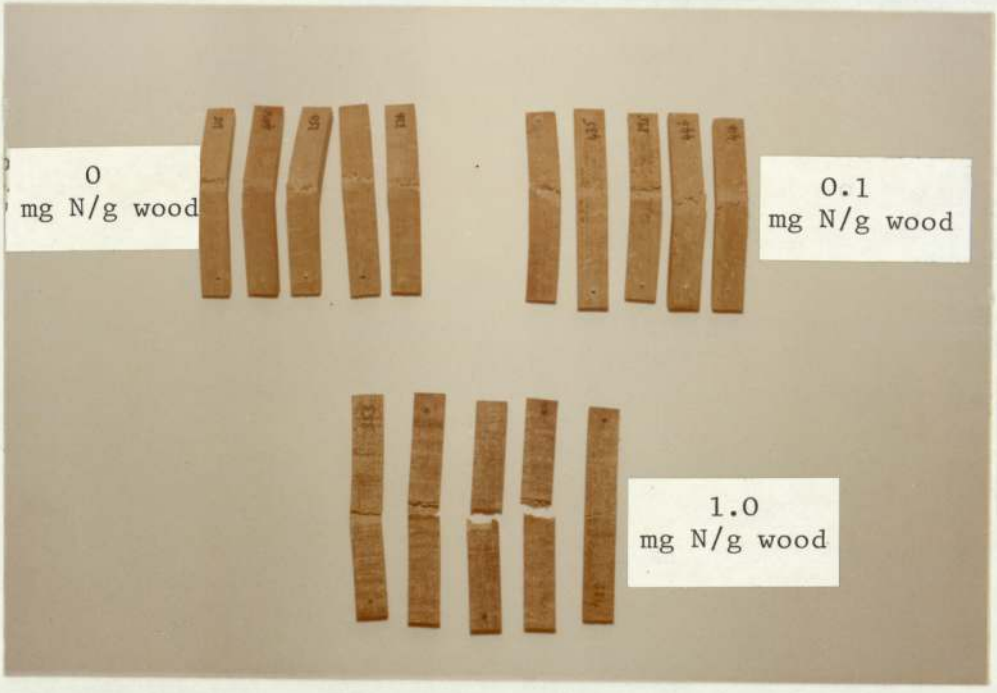


PLATE Va: Surface appearance of beech sapwood test blocks inoculated with Chaetomium globosum spores and incubated for 6 weeks.

PLATE Vb: Beech sapwood test blocks inoculated with Chaetomium globosum spores, incubated for 6 weeks and subjected to the bending strength test.



C. globosum for 6 weeks is shown in Plate Va. Perithecial production increased with the increase in added aspartic acid. Plate Vb demonstrates the effect of increased decay, caused by increased nitrogen addition, on the strength of beech blocks after 6 weeks of incubation. The effect on the surface fibres of the wood was similar to that seen in unleached pine blocks (Plate IV b) i.e. the break became more regular with increased decay.

Analyses of variance on weight loss results recorded in Figure 24 indicated that significant differences occurred in beech at each incubation period sampled but the results for pine, at different nitrogen levels, were only significantly different at the latter two incubation periods sampled. Differences between strength losses at different nitrogen additions (Figure 25) were also only significant at the latter two incubation periods (Appendix I).

7.3.3 Soft-rot decay by Alternaria tenuissima

Figures 26 and 27 summarise the weight and strength losses found in pine and beech blocks inoculated with A. tenuissima. Weight losses, in both woods, increased with increased nitrogen addition and with time. No relationship between strength loss and time or with increase in nitrogen addition was apparent. The strength losses at the end of the incubation period, however, were consistently related to nitrogen additions (Figure 27). Percentage weight losses attained in beech after 6 weeks of incubation were similar to the losses produced in pine after 12 weeks.

The weight losses caused by A. tenuissima in both beech and pine were significantly different between nitrogen

Figure 26: Weight loss in beech and pine sapwood supplemented with aspartic acid and decayed by Alternaria tenuissima.

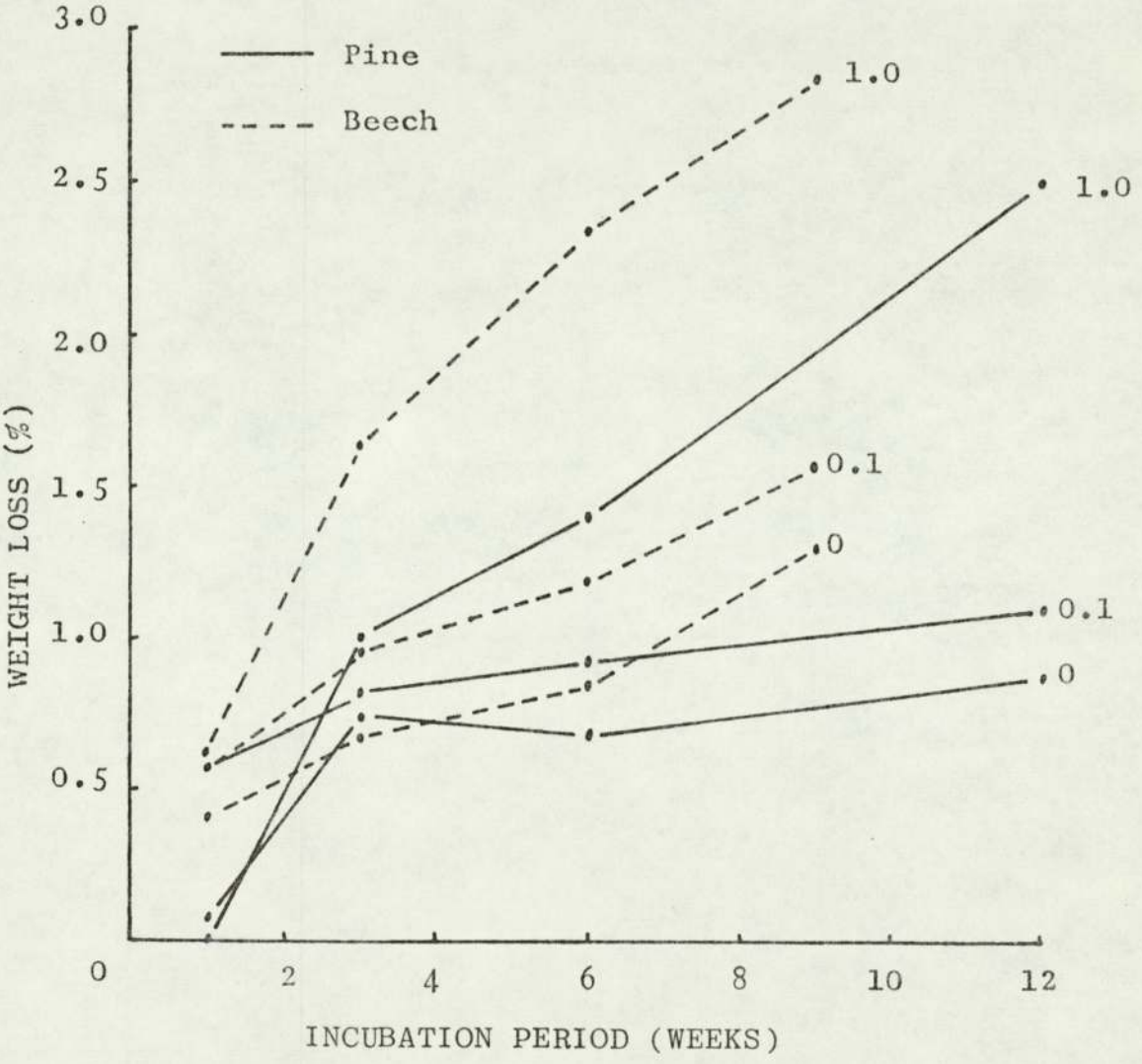
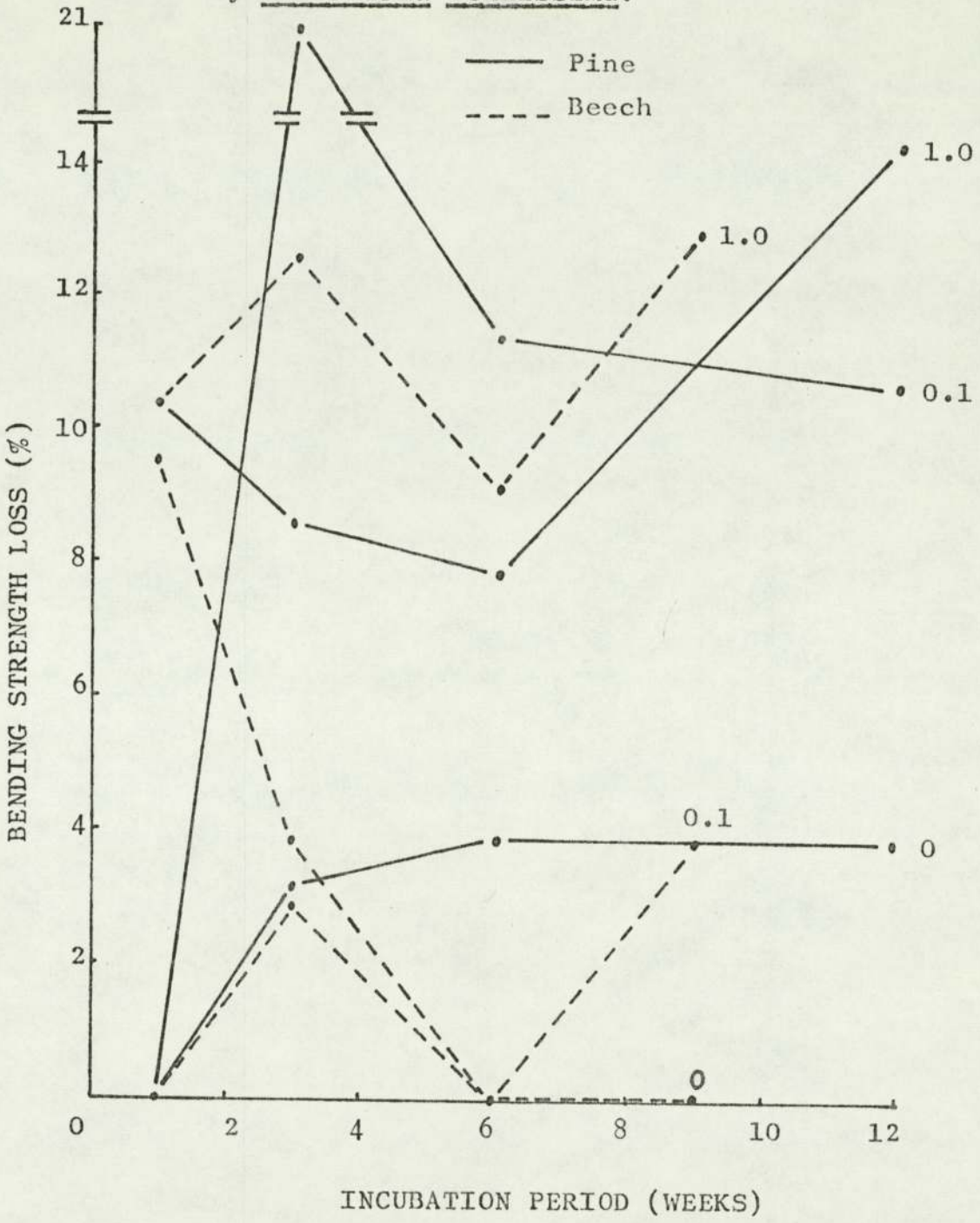


Figure 27: Strength loss in beech and pine sapwood supplemented with aspartic acid and decayed by Alternaria tenuissima.



additions at three of the incubation periods sampled. No significant differences were found in the strength loss results (Appendix I).

7.3.4 Soft-rot decay by Trichoderma viride

The weight and strength losses in wood blocks inoculated with T. viride are recorded in Figures 28 and 29. Weight loss results from both woods incubated for 6 weeks revealed greater decay of pine than beech. The effect of T. viride growth on the surface appearance of pine at the three levels of nitrogen addition can be seen in Plate VI. A general trend of increased weight loss with increased nitrogen addition and with time, similar to that observed with A. tenuissima, occurred. Again, the results for strength loss showed no consistent trend with time or nitrogen addition although, at the end of the incubation period, strength loss was greatest at the higher level of nitrogen addition in both woods.

The relatively small weight losses caused by T. viride and the variation between replicate samples meant that significant differences in losses between the three nitrogen additions were only obtained at the latter incubation periods sampled. Analyses of variance on strength losses recorded in pine blocks inoculated with T. viride showed no significant differences at any of the incubation periods sampled (Appendix I).

7.4 Discussion

Figure 28: Weight loss in beech and pine sapwood supplemented with aspartic acid and decayed with Trichoderma viride.

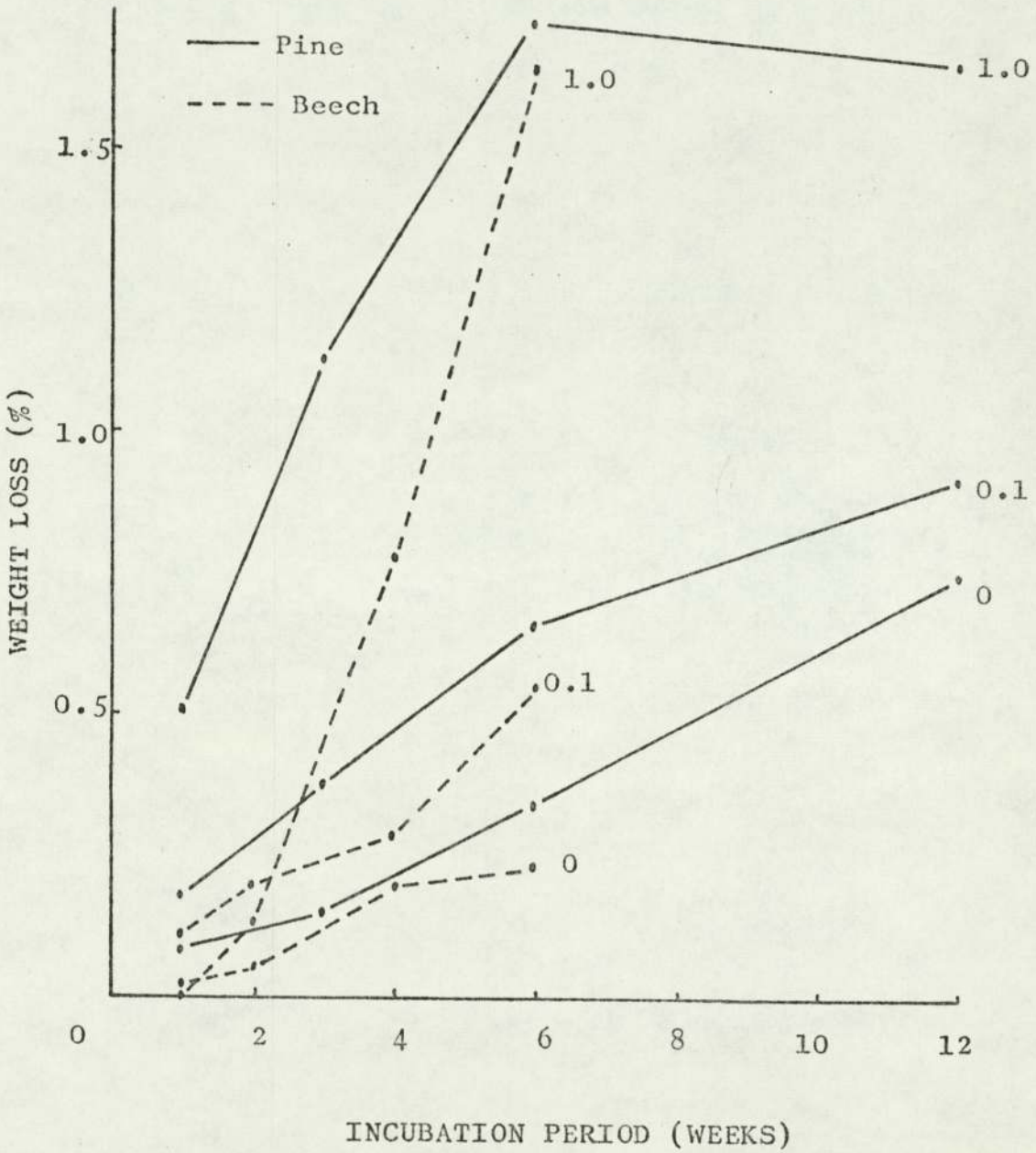
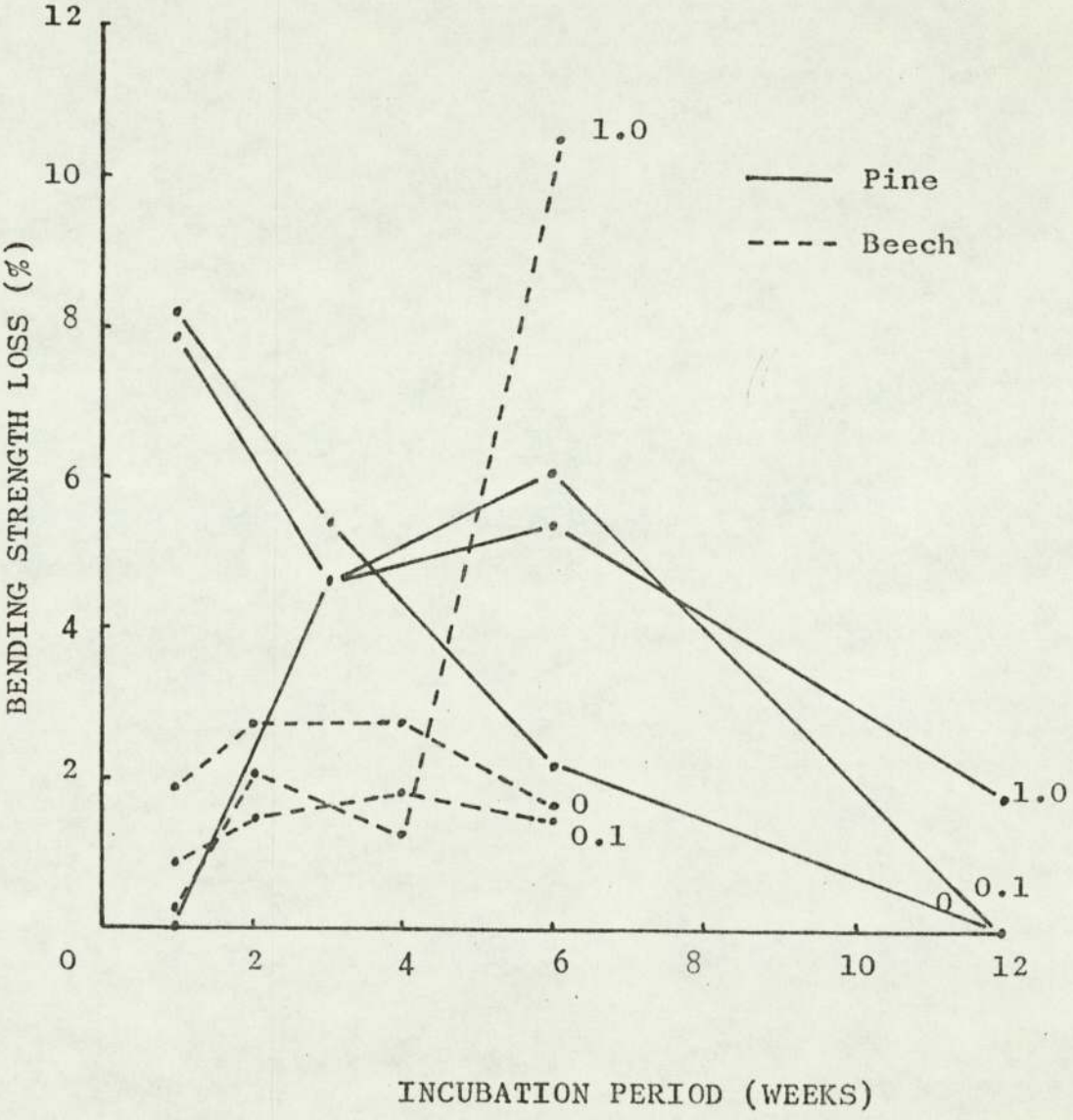


Figure 29: Strength loss in beech and pine sapwood supplemented with aspartic acid and decayed by Trichoderma viride.



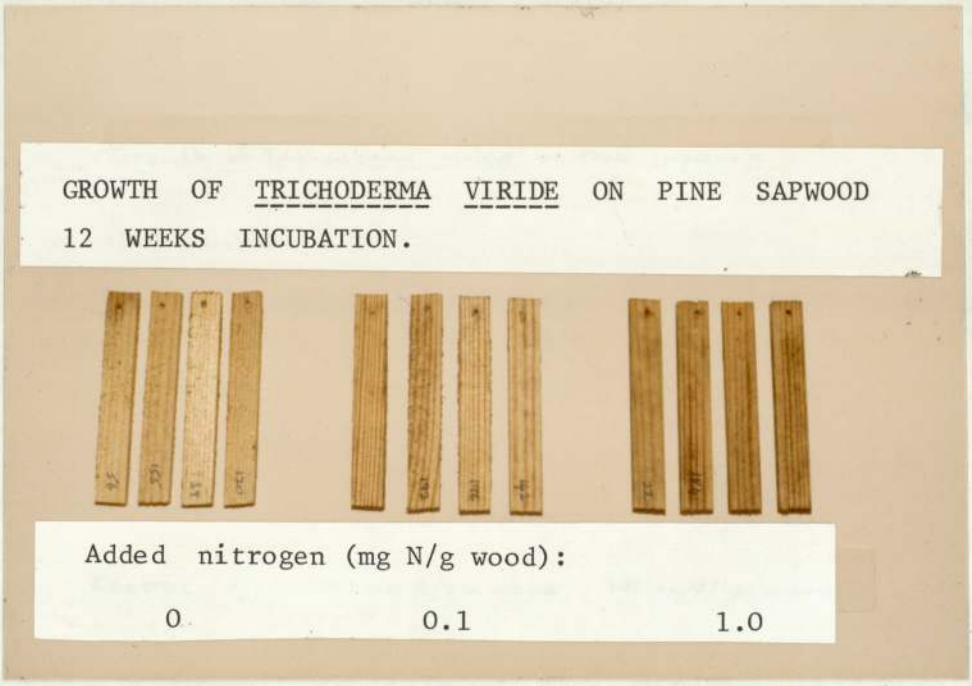


PLATE VI: Surface appearance of pine sapwood test blocks inoculated with Trichoderma viride and incubated for 12 weeks.

7.4.1 Chaetomium globosum decay of unleached beech and pine.

The percentage weight losses attained in pine, at the two lower levels of nitrogen addition, were of similar magnitude after 16 weeks incubation to those found in beech incubated for only 8 weeks. The bending strength losses at these times were also similar. The pine decayed then, at half the rate of beech sapwood at the lower two levels of nitrogen addition and revealed the higher susceptibility of the hardwood to decay by C. globosum. This relationship was also shown by the strength loss data from decayed beech and pine with the highest level of nitrogen addition. However, the weight losses at this level showed the pine decay rate to be much lower than half the rate in beech. The pine blocks suffered a rapid loss of bending strength when weight losses increased from 1.0% to 3.0%, which brought the strength losses, after 16 weeks incubation, to a level similar to those in beech after 8 weeks. The increase in weight loss from 1.0% to 3.0% was critical to the inherent strength of the pine blocks but did not affect the beech in such a dramatic way. The rapid strength loss in pine was not observed when inorganic nitrogen was added to the wood (Chapter 6) but in those experiments, weight losses never exceeded 1.6% and may have been just below the threshold of the increased rate of strength loss.

The effect of decay on beech and pine was illustrated in Plates IVb and Vb. At low levels of decay (low levels of nitrogen addition) the wood elements were not easily broken and instead separated from each

other under pressure, giving rise to an irregular, jagged break line. At higher decay levels (high levels of nitrogen addition) the break lines were clean and regular, because of the ease with which the decayed elements were broken.

7.4.2 *Chaetomium globosum* decay of leached beech and pine

The loss of soluble material from beech during leaching was greater than that in pine by a ratio of 10:1. Amino acid analyses of the leachates were carried out and revealed a greater loss in the beech wood than the pine. If the leaching procedure was assumed to have removed all soluble materials from the wood, then the obvious conclusion would be that beech contained a greater quantity of soluble materials than pine. However, the leaching procedure was less rigorous than that used in Chapter 2 to extract free amino acids from wood, where milled wood was extracted in a series of alcohol/benzene; alcohol/water and water for a total of 65 hours. This rigorous leaching of sawdust can be considered to be exhaustive and the percentage loss of amino acids by the less rigorous leaching of solid wood blocks was 100% in beech and only 11% in pine. A 1:10 ratio of amino acid loss in pine and beech was thus revealed which closely aligned to the 1:10 ratio of weight loss caused by leaching. Leaching of beech blocks had removed all the soluble, or free, amino acids and, because of the close alignment with weight loss, may be presumed to have removed the majority of soluble material from the wood. The leached pine blocks had lost only

11% of their free amino acids and may also have retained a large quantity of other soluble materials.

The difference in ease with which the woods were leached was probably related to the anatomical differences between the woods. Beech contains large vessels which make it more porous and accessible to liquids than the tracheids of pine. Thus, the leaching solution is able to flow through beech wood with less resistance than in pine and so remove a greater quantity of soluble material.

The loss of a large percentage of nitrogenous materials and other soluble nutrients, which would normally be available for fungal nutrition in unleached wood, would be expected to affect the rate of decay in leached wood. This was found to be true in both leached beech and pine when decayed by C. globosum but the effects of leaching were different in each wood. The rates of decay in leached beech blocks were slightly slower than those seen in unleached blocks. The decreases in decay rates were small, however, considering the large loss of amino acids and other soluble material. The leached wood still contained sufficient nutrients for C. globosum to promote decay even with no additional nitrogen source.

A different effect of leaching on decay was observed in pine blocks. Leached pine blocks showed a greater susceptibility to decay than unleached blocks in both weight and strength loss. The relatively small loss of soluble nitrogenous nutrients may not have been great enough to reduce decay rates but, even if this were not so, some factor had overridden the disadvantage of a loss in nitro-

genous materials and enhanced the decay rate. Pine is known to contain resins (Jane, 1970) and compounds fungistatic to some Basidiomycetes (Baker, Miller, Morgan and Savory, 1973) which reduce its susceptibility to decay. Leaching of pine may have removed some, or all, of these materials which adversely affect fungi and so reduced the hostility of the environment for decay fungi.

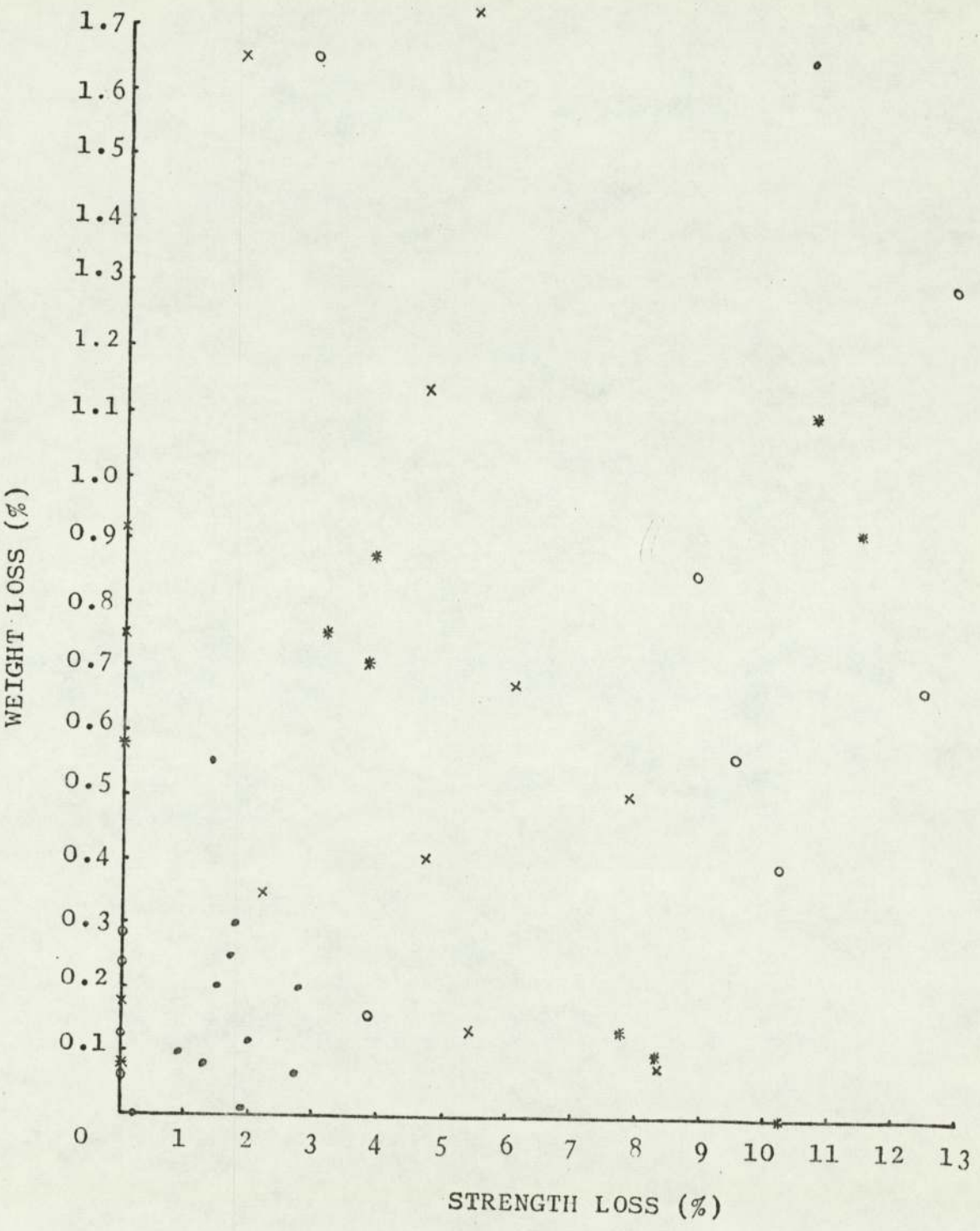
7.4.3 *A. tenuissima* and *T. viride* decay of beech and pine

Fungi Imperfecti have been found to be less aggressive as soft-rot agents than Ascomycetes (Courtois, 1963; Greaves and Savory, 1965; Kerner-Gang, 1965) and the present results confirmed these observations. Weight losses recorded from *A. tenuissima* and *T. viride* inoculated blocks at different nitrogen levels were not significantly different until the later incubation periods. However, the tendency towards increased weight loss with increased nitrogen can clearly be seen in Figures 26 and 28. The results in Figure 28 showed that, after 6 weeks of incubation, weight losses in pine inoculated with *T. viride* were higher than those in beech. Pine blocks inoculated with *C. globosum* or *A. tenuissima* had decayed at a rate approximately half of that which occurred in beech and had reflected the generally accepted view that softwoods are less susceptible to decay than hardwoods. The results from *T. viride* inoculated blocks did not fit in with this view and indicated that pine was as susceptible to decay by this organism as beech. The low significance of some

of the results make this observation insufficient, taken on its own, to state categorically without further long-term trials.

The strength losses from blocks inoculated with the two Fungi Imperfecti (Figures 27 and 29) showed no decay pattern, although if the losses at only the final incubation periods are considered, the greatest nitrogen addition invariably gave the highest strength loss. The intermediate strength losses appeared to vary at random with some losses of approximately 10% after one week of incubation and smaller strength losses in blocks incubated for longer periods. A scatter diagram of strength loss against weight loss was drawn (Figure 30) and the correlation coefficients calculated. The only significant correlation occurred between weight and strength losses observed in beech decayed by T. viride. The significance of these data relied on the results from blocks with the highest nitrogen addition incubated for the longest period. If this set of results were removed, and only the results from blocks with the two lower nitrogen additions and those from 1, 2 and 4 weeks of incubation with the higher nitrogen addition were used, then the correlation coefficient equated to-0.07 and was clearly not significant. Thus, the data at the lower weight losses could not be related to strength losses in T. viride inoculated beech. This lack of relationship between weight and strength loss was true for both woods inoculated with both Fungi Imperfecti. This was not an isolated observation but has also been observed by Zycha (1964), who found that he could not correlate weight

Figure 30: Correlation diagram of weight and strength loss recorded in beech and pine inoculated with Fungi Imperfecti.



o Beech and A. tenuissima
r = -0.57

• Beech and T. viride
r = 0.83

x Pine and T. viride
r = -0.17

* Pine and A. tenuissima
r = 0.42

and strength loss in T. viride decayed beech.

It is possible that the levels of decay recorded in these experiments were not high enough to reveal a relationship between weight and strength loss. The results from pine decayed by C. globosum reported in Chapter 6 also showed inconsistent strength losses, at the two lower levels of nitrogen addition, where the weight losses were below 1.1%.

An alternate, or contributory, explanation may lie in the method of decay by these two fungi. Courtois (1963) conducted a thorough investigation of the infection patterns in beech by a number of soft-rot fungi which included T. viride, C. globosum and A. tenuis. He found that weight losses caused by the Fungi Imperfecti were lower than those caused by C. globosum and that only C. globosum produced typical soft-rot cavities in the S_2 layers of the wood cell walls. A. tenuis and T. viride partially decomposed the S_1 and S_2 cell wall layers in the fibres but no soft-rot cavities were seen in either the fibres or the medullary ray parenchyma cells. The different decomposition symptoms caused by Fungi Imperfecti may explain the difficulty in relating weight loss and strength loss during the initial stages of decay.

7.4.4 Comparison between the effects on decay of inorganic and organic nitrogen addition

A comparison between the effects of inorganic and organic nitrogen on decay by C. globosum can be carried out if the inorganic nitrogen additions used in Chapter 6 are

transformed into units of mg nitrogen/g wood.

	nitrogen/litre medium	mg nitrogen/g wood
	0%	0
Pine	0.035%	0.6
	0.07%	1.2
	0.11%	1.8
	0%	0
Beech	0.035%	0.8
	0.07%	1.6
	0.11%	2.4

Thus, the 1.0mg nitrogen/g wood addition of aspartic acid can be compared approximately with 0.07% nitrogen addition of ammonium nitrate to pine and with 0.035% nitrogen addition of ammonium nitrate to beech.

Comparison of these results showed that the addition of organic nitrogen to both pine and beech promoted a greater degree of decay by C. globosum than the equivalent addition of inorganic nitrogen. This observation was true for both weight and strength loss and supported the results in Chapter 3, in which aspartic acid was shown to promote greater cellulose clearing by C. globosum than when an inorganic source was supplied. These results are also in general agreement with those of Findlay (1934), who worked with Basidiomycete decay fungi, and Sharp (1970) who worked with a variety of soft-rot organisms.

CHAPTER 8

THE EFFECT OF GLUTAMIC ACID ADDITION TO WOOD ON THE RATE OF DECAY BY *CHAETOMIUM GLOBOSUM*.

8.1 Introduction

Glutamic acid was shown to support relatively good growth and cellulose decomposition by Chaetomium globosum when supplied as the major nitrogen source in artificial media (Chapter 3). However, growth and cellulose breakdown were lower than when either aspartic acid or an inorganic nitrogen source was available in the laboratory media. This chapter records the decay rate of beech and pine sapwood to which glutamic acid was added.

8.2 Materials and methods

Beech and pine blocks were numbered, oven-dried at 80°C to constant weight and the dry weights recorded. The blocks were then returned to an oven and heat-sterilised for 18 hours at 110°C. Blocks were treated as described in section 5.3 to bring them to 80% moisture content with nutrient media (see section 6.2) which contained glutamic acid as the nitrogen source. Wood blocks with three levels of nitrogen addition were prepared i.e. 0.0mg additional nitrogen/g wood, 0.1mg additional nitrogen/g wood and 1.0mg additional nitrogen/g wood.

A spore suspension of C. globosum was prepared (see section 4.6) and the pine and beech blocks were inoculated. After inoculation each block was suspended above water in a sterile culture chamber and incubated at 25°C in a humidity chamber (see section 5.2).

A number of blocks were removed from incubation at intervals and the progress of decay assessed by the determination of weight loss and bending strength.

8.3 Results

The results are expressed graphically in Figures 31 and 32. Weight losses attained in pine blocks after 16 weeks of incubation, at the two lower levels of nitrogen addition, were similar to those found in beech blocks after only 9 weeks. Higher weight losses were incurred with the highest nitrogen addition. A 6% weight loss in beech, after 9 weeks of incubation, and a 4% weight loss in pine, after 16 weeks, were observed.

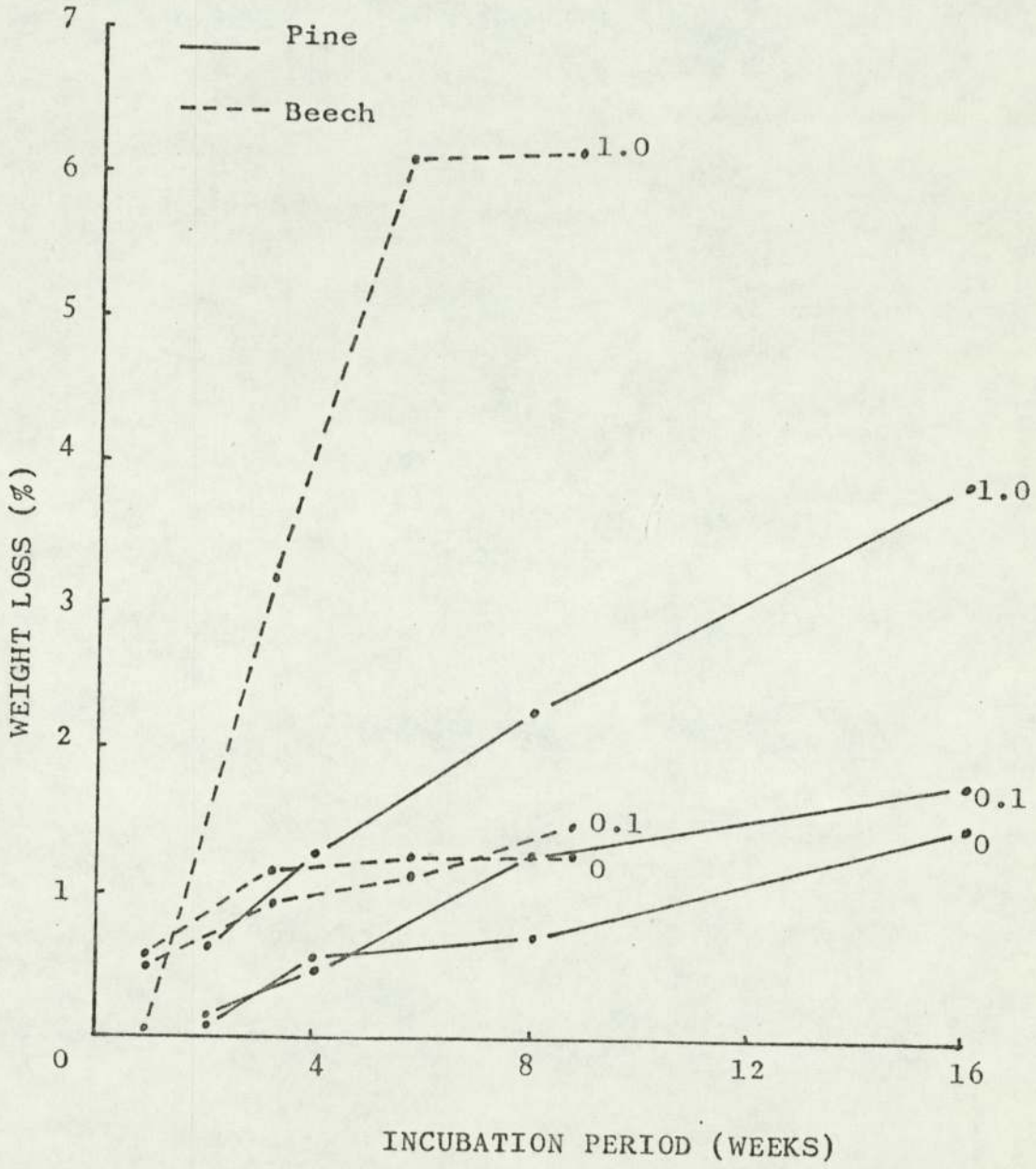
Bending strength losses revealed a 32% loss in beech after 9 weeks and 17% loss in pine after 16 weeks of incubation, at the higher level of nitrogen addition. Strength losses of 12% and below were found in both beech and pine at the two lower nitrogen additions.

Analyses of variance were carried out on sets of results from each incubation period sampled to determine the significance of differences observed between decay levels at different nitrogen additions. The results of the analyses are recorded in Appendix I.

8.4 Discussion

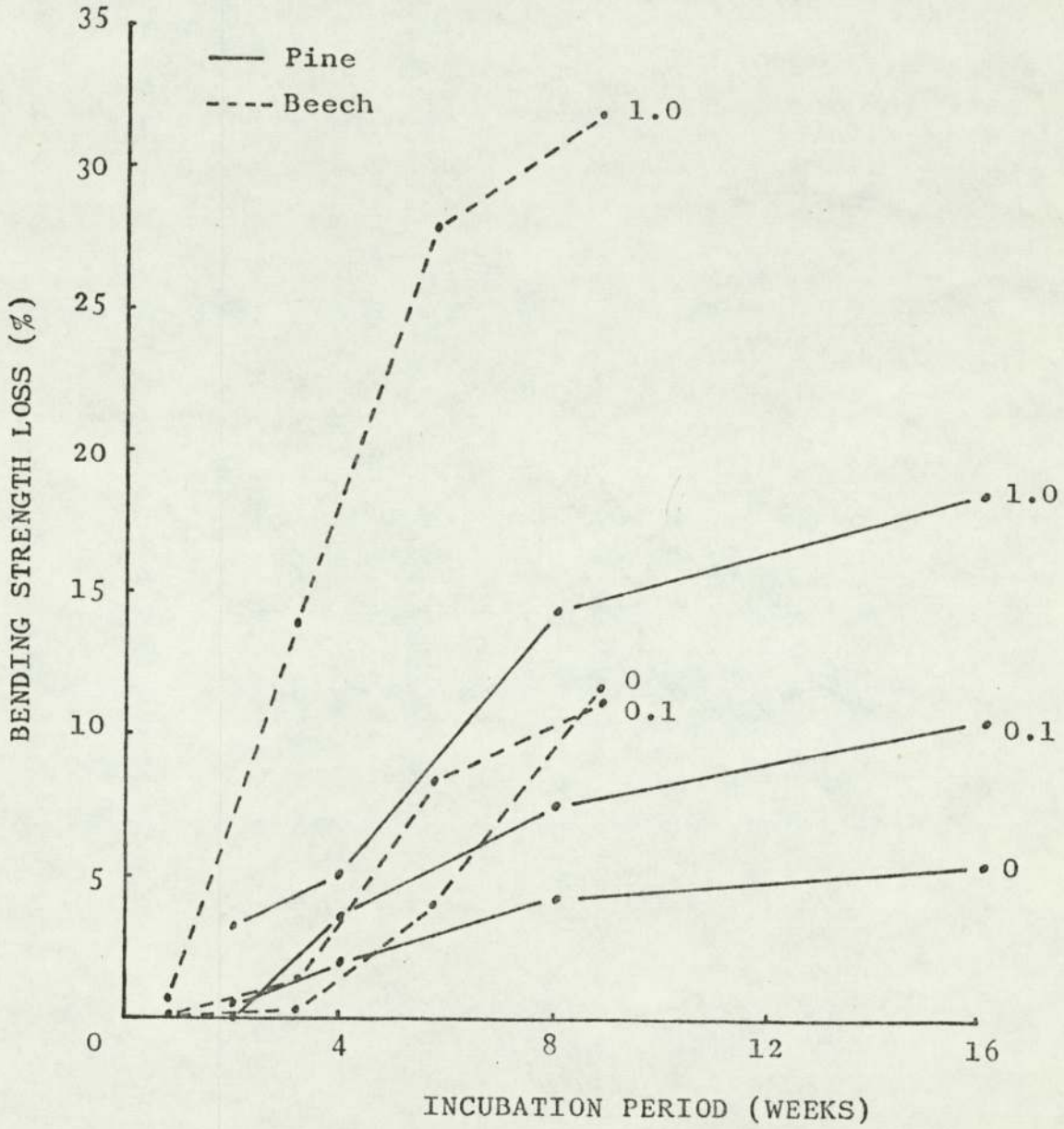
The addition of nitrogen to beech and pine, in the form of glutamic acid, resulted in the same pattern of increased decay with increased nitrogen addition observed when aspartic acid or inorganic nitrogen was added. The addition of a small quantity of glutamic acid (0.1mg nitrogen/g wood) resulted in decay levels similar to those found in blocks with no added nitrogen. The addition of 1.0mg nitrogen/g wood greatly increased the levels of decay over several weeks

Figure 31: Weight loss in beech and pine sapwood supplemented with glutamic acid and decayed by Chaetomium globosum.



0, 0.1 and 1.0 = levels of nitrogen (mg/g wood) added to moisten the blocks.

Figure 32: Strength loss in beech and pine sapwood supplemented with glutamic acid and decayed by Chaetomium globosum.



of incubation. The increased level of decay in beech was greater than that in pine.

The decay observed in beech supplemented with glutamic acid was comparable to that found in beech supplemented with aspartic acid and decayed by C. globosum (Figures 22, 23, 31 and 32). This indicated that C. globosum was capable of utilising both of these organic nitrogen sources to a similar extent during the decay process. The equal utilisation of the two organic sources of nitrogen was also suggested in the results from pine blocks. The weight losses recorded in these blocks were similar (Figures 22 and 31) although the strength losses in pine blocks supplemented with glutamic acid were slightly lower than those in the pine blocks supplemented with aspartic acid (Figures 23 and 32).

In comparisons between experiments the weight losses are likely to be more reliable since the disadvantage of calculating strength loss is that initial and final strengths cannot be measured on the same blocks i.e. the tests are destructive. Instead, a number of control blocks must be subjected to the strength test and the percentage strength loss of decayed samples based on this value. Thus, intra-experimental comparisons based on the same control strengths are subject to less variation than inter-experimental comparisons with different control strengths.

The comparison of the effects of glutamic acid addition with inorganic nitrogen addition on decay reveals that greater decay of wood is caused by C. globosum if glutamic acid is added to the wood (see section 7.3.5 for

conversion of data).

The similarity between C. globosum decay rates when aspartic and glutamic acids were added to the woods, and the reduced level of decay produced with an inorganic nitrogen source addition, could not have been predicted from the data provided from experiments in Chapter 3. There, it was found that C. globosum had greater linear growth and cellulose decomposition when aspartic acid was the nitrogen source than when glutamic acid was available. Cellulose decomposition was also greater when ammonium nitrate was provided than when glutamic acid was available as nitrogen source. The results collated from experiments on artificial media suggested that the order of nitrogen sources for greatest decay by C. globosum would be:- aspartic acid; ammonium nitrate; glutamic acid. The results from Chapters 6-8 have indicated the order to be:- aspartic and glutamic acids; ammonium nitrate. This difference in results emphasises the importance of studying fungal decay on natural substrates and that results from artificial laboratory media may be misleading.

CHAPTER 9

GENERAL DISCUSSION

9. General Discussion

The role of nitrogen in wood decay is known to be one of importance, and the availability of nitrogen in wood influences its susceptibility to fungal decay. The chemical nature of nitrogenous substances in the sapwood of pine, beech and lime was examined and the inherent amino acids of the timbers were classified into three groups:- those found as free amino acids, those incorporated into soluble proteins and those incorporated into insoluble proteins. In the early stages of wood colonisation, the nitrogen compounds most rapidly exploited by the colonising fungi are likely to be the free amino acids and soluble protein. These nitrogen sources are more readily available than those incorporated in the insoluble protein fraction which consisted of proteins bound in the wood cell walls. Several decay fungi were shown to be capable of utilising the individual amino acids found in sapwood for their own nutrition. Henningsson (1968) also showed the ability of Basidiomycete wood decay fungi to grow on amino acids found in timber.

The method of nitrogen utilisation by the fungi was not determined and both direct incorporation of wood amino acids into fungal protein and the conversion of wood amino-nitrogen to ammonium-nitrogen, prior to fungal utilisation, were possible. Further research in this field would be necessary to clarify the form in which nitrogen is utilised by decay fungi.

The similarity in the range and ratio of amino acids found in the timbers and in the mycelium of the

soft-rot organism, Chaetomium globosum, indicated that a direct assimilation of wood amino acids, by the decay organism, would be possible without conversion and resynthesis to its own amino acids. Merrill, Levi and Cowling (1966) revealed that Coriolus versicolor was able to utilise amino acids, dipeptides and proteins as nitrogen sources. This ability to utilise organic nitrogen compounds suggested that decay organisms could satisfy their nitrogen requirements by the utilisation of nitrogen constituents from wood and also by the reutilisation of nitrogen compounds in the cytoplasm of their own lysed cells. Once wood colonisation has begun, the death of early colonisers leaves a concentrated source of nitrogen compounds which may be exploited by secondary colonisers. The continuous recycling of nitrogen compounds, in a situation where the nutrient is sparse, greatly enhances the rate of decay.

Much of the work reported on wood decay has discussed the nitrogen content of the wood in relation to decay susceptibility. However, the majority of the workers recorded the total nitrogen content of wood and did not determine the proportion of this nitrogen which would be available to decay fungi. If the insoluble protein fraction of wood is relatively inaccessible to fungi, it is only the nitrogen content of the free amino acid and soluble protein fraction which will bear any relation to the decay susceptibility of wood. Thus, the timbers with higher quantities of nitrogen contained in these two fractions will be more susceptible to decay by early colonisers, although other timbers may show higher total nitrogen contents because of

relatively higher insoluble protein fractions. King (1975) found that it was only variation in the soluble nitrogen component of wood which influenced soft-rot production and considered that variations in total nitrogen content would not, necessarily, be a guide to soft-rot attack. Many hardwood species have higher total nitrogen contents than softwoods (Merrill and Cowling, 1966) and are more susceptible than softwoods to decay. The present investigation revealed the relatively higher quantities of free amino acids and soluble protein, in two softwoods compared with one hardwood, which may reflect their greater decay susceptibility. A detailed investigation into the distribution of soluble nitrogen in a much wider range of timbers would need to be undertaken to clarify the correlation between soluble nitrogen and soft-rot decay susceptibility.

If the importance of soluble, rather than total nitrogen, is accepted then it also influences the interpretation of the role of total carbon:total nitrogen ratios in the decay process. The cellulase activity of a large variety of fungi was studied at a range of C:N ratios by Levi and Cowling (1966, 1969) and Butcher and Drysdale (1974). In experiments, on laboratory cellulose media, the previously mentioned researchers determined cellulase activity to be increased at C:N ratios in the region of 50:1. This was a much lower ratio than that normally found in wood which has been attacked by soft-rot fungi. The total C:total N ratios of birch wood severely attacked by soft-rot fungi in tests carried out by Lundström (1973) were approximately 140-250:1. Butcher (1975c) claimed that

this illustrated that the optimal conditions for cellulase production were not necessarily optimal for decay. However, it would appear more likely that the discrepancy arises because, in the laboratory media, all of the nitrogen was soluble while, in the wood, the majority of the nitrogen was insoluble. The present investigation showed the levels of soluble nitrogen to be approximately 4% of the total nitrogen content of timbers and available C:soluble N ratios of timber will therefore be very different to the total C:total N ratios and may be much closer to the optimal C:N ratio found for cellulase production on laboratory media. The available C:soluble N ratios in spruce wood have been shown to be lower than the total C:N ratios and similar to the ratios found in laboratory media (King and Oxley, 1976). Thus, the majority of work on wood decay which included discussion on the role of nitrogen in decay susceptibility must be reassessed to consider only the soluble part of the nitrogen fraction.

The individual amino acids found in timber were investigated for their ability to support the growth and cellulolytic activity of decay fungi. The fungi utilised the amino acids as sole nitrogen source to various extents and showed preference for certain amino acids for both growth and cellulase production. Aspartic acid and glutamic acid proved to be the two nitrogen sources which consistently produced greatest fungal growth and cellulase production. These two amino acids were also consistently amongst the amino acids which predominated in the timbers and so provided one of the major forms of nitrogen in timber which

could be utilised by decay fungi. Glutamic and aspartic acids are also the precursors of many of the other amino acids and conversion of an amino acid back to glutamic or aspartic acid may occur, prior to utilisation by the fungi, if direct assimilation of the alternate amino acid is not possible.

Leonian and Lilly (1938, 1940), Steinberg (1942), Pelletier and Keitt (1954), Merrill and Cowling (1968), Henningsson (1968) and Ibbotson (1974) were amongst researchers who carried out work on the growth of fungi on individual amino acids. The work involved a wide range of fungi from different habitats and the amino acids generally found to support good fungal growth included aspartic and glutamic acids. These two particular amino acids were found to support greater growth and cellulase production of the decay fungi studied in the present investigation than other amino acids. They were also amongst the predominant amino acids in sapwood of the three timbers investigated. The correlation between these findings may help to explain the success of certain fungi in wood colonisation and decay. It is recognised, however, that more work is needed on the amino acid content of biodegradable materials and the amino acid nutrition of the fungi which colonise them.

The addition of aspartic and glutamic acids and of inorganic nitrogen and their effects on decay by Chaetomium globosum revealed the relationship between increased nitrogen and increased decay. The results confirmed the work of Findlay (1934), Siu and Sinden (1951) and Sharp (1970) who found that organic nitrogen sources

were more effective than inorganic sources in increasing decay rates. It can thus be assumed that woods which contain higher soluble organic nitrogen contents, particularly in the form of aspartic and glutamic acids, will be more susceptible to decay than woods with lower organic nitrogen contents. The greater decay susceptibility of sapwood compared with heartwood of timbers may also be partially explained by the higher content of organic nitrogen in the sapwood.

The interpretation of results from fungal growth and cellulase production on laboratory media differed from the decay results obtained when wood was used as a substrate. The discrepancies highlighted the difficulties in extrapolating laboratory results to cover natural decay situations.

The burial procedure, used by a great number of workers who have investigated wood decay, was shown to create complications of result interpretation if pure culture work was to be undertaken. Savory and Bravery (1970) reported that burial tests, in both sterile and unsterile soil, gave inconsistent results if carried out in a number of research institutes. One of the major variations between trials in different institutes was the soil organic matter content which affected the test results and gave rise to different toxicity levels of preservatives active against soft-rot organisms. The complexities of soil and the effects of soil chemistry on wood decay have been extensively discussed by Hilditch (1978). Substitute soils were used in decay studies by

Kaune (1970), Takahashi and Nishimoto (1973), Baines et al. (1977), Rubidge (1977) and in the present investigation but complications still existed. The question of how closely the environment within these substitute soils aligned with that found in natural soil was also debatable.

Burial test techniques were abandoned in favour of a system which, although more time-consuming in preparation, was subject to fewer variations of nutrients and moisture. A humidity chamber was devised which would enable the development of pure cultures of soft-rot organisms on wood blocks. Nutrients which affected decay could easily be monitored because only those nutrients added were involved.

The decay criteria used to measure the extent of decay were weight loss and strength loss. Weight loss has been universally used to measure decay but was prone to the disadvantage of long incubation periods, particularly with soft-rot decay, to achieve significant weight losses. In the present study even relatively small weight losses were found to be significant because of the greater similarity between replicates in the humidity chamber than those in soil burial experiments. Strength loss of decayed substrates has been measured in a variety of ways (Hartley, 1958) but the assessment of bending strength was most favoured. Loss of bending strength was found to correlate closely with weight loss of wood decayed by Chaetomium globosum but much less closely in wood decayed by Fungi Imperfecti. The magnitudes of strength loss were high compared with those of weight loss e.g. weight losses of

1-2% corresponded to strength losses of 10-15%. This high susceptibility to strength loss compared with weight loss in decayed wood was also observed by Armstrong and Savory (1959) and Merrill (1965). The large magnitudes of strength losses during the early stages of decay do not, however, make this criterion superior to that of weight loss in all circumstances. In order to reduce standard deviation in wood samples subjected to bending strength measurement, large replicate numbers are needed because of the inherent variability of wood. Wastage of wood was high in sample preparation for strength tests and the cost of this wastage may not be counterbalanced by the subsequent, more rapid completion of experiments. If sample sizes were reduced and the wood blocks made thinner, the techniques outlined in this thesis for investigating soft-rot decay, may be refined to an extent where the measurement of bending strength loss would be more valuable than that of weight loss. Further research is necessary to determine the limits within which strength loss measurement becomes advantageous.

Fungal spore suspensions were used to inoculate wood blocks prior to incubation in the humidity chamber. The role of spores in wood decay has been greatly neglected. Decay is often severe where wood is in contact with actively growing fungal mycelium i.e. in soil contact, and so this situation has been extensively studied. However, fungal decay also occurs in timber which is not in ground contact e.g. dry rot (Serpula lacrimans) and wet rot

(Coniophora puteana). Concern at the extent of decay, particularly in timber joinery, led to laboratory examination of simulated window joinery after outdoor exposure and revealed, not only Basidiomycete decay fungi, but also Ascomycetes and Fungi Imperfecti (Savory, Carey and Stribbling, 1977; Carey, pers. comm.). Some of the fungi found were known soft-rot decay organisms.

A few workers have investigated the germination of fungal spores on wood (Rishbeth, 1951, 1958; Morton and French, 1966; Toole, 1971a; Savory and Carey, pers. comm.) but all have been concerned with Basidiomycete decay fungi. Spores of Chaetomium globosum and conidia of Trichoderma viride and Alternaria tenuissima germinated readily on the sapwood of beech and pine in the present study but lime sapwood contained a water-soluble compound which prevented the germination of Chaetomium globosum spores (Appendix II). Lime sapwood, however, was easily decayed in soil burial experiments (Sharp and Levy, 1974) where the wood was exposed to both fungal mycelia and propagules. The discrepancy between susceptibility of lime wood to decay by spore and mycelial inoculation may also occur in other woods. The presence of fungitoxic or fungistatic substances in woods which affect either the vegetative or reproductive structures of decay fungi deserves further research.

CONCLUSIONS

1. The range and relative quantities of amino acids identified from the three timbers and one fungus investigated were similar although the concentrations of total amino acids were higher in the fungus than in the timbers. The similarity in range between timbers and timber-attacking organism suggested:-
   (a) that the wood-attacking organism may have evolved to, or are the organisms most efficient at, exploiting this particular ecological niche.
   (b) that the wood-attacking organism may be capable of direct assimilation of amino acids from the substrate with reduced need for breakdown and resynthesis of nitrogen compounds.
   (c) that the correspondence between amino acid composition of the substrate and fungus may be particularly helpful to the fungus in enabling it to survive in an environment notably deficient in nitrogen.

2. Physiological experiments provided results in relative growth, cellulolytic activity and carbon utilisation of T. viride, A. tenuissima, Chaetomium globosum and Coriolus versicolor on the individual amino acids identified in timber. The amino acids consistently found to provide greatest activity of the fungi were also those found to be most abundant in the sapwood of the investigated timbers.

3. The addition of organic nitrogen sources, in the form of glutamic and aspartic acids, to pine and beech sapwood blocks was found to increase the rate of soft-rot decay by Chaetomium globosum to a greater extent than the addition

of inorganic nitrogen. Timbers with high concentrations of available amino acids, particularly aspartic and glutamic acids, are likely to be more susceptible to soft-rot decay.

4. Ratio of C:N have frequently been discussed in publications on wood decay because of the high C:N ratios found in timbers. They are usually calculated as total C:total nitrogen ratios although the insoluble protein fraction is likely to be relatively inaccessible to fungi. Since it is only the soluble nitrogen component of wood which influences soft-rot decay rates it would be of greater value to reconsider C:N ratios in terms of available C: soluble nitrogen.

5. Measurement of bending strength loss was found to be a more sensitive decay criterion than weight loss over the initial period of soft-rot attack of pine and beech blocks by Chaetomium globosum. Strength losses of 10 - 15% were found when weight losses were only 1 - 2%. This was not the case, however, in experiments with A. tenuissima and T. viride. Decay of wood by these fungi resulted in strength losses which did not correlate with weight losses obtained over the same time periods. This was possibly due to the low levels of decay observed and the possible different method of attack by these organisms when compared with that of the Ascomycete.

APPENDICES

APPENDIX I

APPENDIX I

Section 3.3.1: The molecular weights and percentage nitrogen of a range of amino acids.

Nitrogen source	Molecular weight	% nitrogen
DL-Alanine $C_3H_7O_2N$	89.10	15.7
L-Arginine $C_4H_{10}O_2N_4.HCl$	210.68	26.6
L-Aspartic acid $C_4H_7O_4N$	133.10	10.5
L-Cysteic acid $C_3H_7O_5NS$	169.16	8.3
DL-Glutamic acid $C_5H_9O_4N$	147.13	9.5
Glycine $C_2H_5O_2N$	75.07	18.6
L-Histidine $C_6H_9O_2N_3$	115.16	27.1
L-Hydroxyproline $C_5H_9O_3N$	131.13	10.7
DL-Isoleucine $C_6H_{13}O_2N$	131.18	10.7
DL-Leucine $C_6H_{13}O_2N$	131.18	10.7
DL-Lysine $C_6H_{14}O_2N_2.HCl$	182.66	15.3
DL-Methionine $C_5H_{11}O_2NS$	149.21	9.4
DL-Ornithine $C_3H_{12}O_2N_2.HCl$	168.62	16.6
DL-Phenylalanine $C_9H_{11}O_2N$	165.19	8.5
L-Proline $C_5H_9O_2N$	115.13	12.2
DL-Serine $C_3H_7O_3N$	105.10	13.3
DL-Threonine $C_4H_9O_2N$	119.12	11.7
L-Tryptophan $C_{10}H_{12}O_2N_2$	204.23	13.7
L-Tyrosine $C_9H_{11}O_3N$	181.20	7.7
DL-Valine $C_4H_{11}O_2N$	117.15	11.9
Ammonium nitrate $H_4O_3N_2$	80.04	35.0
Ammonium sulphate $H_8O_4N_2S$	132.14	21.2

Appendix I - continued

Section 3.3.2: Analyses of variance on growth response to nitrogen source concentration.

Nitrogen source	Chaetomium globosum		Alternaria tenuissima		Trichoderma viride		Coriolus versicolor	
	'F' value	P	'F' value	P	'F' value	P	'F' value	P
Alanine	9.9	0.01	37.4	0.01	24.0	0.01	1.3	N.S
Arginine	81.0	0.01	19.1	0.01	9.3	0.01	10.5	0.01
Aspartic acid	3.4	N.S.	11.2	0.01	4.2	0.05	7.9	0.05
Cysteic acid	567.1	0.01	11.0	0.01	70.0	0.01	204.6	0.01
Glutamic acid	175.0	0.01	1.6	N.S.	5.5	0.05	14.0	0.01
Glycine	0.0	N.S.	74.0	0.01	1.0	N.S.	1.0	N.S.
Histidine	0.6	N.S.	4.4	0.05	0.3	N.S.	2.2	N.S.
Hydroxyproline	6.6	0.05	26.0	0.01	264.0	0.01	456.0	0.01
Isoleucine	10.0	0.01	1.6	N.S.	17.8	0.01	0.6	N.S.
Leucine	35.6	0.01	3.0	N.S.	0.1	N.S.	11.0	0.01
Lysine	8.5	0.01	3.0	N.S.	9.2	0.01	0.4	N.S.
Methionine	5.9	0.05	24.0	0.01	6.2	0.05	1.0	N.S.
Ornithine	16.8	0.01	20.2	0.01	62.7	0.01	0.4	N.S.
Phenylalanine	455.8	0.01	30.0	0.01	0.6	N.S.	6.1	0.05
Proline	3.0	N.S.	13.8	0.01	101.0	0.01	488.0	0.01
Serine	8.3	0.01	27.0	0.01	7.6	0.05	482.0	0.01
Threonine	35.3	0.01	9.5	0.01	5.7	0.05	-	-
Tryptophan	1.5	N.S.	6.2	0.05	6.9	0.05	2.2	N.S.
Tyrosine	401.3	0.01	31.0	0.01	7.0	0.05	110.0	0.01
Valine	13.0	0.01	5.2	0.05	127.0	0.01	16.1	0.01
Ammonium nitrate	301.4	0.01	1.8	N.S.	7.0	0.05	14.0	0.01
Ammonium sulphate	277.7	0.01	4.3	0.05	6.0	0.05	2.0	N.S.

p = probability N.S. = not significant

Appendix I - continued

Section 3.4.1: The C:N ratios of a selection of amino acids.

Carbon and nitrogen source	Molecular weight	%N	%C	C:N
Asparagine $C_4H_8O_3N_2$	150.13	18.6	32.0	1.7:1
Aspartic acid $C_4H_7O_4N$	133.10	10.5	36.1	3.4:1
Glutamic acid $C_5H_9O_4N$	147.13	9.5	40.1	4.2:1
Histidine $C_6H_9O_2N_3$	155.16	27.1	46.4	1.7:1
Leucine $C_6H_{13}O_2N$	131.18	10.7	54.9	5.1:1
Threonine $C_4H_9O_2N$	119.12	11.7	40.3	3.4:1
		Mean		3.2:1
Ammonium nitrate $H_4O_2N_2$	80.04	35.0	-	
Ammonium sulphate $H_8O_4N_2S$	132.14	21.2	-	
Glucose $C_6H_{12}O_6$	180.00	-	40.0	

Appendix I - continued

Section 4.6: Analyses of variance on strength losses in lime sapwood maintained in a substrate at various w.h.c. and decayed by C. globosum.

	Burial substrates		
	Soil	Vermiculite	Perlite
100% w.h.c.	51.0	6.5	7.0
σ	5.2	3.0	2.4
80% w.h.c.	48.0	6.0	6.5
σ	5.5	2.1	2.8
60% w.h.c.	43.0	6.3	5.5
σ	5.1	2.1	2.4
40% w.h.c.	7.0	6.2	4.0
σ	4.4	5.0	2.6
'F' value	41.4	0.9	1.2
probability	0.01	N.S.	N.S.
σ d	2.84	-	-
For p = 0.01 difference of means must be >	16.6	-	-

σ = standard deviation

N.S. = not significant

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 5.3: Levels of media not absorbed by wood blocks.

	Pine	Beech	Lime
Number of samples	10	10	10
% nutrient media remaining in glass tube	3.0 (1.2)*	4.5 (2.3)	2.3 (0.3)
Block moisture content (%)	77 (5.0)	75 (6.0)	79 (9.0)

* standard deviations given in brackets

Appendix I - contined

Section 6.3: Analyses of variance on decay criteria in beech sapwood supplemented with inorganic nitrogen and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N concentration (%)				'F' value	P	σ d	For p=0.01 difference of means must be >
	0.0	0.035	0.07	0.11				
3	0.87	1.21	2.04	2.92	68.4	0.01	0.15	0.47
σ	0.16	0.19	0.26	0.29				
6	0.95	1.46	4.40	5.60	204	0.01	0.22	0.67
σ	0.19	0.05	0.58	0.27				
9	1.48	2.33	4.80	5.99	86.7	0.01	0.32	0.96
σ	0.19	0.21	0.22	0.87				
12	2.13	2.41	5.05	6.87	176.2	0.01	0.23	0.72
σ	0.50	0.30	0.34	0.24				
	Mean bending strength (lbs force)							
3	54.7	52.0	47.9	45.5	9.62	0.01	1.88	5.67
σ	3.10	3.34	2.10	2.41				
6	51.9	50.0	46.7	43.6	13.25	0.01	1.41	4.26
σ	1.80	2.04	2.70	1.70				
9	50.4	48.8	46.0	42.0	5.40	0.05	2.88	8.67
σ	4.60	3.60	3.87	4.76				
12	48.4	48.3	45.2	40.5	8.50	0.01	2.34	4.06
σ	2.64	2.25	4.25	4.30				

Appendix I - continued

Section 6.3: Analyses of variance on decay criteria in lime sapwood supplemented with inorganic nitrogen and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N concentration (%)				'F' value	P	σ d	For P=0.01 difference of means must be >
	0.0	0.035	0.07	0.11				
2	0.20	0.85	0.92	0.92	0.125	N.S.	-	-
σ	0.30	0.26	0.26	0.34				
4	0.45	1.06	2.08	2.19	24.8	0.01	0.26	0.75
σ	0.27	0.23	0.69	0.52				
6	0.77	1.95	2.60	3.50	51.5	0.01	0.27	0.78
σ	0.16	0.57	0.09	0.78				
8	1.48	2.90	3.30	5.00	7.4	0.01	0.76	2.16
σ	0.10	0.91	0.58	0.30				
	Mean bending strength (lbs force)							
2	41.6	41.7	39.7	38.6	1.6	N.S.	-	-
σ	3.30	3.90	2.60	4.70				
4	40.6	39.2	37.0	34.6	11.6	0.01	1.34	3.80
σ	2.50	1.60	2.10	3.10				
6	39.7	38.6	36.3	33.3	6.53	0.01	1.55	4.40
σ	2.50	2.50	3.50	2.40				
8	39.4	38.6	35.8	33.4	4.94	0.01	1.89	4.80
σ	5.00	2.90	2.80	3.30				

Appendix I - continued

Section 6.3: Analyses of variance on decay criteria in pine sapwood supplemented with inorganic nitrogen and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N concentration (%)				'F' value	P	σ d	For p=0.01 difference of means must be >
	0.0	0.035	0.07	0.11				
4	0.26	0.62	0.86	0.90	15.8	0.01	0.10	0.29
σ	0.17	0.27	0.30	0.19				
8	0.35	0.82	0.99	1.05	7.1	0.01	0.17	0.48
σ	0.13	0.27	0.19	0.48				
12	0.61	0.81	1.20	1.55	11.1	0.01	0.17	0.48
σ	0.20	0.17	0.25	0.50				
16	0.70	1.12	1.42	1.51	23.0	0.01	0.13	0.36
σ	0.27	0.16	0.16	0.28				
	Mean bending strength (lbs force)							
4	37.6	39.7	37.3	36.2	1.4	N.S.	-	-
σ	2.27	4.90	0.98	3.47				
8	40.2	36.3	36.4	35.1	5.5	0.05	1.20	3.41
σ	2.36	1.25	2.46	2.27				
12	39.8	36.3	35.1	34.4	5.48	0.05	0.89	2.51
σ	1.29	1.33	1.07	2.31				
16	39.1	38.0	32.2	32.7	12.3	0.01	1.16	3.30
σ	1.46	2.88	2.21	1.25				

Appendix I - continued

Section 7.3.2: Analyses of variance on decay criteria in beech sapwood supplemented with aspartic acid and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
2	0.01	0.52	2.50	752	0.01	0.07	0.19
σ	0.02	0.28	0.20				
4	0.14	0.90	6.54	322	0.01	0.26	0.73
σ	0.20	0.30	0.32				
6	0.23	1.18	6.52	150	0.01	0.18	0.49
σ	0.17	0.31	0.54				
8	0.42	1.55	7.44	1,458	0.01	0.02	0.06
σ	0.22	0.25	0.32				
	Mean bending strength (lbs force)						
2	50.0	46.3	40.0	29	0.01	1.90	5.30
σ	4.91	3.25	3.67				
4	46.4	46.0	34.6	21	0.01	2.04	5.70
σ	3.52	2.10	2.41				
6	45.5	45.0	34.2	19	0.01	1.82	5.23
σ	4.00	3.40	2.37				
8	44.9	43.7	34.0	36	0.01	1.53	4.09
σ	2.19	4.30	1.02				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 7.3.2: Analyses of variance on decay criteria in pine sapwood supplemented with aspartic acid and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
4	0.29	0.61	1.44	28	0.01	0.15	0.42
σ	0.23	0.37	0.35				
8	0.22	0.91	1.80	21	0.01	0.25	0.68
σ	0.11	0.30	0.83				
12	0.35	1.05	1.98	46	0.01	0.19	0.54
σ	0.17	0.33	0.67				
16	0.56	1.30	3.02	70	0.01	0.21	0.59
σ	0.07	0.17	0.27				
	Mean bending strength (lbs force)						
4	28.0	28.7	25.4	11	0.01	1.19	3.35
σ	2.99	3.21	2.90				
8	27.4	26.3	22.2	4	0.05	1.61	4.70
σ	2.92	2.43	2.32				
12	25.9	26.1	20.7	4	0.05	1.43	4.24
σ	1.72	1.66	0.99				
16	24.5	24.2	18.3	114	0.01	0.74	2.10
σ	2.40	2.10	1.04				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 7.3.2: Analyses of variance on decay criteria in leached beech sapwood supplemented with aspartic acid and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
1	0.23	0.15	1.29	30	0.01	0.17	0.48
σ	0.17	0.19	0.48				
3	0.23	0.22	4.18	776	0.01	0.11	0.32
σ	0.23	0.20	0.18				
6	0.25	0.61	5.27	931	0.01	0.13	0.38
σ	0.24	0.16	0.29				
9	0.42	0.93	6.42	1385	0.01	0.13	0.38
σ	0.07	0.11	0.39				
	Mean bending strength (lbs force)						
1	44.6	42.5	40.4	2	N.S.	-	-
σ	3.26	3.29	3.58				
3	44.0	41.8	38.4	1	N.S.	-	-
σ	4.70	2.70	2.28				
6	43.7	39.6	32.9	13	0.01	2.16	6.21
σ	3.30	3.90	2.23				
9	40.9	39.5	32.4	17	0.01	1.68	4.84
σ	3.02	3.72	2.20				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 7.3.2: Analyses of variance on decay criteria in leached pine sapwood supplemented with aspartic acid and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
1	1.49	1.78	1.23	4	N.S.	-	-
σ	0.23	0.34	0.51				
3	1.99	2.10	2.63	1	N.S.	-	-
σ	0.26	0.23	0.22				
6	2.17	2.42	2.99	14	0.01	0.17	0.50
σ	0.30	0.20	0.35				
9	2.46	2.73	3.53	21	0.01	0.12	0.35
σ	0.33	0.39	0.35				
	Mean bending strength (lbs force)						
1	39.6	39.0	38.9	0	N.S.	-	-
σ	3.48	3.34	3.32				
3	39.2	37.5	35.0	2	N.S.	-	-
σ	3.21	3.20	2.01				
6	37.3	36.1	34.3	27	0.01	0.45	1.29
σ	3.16	2.28	2.29				
9	36.5	35.2	33.0	25	0.01	0.42	1.20
σ	0.29	0.31	1.28				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 7.3.3: Analyses of variance on decay criteria in beech sapwood supplemented with aspartic acid and decayed by A. tenuissima.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
1	0.40	0.57	0.61	1	N.S.	-	-
σ	0.37	0.16	0.34				
3	0.67	0.95	1.63	30	0.01	0.13	0.38
σ	0.17	0.19	0.28				
6	0.84	1.18	2.34	23	0.01	0.24	0.71
σ	0.16	0.29	0.58				
9	1.30	1.57	2.83	13	0.01	0.32	0.95
σ	0.67	0.35	0.49				
	Mean bending strength (lbs force)						
1	41.7	47.8	41.4	3	N.S.	-	-
σ	6.40	4.70	12.63				
3	44.3	44.5	40.2	1	N.S.	-	-
σ	15.90	14.70	11.80				
6	47.1	49.4	41.8	6	N.S.	-	-
σ	7.00	6.30	9.00				
9	48.0	44.2	40.0	3	N.S.	-	-
σ	6.20	15.30	6.70				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2x \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 7.3.3: Analyses of variance on decay criteria in pine sapwood supplemented with aspartic acid and decayed by A. tenuissima.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
1	0.09	0.57	0.00	24	0.01	0.09	0.24
σ	0.18	0.26	0.00				
3	0.73	0.81	1.00	2	N.S.	-	-
σ	0.10	0.13	0.53				
6	0.67	0.92	1.40	8	0.01	0.18	0.52
σ	0.35	0.28	0.42				
12	1.02	1.94	3.20	40	0.01	0.24	0.67
σ	0.19	0.28	0.78				
	Mean bending strength (lbs force)						
1	28.5	25.1	28.3	4	N.S.	-	-
σ	9.80	5.95	5.91				
3	27.1	25.6	22.1	3	N.S.	-	-
σ	6.00	4.30	16.00				
6	26.9	23.0	24.8	3	N.S.	-	-
σ	13.70	3.80	4.30				
12	29.0	27.2	24.7	3	N.S.	-	-
σ	8.13	8.54	3.90				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 7.3.4: Analyses of variance on decay criteria in beech sapwood supplemented with aspartic acid and decayed by T. viride.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
1	0.01	0.11	0.00	3	N.S.	-	-
σ	0.02	0.14	0.00				
2	0.05	0.16	0.21	1	N.S.	-	-
σ	0.06	0.15	0.25				
4	0.21	0.29	0.78	3	N.S.	-	-
σ	0.18	0.30	0.66				
6	0.26	0.56	1.66	6	0.05	0.44	1.33
σ	0.28	0.50	0.13				
	Mean bending strength (lbs force)						
1	46.0	45.0	46.8	1	N.S.	-	-
σ	13.70	8.77	3.23				
2	45.6	45.4	45.9	1	N.S.	-	-
σ	15.30	6.60	4.62				
4	43.4	41.0	43.3	1	N.S.	-	-
σ	9.50	8.61	4.75				
6	46.2	47.0	42.0	7	0.01	1.46	4.38
σ	4.60	3.80	4.03				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 7.3.4: Analyses of variance on decay criteria in pine sapwood supplemented with aspartic acid and decayed by T. viride.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
1	0.25	0.17	0.51	1	N.S.	-	-
σ	0.12	0.13	0.26				
3	0.15	0.36	1.13	2	N.S.	-	-
σ	0.10	0.18	0.20				
6	0.35	0.66	1.73	4	N.S.	-	-
σ	0.16	0.38	0.52				
12	0.75	0.92	1.67	7	0.01	0.13	0.37
σ	0.38	0.02	0.12				
	Mean bending strength (lbs force)						
1	25.7	29.6	25.8	5	N.S.	-	-
σ	12.30	3.62	8.50				
3	26.5	26.7	26.7	3	N.S.	-	-
σ	9.21	4.85	12.51				
6	27.4	26.3	26.5	4	N.S.	-	-
σ	3.92	8.17	4.70				
12	29.0	28.1	27.5	4	N.S.	-	-
σ	4.53	6.42	7.53				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 8.3: Analyses of variance of decay criteria in beech sapwood supplemented with glutamic acid and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
1	0.56	0.52	0.04	14	0.01	0.11	0.32
σ	0.13	0.30	0.07				
3	1.13	0.95	3.18	130	0.01	0.20	0.60
σ	0.24	0.21	0.55				
6	1.25	1.12	6.09	209	0.01	0.28	0.82
σ	0.29	0.22	0.75				
9	1.32	1.47	6.16	328	0.01	0.22	0.63
σ	0.51	0.32	0.34				
	Mean bending strength (lbs force)						
1	46.5	47.3	45.7	1	N.S.	-	-
σ	3.93	4.27	2.86				
3	45.9	45.4	39.6	7	0.01	1.88	5.50
σ	4.38	2.61	2.80				
6	43.2	42.2	33.2	13	0.01	2.73	8.12
σ	4.73	2.96	5.19				
9	40.6	40.7	31.4	48	0.01	1.09	3.18
σ	1.84	2.70	1.24				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

Appendix I - continued

Section 8.3: Analyses of variance of decay criteria in pine sapwood supplemented with glutamic acid and decayed by C. globosum.

Incubation period (weeks)	Mean % weight loss Added N (mg/g wood)			'F' value	P	σ d	For p=0.01 difference of means must be >
	0	0.1	1.0				
2	0.10	0.15	0.62	10	0.01	0.12	0.34
σ	0.06	0.10	0.24				
4	0.56	0.50	1.27	18	0.01	0.23	0.64
σ	0.16	0.29	0.21				
8	0.71	1.28	2.26	39	0.01	0.17	0.49
σ	0.24	0.42	0.25				
16	1.51	1.80	3.90	46	0.01	0.28	0.79
σ	0.15	0.41	0.43				
	Mean bending strength (lbs force)						
2	40.6	39.8	38.7	2	N.S.	-	-
σ	3.20	4.80	5.40				
4	38.6	41.7	38.0	4	N.S.	-	-
σ	2.80	3.60	4.00				
8	42.3	39.8	34.3	7	0.01	1.80	5.02
σ	3.26	4.00	1.98				
16	37.6	35.6	32.5	10	0.01	1.65	4.60
σ	0.28	0.20	1.45				

p = probability σ = standard deviation

σ d = standard deviation of the difference of two means

$$= \sqrt{\left(2 \times \frac{\text{residual mean square}}{\text{number of replicates}}\right)}$$

APPENDIX II

GERMINATION OF *CHAETOMIUM GLOBOSUM* ASCOSPORES ON HARDWOODS

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SUMMARY

The sapwood of Tilia europaea L. was found to possess a substance capable of inhibiting germination of ascospores of Chaetomium globosum Kunze ex Fr. The inhibitory substance could be leached from sapwood blocks placed end grain in contact with water agar. Volatile activity of the substance was also demonstrated after oven sterilisation of the wood. Autoclave sterilisation removed the volatile inhibitor but water soluble inhibitors remained. The inhibitor was found to have no effect on conidia of Alternaria tenuissima (Fr.) Wiltsh. and Trichoderma viride Pers. ex Fr. Inhibitors of ascospore germination were also found in oak and idigbo heartwood while enhancers of germination were found in five other hardwoods.

Chaetomium globosum Kunze ex Fr. has been well documented as a soft-rot organism (Levy, 1965). It has been commonly used in tests on the toxicity of wood preservatives to soft-rot organisms (Savory and Bravery, 1970), and is the recommended standard test organism on preservative toxicity (BS 838, 1961). During studies on this fungus in which ascospore inoculation on to a variety of timbers was carried out, it became apparent that spore germination was inhibited on lime sapwood. Changes in ambient temperature, wood moisture content, humidity and aeration did not induce germination.

Several laboratory studies on the effect of wood substrates on spore germination have been published (Morton and French, 1966; Toole, 1971; Baker et al, 1973; Schmidt and French, 1977). Spores from wood-rotting Basidiomycetes were shown to germinate significantly better on some woods than others (Toole, 1971) while inhibitory substances have been found in pine which prevented basidiospore germination of Lenzites trabea (Pers.) Fr. (Baker et al, 1973). The evidence suggests that the type of wood substrate has important effects on germination and early growth of wood-decay fungi.

This paper investigates ascospore germination of C. globosum on water soluble leachates from a variety of hardwoods. Conidial germination of Trichoderma viride Pers. ex Fr. and Alternaria tenuissima (Fr.) Wiltsh., two fungi commonly isolated from soft-rot situations (Levy, 1965), is also briefly studied.

Materials and methods

Three isolates of Chaetomium globosum, coded S70P, S70D and 2g, were obtained from the Building Research Establishment, Princes Risborough. Trichoderma viride and Alternaria tenuissima were obtained from the culture collection of the Biodeterioration Information Centre. Cultures were maintained on 2% malt agar incubated at 25°C. To obtain spore suspensions, 10ml aliquots of sterile distilled water were added to 21 day old, sporulating cultures. A sterile inoculating loop was used to dislodge the spores and the suspension was then filtered through glass fibre fabric. The density was standardised to approximately 2.5×10^5 spores/ml. Spore suspensions were made up immediately prior to use.

Eight species of hardwood and one softwood were used in experiments. Samples of lime (Tilia europaea L.), beech (Fagus sylvatica L.), birch (Betula alba L.) and pine (Pinus sylvestris L.) were supplied by the Building Research Establishment, Princes Risborough. A second sample of lime was obtained from Dr. B. King, Dundee College of Technology. Idigbo (Terminalia ivorensis), ramin (Gonystylus bancanus), oak (Quercus robur L.), walnut (Juglans regia L.) and mahogany (Khaya ivorensis) were supplied by a timber yard in the Birmingham area. All samples were sapwood except oak and idigbo which were heartwood. Samples were cut into 10mm cubes or 10 x 20 x 5mm sections and sterilised either by autoclaving at a pressure of 20 lb/in^2 for 15 minutes or by heating at 100° for 18h in an oven.

Germination tests were performed in 9cm plastic Petri dishes containing 15ml of tap water agar at pH 5.0. In the first experiment, 10mm cubes of four wood samples were placed in the centres of Petri plates, end grain in contact with the agar, and removed after incubation at 25° for two days. The plates were inoculated with 0.1ml aliquots of standard spore suspension spread evenly over the agar. After 24h incubation at 25°, microscopic examination and germination counts of spores within a 2cm radius of the wood/agar contact point were carried out. Germination percentages were calculated by scoring a minimum of 200 spores on each of three replicate plates. The criterion for germination was that the germ tube length should be greater than the length of the spore.

A technique described by J. Carey (pers. comm.) was used in the second experiment where 10mm wide troughs were cut from the agar, to isolate two 3 x 2 cm rectangles (islands) from the rest of the plate. From each of nine hardwood samples four cubes were sterilised by each method. They were then placed end grain in contact with the agar, one per agar island, and incubated at 25° for two days. The cubes of wood were removed and the agar inoculated with a C. globosum (S70D) ascospore suspension. After 24h incubation at 25°C, a minimum 200 ascospores on each agar island and 300 ascospores in the control area were scored.

To test the possible presence of volatiles, samples (10 x 5 x 20mm) of five hardwoods were autoclaved or oven-sterilised and attached to the lids of Petri plates. After incubation at 25° for two days the agar was inoculated

and ascospore germination was scored within an area, 4cm diam., below the wood sample. A minimum of 200 ascospores was counted on each of three replicate plates.

Results

Water agar containing soluble leachates from beech sapwood supported high spore germination percentages of A. tenuissima, T. viride and caused enhanced germination of C. globosum compared with controls (Table 1). Leachates from the softwood, Scots pine, also supported enhanced germination of C. globosum. Lime sapwood leachates were without any effect on A. tenuissima and T. viride but greatly reduced germination of the three C. globosum isolates.

During normal germination, C. globosum ascospores initially produced a globose vesicle from the germ pore. The vesicle then emitted one or more hyaline germ tubes which elongated into hyphae. After 24h incubation, the majority of ascospores on lime leachates had not progressed beyond the germ vesicle stage. Those which had, developed short distorted germ tubes many of which were not sufficently long to score as germinated. Little variation in germination percentages occurred between the C. globosum isolates.

Water soluble leachates from the wood could be seen as a change in colour of the agar. The leachates were concentrated under the wood block and diminished towards the periphery of the plate, as would be expected. In the second experiment, isolation of a rectangle of agar

Table 1: Percentage germination of fungal spores on water soluble wood leachates after 24h incubation at 25°C.

	Chaetomium globosum			*Alternaria tenuissima*	*Trichoderma viride*
	S70D	S70P	2g		
Pine	92 ± 5	84 ± 6	94 ± 9	-	-
Beech	92 ± 5	100 ± 10	98 ± 5	100 ± 6	96 ± 7
Lime 1	6 ± 2	12 ± 2	0 ± 7	88 ± 2	92 ± 6
Lime 2	5 ± 2	10 ± 3	12 ± 2	96 ± 5	89 ± 10
Control	44 ± 3	47 ± 4	48 ± 4	88 ± 5	91 ± 6

Each value is the mean of 3 replicates with standard error

successfully retained the leachates in one area. Germination of C. globosum ascospores on the control areas was 33-50%, and leachates from beech, mahogany, ramin, walnut and birch (Table 2) supported germination in excess of 90% indicating stimulated ascospore germination. Heartwood leachates from oak and idigbo were strongly inhibitory. Leachates from lime sapwood samples also caused inhibition of germination except that from autoclave sterilised lime 1 sapwood. This gave a high germination percentage but microscopic examination showed that the germ tubes were much shorter than those of the controls.

Control areas in the plates with islands containing lime leachates had a slightly depressed germination percentage compared with those of other woods, and those of the controls in the first experiment. Ascospores inoculated on to agar above which walnut, idigbo and oak were suspended, germinated at levels similar to previous controls. Oven-sterilised lime blocks exerted an inhibitory effect on ascospore germination even when not in contact with the agar medium, reducing germination to less than 10% (Table 3). Autoclave sterilised lime blocks, however, reduced percentage germination only slightly and the effect was not significant.

Discussion

Five hardwood species studied contained, in their sapwood, water soluble substances capable of enhancing ascospore germination of C. globosum by approximately 50% compared to controls. Oak and idigbo heartwood inhibited

Table 2: Percentage germination of Chaetomium globosum ascospores on water soluble wood leachates after 24h incubation at 25°C

	Island area		Control area	
	oven sterilised	autoclave sterilised	oven sterilised	autoclave sterilised
Beech	94 ± 6	93 ± 8	39 ± 4	41 ± 3
Mahogany	94 ± 6	98 ± 11	35 ± 3	33 ± 5
Ramin	95 ± 4	97 ± 7	47 ± 7	34 ± 7
Walnut	98 ± 2	98 ± 9	36 ± 1	36 ± 4
Birch	97 ± 3	90 ± 6	41 ± 3	34 ± 3
Oak **	3 ± 2	0 ± 2	38 ± 3	37 ± 2
Idigbo **	5 ± 1	0 ± 2	44 ± 3	50 ± 3
Lime 1	8 ± 6	36 ± 8 *	27 ± 4 *	23 ± 2 *
Lime 2	4 ± 7	6 ± 4	27 ± 5 *	20 ± 5 *

* germ tubes stunted and shorter than other controls

** heartwood samples

Each value is the mean of 3 replicates with standard error.

Table 3: Percentage germination of C. globosum ascospores on water agar below, but not in contact with wood samples.

	oven sterilised	autoclave sterilised
Walnut	38 ± 1	35 ± 3
Idigbo	36 ± 3	39 ± 5
Oak	40 ± 3	34 ± 2
Lime 1	7 ± 3	32 ± 1
Lime 2	3 ± 2	28 ± 3
Control	40 ± 4	39 ± 5

All plates were incubated for 24h at 25°C

Each value is the mean of 3 replicates with standard error.

germination of ascospores almost completely. This was expected since both of these hardwoods have a high durability rating (Ministry of Technology, 1969) with an expected life of 15-20 years when in ground contact.

Among the species studied only lime sapwood contained substances capable of inhibiting ascospore germination. This is surprising, as lime is generally classed as a perishable hardwood (Ministry of Technology, 1969) and is readily attacked by C. globosum when inoculated with active mycelium. The water soluble leachates from lime caused a significant inhibition of C. globosum ascospores but had no effect on T. viride and A. tenuissima conidia. The few ascospores which did germinate on lime leachates had short, stunted germ tubes. Lime has been reported as one of the few hardwoods to possess volatile oils (Mutton, 1962) and the involvement of a volatile in ascospore inhibition was demonstrated. Removal of the volatile was brought about by autoclaving lime blocks although leachates from autoclave sterilised lime were still slightly active in causing inhibition. It appears that lime sapwood, generally considered to be a perishable wood, possesses a water soluble, volatile substance capable of inhibition of C. globosum ascospore germination.

In this study, inhibition has only been shown to occur with C. globosum ascospores on lime sapwood and oak and idigbo heartwood, but substances in living trees with wider inhibitory properties are known (Kirkham, 1954; Irvine, Dix and Warren, 1978) and inhibitory substances in

converted timber may prove to be more widespread than previously thought.

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