EFFECT OF SAPSTAINING FUNGI ON STRUCTURAL WOOD INTEGRITY OF RADIATA PINE (*Pinus radiata* D. DON)

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

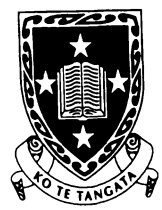
Doctor of Philosophy in Biological Sciences

at

The University of Waikato

by

ANKE SCHIRP



The University of Waikato

Te Whare Wānanga o Waikato

Hamilton, New Zealand

2001

ABSTRACT

Selected sapstaining fungi belonging to the *Ascomycetes* and *Fungi Imperfecti* isolated in New Zealand were evaluated for potential degradation of unseasoned radiata pine sapwood using four independent methods: i) toughness and weight loss measurements, ii) chemical analysis of wood composition, iii) enzyme assays and iv) light and scanning electron microscopy. Several isolates of albino-strains, potential candidates for use as biocontrol agents against sapstain on radiata pine, were included in the experiments.

Sets of side-matched specimens were inoculated with isolates of *Ophiostoma floccosum*, *O. pluriannulatum*, *O. ips*, *O. piceae*, *Leptographium procerum* and *Sphaeropsis sapinea* and incubated for 8 or 16 weeks, along with non-inoculated control samples. Three decay fungi, *Gloeophyllum trabeum*, *Phlebiopsis gigantea* and *Schizophyllum commune*, were included in the tests to serve as references for weight and toughness loss. After incubation, the samples were equilibrated to 14 % moisture content before recording toughness and weight losses. Weight loss was determined by comparing the dry weights of the inoculated samples to the untreated controls rather than expressing weight loss as a percentage of the original weight of a sample.

Neither toughness nor dry weight were significantly ($p<0.05$) different between specimens inoculated with sapstaining fungi and their controls, except for an isolate of *O. ips* in one out of three experiments. This isolate of *O. ips* caused 18% toughness loss without causing weight loss. In one out of four experiments, there was a slight, but statistically significant ($p < 0.05$) overall weight loss of samples inoculated with different sapstaining isolates which was attributed to the degradation of non-lignified parenchyma cells and extractives. In comparison, the brown-rot fungus *G. trabeum* caused up to 61% toughness loss after 4 months' incubation, accompanied by a weight loss of 8%, and the white-rot fungus *S. commune* produced a toughness loss of 32% without any significant weight loss which indicates that toughness loss is a more sensitive indicator of decay than weight loss.

None of the sapstaining fungi tested caused significant reductions of lignin or structural carbohydrates (arabinose, galactose, glucose, xylose and mannose) in radiata pine sapwood after 16 weeks' incubation, but all fungal strains, with the exception of *O. pluriannulatum*, reduced the amount of extractives significantly. Of the isolates tested, *O. floccosum* degraded extractives most effectively (53.6% reduction). The fact that there were no major differences with regard to the composition of the structural cell wall components of sapstained wood and controls complements the results obtained in the weight loss experiments. Significant amylose and extractives consumption reflect the observation of sapstaining fungi growing extensively in the parenchyma cells and resin canals of radiata pine.

Sapstaining fungi secrete hydrolytic enzymes into the growth substrate and subsequently absorb the products of hydrolysis in the fungal mycelium. Enzyme assays demonstrated that all sapstaining isolates tested secreted low amounts of xylanase (up to 1.64 μmoles/min/ml) and pectinase (up to 0.11 μmoles/min/ml) into the growth medium, but extracellular cellulase was not detected. Under the conditions tested, mannanase was secreted only by *O. piceae* (0.29 μmoles/min/ml). The results suggest that although galactoglucomannans are the major hemicelluloses in softwoods, the arabino-4-methylglucuronoxylans are preferably used by sapstaining as well as decay fungi when growing in softwoods. This may be attributed to the accessibility of the xylans in the S_3-layer of the wood cell wall. Amylase activity of the fungal species tested was more significant than xylanase, mannanase and pectinase activities which confirms that sapstaining fungi preferably metabolize easily accessible, non-structural wood components like starch.

A possible function of the cell-wall degrading enzymes may be the facilitation of the colonization of wood cells, specifically, pectinase may assist the fungal penetration of pit membranes. It is likely that the extracellular sheath which has been observed around hyphae of *S. sapinea* contains these enzymes and represents an important source of support and hyphal contact with the host cell wall. In addition, pectinase and xylanase could be involved in the pathogenesis of certain sapstaining fungi, as has been demonstrated for *O. ulmi* and *O. novo-ulmi*. The production of specific antibodies against xylanase and pectinase and the subsequent immunohistochemical localization in the host might resolve this question.

Hyphae of sapstaining fungi pass from one wood cell to another by growing through the pit membranes. No differences were observed with regard to the spatial distribution of the sapstaining fungi. Direct cell wall penetration was not observed. The non-lignified parenchyma cell walls of the wood samples infected with sapstaining fungi appeared to be degraded. The ray tracheids in radiata pine were less colonized than the parenchyma cells.

Although low amounts of hemicellulolytic enzymes and pectinase were detected, the effect of sapstaining fungi on wood quality of radiata pine has to be considered cosmetic and non-degradative which confirms the evaluation of sapstain according to the New Zealand Timber Grading Rules (NZS 3631, 1988). In general, sapstaining fungi do not affect the structural wood integrity of radiata pine. If severe sapstain occurs which obscures the grain, there is a possibility that decay fungi may also be present, and since decay fungi do affect the strength of timber, it seems justified not to allow for the use of severely sapstained timber for structural purposes, consistent with NZS 3631 (1988). The use of selected albino-strains of the naturally occurring sapstain population in New Zealand as biocontrol agents on radiata pine logs and timber can be recommended since these fungi were also not found to decrease toughness nor to cause weight loss in radiata pine.

ACKNOWLEDGEMENTS

They told me to expect
The polka dots of sheep
That blew away as the plane came in to land
And they said there'd be
Outside dunnies
Earthquakes
Attitudes that went out with the ark
And a moon that waxed and waned
The Other Way

But no-one told me of
The Kodak blue
The light that made my forehead ache
The rumpled bedsheet of the country's spine
And the brown muscle of Tahuna Hills

Nor warned me I was coming home

Bridget Musters: Love at First Sight
In: Kapiti Poems 7. Edited by Meg Campbell, Helen Durey and Maxine Montgomery. Rawhiti Press, 1994.

Firstly, I would like to thank my chief supervisor, Prof. Roberta Farrell, for giving me the opportunity to travel across the globe and work in this beautiful country, New Zealand. I guess I can check out any time, but I can never leave!

I would also like to sincerely thank my two supervisors from Forest Research in Rotorua, Dr. Bernhard Kreber and Dr. Adya Singh, for their support and encouragement over the years, as well as my university co-supervisor Dr. Chris Harfoot.

I am grateful for receiving a scholarship from Forest Research, Rotorua, and a research study award, provided by the Foundation for Research, Science and Technology and my supervisor Prof. Roberta Farrell. Thank you also to the Department of Biological Sciences, during my time here headed by Prof. Allan Green, for supporting me financially to attend the 30th Annual Meeting of the IRG (International Research Group on Wood Preservation) in 1999 in Rosenheim, Germany, and to Dr. José Navarrete of Universidad Del Bío-Bío, Concepción, Chile, for inviting me to attend the IX Reunión sobre Investigación y Desarrollo de Productos Forestales y I Congreso Iberoamericano de Investigación y Desarrollo de Productos Forestales.

I would like to thank the following people for providing valuable help and sharing their scientific expertise: Shona Duncan, Arvina Ram and Dr. Robin Janson of the University of

Waikato; the "Thermophiles" at University of Waikato, especially Dr. Judy Bragger, Colin Monk, Lynne Parker and Dr. Gerard Sellek; Jackie van der Waals, Colleen Chittenden, Diahanna Eden, Betty Patterson, Dave Page, Bob Britton, Tripti Singh, Robin Wakeling, Mike Collins, Dr. Bernard Dawson, Dr. Rolf Booker, Dr. Bryan Walford, Dr. Ken Wong, Dr. Ian Suckling, Dr. Shawn Mansfield (now of University of British Columbia) of Forest Research, Rotorua; Annette Ah Chee of HortResearch, Hamilton; Barbara Dow of Dexcel (formerly of HortResearch), Hamilton; Prof. John Walker of the University of Canterbury, Christchurch; Prof. Olaf Schmidt of the University of Hamburg; Prof. Robert Blanchette of the University of Minnesota; and Prof. Tom Harrington and Doug McNew of Iowa State University.

Thank you to Carter Holt Harvey Forests for providing the wood used in the toughness and weight loss experiments of this thesis.

Special thanks to Dr. Thomas Binz of the Federal Office of Public Health, Biosafety Unit, Bern, Switzerland, for many inspiring scientific discussions on those fascinating "Ophis" and their enzymes. I very much appreciate your patience with me and all the interest you showed in my work.

Special thanks also to Dr. Dorothee Götz, now at Portland State University, for helping me to settle in Hamilton during an unpleasant season when it was colder inside than outside, for many memorable dinners and excursions to Raglan, for teaching me all the important things to know in microbiology and not to give up when times are tough.

Another special thanks to Hilke Giles and Marquerite van der Helm for keeping up my spirits as well as sharing spirits like Gin and Tonic with me.

Last but not least, a huge thanks to my family, flatmates and friends here and overseas for their help and encouragement. Even if you may be far away in Europe, I haven't forgotten you!

TABLE OF CONTENTS

		Page
Abstract		ii
Acknowledgements		iv
Table of Contents		vi
List of Figures		viii
List of Tables		xi
CHAPTER 1: INTRODUCTION, OBJECTIVES AND LITERATURE REVIEW		**1**
1.1	**Introduction and Objectives**	1
1.2	**Literature Review: Biology, taxonomy and control of sapstaining fungi**	3
1.2.1	Introduction	3
1.2.2	Fungal morphology and reproduction	3
1.2.3	Taxonomy of sapstaining fungi	9
1.2.4	Sapstain species present in New Zealand	11
1.2.5	Parameters influencing the growth of sapstaining fungi	14
1.2.5.1	Type of wood	14
1.2.5.2	Time of felling	15
1.2.5.3	Wood moisture content	16
1.2.5.4	Oxygen	17
1.2.5.5	Temperature	17
1.2.5.6	Nutrients	17
1.3	**Diseases caused by sapstaining fungi**	19
1.4	**Dissemination of sapstaining fungi**	21
1.5	**Investigations on melanisation of sapstaining fungi**	22
1.6	**Prevention and control of sapstain**	24
CHAPTER 2: MATERIAL AND METHODS – GENERAL		**26**
2.1	**Fungal isolates**	26
2.2	**Cultural isolations**	26
2.3	**Culture conditions**	27
2.4	**Blastospore counts**	27
2.5	**Determination of fungal biomass**	28
CHAPTER 3: GROWTH OF SAPSTAINING FUNGI IN LIQUID MEDIA		**29**
3.1	**Introduction**	29
3.2	**Material and Methods**	29
3.3	**Results and Discussion**	29
CHAPTER 4: CELL WALL DEGRADING ENZYMES OF SAPSTAINING AND DECAY FUNGI		**36**
4.1	**Introduction and Objectives**	36
4.2	**Literature Review**	37
4.2.1	Wood components of radiata pine	37
4.2.2	Mode of enzyme secretion in fungi	39
4.2.3	Enzymes of sapstaining fungi degrading the structural components of wood	41
4.2.3.1	Cellulase	41
4.2.3.2	Xylanase and β-galactosidase	46
4.2.3.3	Mannanase	48
4.2.3.4	Pectinase	49
4.2.3.5	Laccase (Phenoloxidase)	52
4.2.4	Enzymes of sapstaining fungi degrading the non-structural components of wood	53
4.2.4.1	Amylase	53
4.2.4.2	Proteinase and aminopeptidase	54
4.2.4.3	Lipase	55
4.3	**Material and Methods**	56
4.3.1	Determination of enzyme activities	56
4.3.1.1	General	56

	4.3.1.2	PAHBAH-assay	57
	4.3.1.3	DNSA-assay	58
	4.3.1.4	Preparation of PAHBAH-reagent	58
	4.3.1.5	Preparation of DNSA-reagent	59
	4.3.1.6	Preparation of standards used in reducing sugar assays	59
	4.3.1.7	Preparation of McIlvaine citrate phosphate buffer	59
4.3.2		Summary of liquid growth media used in enzyme assays	59
4.3.3		Ultrafiltration of crude enzyme solutions	60
4.3.4		Protein determination	60
4.4		**Results and Discussion**	60
4.4.1		Screening for cellulase activity	60
4.4.2		Screening for xylanase activity	64
4.4.3		Screening for mannanase activity	70
4.4.4		Screening for pectinase activity	72
	4.4.4.1	Pectin as main carbon source in growth medium	72
	4.4.4.2	Polygalacturonic acid as main carbon source in growth medium	76
	4.4.4.3	Pectinase activity and growth characteristics of *Ophiostoma ips*	79
	4.4.4.4	Influence of medium composition on expression of pectinase activity in *O. ips*	82
	4.4.4.5	Determination of cell-bound pectinase activity in *O. ips*	86
4.4.5		Screening for amylase activity	87

CHAPTER 5: PHYSICAL AND CHEMICAL CHANGES CAUSED BY SAPSTAINING FUNGI		**91**
5.1	**Introduction and Objectives**	91
5.2	**Literature Review: Influence of sapstaining fungi on wood properties**	93
5.2.1	Wood structure (general) and characteristics of radiata pine	93
5.2.2	Determination of toughness (impact bending strength)	99
5.2.3	Strength reductions caused by *Ophiostoma* sp. and *Sphaeropsis sapinea*	101
5.2.4	Strength reductions caused by *Botryodiplodia theobromae*	109
5.2.5	Permeability characteristics of wood infected with sapstaining fungi	110
5.2.6	Susceptibility of wood infected with sapstaining fungi to decay	111
5.3	**Material and Methods**	112
5.3.1	Inoculation and incubation of wood samples	112
5.3.2	Toughness and weight loss testing	115
5.3.3	Statistical analysis	115
5.3.4	Chemical analysis of samples used in toughness and weight loss tests	116
5.4	**Results and Discussion**	116
5.4.1	Physical changes caused by sapstaining and decay fungi	116
5.4.2	Chemical changes caused by sapstaining and decay fungi	127

CHAPTER 6: MORPHOLOGICAL ASPECTS OF SAPSTAIN FUNGAL GROWTH IN RADIATA PINE		**132**
6.1	**Introduction**	132
6.2	**Literature Review**	132
6.2.1	Ultrastructural aspects of sapstain development in wood	132
6.2.2	Ultrastructural aspects of brown-rot decay in wood	135
6.3	**Material and Methods**	136
6.4	**Results and Discussion**	137

CHAPTER 7: SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK	**152**
REFERENCES	**162**

Appendices

APPENDIX 1: GROWTH CURVES OF SAPSTAINING FUNGI IN LIQUID MEDIA BASED ON BLASTOSPORE COUNTS AT DIFFERENT TEMPERATURES	**193**
APPENDIX 2: GROWTH CURVES OF SAPSTAINING FUNGI IN LIQUID MEDIA BASED ON FUNGAL BIOMASS DETERMINATIONS AT DIFFERENT TEMPERATURES	**201**
APPENDIX 3 – ORIGINAL DATA FROM TOUGHNESS AND WEIGHT LOSS DETERMINATIONS	**206**

LIST OF FIGURES

		Page
Chapter 1		
1.1	Life cycle diagram of *Nectria*, a member of the Ascomycotina.	5
1.2	Four types of conidiomata.	6
1.3	Synnemata of *Ophiostoma piceae.*	6
1.4	Examples of various types of phialides.	7
1.5	Examples of various types of annellides.	7
1.6	Stalked spore drops of sapstaining fungi.	8
1.7	Discolouration of radiata pine sapwood by sapstaining fungi.	14
Chapter 3		
3.1	Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 25°C during 72 hours incubation.	30
3.2	Blastospore counts for O. piceae #272 and O. ips #281 in liquid media at 25°C during 72 hours incubation.	30
3.3	Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 25°C during 72 hours incubation..	31
3.4	Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 25°C during 72 hours incubation.	31
3.5	Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 25°C during 72 hours incubation.	32
3.6	Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 25°C during 72 hours incubation.	32
3.7	Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 25°C during 72 hours incubation.	32
3.8	Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 25°C during 72 hours incubation.	33
3.9	Biomass accumulation of *S. sapinea* #35 in liquid media at 25°C during 72 hours incubation.	33
3.10	Summary of growth of *L. procerum* #281, *O. piceae* #272, *O. querci* #1069 and *O. pluriannulatum* #5040 in liquid media at different temperatures.	34
3.11	Summary of growth of *O. floccosum* #148, *O. pluriannulatum* #151, *O. ips* #181 and *P. cupulatum* #211 in liquid media at different temperatures.	34
Chapter 4		
4.1	Partial structure of the xylan (arabinoglucuronoxylan) from radiata pine.	39
4.2	Partial structure of radiata pine glucomannan (galactoglucomannan).	39
4.3	Growth curve of *Ophiostoma floccosum* #138 in liquid CMC-medium.	61
4.4	Cellulase activities of *S. commune* and *G. trabeum* during 26 days incubation.	63
4.5	Xylanase activity of *O. piceae* #272 and #170.	66
4.6	Xylanase activity of *O. floccosum* #138 and #148.	66
4.7	Xylanase activity of *O. ips* #308, *O. pluriannulatum* #151 and *L. procerum* #1852.	67
4.8	Xylanase activity of *S. sapinea* #4 and #35.	67
4.9	Xylanase activity of *Schizophyllum commune* at pH 4.6 and pH 6.5 during 186 hours (8 days) incubation.	69
4.10	Xylanase activity of *Gloeophyllum trabeum* during 479 hours (20 days) incubation.	69
4.11	Growth of *O. ips* #308 in liquid medium with xylan or mannan.	71
4.12	Mannanase activity of *S. commune* and *G. trabeum* during 26 days incubation.	72
4.13	Pectinase activity of *S. sapinea* #4 and #35 in growth medium with pectin.	73
4.14	Pectinase activity of *O. ips* #308 and *O. piceae* #272 in growth medium with pectin.	74
4.15	Pectinase activity of *O. floccosum* #138 and *O. pluriannulatum* #151 in growth medium with pectin.	74
4.16	Pectinase activity of *Leptographium procerum* #1852 in growth medium with pectin.	74
4.17	Pectinase activity of *S. commune* and *G. trabeum* in growth medium with pectin.	75
4.18	Pectinase activity of *O. floccosum* #138 and *O. ips* #308 in growth medium with polygalacturonic acid.	77
4.19	Pectinase activity of *O. piceae* #272 in growth medium with polygalacturonic acid.	77
4.20	Pectinase activity of *S. sapinea* #4 and #35 in growth medium with polygalacturonic acid.	78

4.21 Pectinase activity of *S. commune* and *G. trabeum* in growth medium with polygalacturonic acid 78
4.22 Growth of *O. ips* #308 during 574 hours incubation (buffered medium). 80
4.23 Pectinase production by *O. ips* #308 during 574 hours incubation (buffered medium). 80
4.24 Growth of *O. ips* #308 during 266 hours incubation (unbuffered medium). 81
4.25 Pectinase production by *O. ips* #308 during 266 hours incubation (unbuffered medium). 81
4.26 Growth of *O. ips* #308 using different amounts of malt extract in starter cultures. 83
4.27 Pectinase production of *O. ips* #308 using different amounts of malt-extract in starter cultures. 83
4.28 Growth of *O. ips* #308 using washed starter cultures. 84
4.29 Pectinase production of *O. ips* #308 using washed starter cultures. 85
4.30 Pectinase production of *O. ips* #308 using standard starter cultures (controls). 85
4.31 Amylase activities of *O. floccosum* #138 and #148. 87
4.32 Amylase activities of *O. piceae* #272 and #170. 87
4.33 Amylase activities of *L. procerum* #1852 and #281. 88
4.34 Amylase activities of *O. pluriannulatum* #151 and *O. ips* #308. 88
4.35 Amylase activities of *S. sapinea* #4 and #35. 88
4.36 Amylase activities of *S. commune* and *G. trabeum*. 89

Chapter 5

5.1 Structure of cell wall layers in tracheids and ultrastructural arrangement of lignin and carbohydrates in the secondary wall. 95
5.2 Block of sound softwood. 95
5.3 Scanning electron micrograph of bordered pit pair. 97
5.4 DENISON toughness tester in Forest Research Timber Engineering Laboratory, Rotorua. 100
5.5 Preparation of side-matching samples from log bolts. 112
5.6 Incubation of samples used in toughness experiments using resealable plastic bags. 114
5.7 Incubation of samples used in toughness experiments using pickle-jars. 115
5.8 Radiata pine samples after toughness test (experiment I). 117
5.9 Radiata pine samples after toughness test (experiment II). 117
5.10 Radiata pine inoculated with *S. sapinea* and matching control after toughness test. 118

Chapter 6

6.1 Spatial distribution of *S. sapinea* growing in radiata pine (cross section, 200 x). 137
6.2 Spatial distribution of *S. sapinea* growing in radiata pine (radial section, 100 x). 138
6.3 Spatial distribution of *S. sapinea* growing in radiata pine (tangential section, 100 x). 138
6.4 *Sphaeropsis sapinea* growing in ray cells of radiata pine (500 x). 139
6.5 *Leptographium procerum* growing in ray parenchyma cells and, to a lesser extent, in ray tracheids of radiata pine (1000 x). 139
6.6 *Sphaeropsis sapinea* growing in ray cells of radiata pine. 140
6.7 *Leptographium procerum* penetrating bordered pits and pinoid pits (cross-field pits) of radiata pine. 140
6.8 *Leptographium procerum* penetrating bordered pits of radiata pine. 141
6.9 *Ophiostoma ips* crossing from cell to cell via pits. 141
6.10 *Sphaeropsis sapinea* crossing from cell to cell through pits (200 x). 142
6.11 *Ophiostoma ips* growing on tracheid walls without causing degradation. 142
6.12 *Ophiostoma ips* growing in ray and crossing from ray to tracheid without causing degradation (640 x). 143
6.13 Extensive hypha of *Ophiostoma ips* growing through cells of radiata pine. 144
6.14 Detail of Figure 6.13 (left margin) in higher magnification. 144
6.15 *Ophiostoma ips* crossing tracheid lumina. 145
6.16 Thick hyphae of *Sphaeropsis sapinea* growing from cell to cell by penetrating bordered pits. 146
6.17 Slime sheath surrounding hyphae of *S. sapinea* (313 x). 147
6.18 Slime sheath surrounding hyphae of *S. sapinea* (313 x). 147
6.19 Degradation of radiata pine caused by *G. trabeum* (polarized light, 200 x). 150
6.20 Sound radiata pine (polarized light, 200 x). 150

Appendix 1

A1.1 Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 4°C. 193
A1.2 Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 4°C. 193
A1.3 Blastospore counts for *O. querci* #1069 and *O. pluriannulatum* #151 and #5040 in liquid media at 4°C. 193

A1.4 Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 10°C. 194
A1.5 Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 10°C. 194
A1.6 Blastospore counts for *O. querci* #1069 and *O. pluriannulatum* #151 and #5040 in liquid media at 10°C. 194
A1.7 Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 15°C. 195
A1.8 Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 15°C. 195
A1.9 Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 15°C. 195
A1.10 Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 15°C. 196
A1.11 Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 20°C. 196
A1.12 Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 20°C. 196
A1.13 Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 20°C. 197
A1.14 Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 20°C. 197
A1.15 Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 30°C. 197
A1.16 Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 30°C. 198
A1.17 Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 30°C. 198
A1.18 Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 30°C. 198
A1.19 Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 35°C. 199
A1.20 Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 35°C. 199
A1.21 Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 35°C. 199
A1.22 Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 35°C. 200

Appendix 2

A2.1 Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 4°C. 201
A2.2 Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 4°C. 201
A2.3 Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 4°C 201
A2.4 Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 4°C. 202
A2.5 Biomass accumulation of *S. sapinea* #35 in liquid media at 4°C. 202
A2.6 Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 30°C. 202
A2.7 Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 30°C. 203
A2.8 Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 30°C 203
A2.9 Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 30°C. 203
A2.10 Biomass accumulation of *S. sapinea* #35 in liquid media at 30°C. 204
A2.11 Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 35°C. 204
A2.12 Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 35°C. 204
A2.13 Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 35°C. 205
A2.14 Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 35°C. 205
A2.15 Biomass accumulation of *S. sapinea* #35 in liquid media at 35°C. 205

LIST OF TABLES

		Page
Chapter 1		
1.1	Characteristics that distinguish *Ceratocystis* and *Ophiostoma.*	10
1.2	*Ophiostoma* species present in New Zealand (as at June 1999).	11
1.3	Isolations from New Zealand summer survey.	12
1.4	Utilization of carbon and nitrogen sources by *Ophiostoma* spp.	19
Chapter 2		
2.1	List of sapstaining fungi used in this thesis.	26
Chapter 4		
4.1	Approximate chemical composition of radiata pine sapwood and mature bark.	37
4.2	Compounds (as percentage of total extractives) in heartwood and sapwood of radiata pine.	38
4.3	Carbohydrate composition of radiata pine	38
4.4	Cellulolytic activity, type of wood attack and decay capacity of sapstaining fungi.	43
4.5	Relative viscosity units of cellulase and pectinase produced by sapstaining fungi when grown on cellulose (C), cellulose-sodium polypectate (CP) and sodium polypectate (P) media.	44 45
4.6	Xylanase and carboxymethylcellulase activities of *B. theobromae* after 6 and 13 days incubation.	45
4.7	Enzyme activity of *B. theobromae* on various carbon sources after 14 days incubation.	45
4.8	Maximal activities of different cell-wall degrading enzymes secreted by representative isolates of *O. ulmi* and *O. novo-ulmi* when grown in vitro on different carbon sources.	47
4.9	Classification of pectic enzymes.	50
4.10	List of sapstaining fungi used in enzyme assays.	57
4.11	Preparation of McIlvaine citrate phosphate buffer, according to required pH.	59
4.12	Fungal biomass and pH of cultures (original pH 6.7) after 169 h incubation (sapstaining fungi) or 26 days incubation (decay fungi) in media supplemented with CMC.	61
4.13	Fungal biomass produced under conditions of starvation.	62
4.14	Cellulase activities of *Trichoderma viride* (Sigma).	62
4.15	Xylanase activity, fungal biomass and pH of cultures after 184 hours incubation when grown in media supplemented with larchwood xylan.	65
4.16	Influence of assay pH and substrate concentration on xylanase activities of selected cultures (supernatants harvested after 99 hours incubation).	68
4.17	Influence of incubation time in assay on xylanase activity of selected cultures (supernatants harvested after 99 hours incubation).	68
4.18	Mannanase activity, fungal biomass and pH of cultures after 184 hours incubation when grown in media supplemented with locust bean gum.	70
4.19	Pectinase activity, fungal biomass, blastospore formation and pH of fungal cultures after 266 hours incubation in medium II with pectin as main carbon source.	73
4.20	Pectinase activity, fungal biomass, blastospore formation and pH of fungal cultures after 266 hours incubation in medium II with polygalacturonic acid as main carbon source.	77
4.21	Pectinase activities of *O. ips* #294 determined in original supernatant, in supernatant after washing and in the mycelial pellet.	86
4.22	Amylase activities of sapstaining and decay fungi after 72 hours incubation.	89
Chapter 5		
5.1	Typical tracheid dimensions in radiata pine.	96
5.2	Effect of treatment on standard deviation and coefficient of variation in toughness tests.	102
5.3	Results of toughness tests with artificially infected specimens.	103
5.4	Relation between percentage of stained area over the cross-section and toughness.	104
5.5	Strength and weight losses caused by staining fungi after 12 weeks incubation.	106
5.6	Review of the effect of sapstaining fungi on strength properties of wood.	107
5.7	Losses in weight and specific gravity caused by sapstaining fungi.	108
5.8	List of sapstaining fungi used in toughness and weight loss tests.	113
5.9	Inoculum determinations in toughness and weight loss experiments.	113
5.10	Mean toughness and weight loss values observed in inoculated radiata pine after eight and 16 weeks of incubation (experiment I).	119

5.11 Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation (experiment II). 119
5.12 Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation (experiment III). 120
5.13 Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation in plastic bags (experiment IV). 120
5.14 Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation in pickle-jars (experiment IV). 121
5.15 Initial wood moisture content (%) of samples used for inoculation with *G. trabeum* in experiments II and III. 122
5.16 Nominal density (oven-dry weight / volume at test) of samples used for inoculation with *G. trabeum* in experiments II and III. 123
5.17 Initial wood moisture content (%) of samples used for inoculation with *O. ips* #308 in experiments II and III. 124
5.18 Nominal density (oven-dry weight / volume at test) of samples used for inoculation with *O. ips* #308 in experiments II and III. 125
5.19 Nominal density (oven-dry weight / volume at test) and moisture content at test of samples inoculated with *S. sapinea* #35 in experiment III. 126
5.20 Extractives and lignin (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks´ incubation (experiment I). 127
5.21 Extractives and lignin (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks´ incubation (experiments II and III). 127
5.22 Carbohydrates composition (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks´ incubation (experiment I). 127
5.23 Carbohydrates composition (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks´ incubation (experiments II and III). 129
5.24 Pitch reduction in radiata pine by New Zealand sapstaining fungi. 130

Chapter 6
6.1 Action of fungi and bacteria during wood deterioration. 133

Chapter 7
7.1 Summary of extracellular enzyme activities of sapstaining and decay fungi in liquid culture. 152
7.2 Summary of extracellular enzyme activities of *G. trabeum* and *S. commune* in liquid culture. 153

Appendix 3
10-11-98 *O. floccosum* #138; *S. sapinea* #4 (8 weeks)
11-11-98 *O. pluriannulatum* #151 and #5040 (8 weeks)
07-12-98 *O. floccosum* #138; *S. sapinea* #4 (16 weeks)
9-12-98 *O. pluriannulatum* #151 and #5040 (16 weeks)
10-12-98 *O. pluriannulatum* #151 and #5040 (continued)
29-02-00 *L. procerum* #1852, *O. piceae* #272, *O. ips* #308, *S. commune*, *G. trabeum* (16 weeks)
07-09-00 *O. ips* #294, *O. floccosum* #F40, *O. ips* #308, *O. floccosum* #F13 (16 weeks)
26-09-00 *G. trabeum*, *P. gigantea*, *O. ips* #1191, *O. ips* #P36, *O. pluriannulatum* F3410, *S. sapinea* #35 (16 weeks)
23-07-01 *G. trabeum*, *O. ips* #308, *S. sapinea* #4 (16 weeks)
24-7-01 *G. trabeum*, *O. ips* #308, *O. ips* #294, *S. sapinea* #4 (16 weeks)

1 Introduction, Objectives and Literature Review

1.1 Introduction and Objectives

Sapstain, synonymous bluestain, is the discolouration of wood caused by the presence of pigmented fungal cells mainly belonging to the *Ascomycetes* and *Fungi Imperfecti*. It results in downgrade of logs and timber causing large loss in revenues to forest industries. In New Zealand, sapstain represents a major problem to exporters of *Pinus radiata* (D. Don) with an estimated annual loss in revenue of more than NZ$ 100 million (Wakeling, 1996).

New Zealand radiata pine originates from small areas on the west coast of the United States where it is commonly referred to as Monterey Pine. Since the species was introduced into New Zealand, probably sometime between 1830 and 1850, over 1.5 million hectares of commercial plantations of radiata pine have been established, and it has largely replaced other high-quality natural and exotic timbers in the domestic marketplace and also dominates exports (Cown, 1999). The fast growth of plantation radiata pine results in production of commercially mature trees containing a high proportion of sapwood which is highly susceptible to fungal attack and classified as perishable (Butcher and Drysdale, 1991). In addition, radiata pine logs are particularly prone to infection with sapstaining fungi due to the distance from New Zealand to export markets.

If sapstain is commonly occurring, how does it affect the wood quality of radiata pine? Timber graded under the New Zealand Timber Grading Rules (NZS 3631, 1988) is classified into four grade categories. Within each category, timber grades are linked to a range of suitable end uses (New Zealand Ministry of Forestry, 1995):

- Appearance grades: Timber suitable for taking a clear finish or paint finish.
- Structural grades: Timber suitable for building purposes needing strength and stiffness.
- Cuttings grades: Timber suitable for remanufacturing, using clear lengths between whorls of knots.
- Other grades: Timber not meeting the requirements of the other grades but complying with the general provisions of the rules.

Sapstain is permitted in the structural grades of the New Zealand Timber Grading Rules (NZS 3631, 1988) "if it is insufficient to obscure the grain". The same is valid for the merchantable grade of the appearance grades. Examples of end use in the merchantable grade are: shelving, sarking, hidden framing for furniture and doors, fence palings and pallets. This allows for wide use of sapstained wood, unless it is severely darkened, for structural purposes. Stain which obscures grain shows that conditions have been suitable

for decay fungi which affect the strength of timber (New Zealand Ministry of Forestry, 1995). However, in dressing and higher grades of the appearance grades (used e.g. for flooring, architraves, skirting, weatherboards and decking), stain is permitted "if it is insufficient to impair a natural finish". The use of sapstained wood for appearance purposes is limited because it absorbs excessive solution and often finishes unevenly due to increased wood permeability (Zabel and Morrell, 1992). Sapstained material will also absorb water more quickly in service, increasing the development of checks which may provide entry points for decay fungi (Zabel and Morrell, 1992).

The aim of this thesis research was to evaluate the effect of common New Zealand sapstaining fungi on the structural wood integrity of radiata pine. Since the term sapstain comprises a large group of fungi with different physiological and biochemical properties, a wide variation in the behaviour of these microorganisms inside the lignified tissue can be expected. Representative fungal strains were chosen to examine their cell-wall degrading potential using complementary methods. A recent survey of sapstaining fungi in New Zealand identified the presence of *Sphaeropsis sapinea* and 10 species of the genus *Ophiostoma* (Farrell et al., 1997). Between 1997 and 1999, an additional seven *Ophiostoma* species were identified and an additional three species were found in New Zealand which have yet to be identified (Farrell, pers. comm.).

In detail, the objectives of this thesis were the following:

- Examine growth of New Zealand sapstaining fungi in liquid culture;
- Determine the effect of sapstaining fungi on toughness of radiata pine sapwood;
- Determine the weight loss in radiata pine caused by sapstaining fungi;
- Detect and quantify production of cell-wall degrading enzymes, i.e. cellulase, xylanase, mannanase and pectinase, under in-vitro conditions;
- Quantify the utilization of non-structural wood components, i.e. simple sugars, dichloromethane extractives and amylose, by sapstaining fungi, for comparative purposes;
- Determine the possible mode of cell-wall degradation (mechanical or enzymatic penetration or combination) using light and electron microscopy.

Overall, it was intended to clarify the term "degradation" with regard to sapstaining fungi. In general, staining as well as mould fungi have been regarded as passive wood colonisers, causing a cosmetic damage to the wood (Seifert, 1993). However, some staining fungi, f.ex. *Botryodiplodia theobromae* and *Aureobasidium pullulans*, have been reported to cause significant degradation of wood. Several fungal species which have been allocated to the sapstain complex, may have to be transferred to the soft rot group. In any case, from an ecological point of view, staining fungi play an important succession role between early passively colonising organisms (moulds) and wood rotting organisms.

This thesis consists of seven chapters. In Chapter 1, the literature with regard to the taxonomy, biology and control of sapstaining fungi was reviewed to provide essential background knowledge on the mycological aspects of the research topic. A description of wood anatomy and characteristics of radiata pine is included in Chapter 5. Literature reviews relating to more specific aspects of the research objectives are provided at the beginning of the individual chapters. Chapter 2 contains a summary of general material and methods used. More specific information on material and methods is provided in the individual chapters of this thesis. In Chapter 3, experiments on the growth of sapstaining fungi in liquid culture were described and analysed which is regarded as a pre-requisite for the determination of enzyme activities. Cell wall degrading enzymes of sapstaining as well as decay fungi were subsequently investigated, and the results are presented in Chapter 4. Chapter 5 contains investigations on toughness and weight losses caused by sapstaining and decay fungi as well as a chemical analysis of radiata pine sapwood inoculated with these fungi. A microscopical analysis of radiata pine samples used in the toughness and weight loss experiments is presented in Chapter 6. Chapter 7 contains a summary, conclusions and suggestions for future work.

1.2 Literature Review: Biology, taxonomy and control of sapstaining fungi

1.2.1 Introduction

In this chapter, the literature with regard to the taxonomy, biology and control of sapstaining fungi was reviewed. A literature review on wood anatomy and characteristics of radiata pine can be found at the beginning of Chapter 5. Literature reviews relating to more specific aspects of the research objectives are provided at the beginning of the individual chapters. A literature review focussing on cell-wall degrading enyzmes produced by sapstaining fungi is presented at the beginning of Chapter 4. Aspects of chemical changes occurring in wood after infection with sapstaining fungi were reviewed in Chapter 5. Literature relating to the growth of sapstaining fungi in wood and their effect on wood properties are covered in Chapters 5 and 6.

1.2.2 Fungal morphology and reproduction

The following description summarizes the difficulties of explaining the unique fungal morphology: "If it looks like a fungus, if it grows like a fungus, if it lives like a fungus, it has to be a fungus" (modified from Bartnicki-Garcia, 1994). On a more scientific basis, the term fungus includes "eukaryotic, spore-bearing, achlorophyllous organisms that generally reproduce sexually and asexually, and whose usually filamentous, branched somatic structures are typically surrounded by cell walls containing chitin or cellulose, or both of these substances, together with many other complex organic molecules" (Alexopoulos et al., 1996).

The fungal thallus typically consists of microscopic threads or filaments that branch in all directions, spreading over or within the substratum utilized for food. Each of these filaments is known as a hypha (pl. hyphae, from the Greek word *hyphe* = web). A hypha is made of a thin, transparent, tubular wall filled or lined with a layer of protoplasm varying in thickness (Alexopoulos et al., 1996). A hypha only grows at its tip. The hyphal system of many fungi appears to be uniquely adapted to penetrate, externally digest, absorb and metabolize a wide range of plant materials, including wood (Zabel and Morrell, 1992). The mass of hyphae, constituting the thallus of a fungus, is called the mycelium (pl. Mycelia, from the Greek word *mykes* = mushroom). The mycelium of a fungus generally begins as a short germ tube emerging from a germinating spore.

Typically, fungi reproduce both asexually and sexually (Figure 1.1). The essential part of the sexual process is initiated by the fusion of two cells containing one or more nuclei. Nuclear fusion does not occur immediately but the two nuclei form a pair of closely associated compatible nuclei called a dikaryon (Hudson, 1986). The two nuclei then divide together and the products are separated into two daughter cells; by such repeated divisions a dikaryophase is established. In general, asexual reproduction is more important for the propagation of a fungal species because it results in the production of numerous individuals, and particularly since the asexual cycle is usually repeated several times during the season, whereas the sexual stage of many fungi is produced only once a year (Alexopoulos et al., 1996). The asexual methods of reproduction commonly found in fungi can be summarized as follows (Alexopoulos et al., 1996):

1. Fragmentation of the soma, each fragment growing into a new individual;
2. Fission of somatic cells into daughter cells;
3. Budding of somatic cells or spores, each bud producing a new individual;
4. Production of spores, each spore usually germinating to form a germ tube that grows into the mycelium.

The most common method of asexual reproduction in fungi is by means of spores. Fungal spores produced asexually are either borne in sporangia and are then called sporangiospores, or are produced at the tips or sides of hyphae in various ways and are then called conidia which is the case for species of *Ophiostoma*. The asexual spores produced by *Ophiostoma* species are called blastospores and are formed by marked enlargement of a recognizable conidium initial before the initial is delimited by a septum (Hawksworth et al., 1996). Consequently, with regard to species of *Ophiostoma*, the terms blastospores and conidia are used synonymously.

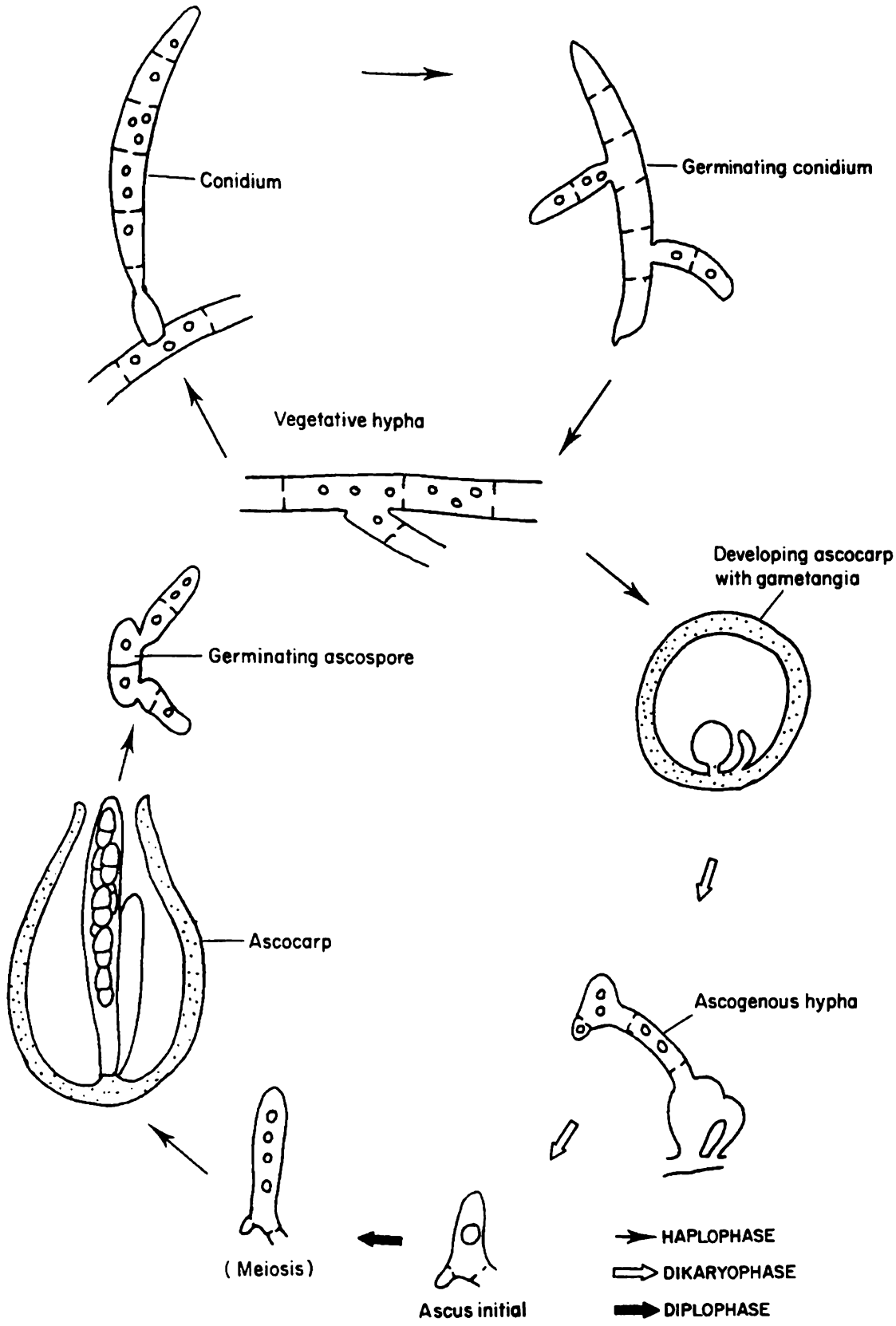


Figure 1.1: Life cycle diagram of *Nectria*, a member of the Ascomycotina. Source: Hudson (1986).

Some *Ophiostoma* species produce conidia on asexual fruiting structures called synnemata, others in a sporothrix-stage in which conidia are borne on hyphae. A synnema consists of a group of conidiophores cemented together by their stalks (Figures 1.2 and 1.3). The

conidiophores comprising a synnema are often branched at the top, with the conidia arising from conidiogenous cells at the tips of numerous branches.

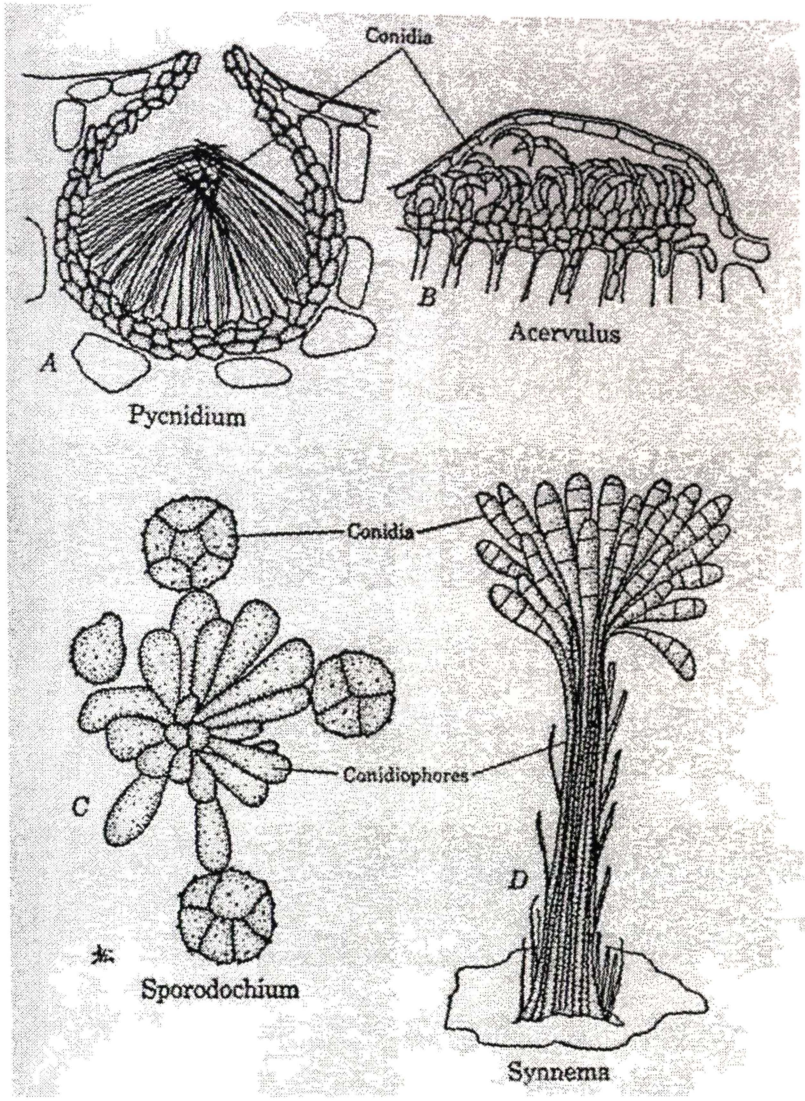


Figure 1.2: Four types of conidiomata. (A) *Septoria*; (B) *Marssonina*; (C) *Epicoccum*; (D) *Arthrobotryum*. Not to scale. Source: Alexopoulos et al. (1996).

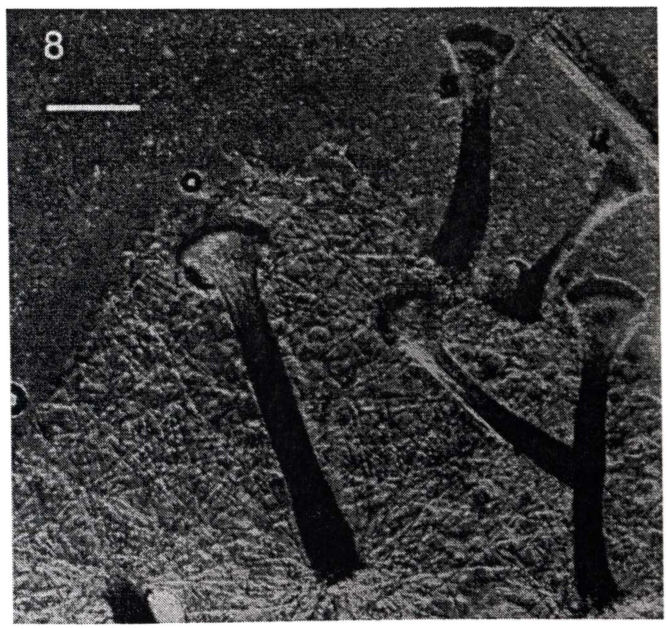


Figure 1.3: Synnemata of *Ophiostoma piceae*. Scale bar: 100 µm. Source: Harrington et al. (2001).

Terms such as phialide and annellide are used to designate different types of conidiogenous cells. A phialide (Figure 1.4) is a cell which develops one or more open ended conidiogenous loci from which a basipetal succession of conidia (phialospores) develops without an increase in length of the phialide itself (Hawksworth et al., 1996). In some fungi, the phialide may be the conidiophore. More frequently, the phialide is either an end cell of a conidiophore, or it is attached to a conidiophore. An annellide (Figure 1.5), on the other hand, is a conidiogenous cell that undergoes repeated proliferation during production of chains of conidia so that the elongating conidiogenous cell becomes marked with a series of scars (Alexopoulos et al., 1996).

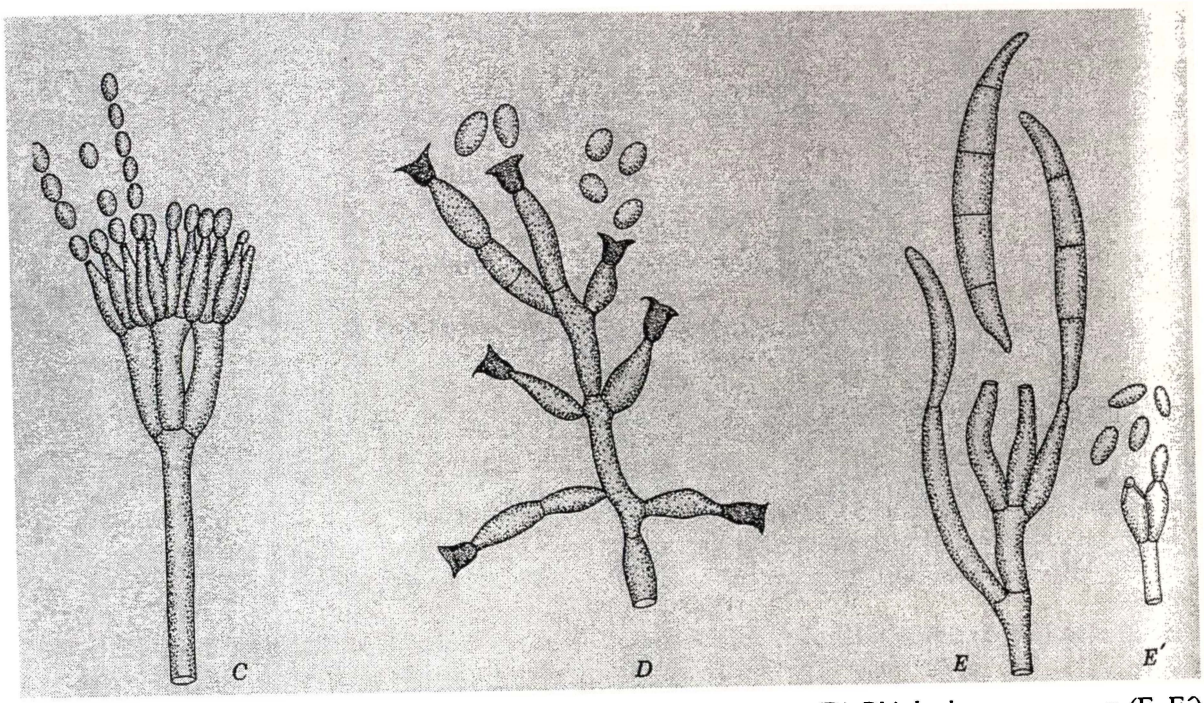


Figure 1.4: Examples of various types of phialides. (C) *Penicillium* sp.; (D) *Phialophora verrucosa*; (E, E') *Fusarium* state of *Nectria desmazierii*: (E) Macroconidia, (E') Microconidia. Not to scale. Source: Alexopoulos et al. (1996).

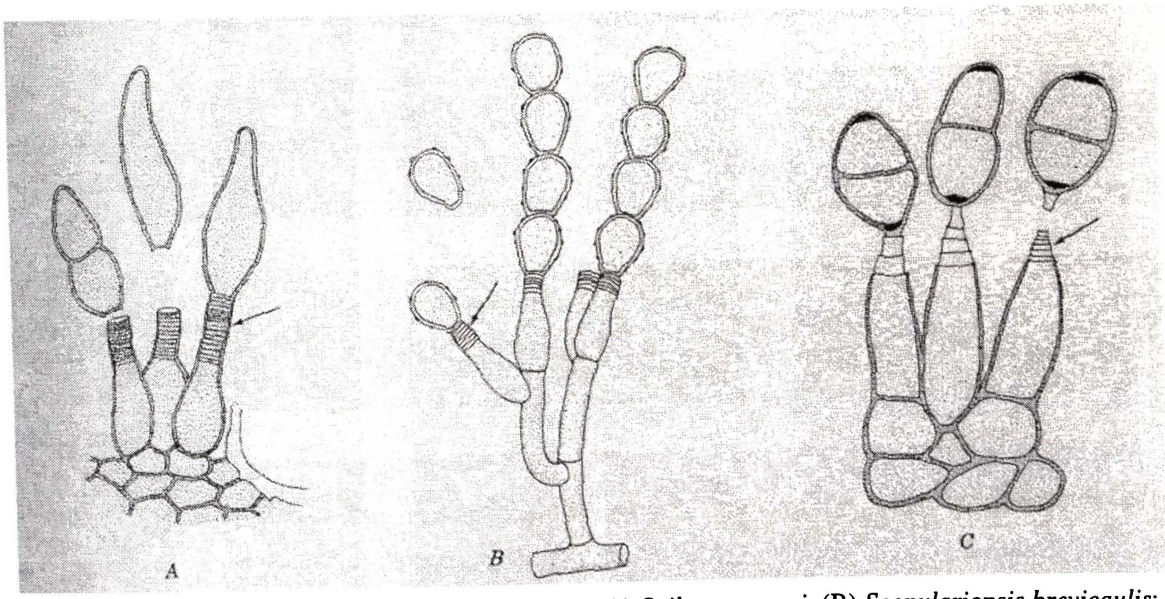


Figure 1.5: Examples of various types of annellides. (A) *Spilocaea pomi*; (B) *Scopulariopsis brevicaulis*; (C) *Oedothea vismiae*. Arrows: annellations. Not to scale. Source: Alexopoulos et al. (1996).

A variety of conidial states is produced (Hudson, 1986). The graphium state has a thick sheath of dark hyphae forming the stalk (Figure 1.6). The component hyphae branch at their tips whereas in the *Leptographium* state, the stalk is a deeply pigmented and very wide single hypha which branches profusely at the apex (Figure 1.6). In *L. procerum*, a conidiogenous apparatus of three to five series of branches terminate in the conidiogenous cells that produce obovoid conidia with truncate ends (Kendrick, 1962). *Leptographium procerum* can also be recognised by its colonies in which conidiophores are arranged to form dark concentric rings on the surface of the agar (Jacobs et al., 2001).

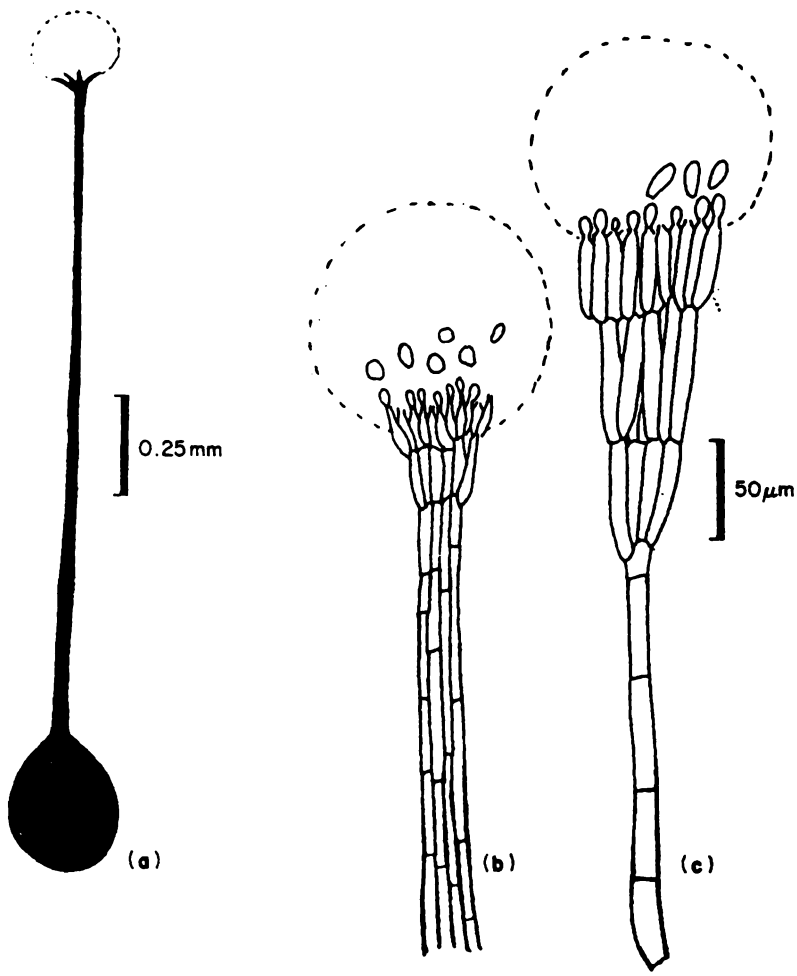


Figure 1.6: Stalked spore drops of sapstaining fungi. (a) *Ceratocystis*; (b) *Graphium*; (c) *Leptographium*. Source: Hudson (1986).

With regard to sexual compatibility, the two following categories are distinguished (Alexopoulos et al., 1996):

1. Homothallic fungi: every thallus is sexually self-fertile and can, therefore, reproduce sexually by itself without the aid of another thallus. Fungi in this category exhibit no mating types.
2. Heterothallic Fungi: every thallus is sexually self-sterile, regardless of whether or not it is hermaphroditic, and requires the aid of another compatible thallus of a different mating type for sexual reproduction.

With the exception of *O. stenoceras* which is homothallic all species of *Ophiostoma* are heterothallic, i. e., in order to produce perithecia, the sexual fruiting structures, both mating types have to be present. The sexual spores, called ascospores, are produced in a mucilagenous matrix and exuded through the long neck of the perithecium into a droplet at the apex (Upadhyay, 1993). Ascospores are hyaline, usually unicellular (sometimes bicellular with a median septum) and variable in shape.

Sphaeropsis sapinea which has formerly been known as *Diplodia pinea* is an anamorphic ascomycete. Its sexual state is *Botryosphaeria* which belongs to the Loculoascomycetes, family *Botryosphaeriaceae*. Several other loculoascomycetes are well known as endophytes, including *Aureobasidium* and *Hormonema* (Alexopoulos et al., 1996). Morphological characteristics of *S. sapinea* include conidia which exude from pycnidia (Birch, 1936). A pycnidium is a globose or flask-shaped pseudoparenchymatous structure which is lined on the inside with conidiophores (Alexopoulos et al., 1996; Figure 1.2). In external appearance, some pycnidia resemble perithecia but can be distinguished from these by the absence of asci and ascospores.

1.2.3 Taxonomy of sapstaining fungi

The observation that stain is caused by a fungus is generally attributed to Hartig (1878). He assumed that all staining of coniferous wood was caused by one species now known as *Ophiostoma piliferum*. Von Schrenck (1903) published the first detailed study of this fungus and its detrimental effects. It was demonstrated by Münch (1908) that more than one species was involved in sapstain. Sapstain of lumber is generally caused by three groups of fungi belonging to the Ascomycetes (Seifert, 1993):

1. Species of *Ceratocystis*, *Ophiostoma* and *Ceratocystiopsis*;
2. Black yeasts such as *Hormonema dematioides*, *Aureobasidium pullulans*, *Rhinocladiella atrovirens* and *Phialophora* spp.;
3. Dark moulds such as *Alternaria alternata*, *Cladosporium sphaerospermum* and *C. cladosporioides*.

In addition to these groups, the ascomycete *Sphaeropsis sapinea* is an important sapstaining fungus on lumber and logs in New Zealand (Farrell et al., 1997) which was first recorded in this country in 1926 (Birch, 1936) and is worldwide known as a pathogen (Waterman, 1943; Harrington and Wingfield, 1998).

Taxonomically, *Ceratocystis* and *Ophiostoma* both belong to the Pyrenomycetes. However, *Ceratocystis* is among the Microascales whereas *Ophiostoma* belongs to the Ophiostomales. Controversy has surrounded the taxonomy of fungi belonging to the genera of *Ceratocystis*, *Ophiostoma* and *Ceratocystiopsis* since their discovery at the end of the last century (Halsted, 1890; Sydow and Sydow, 1919; Upadhyay and Kendrick, 1975). Some taxonomists have considered *Ophiostoma* and *Ceratocystis* to be synonyms (Bakshi,

1951; Upadhyay, 1981) while others have treated them as separate taxa based on morphological and physiological characteristics (Arx, 1952; de Hoog and Scheffer, 1984; Harrington, 1987). Recent analysis of ribosomal DNA sequence data has shown that *Ophiostoma* and *Ceratocystis* are unrelated (Hausner et al., 1993a; Spatafora and Blackwell, 1994), while *Ophiostoma* and *Ceratocystiopsis* might be considered synonymous (Hausner et al., 1993b).

The term *Ceratocystis sensu lato* is commonly used to address the three genera *Ceratocystis sensu stricto*, *Ceratocystiopsis* and *Ophiostoma*. *Ceratocystis sensu stricto* is characterized by *Chalara* anamorphs with phialides (Benade et al., 1995, and references therein). Conidia are produced by ring wall building from the base of the deep cylindrical collarette (i.e. the cup-shaped structure at the apex of a conidiogenous cell; Hawkesworth et al., 1996). In contrast, *Ophiostoma* and *Ceratocystiopsis* have a variety of different anamorphs such as *Graphium*, *Leptographium*, *Hyalorhinocladiella*, *Sporothrix*, *Acremonium* and *Hyalodendron*. Conidium development in these anamorphs is primarily holoblastic (both outer and inner walls of the blastic conidiogenous cell contribute to the formation of the conidium; Hawkesworth et al., 1996), nonphialidic and occurs through apical wall building (Minter et al., 1983; Harrington, 1987). Furthermore, species of *Ceratocystis sensu stricto* are sensitive to low concentrations of the antibiotic cycloheximide. This is in contrast to the tolerance exhibited by species of *Ophiostoma* including *Leptographium* (Harrington, 1981; de Hoog and Scheffer, 1984). Some characteristics that distinguish *Ceratocystis* and *Ophiostoma* are summarised in Table 1.1.

Table 1.1: Characteristics that distinguish *Ceratocystis* and *Ophiostoma*. Source: Alexopoulos et al., 1996.

Characteristics of *Ceratocystis*	**Characteristics of *Ophiostoma***
Enteroblastic anamorphs	Holoblastic anamorphs
Cycloheximide sensitivity	Cycloheximide insensitivity
Cellulose absence	Cellulose presence
Rhamnose absence	Rhamnose presence
Asci line periphery of inner perithecium	Asci line periphery of inner peridium or at peridial base
Primarily parasitic on angiosperms	Primarily saprobic on gymnosperms
Infect through wounds	Infect from bark beetle tunnels

Recent results by Viljoen et al. (2000) support the view that *Ceratocystis sensu stricto* is a distinct genus from *Ophiostoma* using computer aided systematic evaluation of morphological characters. Analysis of equally weighted morphological characters of the ophiostomatoid fungi produced tree topologies reasonably congruent with those from studies using molecular data. In addition, in analyses testing the homology of all morphological characters simultaneously, there does not seem to be any justification for separating species of *Ceratocystiopsis* from *Ophiostoma*.

Over 100 species are included in *Ceratocystis sensu lato*. Käärik (1980) listed a total of 312 species plus 29 mould fungi which have been recorded on wood in temperate regions.

Among these were 59 species of *Ceratocystis sensu lato* many of which are pathogenic fungi associated with stain of living trees. It is important to consider that the term sapstain refers to a series of related phenomena (Seifert, 1993). There is a continuum from truly pathogenic sapstaining organisms that occur in living trees, through pathogenic fungi that grow on weakened trees and may also have a saprophytic phase, to truly saprophytic fungi. Discolourations in lumber are caused mostly by saprophytic fungi (Seifert, 1993). The major agents of sapstain on wood in service are not necessarily the most commonly occurring sapstaining fungi (Eaton and Hale, 1993).

1.2.4 Sapstain species present in New Zealand

A recent survey of sapstaining fungi in New Zealand identified the presence of *Sphaeropsis sapinea* which was formerly known as *Diplodia pinea* and ten species of the genus *Ophiostoma* (Farrell et al., 1997; Farrell et al., 1998; Table 1.2). Between 1997 and 1999, an additional seven *Ophiostoma* species have been identified and an additional three have been found in New Zealand (Farrell, pers. comm.).

Table 1.2: *Ophiostoma* species present in New Zealand (as at June 1999). Source: Farrell, 1999.

***Ophiostoma* species present in New Zealand**	**Other confirmed reports**
*Ophiostoma huntii**	North America
Leptographium procerum	North America, Europe, Asia
Leptographium truncatum	North America, Europe, Asia, Africa
*Ophiostoma galeiformis**	North America, Europe
*Ophiostoma querci**	North America, Europe, Africa, Asia, Australia
Ophiostoma piceae	North America, Europe
*Ophiostoma floccosum**	North America, Europe, Asia, Australia
*Pesotum cupullatum sp. nov.**	North America
Ophiostoma ips	North America, Europe, Asia, Australia
Ophiostoma piliferum	North America, Europe, South America
*Ophiostoma pluriannulatum**	North America, Africa, South America, Asia
Ophiostoma coronatum	North America
*Ophiostoma nigrocarpum**	North America, Australia
*Ophiostoma stenoceras**	North America, Europe, Africa, Asia, South America
Sporothrix sp. A	unknown
Sporothrix sp. B	unknown
Sporothrix sp. C	unknown

* First report from New Zealand.

The results of the survey showed that *S. sapinea* is found throughout the majority of pine plantations in New Zealand and in about one third of the native forest sites sampled (Table 1.3). *S. sapinea* exists extensively as an endophyte on radiata pine at pruned sites and under the bark of healthy trees from age one to about 25 years. This species has also been found on material from the forest floor including branches, cones, leaves and wood. *Ophiostoma* species were found in about one quarter of pine plantations, in about one third of the non-pine sites sampled, and in virtually all samples from pine processing sites. The

most frequently isolated *Ophiostoma* species in New Zealand was *Ophiostoma floccosum*. *Ophiostoma piceae* and *O. querci* were both isolated from radiata pine which makes the New Zealand situation atypical from the European since usually, *O. querci* only colonizes hardwood.

Initial sampling at ports showed that 36% of logs sampled were positive for *S. sapinea* and 64% positive for *Ophiostoma* species. Approximately a quarter of the logs sampled were positive for both *S. sapinea* and *Ophiostoma* isolates.

Table 1.3: Isolations from New Zealand summer survey. Percentage of total number of sites sampled positive for organism*. Source: Farrell et al., 1998.

Fungal organism	Pine plantation sites	Pine processing sites	Non-pine sites
Sphaeropsis	88	60	38
Ophiostoma	28	100	31
Alternaria	69	25	42
Geotrichum	1	0	4
Verticillium	37	40	35

Other common fungi: *Trichoderma*, *Penicillium*, *Rhizopus* and *Fusarium*.

* A positive site can be where the fungal organism is only found on needles or cones and not necessarily on wood.

Earlier studies of sapstain organisms present in New Zealand were conducted by Butcher (1968) and Hutchison and Reid (1988a, 1988b). In these studies, only a few selected areas, mainly on the North Island, were sampled. Samples were collected from both native and introduced tree species (Hutchison and Reid, 1988a, 1988b). Culture descriptions were based on colony morphologies of isolates grown in a controlled environment. The following sapstain organisms were identified: *Sphaeronaemella fimicola*, *Ceratocystiopsis falcata*, *Ceratocystis piceaperda*, C. *ips*, C. *novae-zelandiae*, *C. piceae*, *C. coronata*, *C. rostrocoronata* and *C. pilifera*. The presence of these organisms was not linked with their overall distribution in New Zealand nor their contribution to sapstain in radiata pine.

Ophiostoma piceae and *O. floccosum* are among the most common synnema-forming species in New Zealand and Western North America. Both species belong to the *Ophiostoma piceae* complex which forms a monophyletic group of insect-dispersed ascomycetes with synnema anamorphs (Harrington et al., 2001). *Ophiostoma floccosum* in New Zealand was initially described as a *Graphium* species which could easily be identified by its characteristic red-brown synnema (Farrell et al., 1998). *Ophiostoma piceae* has been reported to be a major stainer on many wood species in Canada (Seifert, 1993, Uzunovic, 1999b). However, in comparison to other species like *O. piliferum*, *O. minus* and *C. coerulescens*, it causes only light stain on wood (Hutchison and Reid, 1988a; Yang, 1999). No correlation in the pigmentation intensity on malt extract agar and on various wood species was found among isolates of *O. piceae* (Yang, 1999).

According to Harrington et al. (2001), members of the *O. piceae* complex form a *Pesotum* anamorph and a *Sporothrix* synanamorph. *Ophiostoma* species outside of the *O. piceae* complex that form synnemata lack the *Sporothrix* state. The nine recognized species within the *O. piceae* complex are delimited by synnema morphology, growth rate at 32°C, mating reactions and sequences of the internal transcribed spacer (ITS) region of the rDNA operon. *Ophiostoma piceae* was originally divided into two reproductively isolated species by Brasier and Kirk (1993). The common conifer form was known as *O. piceae* while the hardwood form, usually isolated from wood of *Quercus*, was referred to as *O. querci*. Further phylogenetic analysis suggests that *O. canum*, *O. floccosum* and the recently discovered *O. setosum* (anamorph *Pesotum cupullatum*) are also members of the coniferous group. In the hardwood group are *O. querci*, *O. catonianum*, *O. ulmi*, *O. novo-ulmi* and *O. himal-ulmi*. *O. piceae*, *O. canum* and *O. setosum* fail to grow at 32°C, whereas the other six members of the *O. piceae* complex grow at this temperature.

O. piceae is saprophytic but has also been described as weakly parasitic in the bark of a wide variety of trees and may be closely related to the other *Ophiostoma* species that cause tree diseases such as *O. ulmi* and *O. roboris* (Brasier and Kirk, 1993).
Optimum temperature for growth was determined to be near 23°C (Abraham et al., 1993). Minimal growth occurred at 4°C after 3 days. An initial pH of 6.1 in liquid culture yielded the greatest biomass but acceptable growth in liquid culture was obtained over a pH range of 3-9. In New Zealand, *O. piceae* has also been recorded on red beech (*Nothofagus fusca*) and silver beech (*Nothofagus menziesii*) by Butcher (1968b) who reported it to be of minor importance on radiata pine. Work done on *O. piceae* as a sapstaining fungus is difficult to interpret today knowing that the fungi used in the earlier studies could have been *O. querci*, *O. floccosum* or *Pesotum cupulatum* (Harrington et al., 2001). Furthermore, as mentioned before, there has been some controversy about the taxonomy of species belonging to *Ophiostoma* and *Ceratocystis*. Species that were formerly addressed as *Ceratocystis* may now have to be transferred to the *Ophiostomataceae*.

Recently, a new species of *Ophiostoma* has been described, *Ophiostoma setosum* (Uzunovic et al., 2000). This fungus is commonly associated with exposed sapwood of *Tsuga heterophylla* in Western North America. *Ophiostoma setosum* as well as its *Pesotum* anamorph have also been isolated from stained radiata pine in New Zealand (Harrington et al., 2001). This species is a member of the *O. piceae* complex and can most easily be distinguished morphologically from the other species of the complex by the dark, seta-like marginal hyphae on the outside of the stipe of the synnematous anamorph.

1.2.5 Parameters influencing the growth of sapstaining fungi

1.2.5.1 Type of wood

As the name implies, growth of sapstaining fungi occurs mainly in the sapwood (Figure 1.7), although the heartwood can be colonized to a lesser extent, depending on the fungal and wood species. Sapstain can occasionally be found in the heartwood of Douglas fir, sitka spruce and pine, especially when contact with green sapwood in a seasoning pile has occurred (Findlay, 1959a). With regard to New Zealand radiata pine, it was observed that the heartwood of this species can be colonized by sapstaining fungi, for example *Leptographium procerum* (Thwaites and Farrell, 2000).

Figure 1.7: Discolouration of radiata pine sapwood by sapstaining fungi.

The sapwood of pine is much more susceptible to stain than that of spruce (Findlay, 1959a). In temperate climates, staining of hardwoods is not as important as staining in softwoods. Among the susceptible hardwood species are aspen (Wang et al., 1997; Hiratsuka and Chakravarty, 1999), poplar (Hiratsuka et al., 1993) and sweet gum (Findlay, 1959a). However, in many light-coloured tropical hardwoods, for example balsa, abachi, celtis, obeche and ramin, sapstain is a serious problem.

Findlay (1959a) suggested that the restriction of sapstaining fungi to colonize the heartwood is mainly due to two factors. Firstly, the sapwood contains easily accessible nutritional sources such as starch and sugars which are not contained in equally high concentrations in the heartwood. Secondly, there are substances present in the heartwood of certain wood species which are toxic to sapstaining fungi. It has been shown that sapwood impregnated with low concentrations of pinosylvin and other phenolic compounds extracted from the heartwood was fairly resistant to sapstaining fungi

(Rennerfelt, 1945). On the other hand, more recent results by Wang et al. (1997) showed that the lack of carbon sources and the inaccessibility of nitrogen were responsible for limited fungal colonization of the heartwood, rather than the presence of wood extractives. Wang et al. (1997) added nutrients to aspen heartwood to determine if *Ophiostoma piceae*, *Lecythophora* sp. and a colourless strain of *Ophiostoma piliferum*, commercially available under the name Cartapip™, grew poorly on this substrate due to lack of nutrients or potentially toxic wood extractives. Nitrogen in aspen heartwood is mainly incorporated in lignified wood components and therefore probably less accessible to some wood-inhabiting fungi. The isolates tested grew well in the heartwood after impregnation with NH_4NO_3 and D-glucose which indicates that the presence of wood extractives was not responsible for preventing growth of sapstaining fungi in the heartwood.

Wood moisture content is a key factor influencing fungal growth. However, Zheng et al. (1994) found that neither moisture content nor pH nor nitrogen could explain why *O. piceae* does not colonize lodgepole pine heartwood. They proposed that the phenolic compounds present in the heartwood are toxic to the fungus.

Tabirih and Seehann (1984) investigated the development of *Botryodiplodia theobromae* on Abachi (*Triplochiton scleroxylon*) wood and found that it can grow on extracts from all stem portions. The inability of the fungus to colonize the physiological heartwood is based neither on a lack of nutrients nor on toxic compounds but most probably on a tight closure of infestation pathways (occlusion of pits and tyloses). This was also shown in the refractory behaviour of Abachi in preservative treatments.

1.2.5.2 Time of felling

It has often been said that softwoods felled in spring and summer are more susceptible to sapstain than those felled in winter but controlled experiments do not support this (Findlay, 1959a). According to Findlay (1959a), it is merely that staining fungi do not attack wood during cold weather, and by the time warmer weather arrives, wood that has been felled and converted in the winter may have partly seasoned and so become less subject to attack. In this context, Rogister (1955; quoted in Findlay, 1959a) carried out a series of experiments on samples taken from poplar trees felled at different seasons of the year. Wood from logs felled in March/April and in July showed the maximum susceptibility to *L. theobromae* whereas wood freshly cut in December, May and June was more or less immune against fungal infection. Trees felled in autumn became much more susceptible to sapstain infection in the following February and March. Presumably, changes in the amount and nature of the reserve materials in the living parenchyma cells of the wood were mainly responsible for these variations in susceptibility.

In places with temperate climates like New Zealand, the length of unprotected exposure of radiata pine logs in the forest after felling is crucial with regard to sapstain infection.

Unless the wood is processed shortly after felling, there is usually a high infection risk (Butcher and Drysdale, 1991). Delays can result in localised loss of moisture, especially from log ends and areas where bark has been removed during extraction. Recently, a mathematical model (Sapstain Danger Index, SDI) was developed and validated which is used to indicate forest sites in New Zealand where felled logs are more susceptible to sapstain infection (Cooper et al., 2000). It also indicates how soon after felling a log should be treated in order to minimise economic losses due to sapstain, by using parameters such as temperature, rainfall and relative humidity. It was shown that in four of five sites monitored in the Central North Island, there was a correlation between the Sapstain Danger Index and the number of days until sapstain infection occurred.

1.2.5.3 Wood moisture content

Sapstaining fungi are aerobic organisms. Consequently, the moisture content of the sapwood in a living tree is too high to allow for fungal growth. However, if the moisture content is reduced due to mechanical injury (wind or harvesting damage) or insect attack, a standing tree might become infected. Local drying of the sapwood is one of the first indicators of pathogenic attack of standing trees.

The limiting maximum moisture content for development of staining fungi can only be determined with regard to the density of the wood. The lower the density, the more air and water the wood contains. The potential area of stain therefore increases during the drying process of wood until fibre saturation is reached. Münch (1907) observed a very slow penetration of *O. minus* into 5-10 cm disks cut from a 40-year-old Scots pine (quoted in Gibbs, 1993). However, with a loss of only 10-20 % of moisture and consequently, an increased availability of oxygen, complete penetration of the sapwood was obtained. Münch´s view that the supply of oxygen is the limiting factor for growth of sapstaining fungi in wood with a high moisture content was confirmed by Lagerberg et al. (1927). Lagerberg et al. (1927), using wood partially sterilized at 50°C, found that *O. minus* was less inhibited by a high moisture content than were *O. piliferum* and *L. lundbergii*.

Minimum moisture content for the growth of most staining fungi is about 27-28%, i.e. around the fibre-saturation point of the wood (Findlay, 1959a; Käärik, 1980). However, a minimum moisture content of about 24% has been determined for stain development in loblolly pine caused by *Ceratocystis pilifera* (Colley and Rumbold, 1930). This result has been confirmed by Lindgren (1942) as well as Björkman (1946). Sapstaining fungi can grow on wood that has been air-dried and re-wetted but generally, they seem to grow more vigorously on wood that is drying for the first time from the green condition (Findlay, 1959a). Some *Ophiostoma* species will not grow on wood that has been dried and then rewetted (Seehann, 1965).

1.2.5.4 Oxygen

As mentioned earlier, sapstaining fungi need oxygen for their development, however, at low levels. Scheffer (1986) sought to establish the O_2 requirements of 48 wood-destroying basidiomycetes and six sapwood-staining fungi, investigating i) growth of the fungi on nutrient agar under various partial pressures of O_2; ii) capacity of the fungi to deplete O_2 when sealed in a closed chamber; iii) capacity of the fungi to survive when so confined. It was found that the six sapstaining fungi lived one to six months without O_2, exhibiting survival comparable to the majority of the basidiomycetes. *Ceratocystis minor*, *C. pilifera*, *Diplodia natalensis* and *Graphium rigidum* were able to survive between one and three months without O_2 whereas *C. ips* survived for up to six months without O_2.

1.2.5.5 Temperature

The temperature minimum for growth of sapstaining fungi, depending on the species, is around 0 to -3°C, the optimum between 18 and 29°C and the maximum between 28 and 40°C (Schmidt, 1994). Only *Ophiostoma clavatum* has been shown to grow at temperatures above 40°C (Henningsson and Lundström, 1974). Under suitable conditions, sapstaining fungi will grow 10-15 mm per week radially and up to 50 mm per week longitudinally in wood (Käärik, 1960). Fluctuating temperatures reduce the growth rate (Reynolds et al., 1972) but serious staining can occur when wood is stored at lower temperatures, in the range 3-8°C (Miller and Goodell, 1981). Pechmann (1965) reports that some fungi which were exposed to short periods of freezing grew at greater than normal rates when the temperature rose. At relative humidities of 10-20%, temperatures of up to 130°C are required before some species are killed (Zimmermann and Butin, 1973). Some *Ophiostoma* species are able to withstand one hour at 200°C (Seehann 1965). The tropical sapstaining fungus *B. theobromae* grows best at about 27°C, and there is hardly any growth below 10°C (Findlay, 1959a).

1.2.5.6 Nutrients

As sapwood inhabiting organisms, sapstaining fungi utilize the easily assimilable nutrients present in the parenchyma cells (protoplasma). These are readily available carbohydrates (sugars and starch) as well as the lipophilic wood extractives (Brush et al., 1994; Gao et al., 1994; Farrell et al., 2000). Extractives are low-molecular weight lipophilic components in wood consisting mainly of triglycerides, waxes, steryl esters, sterols, free long chain fatty acids and resin acids (Fengel and Wegener, 1989). It is generally stated that the major wood components, cellulose, hemicelluloses and lignin, are not the main carbon sources for many sapstaining fungi (Shimada and Takahashi, 1991; Abraham et al., 1993 and 1998; Gao et al., 1994). Since staining fungi use the non-structural components in wood, they consequently produce hydrolases, e.g., proteases, lipases, amylases and esterases to metabolize macromolecules such as proteins, glycerides and starch (Gao et al., 1994).

It was demonstrated by Brush et al. (1994) that *Ophiostoma piliferum* selectively metabolises triglycerides, fatty acids and resin acids which resulted in the application of an albino isolate of this fungus in the manufacturing process of pulp and paper (Blanchette et al., 1992; Farrell et al., 1993; Brush et al., 1994). This strain is commercially available under the tradename Cartapip™. Fungal removal of extractives from wood prior to pulping controls the formation of pitch. Pitch is the technical term used to describe the deposits of wood extractives associated with fibers, inorganic salts and additives which disrupt the runnability of paper-making machinery and reduce strength and brightness of pulp (Allen, 1980). Fischer et al. (1994) reported that *Ophiostoma piliferum* lowered the pitch or resin content of loblolly pine by 18-27 % in two weeks and 33-35 % in four weeks. Gao et al. (1994) examined the utilization of wood lipids in lodgepole pine sapwood by *O. piceae*. After two weeks colonization, the triglycerides decreased by 76% and the resin acids by 62%. Sapstaining fungi also significantly decrease extractives in radiata pine wood chips as has been shown with several albino isolates of *O. floccosum* and *O. piceae* from New Zealand (Farrell et al., 2000).

Recently, Martínez-Inigo et al. (1999) confirmed that two other species of sapstaining fungi, *Ophiostoma ainoae* and *Ceratocystis allantospora*, almost completely degraded triglycerides and long chain fatty acids in Scots pine sapwood. The fungal strains also reduced substantially the amounts of steryl esters and waxes in pine wood. However, sterols and resin acids in sapwood as well in heartwood were not or poorly removed by *Ophiostoma ainoae* and *Ceratocystis allantospora*. The removal of total extractives was higher in sapwood than in heartwood. The highly concentrated extractive fraction in pine heartwood mainly consists of resin acids. The fungal degradation of heartwood extractives was not only limited by the degradative ability of the fungi but also by the inhibitory effect exerted by the extractive fraction.

The growth and sporulation of many different Swedish saprophytic *Ophiostoma* species as well as of *O. ulmi* on synthetic media were studied by Käärik (1960). Different sources of carbon tested included mono-, di- and polysaccharides, sugar alcohols and organic acids. Growth tests were also performed on different sources of nitrogen. The fungi tested grew well on most of the carbon sources tested (Table 1.4). It was found that for mycelial growth, nearly all the *Ophiostoma* species required one or more of the vitamins thiamine, pyridoxine and biotin. The only vitamin-autotrophic species were *O. brunneo-ciliatum* and *O. stenoceras*. Of the Fungi Imperfecti, the insect-associated species were deficient for the same vitamins as the *Ophiostoma* species. In liquid media, only mycelial conidia were produced. The addition of vitamins had variable effects on the production of conidia, and there was no correlation between the numbers of conidia and the amount of mycelium produced. Best production of perithecia was obtained at high concentrations of glucose and low concentrations of nitrogen. The optimum pH for growth of *O. piceae*, *O. canum* and *O. tetropii* were determined to be between 3.5 and 6.5, depending on the nitrogen source used.

Table 1.4: Utilization of carbon and nitrogen sources by *Ophiostoma* spp. Summarized from Käärik (1960).

Carbon sources	Good growth	Poor growth (or variable)
Hexoses	Glucose	Sorbose
	Fructose	
	Mannose	
	Galactose	
Pentoses	Xylose	Ribose
	Arabinose	
	Rhamnose	
Disaccharides	Maltose	Sucrose
	Cellobiose	Lactose
		Raffinose
Polysaccharides	Starch	Inulin
	Pectin	Glycogen
		Cellulose
Sugar alcohols	Glycerol	
	Sorbitol	
	Mannitol	
Nitrogen sources		
Amino acids	Asparagine	
	Glutamine	
	Alanine	
Inorganic	Ammonium sulfate	Nitrate
	Ammonium tartrate	

The growth of *B. theobromae* on different carbon and nitrogen sources was investigated in detail by Seehann and Tabirih (1983). Since *B. theobromae* is a tropical sapstaining fungus which is not present in New Zealand, the nutrient requirements of this species will not be reviewed here. The reader is referred to Seehann and Tabirih (1983) for more information on this aspect.

1.3 Diseases caused by sapstaining fungi

The relationship between sapstaining fungi and their hosts is varied. Some species are non-pathogenic whereas others cause serious diseases. Some fungi are pathogenic on certain tree species but not on others. *Ceratocystis coerulescens*, for example, acts non-pathogenically on Scots pine (*Pinus sylvestris*) and Corsican pine (*P. nigra var. calabrica*) in Europe but it is a pathogen on Norway spruce (*Picea abies*) in Sweden and Norway (Gibbs, 1993). Another example is *Ceratocystis virescens* which causes sapstain on freshly cut oak and sweetgum logs in the Southern United States while it can be a lethal pathogen on sugar maple (Kile, 1993).

There are also species which exhibit pathogenicity towards humans, notably *Sporothrix schenkii*, a conidial fungus causing sporotrichosis which has been linked to *Ophiostoma stenoceras* (Berbee and Taylor, 1992).

Only three species of *Ophiostoma*, *O. ulmi*, *O. novo-ulmi* and *Leptographium wageneri*, have been studied extensively as causal agents of major plant diseases, Dutch elm disease and black stain root disease on conifers, respectively (Harrington, 1993). *Ophiostoma ulmi* is a weak pathogen on most European elm species whereas *O. novo-ulmi* is characterised by high mortality rates among native European elms (Brasier, 1986; 1991). *Ophiostoma novo-ulmi* is further divided into two subgroups. According to their respective geographical distribution, they have been named the Eurasian or EAN and North American or NAN race (Binz, 1996a). *Ophiostoma piceae* has been implicated in oak decline in eastern Europe but pathogenicity of these species has yet to be established (Harrington, 1993). Oak wilt in the United States is caused by *Ceratocystis fagacearum*, a vascular wilt pathogen (Kile, 1993).

Current results indicate that the production of the phytotoxic hydrophobin cerato-ulmin influences virulence in non-aggressive strains of *Ophiostoma* fungi (Del Sorbo et al., 2000). All cerato-ulmin producing transformants used by Del Sorbo et al. (2000) caused an increase in severity of vascular and/or foliar symptoms, the two parameters commonly considered for the evaluation of virulence of Dutch elm disease pathogens (Smalley and Guries, 1993; Sutherland et al., 1997). The expression of the cerato-ulmin gene of *O. novo-ulmi* in a strain of *O. querci* confers the ability to produce cerato-ulmin in culture and to cause symptoms of Dutch elm disease. There is consequently a potential risk that an *O. querci*-like fungus might acquire the cerato-ulmin gene from *O. novo-ulmi* and become a severe pathogen of oak (Del Sorbo et al., 2000).

Two other *Ophiostoma* species have been reported to produce cerato-ulmin (Temple and Horgen, 2000): *O. himal-ulmi* (Brasier and Mehrotra, 1995) and *O. piceae* (Scala et al., 1994). In *O. piceae*, cerato-ulmin could be detected in the cell wall by immunofluorescence (Scala et al., 1994; Tegli and Scala, 1996).

Sphaeropsis sapinea is an opportunistic pathogen of *Pinus* spp. with a cosmopolitan distribution (Waterman, 1943; Harrington and Wingfield, 1998). The fungus is commonly associated with disease symptoms such as shoot blight and collar rot of seedlings (Palmer and Nicholls, 1985), root disease (Wingfield and Knox-Davies, 1980), cankers accompanied by resinosis (Waterman, 1943; Marks and Minko, 1969) and crown wilt (Chou, 1987). Reported hosts of *S. sapinea* include over 30 species of *Pinus* and also species of *Abies*, *Cedrus*, *Juniperus*, *Picea* and *Pseudotsuga* (Waterman, 1943; Punithalingam and Waterston, 1970; Swart et al., 1985; Luley and Gleason, 1988). The pathogen is of relatively minor importance in the United States and Europe compared with the destruction it has caused in *P. radiata* plantations in New Zealand, Australia and South Africa (Swart and Wingfield, 1991, and references therein). *Sphaeropsis sapinea* in New Zealand was extensively characterized by Birch (1936). The earliest record of *Diplodia* dieback of pine in New Zealand was given by Curtis (1930) and it can be inferred from her description that it was a case of crown wilt associated with drought (Chou, 1987).

Sphaeropsis sapinea commonly infects pine cones and seeds (Peterson, 1977; Fraedrich and Miller, 1995) and is the most important source of seedling infections in nurseries (Palmer et al., 1988; Rees and Webber, 1988; Huang and Kuhlman, 1990) while also attacking young pine plantations (Karadzic and Vujanovic, 1992). However, the pathogen is most often associated with wounds on stressed trees (Harrington and Wingfield, 1998). Wounds conducive for infection result from hail, wind damage, pruning and insect feeding. Infections can be minimized or prevented by avoiding pruning during warm and wet weather and avoidance of excessive pruning (Swart and Wingfield, 1991). *Sphaeropsis sapinea* strains show differences in physiology and virulence (Swart et al., 1991, 1993).

The presence of a species of *Leptographium* in New Zealand was first reported in 1980 (Shaw III and Dick, 1980). It was suggested that the species which had been isolated from black stained sapwood of dead and dying *Pinus strobus* in two forests on the North Island may be similar to *L. procerum*. *Leptographium procerum* is known to cause dieback and root decline in *Pinus strobus*. Affected trees have chlorotic, drooping needles, crooking of new shoots, reduced shoot elongation, and retention of needles for a year or more after the tree's death (McCall and Merrill, 1980). Several species of *Leptographium* have been suggested to be at least weak root pathogens (Alexander et al., 1988; Wingfield et al., 1988). The most pathogenic species, *L. wageneri*, causes black-stain root disease, a unique and destructive vascular wilt disease of conifers in the western United States and British Columbia, Canada (Harrington and Wingfield, 1998). Recently, three new species of *Leptographium* have been described, *L. alethinum*, *L. pityophilum* and *L. euphyes*, which have mistakenly been treated as *L. procerum* in the past, and which are expected to be mildly pathogenic or saprotrophic (Jacobs et al., 2001).

Many, but not all of the *Ophiostoma* species associated with tree-killing bark beetles are pathogenic (Harrington, 1993). The fungi are thought to contribute to tree death and arrest host defense rections by causing phloem lesions which expand around the points of beetle-attack and by colonizing the sapwood. Sapwood occlusion and interruption of water flow may be the ultimate cause of death of beetle-attacked trees. Among the numerous *Ophiostoma* species associated with *Ips typographus* on spruce, *O. polonicum* is the most pathogenic. *Ophiostoma clavigerum*, associated with *Dendroctonus ponderosae*, is a pathogen on pine. *O. ips* has been reported to be a substantial pathogen (Basham, 1970; Himelick, 1982; Raffa and Smalley, 1988), but it does not appear to be particularly pathogenic in artificial inoculations (Rane and Tattar, 1987; Parmeter et al., 1989).

1.4 Dissemination of sapstaining fungi

It was thought earlier that air currents play a major part in bringing infection to freshly sawn wood (Findlay, 1959a). The air in timber yards and pulpmills contains large number of fungal spores as well as probably fragments of mycelium from the dust produced during sawing of stained logs (Björkman, 1946; Mathiesen-Käärik, 1955). However, it was found

that dry air is completely ineffective in causing conidium dispersal whereas both mist-laden air and splash droplets disseminate conidia relatively easily (Dowding, 1969; quoted in Gibbs, 1993). Ascospores can also be dislodged by splash droplets but only with difficulty (Dowding, 1969). It should be stressed that intact bark gives effective and prolonged protection against fungal damage (Pearce, 1996). Bark damage suffered by logs during mechanized harvesting increases the probability for sapstain infection (Uzunovic et al., 1999a).

Bark beetles play an important role in disseminating spores in standing trees as well as in logs and timber. The association between bark beetles and staining fungi in Europe was first exhaustively studied by Grosmann (1930; quoted in Findlay, 1959a). Mathiesen-Käärik (1953) showed that while *O. minus* was recorded on several beetles, *O. canum*, *O. brunneociliatum* and *O. clavatum* were associated with a single beetle species only. Siemaszko (1939; quoted in Findlay, 1959a) found that *Ips typographus* was constantly associated with *Ceratocystis penicillata* and certain yeasts which was confirmed by Rennerfelt (1951; quoted in Findlay, 1959a). *Leptographium wingfieldii* and *O. minus* are among the species that have close associations with bark beetles (Gibbs and Inman, 1991; Lévieux et al., 1991). The abundant growth of these two fungi in bark could reflect an evolutionary adaptation to increase the possibility of contact with vector beetles to enhance dissemination (Uzunovic and Webber, 1998). In contrast, *C. coerulescens* and *O. piceae* do not have any recognized specific pine associated insect vector, but their dissemination is linked with non-specific microfauna such as mites, nematodes and flies (Uzunovic and Webber, 1998). This may particularly help *O. piceae* in the superficial colonization of cut wood surfaces. The importance of mites in dissemination of sapstain fungi in small pine logs was first suggested by Dowding (1970) and confirmed more recently by Powell et al. (1995) and Uzunovic et al. (1999a).

As far as it is known, *S. sapinea* is not insect-disseminated. The fungus produces pycnidia in phloem tissue, close to the outer bark surface. Spores extruded from these, through cracks in the outer bark, are then dispersed through water films, rain splash or in mist air (Uzunovic and Webber, 1998). The dispersal of conidia of *S. sapinea* is strongly correlated with the occurrence of rain (Brookhouser and Peterson, 1971; Swart et al., 1987).

1.5 Investigations on melanisation of sapstaining fungi

The term sapstain is misleading in the sense that it implies the release of a substance into the wood by the fungi. However, the colour of stained wood is due to melanin (a polymer synthesized from one of four starting compounds, in fungi typically from 1,8-dihydroxynaphthalene) within the fungal hyphae (Butler and Day, 1998). The mature hyphae are brown in colour but when wood is extensively colonized by sapstaining fungi the wood appears blue to black because of light-diffraction. The possible causes of stain produced by some *Ophiostoma* species in diseased lodgepole pine were identified as 2,3-

dihydroxybenzoic acid (Ayer et al., 1986a, quoted in Seifert, 1993) and ceratenolone chelated in a complex with iron (Ayer et al., 1987, quoted in Seifert, 1993). However, despite the apparent involvement of iron in the staining, no differences were found in the trace element compositions of infected and sound trees (Ayer et al., 1986b).

Melanins from *Ceratocystis coerulescens* and *Alternaria alternata* have been isolated and characterized (Zink and Fengel, 1988). They are associated with carbohydrates and proteinaceous components. The latter are not or only weakly linked by chemical bonds to the melanin and/ or the carbohydrates. It was observed in electron microscopic studies with hyphae of *C. coerulescens* and *A. alternata* that the deposition of melanins is related to the age of the mycelia (Zink and Fengel, 1989). Beginning after about one day, the melanins are deposited in the hyphal walls in the form of globular granules. The deposition of the melanin granules causes a warty and bark-like surface of the hyphal walls. There is evidence that precursors of the melanins are synthesized within the cytoplasma and then transported to the cell walls where they are transformed into melanins (Zink and Fengel, 1989).

Several different pathways for the biosynthesis of fungal melanins have been described (Bell and Wheeler, 1986; Zink and Fengel, 1989, 1990). The DHN (1,8-dihydroxynaphthalene) pathway is thought to be responsible for melanin formation in various Ascomycetes and Fungi Imperfecti. Recent results by Eagen et al. (2001) confirm this suggestion with regard to *Ophiostoma floccosum*. Melanins are not directly essential for growth of sapstaining fungi (Zimmerman et al., 1995; Brisson et al., 1996), however, they have been reported to enhance survival of fungi by (summarized in Kreber et al., 1999) preventing microbial lysis (Bloomfield and Alexander, 1967), insect predation (Brasier, 1978) and by providing protection from ultraviolet light and desiccation (Bell and Wheeler, 1986; Butler and Day, 1998). Recently, it has been demonstrated that melanin plays an important role in building up high turgor pressure for successful host cell wall penetration by pathogenic fungi (Bechinger et al., 1999; Talbot, 1999). Enormous invasive forces are applied by appressoria (specialized infection structures) of fungal pathogens which enable the mechanical infection of plants. The high pressure of appressoria from pathogenic fungi like *Magnaporthe grisea*, the causal agent of the rice blast disease, is due in part to the melanin layer that forms an inner cell wall. Mutants of *M. grisea* that fail to synthesize melanin are nonpathogenic and do not accumulate turgor (Howard and Ferrari, 1989).

Fungal melanins are also necessary for the development of reproductive structures such as perithecia (Zimmerman et al., 1995). Furthermore, nutrients affect production and characteristics of fungal melanin (Juzlova, 1996), and nutrients that occur in wood were shown to induce the darkest melanisation in *O. piceae* (Eagen et al., 1997; Breuil, 1998).

According to Kreber et al. (1999), the development of fungal melanin in unseasoned sapwood is a complex, yet poorly understood process, specifically it is still not fully known what induces biosynthesis of melanins in sapstaining fungi and which factors contribute to their formation. It was found, however, that the development of melanin can be controlled by keeping pre-infected wood in an oxygen-free atmosphere and also by rapidly air-seasoning the wood to below 50% moisture content (Kreber et al., 1999).

1.6 Prevention and control of sapstain

It is much easier to prevent sapstain than trying to remove the evidences of it. It has been tried to reduce the intensity of stain with bleaching agents based on H_2O_2 or with hypochlorites (Findlay, 1959b). Hypochlorites are more effective than H_2O_2 in reducing the contrast between stained and unstained wood. A method was patented by Orth (1950) in Austria that involved treating stained fir, beech, maple or oak with 15 % NaOH or KOH at ordinary temperatures to swell the surface of the wood, then treating with H_2O_2 - solution, and finally rinsing with water (quoted in Findlay, 1959b). The main difficulty when using any bleaching agent is that the latter usually tends to raise the grain of the wood so that further sanding becomes necessary. Sanding, however, removes the bleached surface again and will expose the stained wood beneath.

In order to prevent the occurrence of sapstain in logs, it is necessary i) to process harvested logs rapidly after felling, i.e. sawing and kiln-drying of the wood to a moisture content of below 18 % of its dry weight; or ii) to sprinkle the logs or store them under water until they can be converted since staining fungi are aerobic organisms which cannot penetrate into wood saturated with water (Findlay, 1959a). When these methods are nor feasible, wood is protected by applying a fungicide plus appropriate solvents and surfactants to the surface of the logs. The diluted treatment is applied either by dipping or spraying. Kiln-drying has the significant advantage that once wood is dry, sapstain and other fungal attack is permanently prevented, provided that rewetting of the wood does not occur (Cown, 1999). Sprinkling or ponding logs is a temporary control measure because sapstain will occur if the wood is allowed to dry out.

When conditions for establishment of sapstain are optimal, it is necessary to process logs in New Zealand within 1-3 days after felling (Cown, 1999). The presence of bark gives some protection against the entry of stain. An anti-sapstain chemical should be applied as soon as a fresh log is debarked or when lumber is cut from logs. Anti-sapstain fungicides only provide temporary protection. The maximum period that sapstain can be prevented depends on a number of factors such as climate, handling and storing practices, fungal species, timber species and tree age, season at time of felling, forest location and inherent properties of sapwood such as nutrient and extractive content. In general, reliable protection can be achieved for 4-6 months for sawn timber and 3-4 months for logs (Wakeling, 2000). Control of sapstain in logs is more difficult to achieve than in sawn

timber mainly because of the uneven surface of a log. Sawn timber is also less prone to be exposed to rainfall which will wash off the applied fungicide, and contact with machinery and storage surfaces. Another possibility for fungicide depletion on logs is due to the more severe drying stresses occurring at the surface.

Until the 1970s, salts of pentachlorophenol generally provided excellent protection against sapstain at low costs (Preston, 1999). The use of chlorinated phenols was discontinued in most lumber producing countries after it had been found that the compounds are toxic to fish, potentially carcinogenic and sometimes contaminated with extremely toxic dioxins, as byproducts of the manufacturing process (Seifert, 1993).

A number of biological control organisms, including albino-strains of commonly occurring *Ophiostoma* species (Behrendt et al., 1995a, 1995b; Schmidt and Müller, 1996), other sapwood inhabiting fungi (Seifert et al., 1988; Hiratsuka and Chakravarty, 1993; Croan and Highley, 1996; Kay, 1997), mycorrhizal fungi (Benko, 1987) and bacteria (Kreber and Morrell, 1993; Kim and Morrell, 1998; Payne and Bruce, 1999) have been investigated for their efficacy against sapstain. To date, none of these alternative antisapstain treatments is routinely used by the wood processing industry. However, as field and laboratory trials have shown, there is a potential for using various biological agents to help alleviate the sapstain problem (Farrell and Thwaites, 2001). The use of integrated strategies that employ cultural, chemical and biological procedures offers the most immediate opportunities for protecting wood from discolouration while reducing the need for chemicals (Morrell, 1999).

2 Material and Methods – General

2.1 Fungal isolates

All sapstaining fungi used in this study were isolated in New Zealand and belong to the mycological culture collection at the University of Waikato, Hamilton (Table 2.1). Strains for this thesis research were chosen according to their overall occurrence in New Zealand and from different geographical origins in the North and South Islands. Two white-rot fungi, *Schizophyllum commune* (isolate #3 from HortResearch, Hamilton, New Zealand) and *Phlebiopsis gigantea* (isolate #104 from *Forest Research*, Rotorua, New Zealand), and a brown-rot fungus, *Gloeophyllum trabeum* (isolate BAM Ebw. 109 from *Forest Research*) were included to serve as positive controls.

Table 2.1: List of sapstaining fungi used in this thesis.

Species	Isolate no.	Origin	Date isolated
Ophiostoma floccosum	138*	Riverhead Forest, North Island	29-1-1997
O. floccosum	148°	Hanmer Springs, South Island	23-1-1997
O. floccosum	F13*	Albino strain	-
O. floccosum	F40*	Albino strain	-
O. piceae	170	Greymouth, South Island	18-2-1997
O. piceae	272*°	Rotorua, North Island	21-4-1997
O. querci	1069°	Tasman Pulp & Paper, Kawerau, North Island	6-8-1997
O. pluriannulatum	151*°	Abel Tasman National Park, South Island	30-1-1997
O. pluriannulatum	5040*°	Albino strain	-
O. pluriannulatum	F3410*	Albino strain	-
O. ips	308*	Waipa sawmill, Rotorua, North Island	9-4-1997
O. ips	294*	Nelson, South Island	1-5-1997
O. ips	1191*	Maharangi Forest, North Island	28-8-1997
O. ips	P36*	Kinleith Forest, North Island	12-1-2000
O. ips	181°	Port Tauranga, North Island	7-4-1997
Leptographium procerum	1852*	Whitford Forest, North Island	29-1-1998
Leptographium procerum	281°	Waipa sawmill, Rotorua, North Island	9-4-1997
Pesotum cupulatum	211°	Kaingaroa Processing Plant, North Island	14-3-1997
Sphaeropsis sapinea	4*	Dome State Forest, North Island (pine cone)	Spring 1996
Sphaeropsis sapinea	35*°	Kinleith Forest, North Island (live tree)	13-12-96

* = refers to fungi used in toughness and weight loss tests.

° = refers to fungi used in growth experiments.

2.2 Cultural isolations

For fungal isolations, the bark of a wood sample was carefully separated from the underlying sapwood surface, and slivers from the inner face of the bark and the wood surface were isolated aseptically. Sapwood was sampled from the exterior to the internal surface, soaked in 2% hypochlorite solution for one minute and rinsed twice in sterile

water. Slivers were then taken from the samples with a sterile scalpel and placed onto two different plates both consisting of yeast-malt agar (0.2% yeast extract, 2% malt extract and 2% agar), supplemented with 200 micrograms per ml chloramphenicol and 100 micrograms per ml streptomycin sulphate and one set also supplemented with 400 micrograms per ml cycloheximide. *Ophiostoma* species are cycloheximide resistant (Harrington, 1981) whereas other sapstaining species are not.

Inoculated plates were incubated in a darkened growth chamber at 25°C for 4 to 21 days. Any resulting cultures were aseptically transferred onto fresh plates with and without cycloheximide. All cultures suspected of being *S. sapinea* were inoculated onto sterile radiata pine needles and maintained under ultraviolet light at ambient temperature for two weeks. They were then examined microscopically in sterile water for the presence of pycnidia and spore release. Cultures are maintained at -70°C in 20% glycerol.

Ophiostoma species were identified on the basis of morphological features into putative species. Molecular characterizations, particularly DNA sequences of the internal transcribed spacer regions of ribosomal DNA and mating compatibilities with tester strains have been used to confirm species identifications (Farrell et al., 1998).

2.3 Culture conditions

Ophiostoma spp. cultures were maintained on Petri dishes containing 2 g yeast extract (Becton Dickinson and Company, U.S.A.), 15 g malt extract (Difco, U.S.A.), 18 g agar (Germantown, New Zealand), 200 mg chloramphenicol (Sigma, U.S.A.), 100 mg streptomycin sulphate (Sigma, U.S.A.) and 400 mg cycloheximide (Sigma, U.S.A.) per one litre of water. *Sphaeropsis sapinea*, *Schizophyllum commune* and *Gloeophyllum trabeum* were maintained on the same medium but cycloheximide was omitted. Liquid starter cultures were prepared by transferring a mycelial plug (one cm diameter) taken from the edge of an actively growing colony into universal flasks containing 10 ml yeast-malt extract broth (2 g yeast extract, 15 g malt extract and one liter of water; medium I). After inoculation, starter cultures were incubated using a rotary shaker set at 120 rpm and 25°C for 24 hours.

2.4 Blastospore counts

Blastospores were counted using a haemocytometer in a light microscope. Approximately 10 µl of cell suspension were transferred onto the haemocytometer, and a cover slip was placed on top. All fungal cells in the five sub-squares of the total 25 squares were counted. The optimum dilution for counting is 10-20 cells per sub-square. The total number of spores per ml is obtained using the following equation: no. of cells·5·10^4.

2.5 Determination of fungal biomass

Cultures of a known volume were spun in a Beckman centrifuge for 30 minutes at 10,000 rpm and 4°C. Supernatants were filtered under vacuum through pre-dried and weighed filter-papers. The mycelial pellets were resuspended in distilled water and filtered using the same procedure. The filter papers were subsequently dried in an oven at 80°C until constant weight was obtained.

For isolates of *S. sapinea* and the two decay fungi, Whatman filter paper #4 (particle retention 20-25µm) was used. For isolates of *Ophiostoma*, Whatman filter paper #5 (particle retention 2.5µm) and subsequently Schleicher and Schüll glass fibre filterpaper GF6 (particle retention 1.2µm) were used.

For enzyme determinations, fungal biomass was determined after the last supernatants of the cultures were harvested, unless stated otherwise.

3 Growth of sapstaining fungi in liquid media

3.1 Introduction

Among the many publications on sapstain, only few deal with fungal physiology and specifically fungal growth kinetics in liquid media. Käärik (1960) mentions several earlier investigations on the physiology of two of the most widely studied pathogenic sapstaining species, *O. ulmi* and *Ceratocystis fagacearum*. The growth and enzyme production of *O. multiannulatum* has also been extensively studied (f.ex. v. Hofsten, 1956; for further publications see Käärik, 1960).

The most comprehensive study on the growth and sporulation of sapstaining fungi was published by Käärik (1960; for a list and description of sapstaining fungi see also Käärik, 1980). She studied 18 species of *Ophiostoma* and six species of Fungi Imperfecti on synthetic media. More recently, Binz (1996a) determined the effect of growth substrates on the secretion of cell-wall degrading enzymes by *O. ulmi* and *O. novo-ulmi* and related enzyme activities to fungal dry weight.

In this thesis, growth of several sapstaining fungi was measured at different temperatures by counting blastospores and determining accumulated fungal biomass in order to obtain the temperature optima for fungal growth. In addition, growth curves were a prerequisite for the determination of enzyme activities. It is necessary to know when fungal growth is likely to peak so that an appropriate incubation time can be chosen for enzyme screenings. Lastly, growth was determined in liquid media because the isolates chosen were representative for the behaviour of candidate strains for use in large-scale fermentations in the development of an albino-biocontrol agent.

3.2 Material and Methods

Liquid starter cultures (in duplicates) were prepared as described in Chapter 2. Starter cultures were transferred into medium I (80 ml in 250 ml-flasks) consisting of 2 g yeast extract, 15 g malt extract and one liter of water. The cultures were further incubated in a rotary shaker set at 120 rpm and different temperatures (4°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C) for up to 90 hours. Blastospores formed were counted in intervals using a haemocytometer as described in Chapter 2. In addition, fungal biomass of the cultures in the experiments with an incubation temperature of 25°C was determined (see Chapter 2) in order to correlate blastospore formation and biomass accumulation.

3.3 Results and Discussion

The results of the growth experiments are presented in Figures 3.1-3.4 (blastospore counts over incubation time at 25°C), Figures 3.5-3.9 (fungal biomass accumulated over

incubation time at 25°C), Figures 3.10-3.11 (summary of results for different incubation temperatures) and in Appendix 1 and 2 (growth curves at 4°C, 10°C, 15°C, 20°C, 30°C and 35°C). In all figures, vertical bars represent the range of duplicate values obtained for an isolate at a specific temperature and time after inoculation.

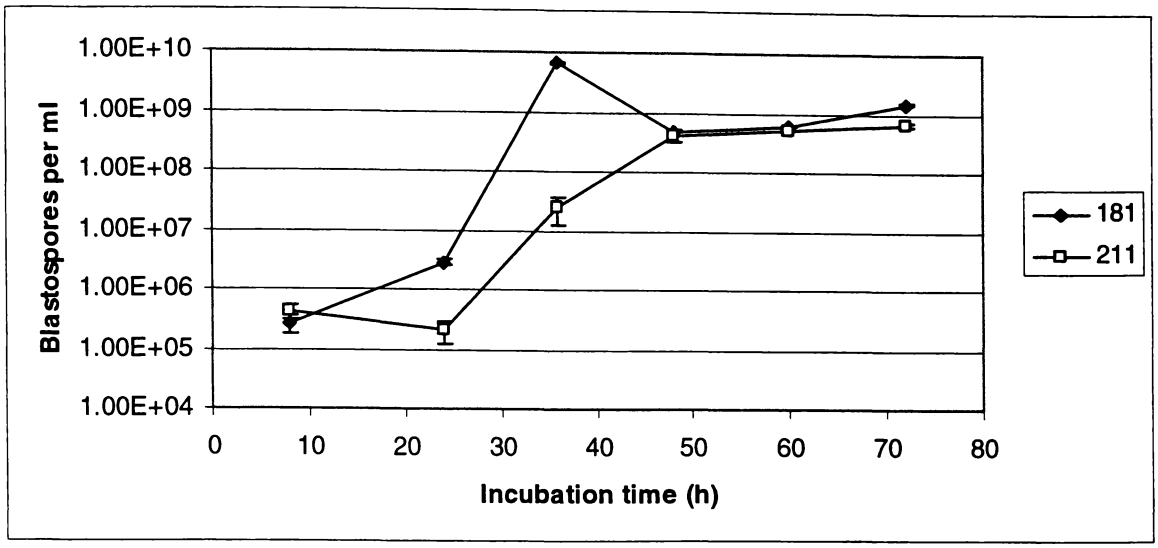


Figure 3.1: Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 25°C during 72 hours incubation. Vertical bars represent the range of values obtained for duplicate cultures.

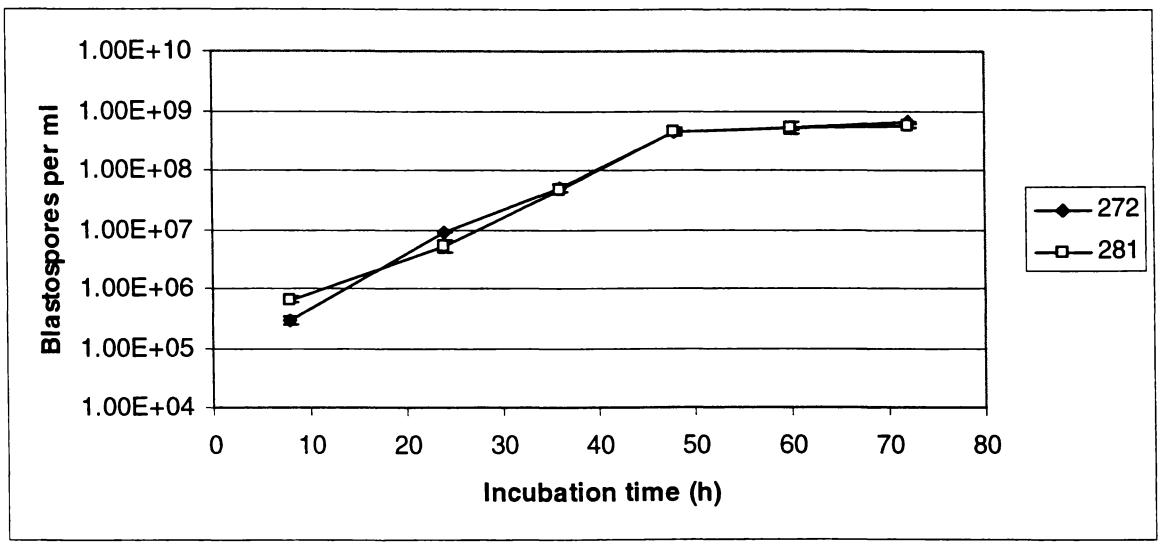


Figure 3.2: Blastospore counts for *O. piceae* #272 and *O. ips* #281 in liquid media at 25°C during 72 hours incubation.

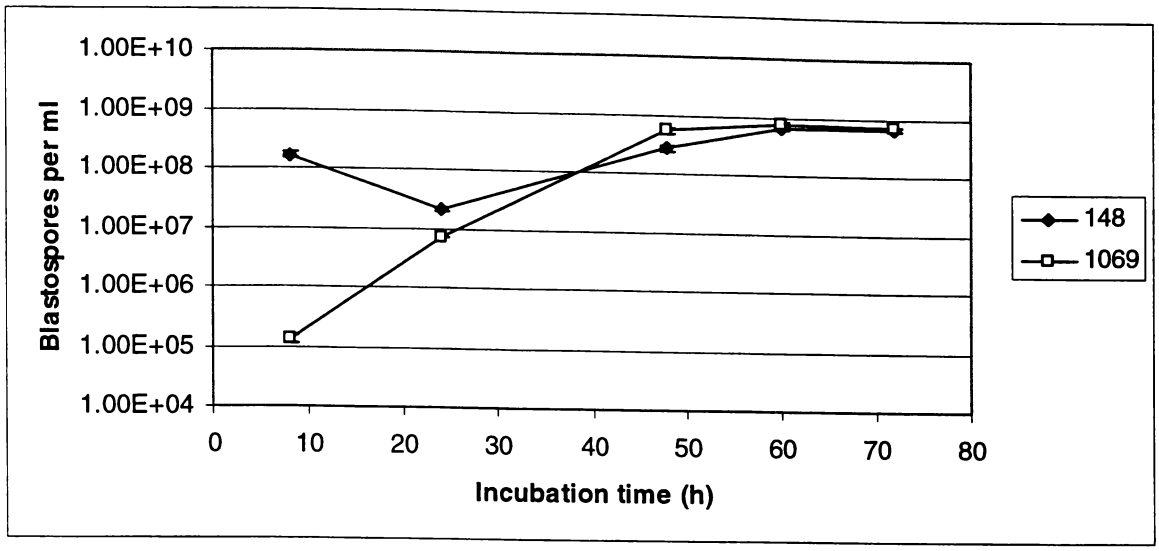


Figure 3.3: Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 25°C during 72 hours incubation.

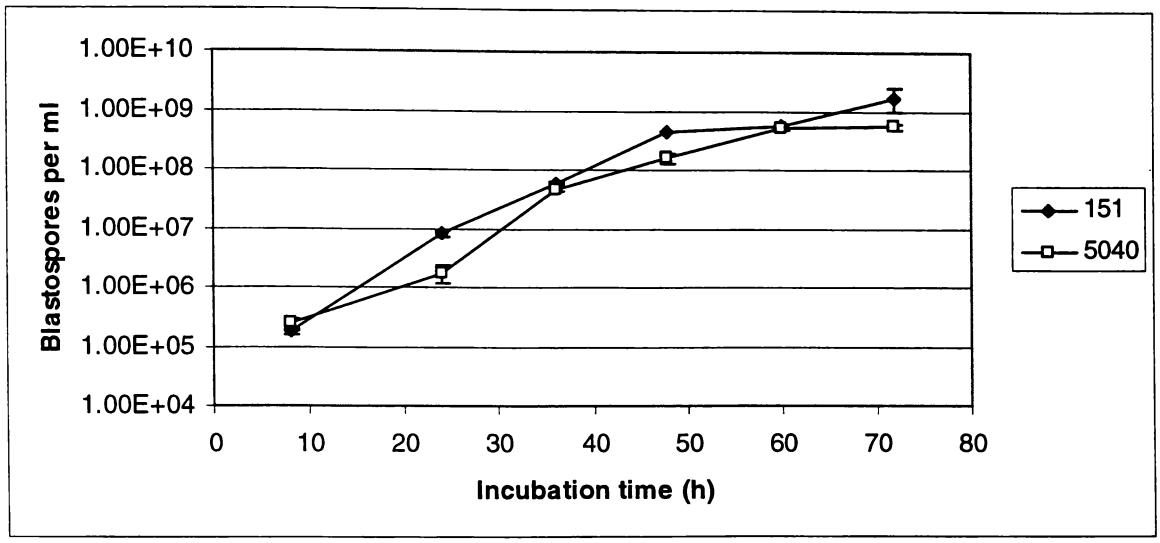


Figure 3.4: Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 25°C during 72 hours incubation.

Over an incubation period of 72 hours, there was a correlation between blastospore (conidia) formation in species of *Ophiostoma* and biomass accumulation (*S. sapinea* does not produce blastospores; see Chapter 1). However, after 72 hours, the exponential growth phase of the fungal strains as measured in blastospore counts was finished whereas fungal growth as determined in total biomass (mycelia and blastospores) was still increasing. At this stage, autolysis was observed when the cultures were examined under polarised light.

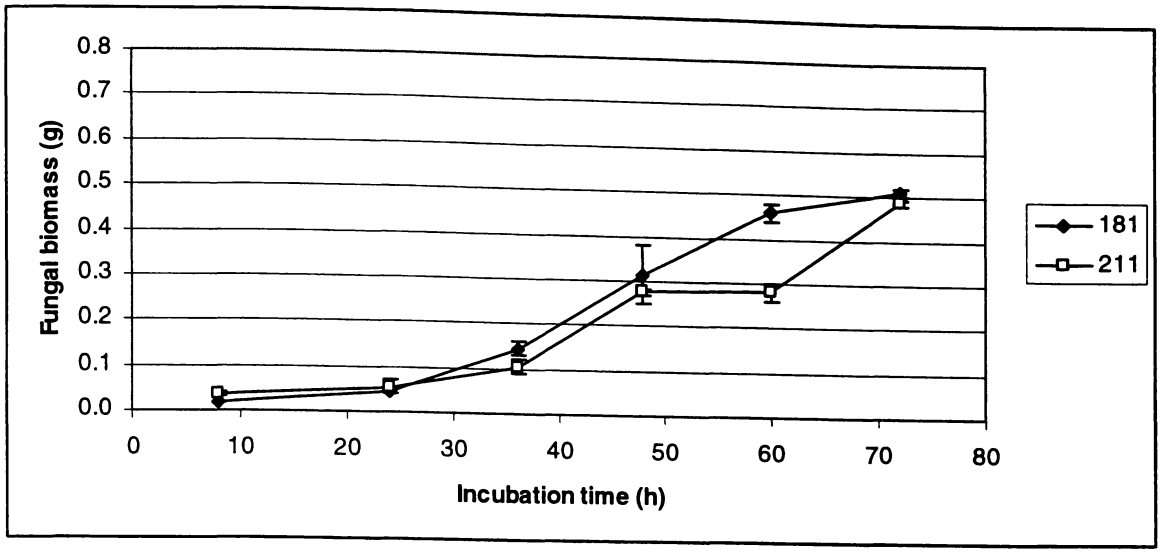


Figure 3.5: Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 25°C during 72 hours incubation.

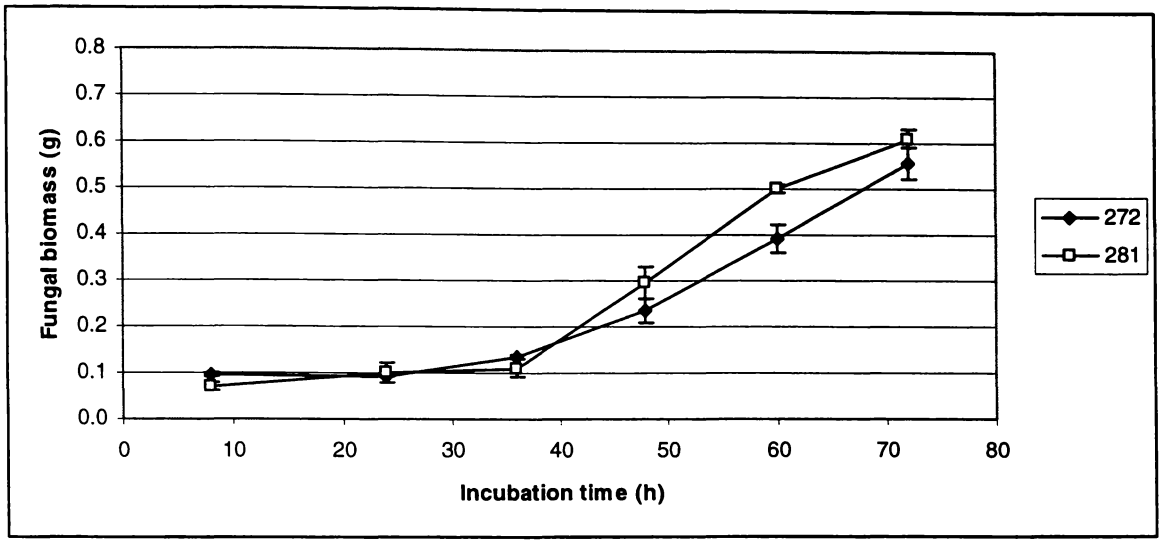


Figure 3.6: Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 25°C during 72 hours incubation.

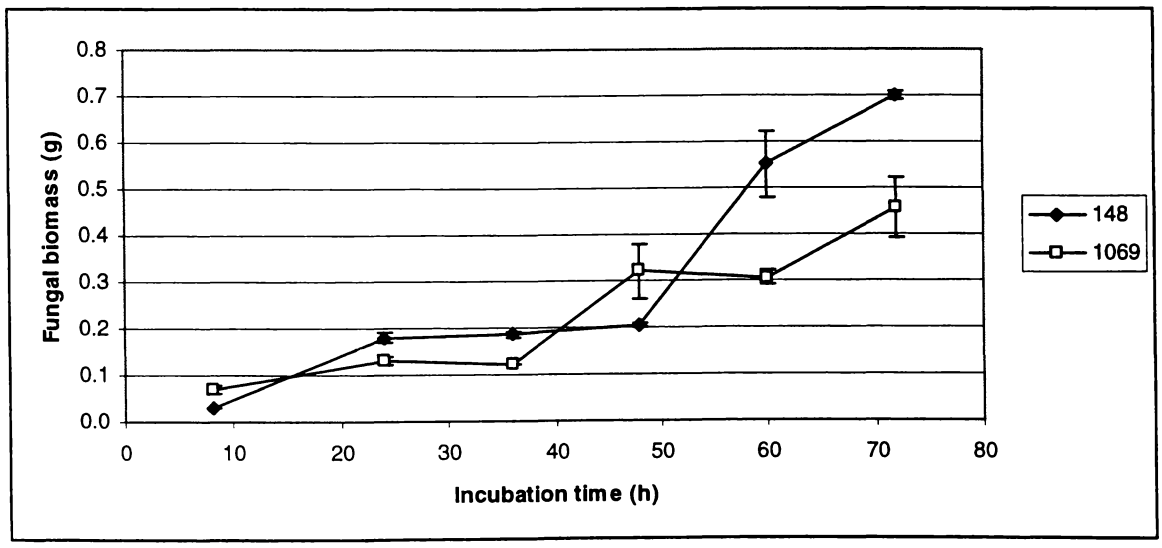


Figure 3.7: Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 25°C during 72 hours incubation.

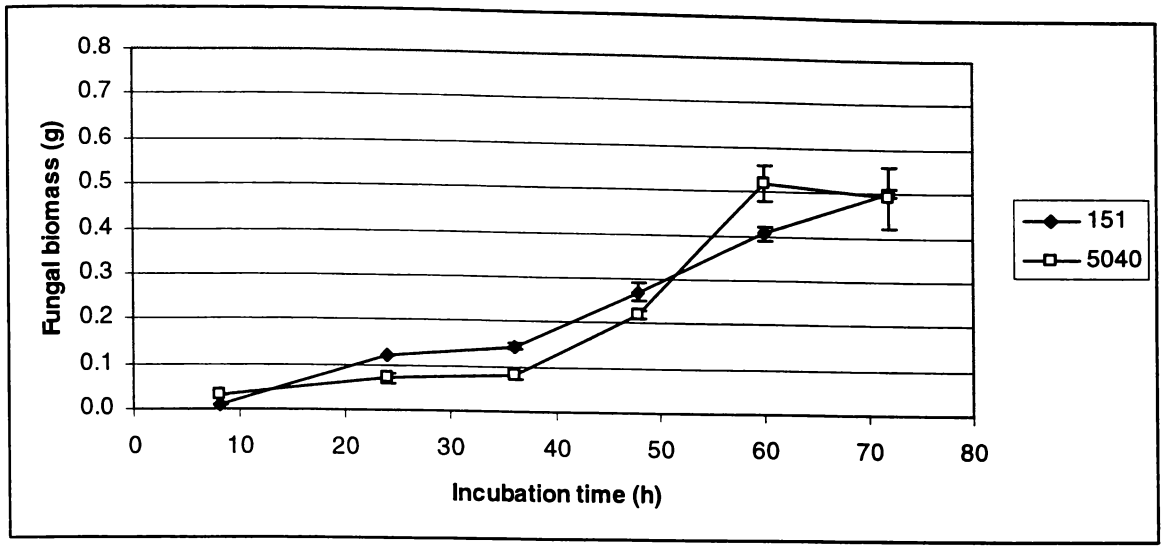


Figure 3.8: Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 25°C during 72 hours incubation.

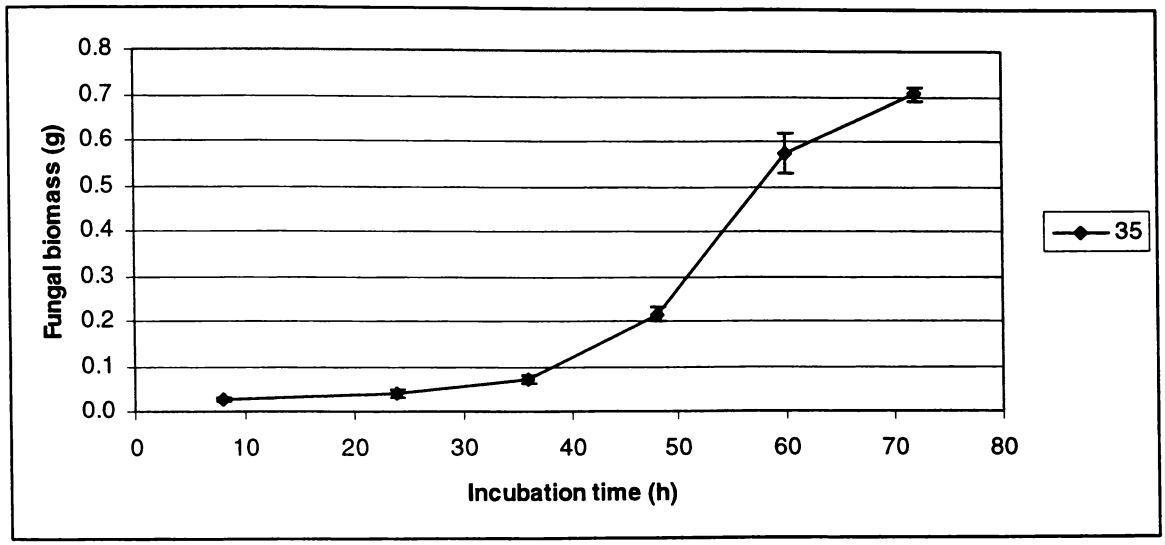


Figure 3.9: Biomass accumulation of *S. sapinea* #35 in liquid media at 25°C during 72 hours incubation.

When plotting the results for maximal blastospore counts at different temperatures in one graph, it becomes obvious that the fungal isolates investigated grew at a wide temperature-range (Figures 3.10 and 3.11). This is consistent with reports in the literature. According to Schmidt (1994), the temperature minimum for growth of sapstaining fungi is around 0 to minus 3°C, the optimum between 18 and 29°C and the maximum between 28 and 40°C. All fungi tested produced blastospores at 4°C and 10°C with the exception of *O. floccosum* #148 which is sensitive to lower temperatures (see Appendix 1). All isolates grew well at 35°C (see Appendix 1 and 2). Four isolates grew best at 25°C (*O. piceae* #272, *O. floccosum* #148, *O. ips* #181 and *Pesotum cupulatum* #211). *Leptographium procerum* #281 showed maximum growth at 30°C, and the two isolates of *O. pluriannulatum* (#151 and #5040) as well as *O. querci* #1069 grew best at 20°C (see Appendix 1).

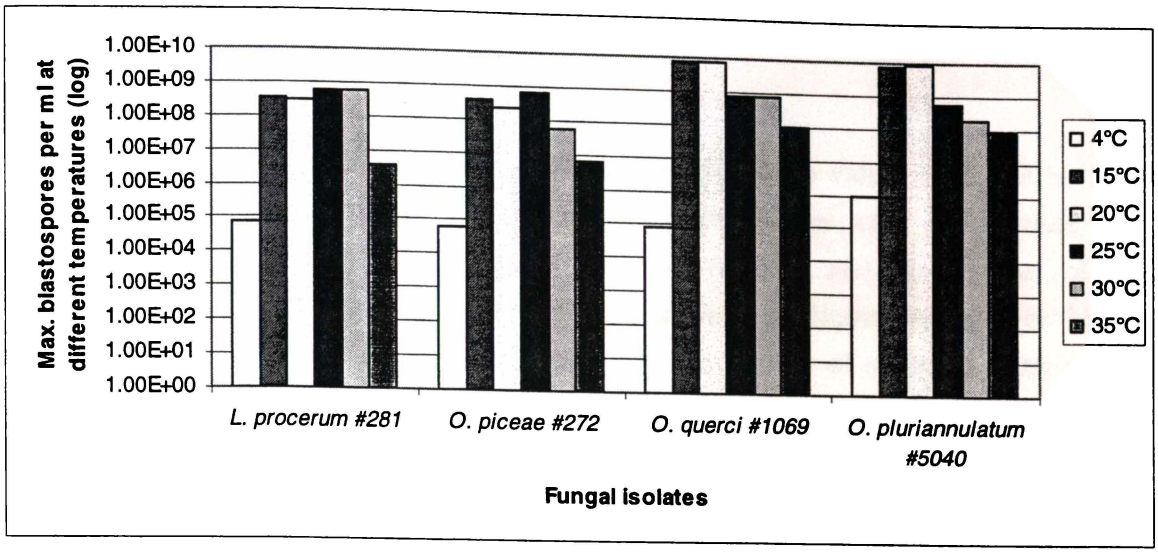


Figure 3.10: Summary of growth of *L. procerum* #281, *O. piceae* #272, *O. querci* #1069 and *O. pluriannulatum* #5040 in liquid media at different temperatures.

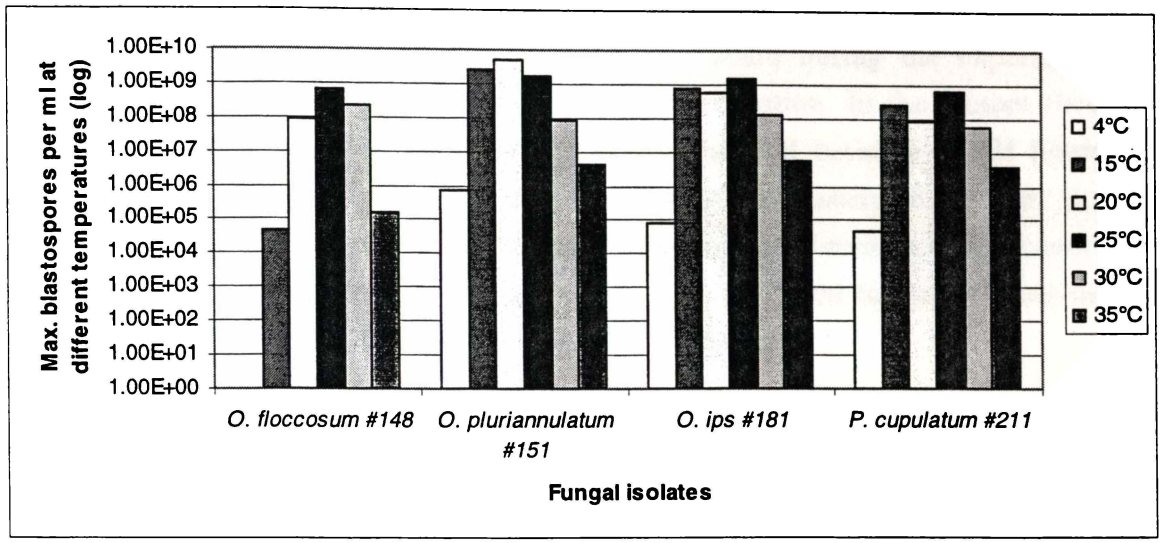


Figure 3.11: Summary of growth of *O. floccosum* #148, *O. pluriannulatum* #151, *O. ips* #181 and *P. cupulatum* #211 in liquid media at different temperatures.

As mentioned earlier, there are not many publications dealing with growth characteristics of sapstaining fungi in liquid media which makes it difficult to discuss the results obtained in this thesis. However, in a publication by Abraham et al. (1993), the growth of *O. piceae* in liquid medium was investigated. They found that the dry weight growth curve showed an initial 24h lag phase, followed by a rapid biomass increase that corresponded to nitrogen depletion. After day 3, growth slowed down and reached a maximum dry weight of 5 mg/ml at day 4. The carbon source was completely depleted after 6 days. In the present thesis, a similar growth curve based on biomass measurements was obtained for an isolate of *O. piceae*. Here, the dry weight growth curve showed an initial lag phase which lasted about 35 hours (Figure 3.6). At the same time, however, when only blastospore counts are taken into account, growth was exponential for up to 50 hours from the start of incubation (Figure 3.2). With regard to dry weight measurements, the exponential growth phase started after 35 hours incubation and lasted until the end of incubation, i.e. 72 hours.

At this time, autolysis was observed in the cultures. Maximum dry weight of *O. piceae* #272 was 0.56 g/70 ml.

It is difficult to compare the results from the present thesis to values obtained by Käärik (1960) because she used different species of *Ophiostoma* and a different media composition. Käärik (1960) investigated the growth rate and sporulation of three *Ophiostoma* species (*O. pini*, *O. tetropii* and *O. canum*) in liquid media over 30 days. Measurements of the dry weight of the mycelium, the number of spores and the pH were made every two days. It was found that *O. canum* and *O. tetropii* reached their maximum weight at the 14th day of incubation whereas the growth of *O pini* continued up to the 30th day of incubation. With *O. canum* and *O. tetropii* the phase of autolysis was attained already after 18-20 days of incubation so from day 20 onwards, great differences were observed in parallel flasks with regard to the dry weight of the mycelia. In *O. canum*, the number of conidia reached a maximum after 8-12 days incubation. In *O. pini*, the maximum number of conidia was found later, after 18-20 days. In contrast, with *O. tetropii*, the number of conidia remained fairly constant during the experiment with maximum values from the 8th to the 22nd day of incubation. In the present thesis, the maximum number of blastospores was generally obtained between 48-60 hours after incubation. After about 60 hours, growth as measured in blastospore numbers declined. The fact that Käärik (1960) measured the maximum number of conidia after a much longer incubation period than in this thesis may have to be attributed to the fact that different species were investigated and different culture conditions used.

Gagnon (1961) grew *Ceratocystis ulmi* in liquid media and used fungal dry weight as an indicator of growth. The liquid media contained four different nitrogen compounds, eight sugars and four concentrations of glucose. The fungus was grown at room temperature in the dark as still cultures and in daylight as still and as shake cultures. Although the fungus grew well in still and shaken cultures, the growth was twice as great in the latter. It was found that the dry weight of the fungus fluctuated regularly with age possibly due to autolysis, as had been found by Käärik (1960). The most marked reductions in dry weight in the shaken cultures occurred on days 9 and 14 after incubation.

Growth rates in liquid media cannot be compared to results obtained on solid media. However, it should be mentioned that on malt extract agar, *O. piceae* and *O. querci* can easily be distinguished by their ability to grow at 32°C (Harrington et al., 2001): *O. querci* grows at this temperature whereas *O. piceae* does not. *O. floccosum* also grows at 32°C but not as profoundly as *O. querci*.

4 Cell wall degrading enzymes of sapstaining and decay fungi

4.1 Introduction and Objectives

Wood-inhabiting fungi can be classified on the basis of their enzyme systems and ability to degrade wood into the following categories (Subramanian, 1983):
a) Moulds and sapstaining fungi that normally do not degrade lignified cell walls but derive their nutrition from contents of dead cells.
b) Soft rot fungi capable of degrading lignified cell walls enzymatically to a limited extent.
c) Decay fungi with a high ability to degrade wood and causing brown or white rot.

In category a, the term "normally" implies that there are exceptions. The common tropical sapstaining fungus *B. theobromae* produces extracellular cellulase, xylanase and pectinase (Umezurike, 1968, 1969, 1970, 1971; Seehann and Tabirih, 1983), and is generally regarded as a sapstaining organism but in its ability to degrade cellulose and produce cavities in the wood cell walls it strongly resembles a soft-rot fungus (Krapivina, 1960). *Botryodiplodia theobromae* possesses a multicomponent system for cellulose degradation, i.e. one or more endoglucanases which produce cleavage of ß-1,4-bonds in polymers, one or more exoglucanases for cleavage of cellobiose units from the non-reducing free ends liberated by the endoglucanases and ß-glucosidases for removal of cellobiose (Umezurike, 1969, 1970a, 1970b, 1971).

It has also been shown that the causative agents of the Dutch elm disease, *O. ulmi* and *O. novo-ulmi*, produce cellulase and pectinase (Beckman, 1956; Biehn and Dimond, 1971; Elgersma, 1976; Svaldi and Elgersma, 1982). However, recent studies by Binz (1996a) did not confirm the presence of cellulase in these species. *Aureobasidium pullulans* produces carboxymethylcellulase, xylanase, ß-galactosidase, mannanase, polygalacturonase and laccase (Deshpande et al., 1992). *Ceratocystis minor* secretes carboxymethylcellulase, polygalacturonase, pectinesterase and laccase (Rösch et al., 1969). Laccase has been detected in several sapstaining species (Rösch and Liese, 1971). Sapstaining fungi produce enzymes which act intra- and extracellularly (Rösch et al., 1969; Rösch and Liese, 1971; Berndt and Liese, 1971).

In general, there is little quantitative information available on cell wall-degrading enzymes of other common sapstaining fungi. The differences in reports regarding the presence or absence of certain enzymes can partially be explained with the sensitivity of the assay methods used as well as the substrates employed for their detection. Highly sensitive assays are based on the detection of reducing sugars (Miller, 1959; Lever, 1973). Inconclusiveness of enzyme data may also be attributed to differences between strains of one fungal species tested as well as differences in the wood species.

With regard to the non-structural components of the sapwood, sapstaining fungi, including *O. piceae* and *O. piliferum*, have shown to produce extracellular enzymes to utilize sugars, proteins and extractives (Abraham et al., 1993 and 1998; Brush et al., 1994; Breuil and Huang, 1994; Breuil et al., 1995; Gao and Breuil, 1995; Abraham and Breuil, 1996; Gao and Breuil, 1998).

In the following sections, structure and function of wood components of radiata pine were illustrated and important aspects of the degradative potential of sapstaining fungi were reviewed in detail. The objectives of this chapter were to detect and quantify the accumulation of cell-wall degrading enzymes, i.e. cellulase, xylanase, mannanase and pectinase, under in-vitro conditions, and to determine the utilization of non-structural wood components (amylose, extractives) for comparative purposes.

4.2 Literature Review

4.2.1 Wood components of radiata pine

In radiata pine, as in other softwoods, cellulose is the principal component of the wood cell wall. Microfibrils of cellulose constitute the structural framework of the tracheid and are surrounded and permeated by the cell wall matrix which is composed of lignin and hemicelluloses (see Figure 5.1 in Chapter 5). Extractive-free wood of radiata pine has the approximate chemical composition of 40% cellulose, 27% lignin, 31% hemicelluloses and 2% other components (Table 4.1). The major difference between sapwood and bark of radiata pine is that the sapwood contains considerably more cellulose and hemicelluloses than the bark, and only a small percentage of extractives.

Table 4.1: Approximate chemical composition of radiata pine sapwood and mature bark*.
Source: Uprichard (1991).

Component	**Composition (%)**	
	Wood	Bark
Extractives		
Non-polar	2	3
Lower molecular weight phenols	(0.1)	4
Condensed tannins	-	18
Tannins/phenolic acids	-	40
Subtotal	2	65
Cellulose	40	12
Lignin	27	15
Hemicelluloses and other compounds	31	6
Ash	(0.2)	2

*Based on weight of oven-dry material.

Wood extractives are a mixture of substances extracted from wood using dichloromethane and consist mainly of glycerol esters (mono-, di- and triglycerides), fatty and resin acids, sterols, steryl esters, waxes, fatty alcohols and other volatile compounds (Gao et al., 1995). The extractives present in the heartwood differ from those in the sapwood. The sapwood is rich in both fatty acid glycerides and resin acids whereas the heartwood resin contains mainly resin acids (Table 4.2). Except for the inner heartwood zone at the base of mature trees which contains 5 to 10% resin, the resin content of radiata pine is generally no more than 2-3% (Uprichard, 1991).

Table 4.2: Compounds (as percentage of total extractives) in heartwood and sapwood of radiata pine. Source: Uprichard, 1991.

Compounds	Heartwood	Sapwood
Fatty acids (free)	2	1
Fatty acid esters	11	41
Resin acids	71	41
Phenols	6	3
Unsaponifiables (neutrals)	10	14

The carbohydrate components of radiata pine are summarized in Table 4.3. Cellulose is a linear polymer of ß-1,4-linked anhydroglucose units. An important feature of cellulose, and of the other polysaccharides present in radiata pine, is that they all possess a reducing end-group and have potentially aldehydic or reducing properties.

Table 4.3: Carbohydrate composition of radiata pine. Source: Smelstorius (1974).

Component	Percentage of oven-dry wood
Carbohydrate	
Glucan	41.6
Mannan	12.1
Galactan	2.8
Xylan	6.5
Arabinan	2.7
4-O-methylglucuronic acid anhydride	1.6
D-galacturonic acid anhydride	0.9
O-acetyl	1.9
Total carbohydrate	70.1
Lignin	27.4
Extractives (approx.)	2.5

The hemicelluloses are shorter chain polysaccharides, such as the arabinoglucuronoxylans, galactoglucomannans and arabinogalactans which are composed of simple sugar monomers or related acidic compounds. The predominant hardwood hemicellulose is partially acetylated 4-O-methylglucuronoxylan (Fengel and Wegener, 1989; Figure 4.1). A xylan, similar but not identical to that found in hardwoods, forms a small, but substantial part of softwoods. The main sugar component of xylan is D-xylose. Depending on the

source, xylan structures vary from linear 1,4-ß-D-polyxylose main chain to highly branched heteropolysaccharides (Timell, 1967; Puls and Schuseil, 1993).

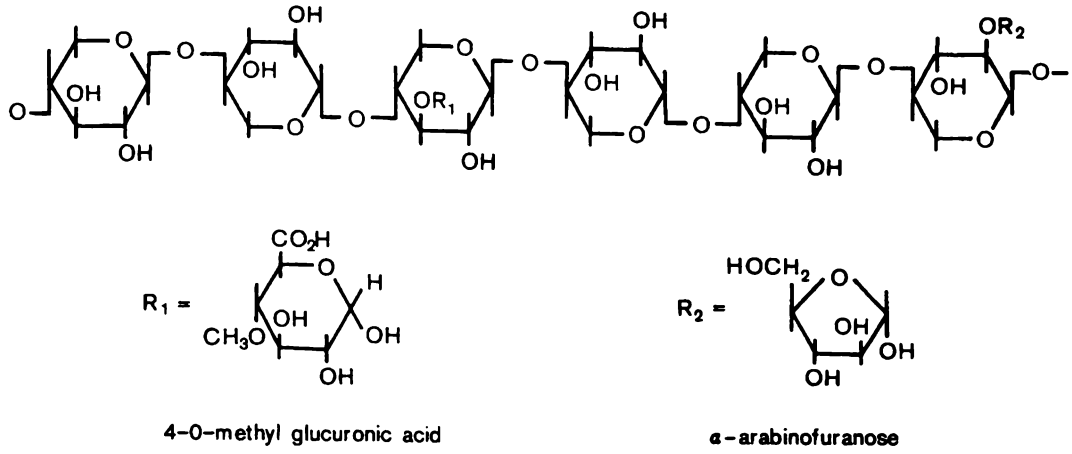


Figure 4.1: Partial structure of the xylan (arabinoglucuronoxylan) from radiata pine.
Source: Uprichard (1991).

The predominant softwood hemicellulose is galactoglucomannan which is partially acetylated (Figure 4.2). In some softwoods, it can comprise up to 20% of the wood (Timell, 1967; Puls and Schuseil, 1993). A small percentage (2-5%) of hardwoods is composed of glucomannan (Eriksson et al., 1990). Both glucomannans and galactoglucomannans are composed of a backbone of ß-1,4-linked D-glucopyranose and D-mannopyranose residues. The ratio of these monosaccharides is between 1:1 and 1:4, depending on the source. D-galactose in galactoglucomannans is α-1,6-linked as a single unit side-chain (Eriksson et al., 1990).

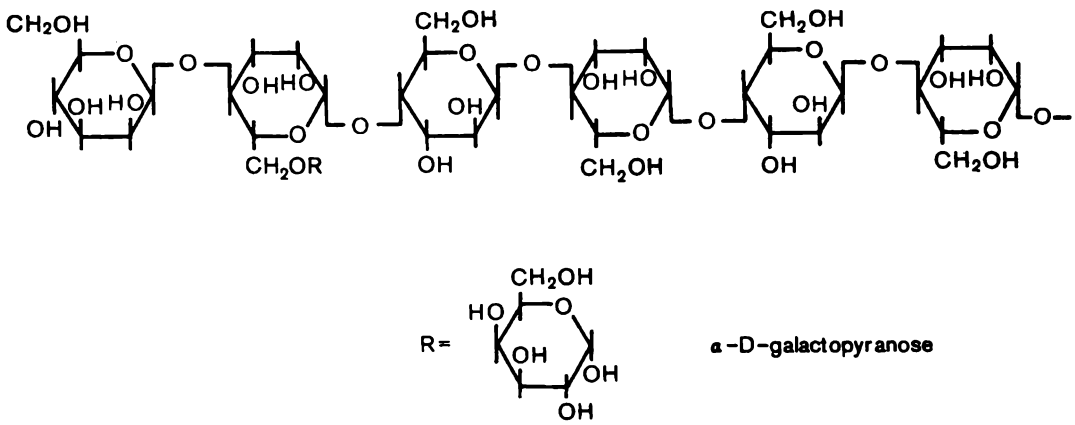


Figure 4.2: Partial structure of radiata pine glucomannan (galactoglucomannan). Source: Uprichard (1991).

4.2.2 Mode of enzyme secretion in fungi

Lytic enzymes which are excreted at growing hyphal tips digest external polymers while the hyphae are advancing, converting the external polymers into small molecules that can

be taken up and used as nutrients. Turgor is another important factor in penetration of wood by hyphae. Because hyphal walls form a diffusion barrier for proteins, a mechanism different from diffusion probably exists to transport proteins across the cell wall (Sietsma et al., 1995). This mechanism, according to Sietsma et al. (1995), will be explained in the following paragraph.

It has been shown that in *S. commune*, a white-rot fungus, the wall at the hyphal apex grows by apposition of plastic wall material so that proteins excreted at the apex may pass the wall by being carried with the flow of wall material (bulk flow theory), creating pores in the wall. A large portion of excreted proteins leaves hyphae at the growing apices, another portion is retained by the wall and slowly released from the mature wall into the environment. Among proteins that can be permanently retained by the wall are the hydrophobins that self-assemble at the outer wall surface when confronted with a hydrophilic-hydrophobic interface. The bulk flow theory would seem to solve the paradox that the measured porosity of the fungal wall does not permit the passage of large proteins. In ascomycetes and basidiomycetes the matrix component of the cell wall consists of 1,3-/1,6-ß-glucan which is by itself soluble but obtains its alkali insolubility from covalent linkage to chitin. The apical zone of growing hyphae is filled with vesicles which are thought to arise in the endoplasmatic reticulum far behind the tip. They are transported to the apex, pass through Golgi equivalents and are then subjected to exocytosis. In the lumina of these apical vesicles may, among other components, be located the lytic enzymes which are destined for secretion to digest polymeric substrates (Brada and Schekman, 1988; Gooday and Gow, 1990).

Exocytosis of proteins and wall expansion are tightly coupled (Sietsma et al., 1995). At the growing apex, wall polymers and proteins are excreted together. Several proteins are retained in the wall and may not be released into the medium after a certain period of time. A portion of the enzyme may be caught in inner wall layers from which release into the medium is slow. This may apply to enzymes, f.ex. cellulase, secreted by sapstaining fungi. Some of these proteins can be easily solubilized by detergents or chaotropic agents. Several low molecular mass proteins were released from the wall of *Saccharomyces cerevisiae* and *Candida albicans* with hot sodium dodecyl sulfate (SDS) (Valentin et al., 1984; Marcilla et al., 1991). Some high-mannan high molecular mass proteins can only be solubilized by digestion of the wall with polysaccharases. These proteins are thought to be entrapped in the three-dimensional network of wall polysaccharides or cross-linked to these polysaccharides; some of these high molecular mass proteins are also suggested to be anchored to the cytoplasmic membrane (Sietsma et al., 1995).

4.2.3 Enzymes of sapstaining fungi degrading the structural components of wood

4.2.3.1 Cellulase

Cellulose is decomposed through the synergistic action of hydrolytic enzymes, specifically endo-ß-1,4-glucanase, exo-ß-1,4-glucanase and 1,4-ß-glucosidase. Endo-ß-1,4-glucanase randomly attacks and splits ß-1,4-glucosidic linkages of the cellulose chain. Exo-ß-1,4-glucanase splits off glucosidic linkages only from the non-reducing end of the cellulose, resulting in cellobiose or glucose. 1,4-ß-glucosidase catalyzes the hydrolysis of ß-glucosidic linkage of cellobiose, yielding D-glucose.

Cellulase activities of *O. ulmi* and *O. novo-ulmi*, the causative agents of the Dutch elm disease, have frequently been reported (Beckman, 1956; Elgersma, 1976; Svaldi and Elgersma, 1982). *Ophiostoma ulmi* is a weak pathogen on most European elm species whereas *O. novo-ulmi* is characterised by very high mortality rates among native European elms (Brasier, 1986 and 1991; quoted in Binz, 1996a). Recent findings by Binz (1996a, 1996b) could not confirm the presence of endocellulase activity in *O. ulmi* and *O. novo-ulmi*. This result is surprising considering that both species produce glucosidases which complement cellulase activity and may also remove side groups of polysaccharides in order to facilitate the action of endoenzymes (Walton, 1995). Using immunocytochemistry, Benhamou et al. (1987) found well-delineated digested host wall areas along the fungal periphery of *O. ulmi* or often away from the point of penetration which strongly suggests that this fungus produces specific enzymes that may diffuse through the wall. Since the presence of β-(1→4)-D-glucans was not observed in these areas, as revealed by the total absence of gold labeling, it is suggested that *O. ulmi* produces extracellular cellulase complexes which are able to degrade crystalline and amorphous cellulose (Benhamou et al., 1987).

The production of carboxymethylcellulase has also been demonstrated in culture filtrates of *Ceratocystis minor*, *Aureobasidium pullulans* and *Alternaria humicola* (Rösch et al., 1969). The production of cellulase was higher in a medium with glucose as carbon source than with carboxymethylcellulose. It has to be stressed, however, that the presence of cellulase was determined viscosimetrically. The method used does not reveal if in addition to endoglucanase, there was exo-ß-1,4-glucanase present which splits off glucosidic linkages from the non-reducing end of the cellulose.

Due to the degradation of the cellulose derivatives, the existence of a C_x-component of the cellulase (capable of hydrolyzing modified celluloses but not native cellulose) was thus proven but the presence of the C_1-component necessary for the enzymatic degradation of native cellulose was not conclusively established (Liese, 1970b). A C_1-type enzyme is capable, on its own, of solubilizing crystalline cellulose (Reese et al., 1950).

Cellulose activity has earlier been indirectly attributed to *A. pullulans* by Seifert (1964). The treatment of Scots pine samples with *A. pullulans* for 10 weeks resulted in a loss of cellulose of approximately 7%. Total hemicelluloses were reduced by up to 4%. Alkali-insoluble pentosans, however, decreased up to 29%. From these results it can be seen that, as far as the cell wall components are concerned, the main action of sapstaining fungi is a splitting of the polyose molecules which become soluble in alkali.

However, other reports state that *A. pullulans* is non-cellulolytic (White et al., 1948; Reese and Levinson, 1952). Levy (1969) reported that no effect occurred in wood of *Betula* sp. and *Pinus sylvestris* except staining. Butcher (1968a) obtained no weight losses of beech (*Fagus sylvatica*) wood veneers after incubation for eight weeks. Greaves and Savory (1965) reported clearing of cellulose agar by *A. pullulans* but no soft rot cavities were found in birch wood.

Growth and metabolism of *Ophiostoma multiannulatum* on different carbon sources were studied by v. Hofsten (1956). Tests for cellulolytic activity of this fungus were negative, but β-glucosidase and β-galactosidase were detected. The enzymes were partially purified and shown to have pH-optima around 5.

Nilsson (1973) investigated the cellulolytic activity and wood-degrading ability of 160 species of microfungi some of which belong to the sapstaining fungi. The cellulolytic activity was determined by two types of cellulose clearing media, the Rautela-Cowling medium (R-C medium; Rautela and Cowling, 1966) and the Bravery-VII medium (B-VII medium; Bravery, 1968). The main difference between the two media is that the Bravery-VII medium contains no carbon source except for the cellulose. The Rautela-Cowling medium contains 0.5g/l yeast extract in addition to the cellulose. Of the sapstaining fungi, only *Ceratocystis cana*, *C. piceae* and *C. tetropii* showed cellulolytic activity (Table 4.4). Abraham et al. (1993) also report that *O. piceae* degrades carboxymethylcellulose but no quantitative data are shown. *Ceratocystis minor* produced no clearing on the R-C medium and no attack on birch wood (Nilsson, 1973) which is in contrast to results by Rösch et al. (1969) who found that carboxymethylcellulase was produced in nutrient solutions with glucose or carboxymethylcellulose. This might indicate that the cellulose clearing method used by Nilsson (1973) is not as sensitive as the viscometric method used by Rösch et al. (1969).

Ceratocystis pilifera and *L. lundbergii* did not cause any clearing of cellulose whereas King and Eggins (1972) reported that these fungi produced clearing in cellulose agar. The strain of *C. pilifera* tested by Nilsson (1973) produced no clearing on the R-C medium nor on the B-VII medium. *L. lundbergii* has not been tested on the B-VII medium and may possibly produce clearing on this medium.

The wood-degrading ability was determined microscopically on sections from decayed wood blocks (Nilsson, 1973). Generally, two morphologically distinct types of attack were observed, cavity formation (type 1) and erosion of cell walls (type 2). Of the sapstaining fungi tested, only *C. piceae* showed type 1 decay on birch and pine wood. *Graphium fragrans* failed to produce cellulase but exhibited type 1 decay on birch and pine wood as well. None of the sapstaining fungi tested produced erosion of the cell walls or caused significant weight loss. It was noticed that all cavity-forming species, not only the sapstaining species, formed cavities in both hardwoods and softwoods. In addition, all the species which produced type 2 or type 1 and type 2 decay in birch wood showed cellulolytic activity in the cellulose clearing test. Obviously, the inability to produce erosion of the cell walls distinguishes most sapstaining fungi from other wood-inhabiting microfungi even though some of them may produce cellulase.

Table 4.4: Cellulolytic activity, type of wood attack and decay capacity of sapstaining fungi. Summarized from Nilsson (1973).

Fungal species	**Wood species (Origin)**	**Cellulolytic activity***		**Type of attack**	
		R-C medium	B-VII medium	Wood species	Type**
Aureobasidium pullulans	Birch	-		Birch	0
Ceratocystis brunneo-ciliata	Pine	-		Birch	0
Ceratocystis cana	Pine	+		Birch	0
Ceratocystis clavata	Pine	-		Birch	0
Ceratocystis coerulescens	Spruce	-		Birch	0
Ceratocystis crassivaginata	Aspen	-		Birch	0
Ceratocystis ips	Pine	-		Birch	0
Ceratocystis minor	Pine	-		Birch	0
Ceratocystis minuta	Pine	-		Birch	0
Ceratocystis olivacea	Spruce	-		Birch	0
Ceratocystis piceae	Beech	+		Birch	1
				Pine	1
Ceratocystis pilifera	Pine	-	-	Birch	0
Ceratocystis tetropii	Spruce	+		Birch	0
Graphium fragrans	Pine	-	-	Birch	1
				Pine	1
Leptographium lundbergii	Pine	-		Birch	0

* + = cellulolytic activity present; - = cellulolytic activity absent. R-C medium = Rautela-Cowling medium; B-VII medium = Bravery-VII medium. Explanation in text.

** 0 = no attack; 1 = cavities; (2 = erosion).

Two additional species of *Ceratocystis*, *C. albida* and *C. stenoceras*, were included in further tests by Nilsson (1974). Additionally, *Ceratocystis olivacea* was tested again. None of the species tested produced cellulase.

King and Eggins (1973) tested 33 species of mould and sapstaining fungi associated with colonization of green timber for degradation of cellulose, pectin and amylose in vivo and in vitro. In vivo, cellulose degradation was assessed by the method of Rautela and Cowling (1966) as well as by loss of tensile strength of fibrous cellulose strips. In vitro, cellulolytic activity was determined viscosimetrically on carboxymethylcellulose, cellulose-sodium polypectate and sodium polypectate solutions. Using the cellulose clearing method, of the sapstaining fungi, only *Botryodiplodia theobromae*, *Ceratocystis pilifera*, *Leptographium lundbergii* and *Graphium* sp. produced cellulolytic activity. Strength loss in cellulose strips was caused by all of these species and in addition by *Diplodia natalensis*, *Diplodia pinea*, *Diplodia sapinea*, *Alternaria tenuis*, *Ceratocystis picea*, *Aureobasidium pullulans*, *Ceratocystis ulmi* and *Ceratocystis coerulescens*. Activities assessed in vitro produced broadly similar results to the in vivo studies. Carboxymethylcellulase activity was correlated with cellulolytic activity as determined by tensile strength loss. The results of the viscometric measurements for some of the sapstaining isolates among the test organisms are presented in Table 4.5.

Table 4.5: Relative viscosity units of cellulase and pectinase produced by sapstaining fungi when grown on cellulose (C), cellulose-sodium polypectate (CP) and sodium polypectate (P) media. Summarized from King and Eggins, 1973.

Fungal species	**Cellulase**			**Pectinase**		
	C	CP	P	C	CP	P
Botryodiplodia theobromae	26.7	44.7	3.7	*	0.1	0.3
Diplodia natalensis	39.1	40.0	3.8	*	*	*
Ceratocystis pilifera	2.0	40.0	3.7	0	*	0.3
Ceratocystis coerulescens	9.5	16.0	1.3	0	*	*
Alternaria tenuis	8.5	5.9	*	0.9	0.6	0
Graphium sp.	1.4	2.5	0.6	*	0	0.1
Diplodia sapinea	0.7	2.4	*	*	*	*
Diplodia pinea	0.5	0.6	0.2	0	*	0
Ceratocystis picea	2.0	0.3	0.2	0	0	0
Ceratocystis ulmi	0.8	0.3	*	0	0	*
Aureobasidium pullulans	0.9	*	*	0	0.1	0
Leptographium lundbergii	*	0	0	*	*	*

* indicates distinct but not measurable viscosity losses of the test substrates.

Except for *Leptographium lundbergii*, all sapstaining organisms secreted measurable amounts of cellulase on at least one of the three substrates tested (King and Eggins, 1973). Significant amounts of pectinase were produced only by *Botryodiplodia theobromae*, *Ceratocystis pilifera* (not on cellulose medium), *Aureobasidium pullulans* (only on cellulose sodium polypectate) and *Graphium sp.* (not on cellulose sodium polypectate). It seems that under the test conditions, the cellulase system was the dominant enzyme system. While many of the cellulolytic species did not produce pectinase, all of the pectinolytic species produced cellulase. While the results obtained by King and Eggins (1973) are only relative to the organisms tested, a general trend is indicated that certain

staining fungi are to some extent capable of both cellulose and pectin breakdown in addition to the starch degradation which is commonly associated with their growth. Unfortunately, in the study of King and Eggins (1973), no true wood-decaying fungi were included in the tests to provide a reference value for the measurements.

Seehann and Tabirih (1983) investigated carboxymethylcellulase and xylanase activities of *B. theobromae* after 6 and 13 days incubation in liquid culture (Table 4.6). The accumulation of reducing sugars in the xylan and carboxymethylcellulose substrate solutions as well as the growth of mycelium was measured. After 6 days, all strains had exuded xylanase into the culture medium, with activities varying between 169 and 258 U/ml. After 13 days, only one strain out of four still produced some xylanase. However, this strain produced low quantities of mycelium until 6 days incubation and reached the best mass development after 13 days. Carboxymethylcellulase was produced on the 6th day of incubation only by two strains, and on the 13th only by one. The isolates of *B. theobromae* tested produced more xylanase than carboxymethylcellulase which confirms observations on several fungi by Lyr (1959) and Schmidt and Liese (1980).

Table 4.6: Xylanase and carboxymethylcellulase activities of *B. theobromae* after 6 and 13 days incubation. Source: Seehann and Tabirih (1983).

Isolate	Growth of mycelium (mg/day)		Enzyme activity (U/ml)			
			Xylanase		CM-cellulase	
	6	13	6	13	6	13
A	176	225	169	0	47	0
B	172	212	201	0	0	0
C	115	195	258	15	30	20
D	104	134	220	0	0	0

Umezurike (1969, 1970a, 1970b) demonstrated that *B. theobromae* produces cellulase and ß-glucosidase. Cellulase synthesis was apparently delayed when wood powder of *Bombax buonopozense* used in the growth medium contained starch and saccharides (Umezurike, 1969). The formation of CM-cellulase and ß-glucosidase was repressed in the presence of glucose (Umezurike, 1970b; Table 4.7). A high molecular weight ß-glucosidase from culture filtrates of *B. theobromae* has been purified (Umezurike, 1971).

Table 4.7: Enzyme activity of *B. theobromae* on various carbon sources after 14 days incubation (µg reducing sugar per ml culture filtrate in 2 hours at 40°C). Source: Umezurike (1970b).

Carbon source	CM-cellulase	ß-glucosidase	Amylase
Glucose	0	0	0
Cellobiose	23.5±4.5	98.5±6.5	0
Cellulose	888.5±28.5	160.0±11.0	0

4.2.3.2 Xylanase and ß-galactosidase

Xylans are predominantly found in the secondary wood cell walls and represent only a minor fraction in primary walls. They are composed of xylose residues substituted by various side groups depending on the origin of the native polymer. They are degraded to short oligosaccharides by endoxylanases (endo-ß-1,4-xylanases) which act randomly on the xylan backbone, and ß-xylosidases which hydrolyze the oligosaccharides to D-xylose. In addition to these two enzymes, several accessory enzyme activities are necessary for debranching the substituted xylans (Poutanen et al., 1991).

Information on xylan-degrading enzymes from yeast-like fungi has been limited to only three organisms, i.e. *Aureobasidium pullulans*, *Cryptococcus* sp and *Trichosporon beigelii.* (Deshpande et al., 1992). However, recently, xylanases from the Dutch elm disease pathogens *O. ulmi* and *O. novo-ulmi* have been described (Binz, 1996a, 1996b). Previously, Svaldi and Elgersma (1982) failed to detect xylanase activity in three times concentrated culture filtrates of several *O. ulmi* and *O. novo-ulmi* isolates (formerly referred to as non-aggressive and aggressive strains) using viscometry. They were able to show, however, that the culture filtrates released xylose and other monosaccharides, particularly arabinose, from elm wood cell walls. Binz and Canevascini (1996b) suggest that the failure of Svaldi and Elgersma (1982) to detect xylanase activity may have been due to the use of an inadequate substrate. Both *O. ulmi* and *O. novo-ulmi* secreted similar amounts of xylanase into the culture medium when grown on birchwood xylan, water-insoluble xylan or elm sapwood (Binz, 1996b). On elm-sapwood, the amount of enzyme activity was 3-4 times lower than on the xylans. Xylanase production increased by a factor of two in the presence of arabinose and when the organisms were grown in the mycelial form. *O. novo-ulmi* was shown to form two distinct xylanase enzymes which were purified to homogeneity (Binz, 1996b). It was suggested that the two enzymes are distinct gene products since the amino acid compositions and isoelectric points of the xylanases diverge to a a significant extent. The pH of the culture medium strongly influenced the accumulation of fungal biomass and xylanase activity. Under the specific assay conditions, optimum activity was obtained at pH 5.2 for xylanase I and at pH 4.0 for xylanase II whereas the temperature optimum was 60°C for both enzymes (Binz, 1996b).

Binz (1996a) also compared secretion of several other cell-wall degrading enzymes by *O. ulmi* and *O. novo-ulmi* (Table 4.8). Under all conditions tested, the growth of the mycelial form was faster for both organisms and the enzyme secretion higher than growth of the yeast form. The only exception was observed with ß-galactosidase which was produced in equal amounts in the yeast and mycelial forms. The enhanced enzyme secretion by the mycelial form supports the view that the degradation and penetration of pit membranes (which are associations of different pectins and hemicelluloses) in the vascular tissue of the host during infection may be achieved by the mycelial stage (Binz, 1996a). In

contrast, the yeast form may be responsible for the systemic spread of the pathogen within the tree.

Table 4.8: Maximal activities of different cell-wall degrading enzymes secreted by representative isolates of *O. ulmi* and *O. novo-ulmi* when grown in vitro on different carbon sources. Source: Binz (1996a).

Enzyme	Best inducer	Maximal activity / Organism	
		O. ulmi H200	*O. novo-ulmi* CKT-11
Endo-Xylanase (µmol/h/ml)	Xylan	85.2	83.4
Polygalacturonase (µmol/h/ml)	Pectin	7.1	6.4
ß-galactosidase (nmol/min/ml)	Sodium pectate	61.9	62.9
α-arabinosidase (nmol/min/ml)	Pectin	27.2	16.1
ß-glucosidase (nmol/min/ml)	Elm sapwood/starch	92.5/94.5	52.1/60.7
ß-xylosidase (nmol/min/ml)	Elm sapwood	5.3	3.9
Laccase (U/ml)	Arabinose	0.03	0.35

The involvement of ß-galactosidase in pathological processes has been investigated only superficially (Binz et al., 1997). ß-galactosidases are produced by a large number of plant pathogens including the two vascular wilt fungi *Fusarium oxysporium* and *Verticillium albo-atrum*. *O. novo-ulmi* was shown to produce two ß-galactosidases, named ß-galactosidases I and II (Binz et al., 1997). In cultures grown on galacturonic acid, ß-galactosidase I accounted for approximately 75% of the total activity in the culture filtrate. This enzyme was further purified to apparent electrophoretic homogeneity and characterized. The induction of ß-galactosidase in *O. ulmi* and *O. novo-ulmi* by sodium pectate, pectin, galactan and the monomers of rhamnogalacturonan, galacturonic acid and rhamnose, but not by lactose, supports the hypothesis that this enzyme may be involved in the degradation of the pectinic fraction of the cell wall and of pit membranes. Function of ß-galactosidase would thus be the digestion of galactose-containing polysaccharides (galactans) or galactosyl side chains of the rhamnogalacturonan backbone polymer. This hypothesis is strengthened by the fact that the pure ß-galactosidase I also degrades ß-1,4-galactan.

Abraham et al. (1993) report that a strain of *O. piceae* was able to degrade xylan as well as carboxymethylcellulose in liquid culture but data were not shown.

Xylanases from *Aureobasidium pullulans* were studied by Leathers (1986) and Leathers et al. (1984, 1986). Certain isolates of *A. pullulans* were found to be extraordinary producers of xylanase. These strains were previously reported as colour variants of *A. pullulans*. Leathers (1986) found that typical strains of *A. pullulans* produce xylanase constitutively.

The natural inducers of xylanase appear to be specifically derived from arabinoxylan (Leathers et al., 1986). The induction of xylanase was subject to glucose repression. Significant extracellular xylosidase activity was also detected, apart from xylanase activity. Typical and colour-variant strains were found to differ in both the levels and the regulation of these activities. The xylanase activity of the typical strain Y-2567 was about 5 IU/ml in comparison to 373 IU/ml produced by colour variant strain Y-2311-1 (Leathers, 1986).

Nilsson (1974) assayed xylanase and other enzyme activities of 36 species of wood-inhabiting microfungi among which were three isolates of *Ceratocystis*. *Ceratocystis stenoceras* produced xylanase in birch wood blocks (according to the method of Stranks and Bieniada, 1971) but not in liquid culture. No xylanase activity could be detected for the other two species, *C. albida* and *C. olivacea*.

Another species of *Ceratocystis*, the pathogenic fungus *Ceratocystis paradoxa*, produces extracellular endoxylanases (Dekker and Richards, 1975a, 1975b). Structures of the oligosaccharides obtained after hydrolysis by these enzymes have been described and an endoxylanase was purified.

Xylanase activities of *B. theobromae* which have been determined by Seehann and Tabirih (1983) were described in the previous section (see Table 4.6).

4.2.3.3 Mannanase

The crucial enzyme for mannan depolymerization is ß-1,4-mannanase which hydrolyzes the main chain of ß-1,4-mannans, generating linear or branched oligosaccharides. The oligosaccharides are further cleaved by the corresponding glycosidases and esterases: α-galactosidase, ß-glucosidase, ß-mannosidase and acetylmannanesterase (Dekker and Richards, 1976; Tenkanen et al., 1993).

Endomannanases of microbial origin have been reported to be both inductive and constitutive enzymes (Eriksson et al., 1990). There are only few reports dealing with endo-ß-1,4-mannanases originating from yeasts and yeast-like microorganisms. According to Kremnický et al. (1996), the only mannanolytic yeasts mentioned in the literature belong to the genera *Aureobasidium* (Berndt and Liese, 1971), *Hormoascus*, *Guilliermondella* and *Endomyces* (Kocková-Kratochvílová et al., 1983). *Ceratocystis stenoceras*, *C. albida* and *C. olivacea* were tested for mannanase activity by Nilsson (1974). Tests were negative for all isolates using various methods with liquid and solid cultures (test tubes and agar plates). Berndt and Liese (1971) detected mannanase produced by *Aureobasidium pullulans* using viscometry and polygalactomannan (polymer of ß-1,4-mannopyranose with monomeric α-1,6-galactopyranose side-chain) as a substrate. Enzyme activity had the highest turnover number at pH 4.

Kremnický et al. (1996) determined endo-ß-1,4-mannanase activities for several strains of *Aureobasidium pullulans* in liquid media measuring the amount of reducing sugars produced. Activities of the strains tested were between 48 and 900 mU/ml after 3 and 6 days. The best producers of endo-ß-1,4-mannanase are endo-ß-1,4-xylanase hyperproducing strains of *A. pullulans* (Leathers et al., 1984). Interestingly, these strains secrete only negligible amounts of cellulolytic enzymes (Leathers, 1986; Deshpande et al., 1992). In addition to extracellular endo-ß-1,4-mannanase, a xylanolytic strain of *A. pullulans* was also found to produce intracellular ß-mannosidase as well as α-galactosidase and ß-glucosidase which were found both extracellularly and intracellularly (Kremnický and Biely, 1997). Among these enzyme components, only extracellular ß-mannanase and intracellular ß-mannosidase were inducible.

4.2.3.4 Pectinase

Pectic substances are complex structural polysaccharides that occur mainly in the middle lamella and primary cell wall of higher plants. In wood, they are also deposited in the tori of bordered pit-membranes. The amount of pectin in wood is about 1% (Fengel and Wegener, 1989). Pectins consist of a main backbone containing a variable but normally large proportion of partially methyl-esterified galacturonic acid subunits linked by α-1,4-glycosidic linkages (Blanco et al., 1999). This compound is known as pectin while the demethylated compound is known as pectic acid or polygalacturonic acid. Several L-rhamnopyranosyl residues can be linked through their C-1 and C-2 atoms in the main chain. Moreover, galacturonate residues may be acetylated at positions C-2 and C-3, and side chains of neutral sugar residues (f.ex. D-galactose, L-arabinose and D-xylose) can be linked to galacturonic acid or to C-4 of the rhamnose residues in the main chain.

The enzymes that hydrolyze pectic substances are known as pectic enzymes, pectinases or pectinolytic enzymes. They are classified in two main groups, pectinesterases which are able to de-esterify pectin by removal of methoxyl residues and depolymerases which readily split the main chain. The depolymerases are divided into polygalacturonases, enzymes that cleave the glycosidic bonds by hydrolysis, and lyases which break the glycosidic bonds by ß-elimination (Table 4.9). In addition, the latter two types of enzymes are classified on the basis whether they exhibit a preferential hydrolytic power against pectin, pectic acid or oligogalacturonate as the substrate and whether the pattern of action is random (endo-) or terminal (exo-) (Fogarty and Kelly, 1983). A common feature among pectic enzymes is their glucose repressible synthesis.

Table 4.9: Classification of pectic enzymes. Source: Blanco et al. (1999).

A.	**Pectinesterases (EC 3.1.1.11): de-esterify pectin by the removal of methoxyl residues.**	
B.	**Depolymerases: split the main backbone:**	
1.	By hydrolysis of α-1,4-linkages	
	1.1	Polygalacturonases, acting on pectate
		Exo-polygalacturonase (EC 3.2.1.67
		Endo-polygalacturonase (EC 3.2.1.15)
	1.2	Polymethylgalacturonases, acting on pectin (EC 3.2.1.15)
2.	By ß-elimination of glycosidic bonds	
	2.1	Lyases, acting on pectate
		Exo-lyase (EC 4.2.2.9)
		Endo-lyase (EC 4.2.2.2)
	2.2	Pectin methyl-lyase, acting on pectin (EC 4.2.2.10)

The production of pectic enzymes has been thoroughly studied in bacteria and filamentous fungi because they play an essential role in phytopathogenesis. These enzymes can elicit the cascade of defence reactions in plant cells which in favourable cases contain the growth of the pathogen (Jarvis, 1984). The involvement of pectinases in the pathogenesis of the Dutch elm disease has been demonstrated by Gagnon (1967) using histochemical tests. He showed that pectins were the main constituents of the plugging material in the vessels of infected elm trees. The alteration of pectins in the vessel walls may change their permeability and affect the movement of water. The action of pectinases on vessel pits allows the flow of protoplasm from adjoining parenchyma cells into the vessels and contributes to plugging of the vessels.

Recently, Rioux et al. (1998) emphasized that fungal infections as well as injuries and aging processes are probably not the main cause for the formation of vessel occlusions in angiosperm trees. It is suggested that partial to complete embolism which almost always accompanies these events, might be the main factor triggering blocking of the vessels. Tylosis and gel (gum) formation is a response to embolism in order to prevent desiccation of adjacent healthy tissue and limit the spread of the invading microorganism. The low pectin content in wood may thus be misleading in terms of the importance of pectin during fungal attack. Solubilization of the bordered pit membranes of tracheids not only provides a non-lignified and easily accessible carbon source for the attacking microorganism but it also gives relatively fast access to adjacent tracheids (Green et al., 1995).

Using viscometry, King and Eggins (1973) screened for pectinolytic activities of 33 fungal species which included several sapstaining fungi (see Table 4.5). Of the sapstaining species, only *Botryodiplodia theobromae*, *Ceratocystis pilifera*, *Aureobasidium pullulans* and *Graphium* sp. produced pectinase. No pectinase or only insignificant amounts of this enzyme were secreted by *Leptographium lundbergii*, *Diplodia natalensis*, *Diplodia pinea*, *Diplodia sapinea*, *Ceratocystis picea*, *Ceratocystis ulmi* and *Ceratocystis coerulescens*.

In contrast to the results by King and Eggins (1973), there is strong evidence that *Ophiostoma ulmi* does in fact produce pectinases (Beckman, 1956; Biehn and Dimond, 1971; Elgersma, 1976; Binz, 1996a). Elgersma (1976) reported that polygalacturonase activity was much higher when *O. ulmi* was grown on freeze-dried wood than on ethanol-extracted wood, especially when the initial pH was low. The most significant polygalacturonase activity was obtained at pH 3.0 after 26 hours incubation. Enzyme activity was either low or negative in cultures grown at pH 7.0 or when the pH became even higher during incubation in cultures with weak buffering capacities. No correlation was found between polygalacturonase production and pathological aggressiveness of strains of *O. ulmi*. No pectate lyase could be detected.

Binz (1996a) also failed to detect pectate lyase activity in *O. ulmi* but measured polygalacturonase activity which was best induced with pectin. Maximal activities were 7.1 µmol/h/ml for *O. ulmi* and 6.4 µmol/h/ml for *O. novo-ulmi* (Table 4.8). The pH of the culture medium strongly affected the accumulation of biomass and polygalacturonase activity. Growth of the fungus was impaired on sodium pectate at pH 7.0 (compared to growth at pH 4.0 and 5.5) as was the production of polygalacturonase. A similar behaviour was observed for *Aureobasidium pullulans* which shows its maximum polygalacturonase activity at pH 4.5 (Berndt and Liese, 1971; see later in this chapter).

The secretion of polygalacturonase and pectinesterase by *Aureobasidium pullulans*, *Alternaria humicola* and *Ceratocystis minor* was demonstrated by Rösch, Liese and Berndt (1969) using viscometric methods. There was no conclusion if the enzymes were of the endo- or exo-type. Activity of the polygalacturonases was highest between pH 4 and 6, and in general, during the course of incubation the pH was reduced. Polygalacturonase activity of *Aureobasidium pullulans* was most significant after 18 days and of *Ceratocystis minor* after 12 days but activity of the latter species stayed at the same level until the end of incubation time, i.e. 38 days.

Berndt and Liese (1971) showed that in addition, *Aureobasidium pullulans* produces pectin-transeliminase. They found that the pectin-transeliminase has a pH optimum at 6.5 and splits only pectin whereas polygalacturonase degrades pectin as well as polygalacturonic acid. With polygalacturonic acid as a substrate, polygalacturonase shows its maximum activity at pH 4.5. The hydrolysis of pectin by *Aureobasidium pullulans* in a wide pH range (3.5-7.5) is thus made possible by the presence of at least two enzymes, i.e. polygalacturonase and pectin-transeliminase.

Further studies on the pectinolytic system of *Aureobasidium pullulans* were recently done by Biely et al. (1996). They found that a strain of this species which is a hyperproducer of endo-1,4-ß-xylanase, secreted exo- and endopolygalacturonase. Both enzymes are not produced on D-glucose or under carbon starvation conditions. The enzymes can be induced in glucose-grown cells by D-galacturonic acid and its oligomers. Polygalacturonase

activity was expressed when the organism was grown over a wide pH-range (pH 3.3-6.1). Production of exopolygalacturonase was found to be highest at pH 5 and 6. Biely et al. (1996) could not obtain any evidence for the production of pectin lyase although this enzyme had previously been reported by Manacini et al. (1988).

The presence of genes encoding pectolytic enzymes and their synthesis and secretion have been demonstrated in a range of pathogenic fungi (Mendgen and Deising, 1993; Deising et al., 1996; quoted in Hardham and Mitchell, 1998). Endopolygalacturonases are one category of pectinolytic enzymes whose activity *in planta* during fungal infection has been assessed by labeling its substrate, non-methyl-esterified pectin, with a monoclonal antibody (Hardham and Mitchell, 1998).

4.2.3.5 Laccase (Phenoloxidase)

The presence of lignin-degrading and modifying enzymes from sapstaining fungi has not been reported but there are studies on laccase (phenoloxidase) activity of a few species. Rösch et al. (1969) detected laccase in cultures of *Alternaria humicola*, *Aureobasidium pullulans* and *Ceratocystis minor*. However, it remained unclear if laccase activity was extracellular or intracellular. In a consecutive study, Rösch and Liese (1971) investigated this problem further and concluded that in sapstaining fungi, laccase apparently occurs more intracellularly than extracellularly. They tested 19 species of sapstaining fungi for the presence of laccase using test agar, nutrient solutions and the ring dish method with guaiacol or tannin (Rösch and Liese, 1970). Among the species were *Ceratocystis coerulescens*, *C. floccosa*, *C. ips*, *C. minor*, *C. penicillata*, *C. piceae*, *C. pilifera* and *Leptographium lundbergii*. In culture filtrates and partially purified enzyme fractions obtained from mycelial extracts and culture-filtrates, extracellular laccase was found in *Discula pinicola* only. In *Ceratocystis coerulescens*, intracellular laccase as well as tyrosinase (o-diphenol-oxidoreductase) were detected.

Laccase is also secreted by *O. novo-ulmi* (Binz, 1996a). *O. ulmi* and *O. novo-ulmi* can be differentiated by their potential to produce constitutive extracellular laccase. *O. novo-ulmi* secreted ten times more of this enzyme in comparison to *O. ulmi* (Binz, 1996a; see Table 4.8). However, no laccase activity could be detected in *O. novo-ulmi* in its yeast phase. The best inducer for laccase activity was arabinose, yielding 0.35 U/ml, and maximal activity was obtained when the organism was cultivated at pH 5.5. The extracellular enzyme was purified and partially characterized (Binz and Canevascini, 1997). In addition, two distinct intracellular laccase activities were detected in *O. novo-ulmi* in the early growth phase in liquid culture. The physicochemical and kinetic properties of the laccase suggest that it is, like all fungal laccases described so far, a copper-containing glycoprotein. A possible function of this enzyme in the pathogenesis of Dutch elm disease could be the detoxification of phenols in elm bark. It is known that the sapwood of diseased elms shows a brown discolouration in response to infection by

O. ulmi and *O. novo-ulmi*. This browning probably originates from the enhanced production and subsequent enzymatic oxidation of phenols by parenchyma cells within the xylem. Therefore, the secretion of high levels of phenol-detoxifying laccase could protect *O. novo-ulmi* from the damaging effect of host-defence related phenolics.

4.2.4 Enzymes of sapstaining fungi degrading the non-structural components of wood

4.2.4.1 Amylase

Starch and lipids are the principal nutrition sources in the storage tissue of wood (Zabel and Morrell, 1992). Starch is a readily available food source for sapstaining fungi because it is present unmasked by any encrusting material in the cell. Sapstaining fungi express amylases in order to use starch as a carbon source. King and Eggins (1973) tested 33 species of mould and sapstaining fungi associated with colonization of green timber for degradation of amylose. All of the species tested were considerably amylolytic. Nilsson (1974) assayed 36 species of wood-inhabiting microfungi for wood-degrading enzymes and amylase. With one exception (*Phialophora verrucosa*), all species were able to degrade starch.

Amylase production was also demonstrated for *Aureobasidium pullulans* (Deshpande et al., 1992) and *B. theobromae* (Umezurike, 1969; Tabirih and Seehann, 1984; Encinas and Daniel, 1999). Umezurike (1969) showed that *B. theobromae* uses starch and other saccharides present in the wood of *Bombax buonopozense* as initial substrates before degrading the cellulose and hemicellulose components of the wood. Amylase activity was also detected in culture filtrates of *B. theobromae*. Cellulase synthesis was delayed when the wood powder in the medium contained starch and saccharides. Amylase behaved like an inducible enzyme and was not detectable in the cultures after exhaustion of starch.

Tabirih and Seehann (1984) measured starch depletion caused by *B. theobromae* attack. They found that mass losses of 7 to 8% and a slight density reduction in Abachi wood infected with *B. theobromae* were in accordance with the consumption of accessory compounds, especially starch, in the parenchymatous tissue.

Encinas and Daniel (1999) studied the depletion of non-structural soluble sugars, starch, lipids and nitrogen from kiln-dried Caribbean pine, Scots pine and birch sapwood blocks over a five months incubation period with *B. theobromae*. Depletion of non-structural compounds was correlated with fungal growth and total dry weight losses. In birch, after 30 days incubation almost all starch, glucose and fructose had been removed which corresponded to a weight loss of about 2%. In Scots and Caribbean pine however, starch, sucrose and fructose were reduced to about 10% of their original values when 2% weight loss was reached. This also corresponded to a 30-day incubation period. Glucose in the conifers was not depleted to the same extent as the other sugars.

4.2.4.2 Proteinase and aminopeptidase

Wood-colonizing fungi obtain most of their nitrogen from proteins in the sapwood of host trees. In order to retrieve the nitrogen, fungi require extracellular proteinases to break down these proteins. Abraham et al. (1993) initially showed the production of proteinase of one strain of *O. piceae* in protein-supplemented liquid culture. On solid media, proteolytic activity was shown by clear zones on skim milk agar after 5 days incubation at 23°C. Proteinase activity was minimal in liquid media supplemented with easily assimilable nitrogen (e.g., ammonium, urea and single amino acids). The activity started to increase when ammonia was depleted. Activity was highest when protein was used as the source of organic nitrogen, f.ex. as bovine serum albumin and collagen, and at pH 8. Total proteinase activity in the culture filtrate increased from day 1 to day 9. However, when the activity was expressed as a function of the biomass present, the maximum activity was observed at days 2 and 3.

Subsequent work showed that proteinase production also occurred in other staining fungi when they were grown under similar conditions in liquid culture (Breuil and Huang, 1994), i.e. *O. ainoae, O. piliferum, O. populinum, Ceratocystis adiposa, Alternaria tenuis, Aureobasidium pullulans, Cladosporium cladosporioides* and *Trichoderma harzianum.* The extracellular proteolytic enzymes were mainly synthesized and released into the medium when the fungi were actively growing during the exponential phase, i.e. up to about four days after inoculation. Consequently, it was suggested that the major role of these enzymes is to hydrolyze protein and provide easily assimilable breakdown products. Temperature tests showed maximum activities around 37-40°C. Proteinase-inhibition studies suggested that the majority of the enzymes were metal-activated serine-type proteinases. Proteinase activity in wood has been determined for *O. piceae*, *O. ainoae*, *Alternaria tenuis* and *Trichoderma harzianum* (Breuil et al., 1995). As in liquid culture, proteinase activity increased during the active growth phase of the fungi, as shown by ergosterol production.

Banerjee et al. (1995) were the first to report aminopeptidase activity in sapstaining fungi, namely *O. piceae* and *O. ainoae*, as well as in the surface mould *Trichoderma harzianum.* Aminopeptidases catalyze the hydrolysis of amino acid residues from the amino terminus of protein and peptide substrates. They are associated with a myriad of physiological and pathological processes. Degradation of peptides for nutritional purposes is one of the many functions ascribed to aminopeptidases. Most of the aminopeptidase activity in *O. piceae* was observed in the cell pellet and only a small amount detected in the culture supernatant. As for extracellular proteinases, maximum activity was obtained at 37°C.

In order to further characterize the major proteinase of *O. piceae*, polyclonal antibodies were raised against the commercially available proteinase K produced by *Tritirachium album* (Hoffert et al., 1995). These antibodies were characterized and used to

immunolocalize the extracellular proteinase of *O. piceae* when grown in both protein-supplemented liquid medium and in lodgepole pine sapwood. Immunodot blotting showed that the antibodies recognized both enzymes but reacted more strongly with proteinase K than with the *O. piceae* proteinase. Immunogold labelling and transmission electron microscopy revealed that the *O. piceae* proteinase was localized in the cell walls of *O. piceae*. The growth and proteolytic activity of *O. piceae* was also characterized in the wood of three other tree species, aspen, Douglas fir and Western hemlock (Gharibian et al., 1996). Proteolytic activity and growth were most significant in aspen which might be due to its high nitrogen content. Immunolocalization of the proteinase after fungal growth in lodgepole pine and aspen again revealed that the enzyme was localized mainly in the cell wall of *O. piceae*. Lower levels of binding of the enzyme to the sheath surrounding the hyphae were found. These results support the view that the proteinases are located extracellularly so the products of degradation can diffuse across the fungal cell wall and be taken up by the pathogen.

The 33-kDa proteinase secreted by a strain of *O. piceae* was isolated and characterized (Abraham and Breuil, 1996). The purified proteinase degraded a wide range of proteins from animals and plants as well as proteins isolated from wood (Abraham et al., 1998). This demonstrates the major role of proteinase in the primary retrieval of nitrogen for fungal growth and the broad specificity of this enzyme.

Further characterization of the stability and mode of action of proteinases might permit specific inhibition or disruption of proteolytic activity and consequently prevention of sapstain fungal growth on wood. Various enzyme inhibitors tested on wood and in artificial media illustrated the potential to identify specific anti-sapstain compounds (Abraham et al., 1997).

In summary, a lot of information has become available on proteinases from *O. piceae* whereas there are virtually no reports on proteinases from other sapstaining fungi. Attempts to detect proteinases in cultures of *O. ulmi* and *O. novo-ulmi* were not successful (Binz, pers. comm.). It would be desirable to compare the activities of the *O. piceae* proteinase to proteinases possibly secreted by other species of *Ophiostoma* since in nature, there is hardly one sapstaining species present on wood exclusively. This is especially important in order to identify specific anti-sapstain compounds.

4.2.4.3 Lipase

Lipids constitute most of the total extractives in a tree and are much more abundant than nitrogen (Abraham et al., 1998). The total lipid content in the sapwood of most tree species is approximately 2% of the total dry weight of the wood (Gao et al., 1995). Commonly, triglycerides account for 40-50% of the total extractives and are one of the largest energy reserves within a tree (Chen et al., 1994; Gao et al., 1995). Triglycerides are easily

hydrolyzed by lipases (glycerol ester hydrolases) into fatty acids and glycerol which can then be used as a carbon source by the fungi (Käärik, 1960; Gao et al., 1994; Zheng et al., 1994). Fatty acids and glycerol also play several other roles in the physiology of the fungi (Abraham et al., 1998). Glycerol has been shown to induce pigmentation, suggesting that it might play a role in melanin synthesis, while fatty acids have been shown to enhance the production of perithecia (Seifert, 1993; Eagen et al., 1997).

Extracellular lipase activity was detected in an albino strain of *Ophiostoma piliferum* (McNaughton, 1997) which is commercially available under the tradename Cartapip®97, and in a wild-type strain of *O. piceae* (Gao and Breuil, 1995). In addition, the effect of different carbon and nitrogen sources on the production of extracellular lipase by *O. piceae* in liquid media was investigated (Gao and Breuil, 1995). The greatest accumulated lipase activity was obtained in a medium with olive oil as carbon source and a combination of $(NH_4)_2SO_4$ and peptone as nitrogen source. Optimum conditions for lipase production were pH 5.5 and 37°C. The lipase was subsequently purified to homogeneity and characterized (Gao and Breuil, 1998). It was found to be specific for triglycerides with intermediate to long (12-20 carbons) chain fatty acids. The lipase from *O. piceae* was also shown to be able to hydrolyze triglycerides isolated directly from wood which are mainly composed of long chain fatty acids (Abraham et al., 1998). Like proteinase inhibitors, lipase inhibitors may offer potential for prevention of sapstain fungal growth or pigmentation in wood.

4.3 Material and Methods

4.3.1 Determination of enzyme activities

4.3.1.1 General

The sapstaining isolates used in the enzyme assays are listed in Table 4.10. A white-rot fungus, *Schizophyllum commune* (isolate #3 from HortResearch, Hamilton, New Zealand), and a brown-rot fungus, *Gloeophyllum trabeum* (isolate BAM Ebw. 109 from *Forest Research*) were included in the experiments to serve as positive controls.

Starter cultures (for preparation see Chapter 2) were inoculated into medium II (80 ml in 250 ml-flasks) consisting of 0.9 g NaCl, 0.2 g $MgCl_2 \cdot 6H_2O$, 1.5 g K_2HPO_4, 0.75 g KH_2PO_4, 0.9 g NH_4Cl, 0.05 g $CaCl_2 \cdot 2H_2O$, 0.05 g yeast extract, 0.1 g tryptone, 5 g of carboxymethylcellulose (CMC; medium viscosity; Sigma) or larchwood xylan (Sigma) or locust bean gum (Sigma) or citrus fruit pectin (Sigma) and one litre of distilled, deionized water. The pectin was moistened with ethanol prior to dissolving in water.

Table 4.10: List of sapstaining fungi used in enzyme assays.

Species	Isolate #	Origin	Date isolated
Ophiostoma floccosum	138	Riverhead Forest, North Island	29-1-1997
O. floccosum	148	Hanmer Springs, South Island	23-1-1997
O. piceae	170	Greymouth, South Island	18-2-1997
O. piceae	272	Rotorua, North Island	21-4-1997
O. pluriannulatum	151	Abel Tasman National Park, South Island	30-1-1997
O. ips	308	Waipa sawmill, Rotorua, North Island	9-4-1997
O. ips	294	Nelson, South Island	1-5-1997
Leptographium procerum	1852	Whitford Forest, North Island	29-1-1998
Sphaeropsis sapinea	4	Dome State Forest, North Island (pine cone)	Spring 1996
S. sapinea	35	Kinleith Forest, North Island (live tree)	13-12-96

For the detection of amylase, ten ml of the starter cultures were inoculated into medium III (80 ml in 250 ml-flasks) consisting of 0.5 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g$CaCl_2$, 0.5 g yeast extract, 10 g potato starch (Sigma), 18 g agar and one liter of water. Two flasks were used for each isolate. The cultures were incubated in a rotary shaker at 120 rpm and 25°C for up to 11 days (sapstaining species) or 26 days (decay fungi). Two ml of the cultures were harvested at defined time periods, spun down in a bench centrifuge at 13,000 rpm and immediately frozen at -20°C until usage.

Enzyme activities were spectrophotometrically determined by measuring the reducing sugars released during the reaction with a substrate. For the detection of endocellulase activity, p-hydroxybenzoic acid hydrazide (PAHBAH, Sigma) reagent was used (Lever, 1973). Xylanase, mannanase, pectinase and amylase were determined using the dinitrosalicylic acid (DNSA) reagent (Miller, 1959). During the assays for the detection of cellulase, the amount of glucose in the medium was monitored using reagent strips for urinanalysis (Clinistix, Bayer Diagnostics, Australia).

4.3.1.2 PAHBAH-assay

For the detection of endocellulase activity of the sapstaining isolates, p-hydroxybenzoic acid hydrazide (PAHBAH, Sigma) reagent was used (Lever, 1973; for preparation see below). The reaction mixture of the assay consisted of 300 µl substrate and supernatant in different dilutions to bring the total volume to 500 µl in a 1.5 ml Eppendorf tube. Glucose in different concentrations was included in each assay to calculate a standard curve (for the preparation of the standards, see below). A commercial cellulase from *Trichoderma viride* (Sigma) was included to serve as a positive control. Samples, controls and standards were assayed in duplicates. CMC was used in two different concentrations (0.2 % and 1 %) in a 100 mM MOPS (3-[N-Morpholino]propane-sulfonic acid) buffer at pH 6 and 7, respectively. After incubation for 15 or 60 minutes at 30°C, 40°C or 50°C, the tubes were transferred to an ice-water bath. The reaction was stopped with 1 ml of PAHBAH reagent. The tubes were mixed by inversion, boiled for 6 minutes, cooled in ice-water and

centrifuged for 3 minutes at 11,000 rpm in a Beckman Microfuge to remove suspended substrate from the supernatant. Blanks consisted of substrate buffer incubated identically to the sample to which enzyme was added after the PAHBAH-reagent. Absorbance was read at 420 nm in a spectrophotometer (Pharmacia Biotech Ultrospech 3000) after calibration with a solution of 300 µl substrate, 200 µl buffer and 1 ml of PAHBAH reagent. Absorbance was calculated by subtracting the value of the blanks from the incubated samples. Using the standard line, absorbance was converted to enzyme activity expressed as µmoles of glucose equivalents released per minute per ml at a defined temperature.

4.3.1.3 DNSA-assay

Reducing sugars were also determined using the dinitrosalicylic acid reagent (DNSA; Miller, 1959). The reaction mixture for the xylanase assays consisted of 300 µl larchwood xylan (1.33 % in McIlvaine citrate phosphate buffer at pH 4.6; Binz, 1996a), 180 µl buffer and 20 µl supernatant in a 1.5 ml Eppendorf tube. For detection of mannanase, 300 µl locust bean gum (0.2% in McIlvaine citrate phosphate buffer at pH 4.6), 100 µl buffer and 100 µl supernatant were used. The xylan or locust bean gum was dissolved in buffer, heated to boiling point, cooled and centrifuged at 10,000 rpm and 4 °C for 15 minutes; only the supernatant was used in the assay. In the pectinase assays, the standard reaction mixture consisted of 300 µl polygalacturonic acid (0.33% in McIlvaine citrate phosphate buffer at pH 4), 100 µl buffer and 100 µl supernatant. For determination of amylase activity, 300 µl potato starch (1% in 100 mM MOPS buffer at pH 7), 100 µl sterile distilled water and 100 µl supernatant were used.

Glucose, xylose, mannose or galacturonic acid (all from Sigma) in different concentrations were included in each assay to calculate a standard curve (see below). Samples, controls and standards were assayed in duplicates. After incubation for 60 minutes at 40°C (30°C for pectinase), the tubes were transferred to an ice-water bath. Amylase samples were incubated at 40°C for 30 minutes. One ml of DNSA was added to each tube, and the supernatant was added to the blanks. The tubes were mixed by inversion, boiled for six minutes, cooled in ice-water and centrifuged for three minutes at 13,000 rpm. Absorbance was read at 575 nm in a spectrophotometer (Pharmacia Biotech Ultrospech 3000) after zeroing the absorbance with a solution of 300 µl substrate, 200 µl buffer and 1 ml of DNSA. Enzyme activity is expressed as µmoles of reducing sugar equivalents released per minute per ml at 30°C or 40°C, respectively.

4.3.1.4 Preparation of DNSA reagent

13.6 g of dinitrosalicylic acid (Sigma) were dissolved in 850 ml distilled water, and 13.6 g sodium hydroxide were added to aid dissolution. 0.68 g sodium sulphite (anhydrous), 273 g potassium sodium tartrate were added and dissolved. The volume was adjusted to one liter. The solution was filtered through a 0.8µm filter, and 2.7 g phenol were added.

4.3.1.5 Preparation of PAHBAH-reagent

The reagent was prepared by adding 10 ml of each of 0.5 M Na_3 Citrate·$2H_2O$ (36.76 g per 250 ml), 1 M Na_2SO_3·$7H_2O$ (63.05 g per 250 ml), 0.2 M $CaCl_2$·$2H_2O$ (7.35 g per 250 ml) and 5 M NaOH (50 g per 250 ml) to 50 ml of single-distilled water, mixing well between additions, and adding 1.52 g PAHBAH. The volume was then made up to 100 ml. The solution was used within 24 hours.

4.3.1.6 Preparation of standards used in reducing sugar assays

Standards were prepared according to Bailey et al. (1992). The standard was either D-glucose, D-xylose, mannose or galacturonic acid (all from Sigma), depending on the assay. A 0.01 M (= 0.15 g per 100 ml buffer) stock solution was prepared and diluted in buffer as follows:

1:1 = 10.0 µmol per ml (2 µmol per 200 µl);

1:2 = 5.0 µmol per ml (1 µmol per 200 µl);

1:3 = 3.33 µmol per ml (0.66 µmol per 200 µl);

1:5 = 2.0 µmol per ml (0.4 µmol per 200 µl).

The dilutions were stored in aliquots at -20°C. After thawing, the tubes were mixed well because the solution becomes layered on freezing. To each tube containing 200 µl standard, 300 µl substrate in buffer were added in each assay.

4.3.1.7 Preparation of McIlvaine citrate phosphate buffer

A 0.1M solution of citric acid monohydrate ($C_6H_8O_7$·H_2O; 21.01 g/l) and a 0.2M solution of di-sodium-hydrogenphosphate-2-hydrate (Na_2HPO_4·$2H_2O$; 35.61 g/l) were prepared. These two solutions were mixed adequately to obtain pH 4.0, 4.6 or 6.6 (Table 4.11).

Table 4.11: Preparation of McIlvaine citrate phosphate buffer, according to required pH.

Required pH	0.1M $C_6H_8O_7$·H_2O (ml)	0.2M Na_2HPO_4·$2H_2O$ (ml)
6.6	27.25	72.75
4.6	53.25	46.75
4.0	61.45	38.55

4.3.2 Summary of liquid growth media used in enzyme assays

In summary, the composition of growth media used in the enzyme screenings were as follows:

Medium I: 2 g yeast extract, 15 g malt extract and one liter of water.

Medium II: 0.9 g NaCl, 0.2 g $MgCl_2$·$6H_2O$, 1.5 g K_2HPO_4, 0.75 g KH_2PO_4, 0.9 g NH_4Cl, 0.05 g $CaCl_2$·$2H_2O$, 0.5 g yeast extract, 0.1 g tryptone, 1 g or 5 g of carbon source and one liter of water.

Medium III: 2 g L-asparagine, 2 g K_2HPO_4, 10 g CMC, 10 mg thiamine, 1 mg biotin, 5 mg pyrodoxine and one liter of water.

Medium IV: 2 g L-asparagine, 2 g K_2HPO_4, 10 g CMC, 10 mg thiamine, 1 mg biotin, 5 mg pyrodoxine and one liter of water.

4.3.3 Ultrafiltration of crude enzyme solutions

Enzyme supernatants harvested at the end of the cultivation were concentrated 5- to 10-fold by ultrafiltration using Centricon-10 concentrators (Amicon, Beverly, MA, U.S.A.) with a molecular weight cut-off of 10,000 daltons.

4.3.4 Protein determination

Total extracellular protein of selected cultures grown in xylan medium was determined using a commercial protein assay kit (Bio-Rad Laboratories) which is based on the method described by Bradford (1976). Bovine serum albumin (Sigma) was used as the standard at a series of concentrations between 0 and 0.2 mg/ml.

4.4 Results and discussion

4.4.1 Screening for cellulase activity

The sapstaining fungi tested grew in medium II containing CMC (Table 4.12; Figure 4.3) but none of the isolates tested in this study produced extracellular cellulase under the various conditions tested. The pH of the cultures was not changed significantly during the incubation. At the end of the cultivation, there were far more blastospores than mycelia present in the cultures of *Ophiostoma* which resulted in lower fungal dry weights compared to growth of these cultures in media containing xylan or amylose. In the culture of *O. ips* #308, blastospores developed only after 128 hours (5 days) of cultivation.

Growth of *Ophiostoma floccosum* #138 was also measured by counting blastospores in intervals after inoculation. Growth of this isolate peaked at approximately 84 hours of incubation with $3.8 \cdot 10^9$ blastospores per ml and apparently started to decline thereafter (Figure 4.3).

Table 4.12: Fungal biomass and pH of cultures (original pH 6.7) after 169 h incubation (sapstaining fungi) or 26 days incubation (decay fungi) in media supplemented with CMC. Values represent the average of duplicate flasks.

Isolate	Fungal biomass (g)	pH
O. floccosum #138	0.06	6.5
O. floccosum #148	0.07	6.6
O. piceae #272	0.07	6.5
O. piceae #170	0.08	6.4
O. ips #308	0.04	6.7
O. pluriannulatum #151	0.06	6.6
L. procerum 1852	0.05	6.6
S. sapinea #4	0.12	6.5
S. sapinea #35	0.11	6.7
S. commune	0.09	5.9
G. trabeum	0.04	4.5

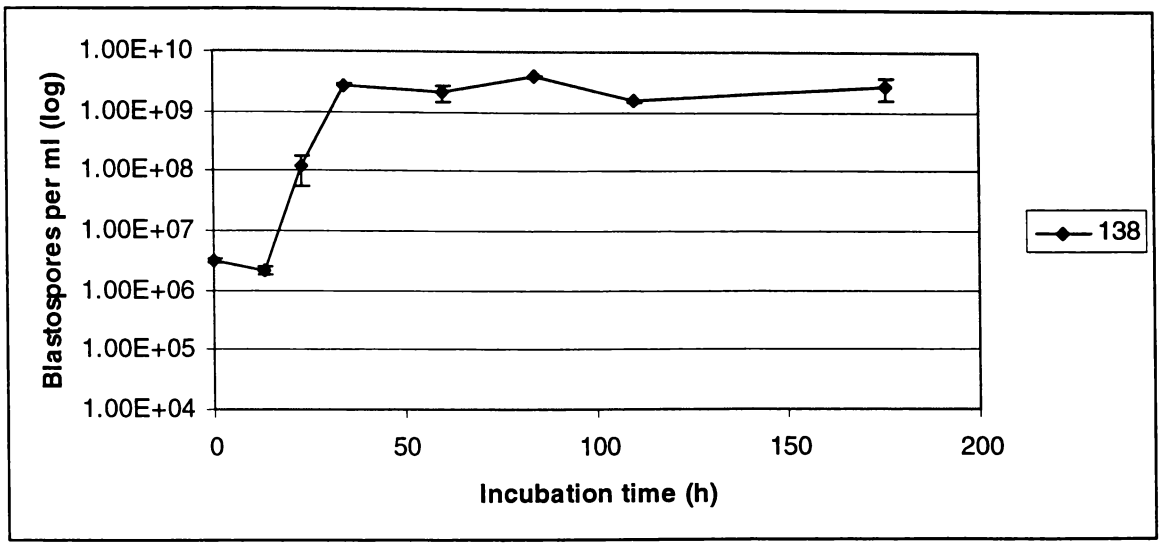


Figure 4.3: Growth curve of *Ophiostoma floccosum* #138 in liquid CMC-medium. Vertical bars represent the range of values obtained for duplicate cultures.

The amount of glucose in the starter cultures after 12 hours incubation was more than 28 mmol/l. Residual glucose was not detected in the cultures supplemented with CMC at 23 hours after inoculation.

Isolates of *S. sapinea* and *O. piceae* were tested to determine if cellulase was produced in a minimal medium. After 24 hours, one ml of each starter culture was transferred into nine ml of medium II, adjusted to pH 5 and pH 7, respectively. After further incubation for 48 hours in a rotary shaker set to 25°C, the cultures were inoculated into 80 ml of medium IV (2 g/l L-asparagine, 2 g/l K_2HPO_4, 10 g/l CMC, 10 mg/l thiamine, 1 mg/l biotin, 5 mg/l pyrodoxine; adjusted to pH 5 and 7, respectively) in a 250 ml-flask. The vitamins were added to the medium after autoclaving. Three flasks per culture and pH were used. The cultures were incubated in a rotary shaker at 25°C and supernatants harvested after 24, 48, 72 and 96 hours. Fungal biomass of the cultures was determined after 96 hours of growth.

In the PAHBAH-assay, samples were incubated with 1 % CMC in a 100 mM MOPS buffer at pH 5 for 15 minutes at 50°C.

The fungi tested grew well even under conditions of starvation in medium III. It is likely that there was enough yeast-malt extract present in the starter cultures which allowed the fungi to continue to grow in a minimal nutritive medium. The pH of the culture medium did not have an effect on the growth of the fungi nor did it stimulate the production of cellulase (Table 4.13). After 96 hours of incubation, the pH had increased on average to 8 for all cultures.

Table 4.13: Fungal biomass produced under conditions of starvation. Values represent the mean of three flasks per culture and pH.

Isolate	Fungal biomass (g)	
	Original pH 5	Original pH 7
O. piceae #170	0.17	0.15
S. sapinea #4	0.12	0.11

There was slight cellulase activity in the supernatants of *S. sapinea*, harvested after 18 and 169 hours of incubation in medium II (up to 0.05 µmoles/min/ml). Following ultrafiltration, the supernatants harvested after 18 hours showed no cellulase activity whereas in the samples harvested after 169 hours, there was still slight enzyme activity (0.03 µmoles/min/ml) which may have occurred when the fungus was digesting its own cell walls, i.e. when autolysis occurred. The presence of cellulosic glucans in cell walls of fungi belonging to the *Ophiostomataceae* was shown to occur first in *O. ulmi* by X-ray techniques (Rosinski and Campana, 1964).

Cellulase activities of a commercial enzyme preparation of *Trichoderma viride* (Sigma) which was included to serve as a positive control, were determined using the same conditions as for the other fungal cultures, i.e. incubation for 15 min. at 30°C, and including 300µl substrate (0.2% CMC, medium viscosity) in MOPS buffer at pH 7 in the reaction mixture (Table 4.14).

Table 4.14: Cellulase activities of *Trichoderma viride* (Sigma).

Amount of cellulase in reaction mixture (µg)	Cellulase activity (µmoles/min/ml)
5	0.004
10	0.070
20	0.140

Cellulolytic activity has been reported for some sapstaining fungi, namely *Aureobasidium pullulans* (Greaves and Savory, 1965; Rösch et al., 1969), *Ceratocystis coerulescens* (King and Eggins, 1972), *Ceratocystis piceae* (Nilsson, 1973), *Ceratocystis minor* (Rösch et al., 1969), *Ceratocystis pilifera* (King and Eggins, 1972) and *Botryodiplodia theobromae*

(Umezurike, 1969). Some of these organisms produce detectable activity only in vitro, and occasionally there are contradictory reports on measurements in vitro and in vivo. In addition, differences between fungal strains and tested wood species have to be considered when comparing results. The *Ophiostoma* species best studied with regard to cellulolytic activity is *O. ulmi* although the results are inconclusive. In-vitro-activity has been reported by Beckman (1956) and Elgersma (1982), but more recently Binz (1996b) was not able to confirm this result.

It is very unlikely that the sapstaining fungi tested in this thesis produce cellulase because a variety of assay parameters have been considered (substrate concentration, incubation time, type of buffer etc.). Cellulose was used as the substrate in the enzyme assays since in virtually all microorganisms examined, the synthesis of cellulases is induced by the presence of cellulose (Eriksson et al., 1990). Carboxymethylcellulose was chosen because it is known that some pseudo-cellulolytic fungi (mostly Ascomycetes and Fungi Imperfecti) attack water-soluble carboxymethylcellulose and amorphous cellulose substrates in preference to crystalline substrates (Shimada and Takahashi, 1991).

In the cellulase assays involving the basidiomycetes *S. commune* and *G. trabeum* which also served as positive controls, extracellular supernatants of the cultures were incubated for 60 minutes at 40°C. The reaction mixture consisted of 20 µl (*S. commune*) or 50 µl supernatant (*G. trabeum*), 300 µl substrate (1 % CMC) and McIlvaine citrate phosphate buffer at pH 4.6 to bring the total volume to 500 µl. Cellulase production of both *S. commune* and *G. trabeum* was highest after 19 days incubation (Figure 4.4). Cellulase activities were 1.31 µmoles/min/ml for *S. commune* and 0.95 µmoles/min/ml for *G. trabeum*.

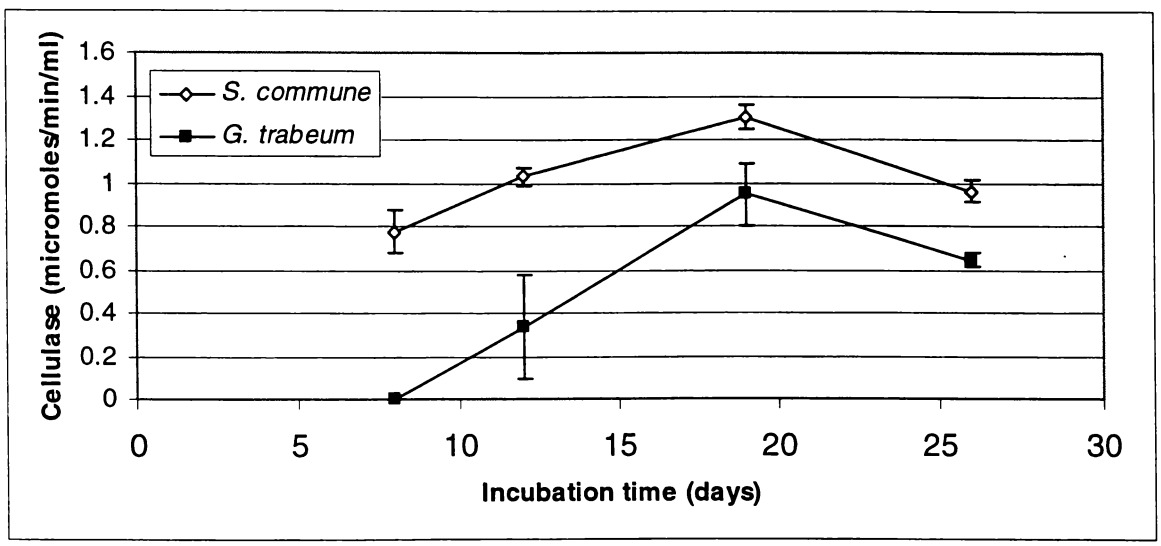


Figure 4.4: Cellulase activities of *S. commune* and *G. trabeum* during 26 days incubation. Vertical bars represent the range of values obtained for duplicate cultures.

It is surprising that *S. commune* demonstrated more significant cellulase activity than *G. trabeum* (Figure 4.4) because *Schizophyllum* is known to be a rather weak wood-destroyer (Lyr, 1959; Schmidt and Liese, 1980). Schmidt and Liese (1980) investigated a total of 49 isolates of *S. commune* isolated from different wood species and environments for their capability to produce wood-degrading enzymes. Carboxymethylcellulase activities measured differed considerably among the strains tested, ranging from 0.01 to 0.7 µmoles/min/ml.

The same isolate of *S. commune* as used in this thesis has been shown to secrete 0.35 µmoles/min/ml cellulase (Ah Chee et al., 1998). However, it has to be taken into account that different growth media and assay conditions were used.

The results obtained in this thesis for *G. trabeum* confirm findings by Ritschkoff et al. (1992) who detected low total cellulase activity in the same strain (isolate BAM Ebw. 109). The amount of endo-β-1,4-glucanase produced was highest after 5-6 weeks cultivation (less than 1.8 nkat/ml or 0.11 µmoles/min/ml). Kreber et al. (2000) also measured enzyme activities of *G. trabeum* isolate BAM Ebw. 109. The strain yielded cellulase activities of 0.37 nkat/ml (0.02 µmoles/min/ml) after six days cultivation, but no activity was detected after 18 and 36 days incubation. Mansfield et al. (1998) determined much higher endoglucanase levels for a different strain of *G. trabeum* when grown in a liquid CMC-medium for 11 days (360 nkat/ml or 21.56 µmoles/min/ml).

In this thesis, *G. trabeum* decreased the pH in the growth medium significantly, i.e. from 6 to 4.7 with CMC as carbon source and from 6.9 to 4.1 with mannan as carbon source. Ritschkoff et al. (1992) observed a similar effect after two weeks´ cultivation of this isolate in a liquid sawdust medium. They found that *G. trabeum* decreased the pH from 5.5 to 3.8 which is apparently due to the production of organic acids. It has been proposed that oxalic acid is a physiological key metabolite in cellulose degradation by brown-rot fungi (Akamatsu et al., 1991). The low pH maintained by brown-rot fungi might play a role in solubilizing and reducing iron present in the wood (Koenings, 1974). Oxalic acid might reduce the ferric ions to ferrous ions which is necessary for Fenton-type reactions.

4.4.2 Screening for xylanase activity

All sapstaining fungi tested produced xylanase (Figures 4.5 to 4.8). Following enzyme production over time, the fungi investigated can be divided into two groups. In both cultures of *S. sapinea* as well as *O. floccosum* #138, activities measured in the extracellular supernatants increased with incubation time. In the other cultures, enzyme production increased during approximately the first 80 hours after inoculation, dropped and increased again which is likely to occur due to autolysis of the fungi. Gagnon (1961) noticed that when *Ceratocystis ulmi* was grown in liquid media, the dry weight of the fungus fluctuated regularly with age and attributed this to autolysis. The same phenomenon had earlier been

reported by Käärik (1960). The sharp reductions in fungal dry weight were not attributed to a sugar depletion of the culture medium by Gagnon (1961) since they occurred at the same time for cultures which had been given different concentrations of glucose.

The two isolates of *S. sapinea* produced more xylanase (up to 1.6 µmoles/min/ml or 11.4 µmoles/min/ml/g) than the *Ophiostoma* species (up to 0.5 µmoles/min/ml or 3.9 µmoles/min/ml/g) and the two decay fungi (up to 0.3 µmoles/min/ml or 2.1 µmoles/min/ml/g). Xylanase activities determined for *S. sapinea* are comparable to values obtained for an isolate of *O. ulmi* which released 1.42 µmol/min/ml on birch wood xylan 62 hours after inoculation (Binz, 1996a).

Ophiostoma ips and *L. procerum* produced low amounts of xylanase. *Ophiostoma ips* produced mainly blastospores when grown in liquid xylan medium (see Figure 4.11) and the least biomass of all isolates tested. Assuming that cell-wall degrading enzymes of *Ophiostoma* species are preferably secreted in the mycelial stage (Binz, 1996a), *O. ips* might produce more xylanase under culture conditions that induce mycelial growth. There was also hardly any xylanase activity in the extracellular supernatant of the culture of *Leptographium procerum*, despite the fact that the fungus grew well in xylan medium (Table 4.15). It may be that xylanase activity in this species is bound to the hyphal sheath, however, cell-bound xylanase activity in a related species, *O. ulmi*, was not detected (Binz, pers. comm.). The possibility that enzyme activity of *Ophiostoma* species is cell-bound, has been tested in this thesis research with regard to pectinase in *O. ips* #294 (see later in this chapter).

Table 4.15: Xylanase activity, fungal biomass and pH of cultures after 184 hours incubation when grown in media supplemented with larchwood xylan. Values represent the average of two (sapstaining species) and three (decay fungi) flasks.

Isolate	Xylanase activity (µmoles/min/ml)	Xylanase act. after 5-fold conc. (µmoles/min/ml)	Fungal biomass (g)	Xylanase activity (µmoles/min/ml/g)	pH
O. floccosum #138	0.28	1.66	0.12	2.33	6.4
O. floccosum #148	0.33	2.70	0.12	2.75	6.2
O. piceae #272	0.08	0.70	0.15	0.53	6.3
O. piceae #170	0.17	1.18	0.14	1.21	6.3
O. ips #308	0.03	0.04	0.05	0.60	6.7
O. pluriannulatum #151	0.51	2.81	0.13	3.92	6.6
L. procerum #1852	0.04	0.05	0.11	0.36	6.7
S. sapinea #4	1.59	3.67	0.14	11.36	6.2
S. sapinea #35	1.64	3.72	0.17	9.65	6.1
S. commune	0.31	1.91	0.15	2.07	6.2
G. trabeum	0.10	0.64	0.08	1.25	4.6

The pH of the growth medium did not change significantly during incubation (Table 4.15; the original pH was 6.7). The effect of the culture pH on the secretion of xylanase was not tested in this study but results by Binz (1996a) show that xylanase activity and biomass production on xylan at pH 4.0 by *O. ulmi* and *O. novo-ulmi* were inferior to those obtained at pH 5.5 and 7.0.

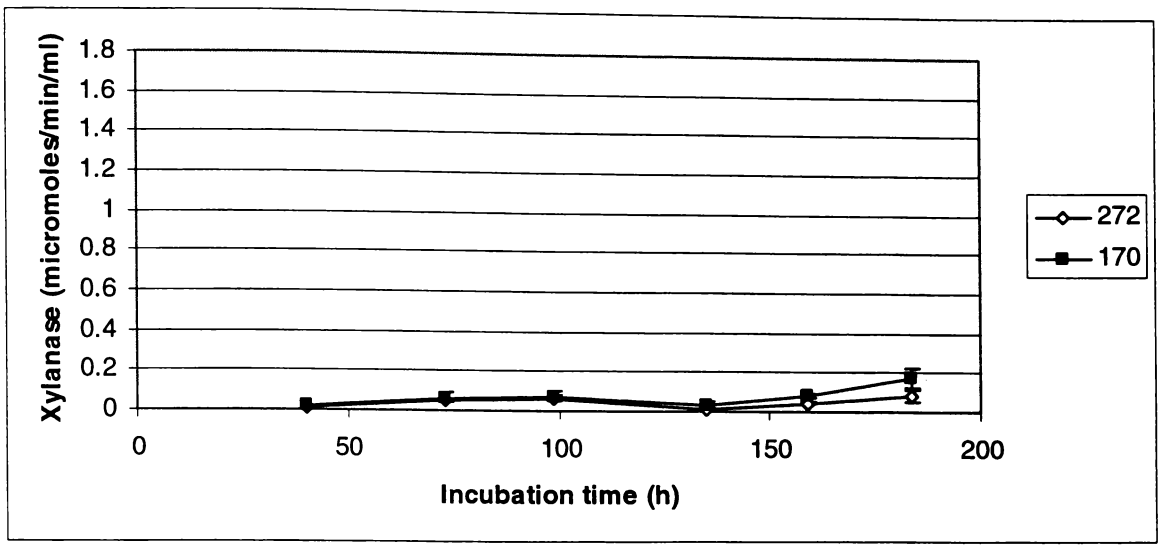


Figure 4.5: Xylanase activity of *O. piceae* #272 and #170. Vertical bars represent the range of values obtained for duplicate cultures.

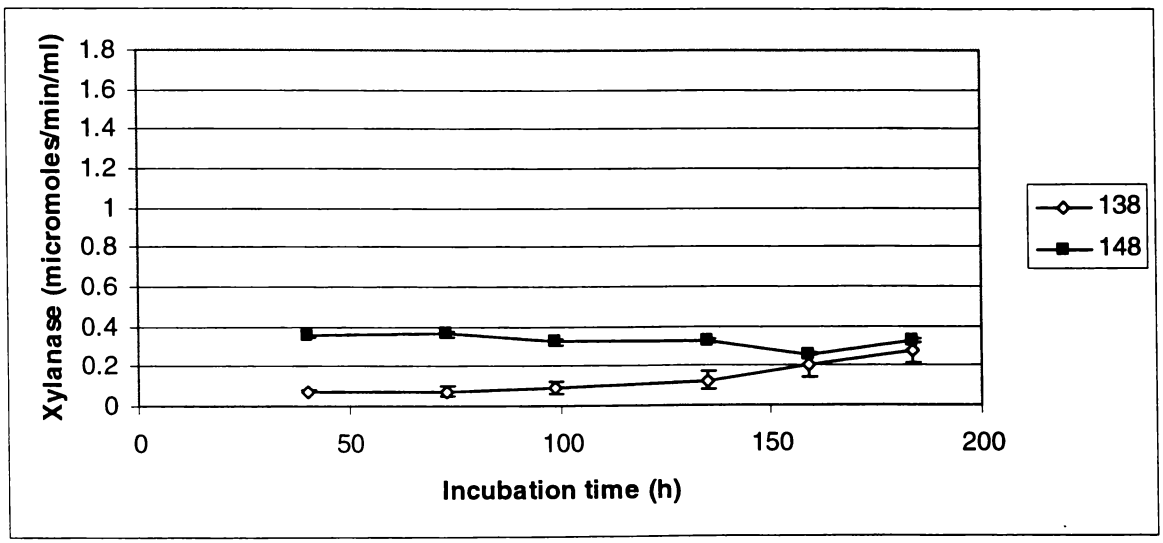


Figure 4.6: Xylanase activity of *O. floccosum* #138 and #148.

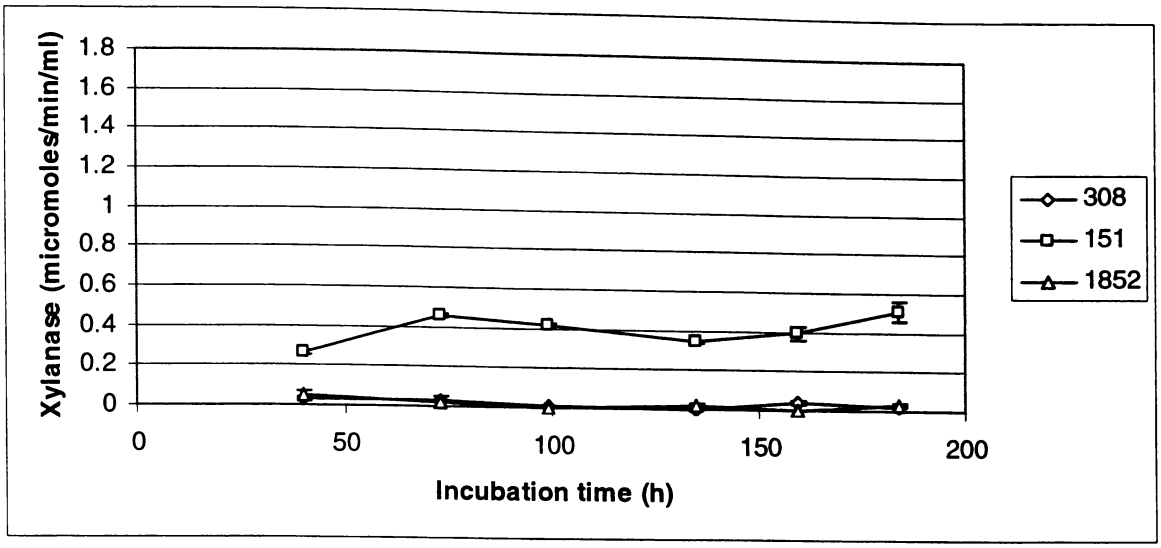


Figure 4.7: Xylanase activity of *O. ips* #308, *O. pluriannulatum* #151 and *L. procerum* #1852.

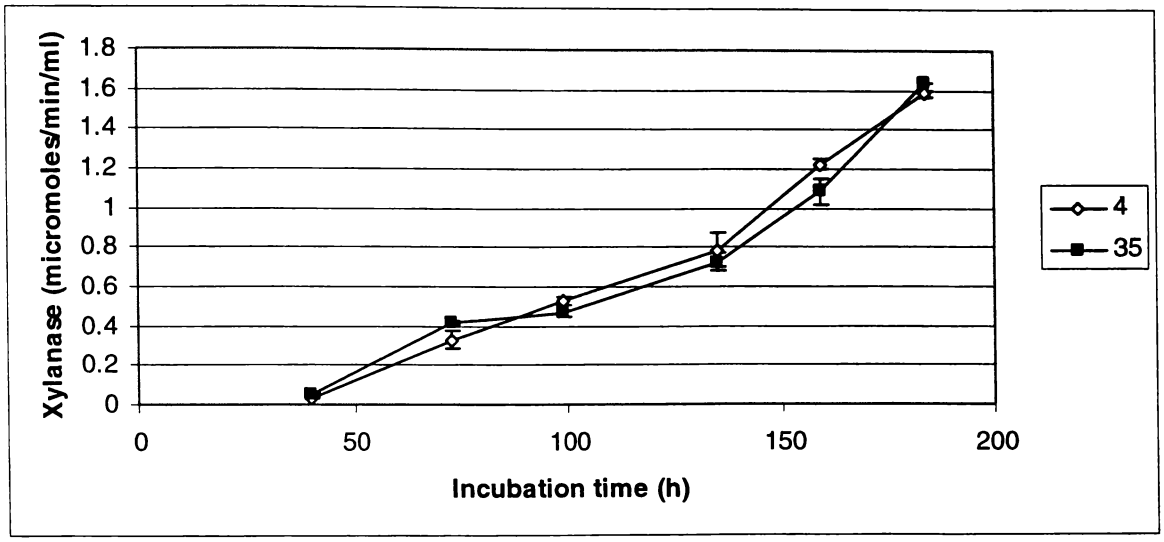


Figure 4.8: Xylanase activity of *S. sapinea* #4 and #35.

Total extracellular protein concentration of the supernatants which were harvested after 184 hours incubation was less than 20 µg per ml. This confirms values obtained for *O. ulmi* (17 µg per ml; Binz, pers. comm.).

It was also tested with selected cultures whether supernatants harvested after 99 hours incubation demonstrated more xylanase activity when MOPS-buffer at pH 6.5 was used in the assay, instead of pH 4.6. It was found that there was less xylanase activity in two isolates of *O. piceae* (#272 and #170), in *O. floccosum* #148 and *O. pluriannulatum* #151 when buffer at pH 6.5 was used (Table 4.16). Slightly more xylanase activity was demonstrated by *S. sapinea* #4 when buffer at pH 6.5 was used.

In order to determine substrate-limitation, supernatants harvested after 99 hours incubation were assayed using 3 % (instead of 1.33 %) larchwood xylan in McIlvaine buffer pH 4.6. It was found that the activities obtained were substrate-independent (Table 4.16).

Table 4.16: Influence of assay pH and substrate concentration on xylanase activities of selected cultures. Supernatants harvested after 99 hours incubation.

Isolate	Xylanase (µmoles/min/ml)		
	pH 4.6; 1.33 % xylan	pH 6.5; 1.33 % xylan	pH 4.6; 3 % xylan
O. piceae #272	0.07	0.02	0.03
O. piceae #170	0.08	0.05	0.10
O. floccosum #148	0.33	0.18	0.42
O. pluriannul. #151	0.42	0.27	0.35
S. sapinea #4	0.46	0.51	0.58

A significantly longer incubation time of four hours in the reducing sugar assay did not increase xylanase activity (Table 4.17) which may be due to the accumulation of proteases in the extracellular supernatant and their interference with xylanase activity. For *O. pluriannulatum*, a four-times longer incubation time reduced xylanase activity by almost 50%.

Table 4.17: Influence of incubation time in assay on xylanase activity of selected cultures. Supernatants harvested after 99 hours incubation.

Isolate	Xylanase (µmoles/min/ml)	
	pH 4.6; 1.33 % xylan; incubation time one hour	pH 4.6; 1.33 % xylan; incubation time four hours
O. piceae #272	0.07	0.05
O. piceae #170	0.08	0.09
O. floccosum #148	0.33	0.27
O. pluriannulatum #151	0.42	0.24
S. sapinea #4	0.46	0.45

In the xylanase assays involving the decay fungi *S. commune* and *G. trabeum*, extracellular supernatants of the cultures were incubated for 60 minutes at 40°C. The standard reaction mixture (same as for the sapstaining isolates) consisted of 20 µl supernatant, 300 µl substrate (1.33 % xylan in buffer) and McIlvaine citrate phosphate buffer at pH 4.6 to bring the total volume to 500 µl. Additionally, all samples were assayed using MOPS-buffer at pH 6.5. Activities of the culture supernatants of *S. commune* harvested after 186 hours incubation were also determined using 3 % xylan in buffer at pH 4.6. This resulted in higher xylanase activities (up to 0.68 µmoles/min/ml) compared to when the standard substrate concentration (1.33 %) was used (data not shown). Final pH of the *S. commune*-cultures in xylan medium was between 6.0 and 6.4, and fungal biomass after 186 hours incubation was between 0.13 g and 0.17 g.

Xylanase activity of *S. commune* increased continously during incubation and reached 0.31 µmoles/min/ml after 8 days (Figure 4.9). There was no significant difference in enzyme activity whether McIlvaine citrate phosphate buffer at pH 4.6 or MOPS buffer at pH 6.5 were used in the assay.

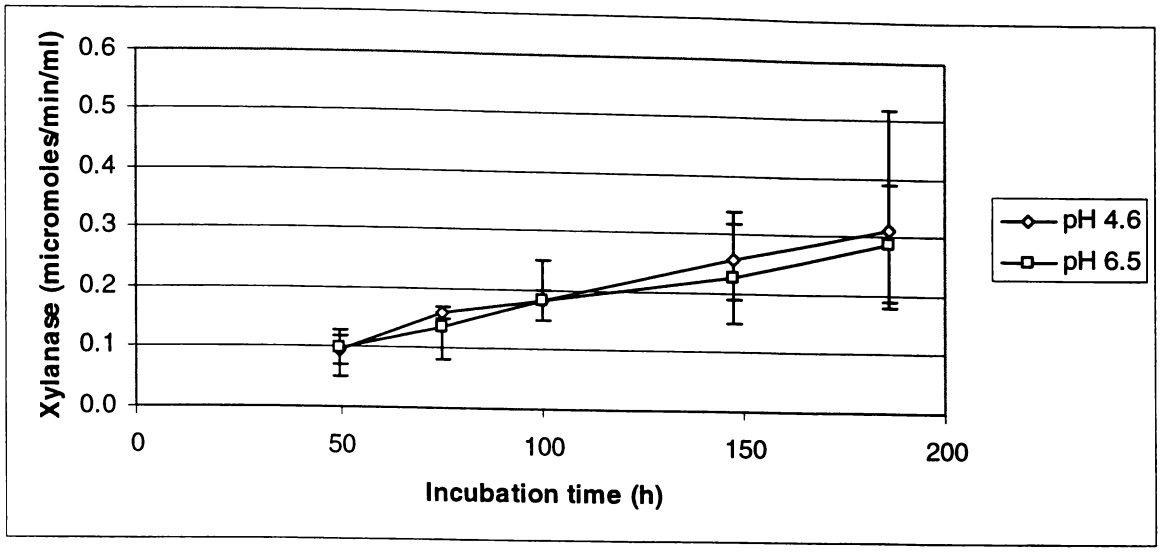


Figure 4.9: Xylanase activity of *Schizophyllum commune* at pH 4.6 and pH 6.5 during 186 hours (8 days) incubation. Vertical bars represent the range of values obtained for triplicate cultures.

Xylanase activities of *G. trabeum* were monitored over a longer incubation period (479 hours or 20 days) because enzyme activity after 148 hours accumulated to only 0.02 µmoles/min/ml (Figure 4.10). After 280 hours incubation, xylanase activity had increased significantly (0.1 µmoles/min/ml) and stayed at the same level until the end of incubation.

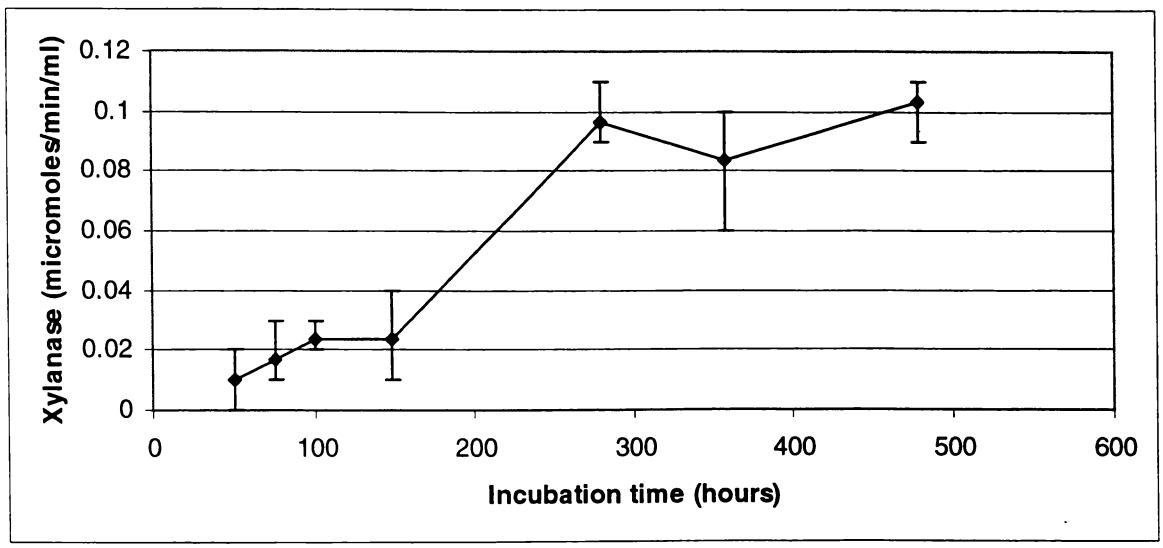


Figure 4.10: Xylanase activity of *Gloeophyllum trabeum* during 479 hours (20 days) incubation. Vertical bars represent the range of values obtained for triplicate cultures.

The same isolate of *S. commune* as used in this thesis produced more significant xylanase activity (4.67 µmoles/min/ml) in independent experiments (Ah Chee, 2001) but different growth and assay conditions were used.

Haltrich et al. (1996) reported maximal xylanase activities of different strains of *Schizophyllum commune* in the range between 0.37 and 4839 IU/ml in submerged cultivations with different conditions. The xylanase activities measured in this thesis are

comparatively low but reflect the wide range of enzyme activities detected for different isolates of one particular species.

Ritschkoff et al. (1992) determined xylanase activity of the same isolate of *G. trabeum* as used in this thesis. When *G. trabeum* was grown on sawdust medium, enzyme activity was 1.2 nkat/ml (0.07 µmoles/min/ml), whereas on crystalline cellulose, less than half of that activity (0.05 nkat/ml) was measured. The xylanase of *G. trabeum* was further purified and characterized (Ritschkoff et al., 1994).

4.4.3 Screening for mannanase activity

Although mannan is the main constituent of hemicelluloses in softwood, it is significant that mannanase activity on galactoglucomannan (locust bean gum) was detected only in one sapstaining species in this thesis research, namely *O. piceae* (activity of the crude supernatant ≤0.3 µmoles/min/ml; Table 4.18). To our knowledge, this is the first report of mannanase activity quantified for an *Ophiostoma* species. There is little information available on production of mannanase by sapstaining fungi. According to Kremnický et al. (1996), the only mannanolytic yeasts mentioned in the literature belong to the genera *Aureobasidium* (Berndt and Liese, 1971), *Hormoascus*, *Guilliermondella* and *Endomyces* (Kocková-Kratochvílová et al., 1983). A possible reason for not detecting mannanase activity in cultures other than *O. piceae* in this thesis research is that the physiological conditions of cultivation in the screen were not suitable for enzyme production. Locust bean gum was the substrate of choice in the enzyme screen because it is has been successfully used for the detection of mannanase in yeasts and yeast-like microorganisms (Kremnický et al., 1996).

Table 4.18: Mannanase activity, fungal biomass and pH of cultures after 184 hours incubation when grown in media supplemented with locust bean gum. Values represent the average of two (sapstaining species) and three (decay fungi) flasks.

Isolate	Mannanase activity (µmoles/min/ml)	Mannanase act. after 5-fold conc. (µmoles/min/ml)	Fungal biomass (g)	Mannanase activity (µmoles/min/ml/g)	pH
O. floccosum #138	0	0	0.03	0	6.8
O. floccosum #148	0	0	0.11	0	6.8
O. piceae #272	0.04	0.50	0.06	0.67	6.5
O. piceae #170	0.29	0.75	0.17	1.71	6.2
O. ips #308	0	0	0.08	0	6.7
O. pluriannulatum #151	0	0	0.09	0	6.8
L. procerum #1852	0	0	0.08	0	6.6
S. sapinea #4	0.06	0.09	0.22	0.27	6.4
S. sapinea #35	0.01	0.03	0.16	0.06	6.8
S. commune	0.19	not determined	0.26	0.73	6.3
G. trabeum	0.02	not determined	0.12	0.17	4.1

The initial pH of the culture medium (6.9) was not changed significantly by the sapstaining fungi during 11 days incubation (Table 4.18).

Growth of *O. ips* #308 was also monitored by counting blastospores in liquid mannan medium (Figure 4.11). There is obviously not much difference in the growth behaviour of *O. ips* #308 whether the liquid medium contains mannan or xylan as a carbon source. In both media, there was an exponential growth phase during the first 40 hours after inoculation. After about 120 hours, growth started to decline. However, no mannanase was detected in the medium containing mannan whereas a slight amount of xylanase was present in the medium containing xylan.

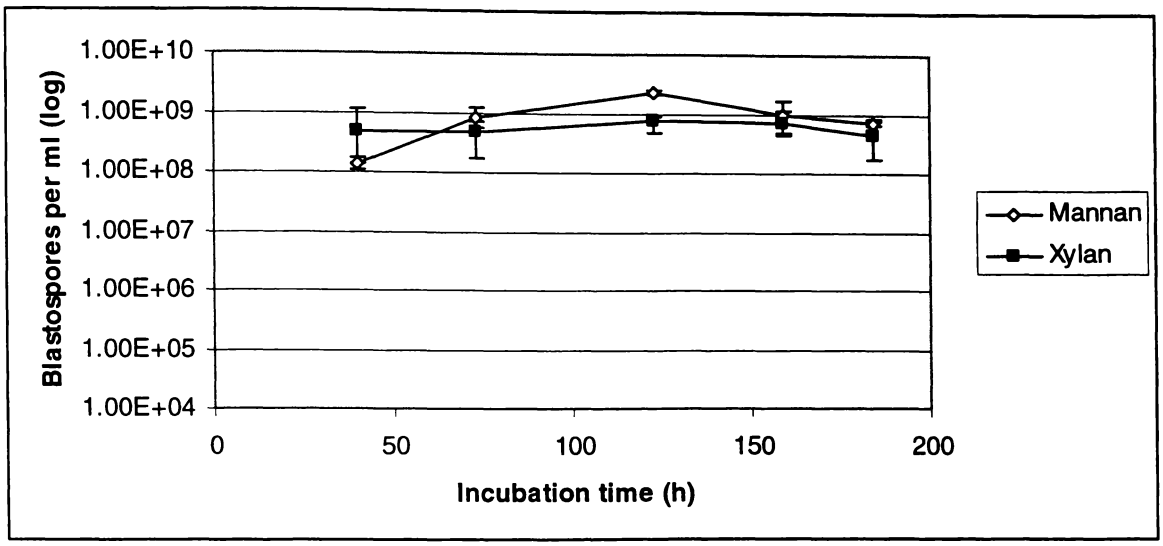


Figure 4.11: Growth of *O. ips* #308 in liquid medium with xylan or mannan. Vertical bars represent the range of values obtained for one isolate (triplicate flasks).

In addition, it was tested if a longer incubation time produced mannanase activity. Supernatants from *O. floccosum* #138, *O. piceae* #170 and #272, *O. pluriannulatum* #151, *S. sapinea* #4 and *S. commune*, harvested after different timepoints, were incubated for 17 hours at 40°C. There was slight activity only in the cultures of *S. sapinea* and *S. commune* (0.02 µmoles/min/ml).

Maximal mannanase activities were 0.19 µmoles/min/ml (after 26 days incubation) for *S. commune* and 0.02 µmoles/min/ml (after 8 and 12 days incubation) for *G. trabeum* (Figure 4.12). No mannanase activity was detected in *G. trabeum* on days 19 and 26 of incubation.

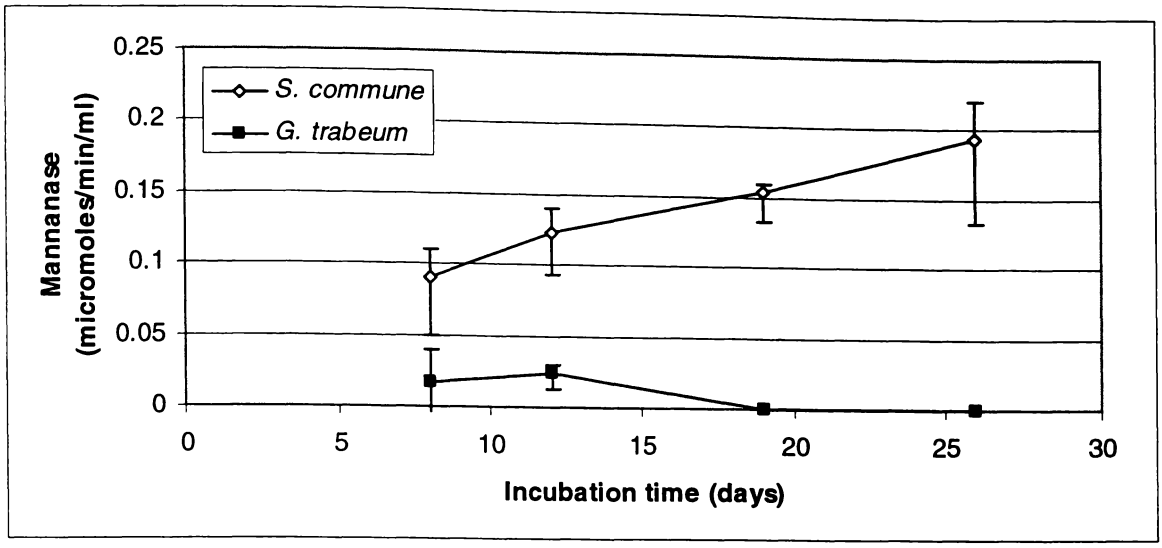


Figure 4.12: Mannanase activity of *S. commune* and *G. trabeum* during 26 days incubation. Vertical bars represent the range of values obtained for triplicate cultures.

Ritschkoff et al. (1992) also determined endo-β-1,4-mannanase activities of *G. trabeum* isolate BAM Ebw. 109 on sawdust medium and on crystalline cellulose. Activities were very low and constant during a 9-week-cultivation, as has been found in this thesis. Mannanase activity was about three times higher on crystalline cellulose medium (0.06 nkat/ml or 0.035 μmoles/min/ml) compared to activity on sawdust medium.

The same isolate of *S. commune* as used in this thesis produced slightly more mannanase (0.3 μmoles/min/ml) in independent experiments (Ah Chee, 2001) which may be due to different assay and growth conditions.

4.4.4 Screening for pectinase activity

4.4.4.1 Pectin as main carbon source in growth medium

All strains tested produced low amounts of pectinase (Table 4.19; Figures 4.13 to 4.16). Maximal pectinase activity was 0.04 μmoles/min/ml which was determined for *O. ips* #308, *O. pluriannulatum* #151 and *L. procerum* #1852. After 266 hours incubation, activity in all cultures was still increasing, except for *O. floccosum* #138 and *L. procerum* #1852. Biomass produced by the sapstaining fungi was between 0.15 g (*S. sapinea* #4) and 0.27 g (*L. procerum* #1852). There was no correlation between fungal biomass and blastospore formation in the cultures of *Ophiostoma* (Table 4.19). The initial pH of the growth medium (5.6) was decreased significantly, except for the culture of *S. commune*.

Table 4.19: Pectinase activity, fungal biomass, blastospore formation and pH of fungal cultures after 266 hours incubation in medium II with pectin as main carbon source. Data represent mean of duplicates (range of values included in Figures 4.13 to 4.16).

Isolate	Pectinase activity (µmoles/min/ml)	Fungal biomass (g)	Pectinase activity (µmoles/min/ml/g)	Blastospores per ml	pH
S. sapinea #35	0.03	0.18	0.17	n.a.	3.7
S. sapinea #4	0.03	0.15	0.20	n.a.	3.1
O. floccosum #138	0.02	0.26	0.08	$1.35 \cdot 10^8$	3.2
O. floccosum #148	n.d.	0.19	n.d.	$48.0 \cdot 10^8$	3.3
O. pluriannulatum #151	0.04	0.23	0.17	$1.39 \cdot 10^8$	3.6
O. piceae #272	0.03	0.25	0.12	$1.35 \cdot 10^8$	3.1
O. piceae #170	n.d.	0.16	n.d.	$26.0 \cdot 10^8$	3.5
O. ips #308	0.04	0.19	0.21	$0.37 \cdot 10^8$	3.3
L. procerum #1852	0.02	0.27	0.15	$2.35 \cdot 10^8$	2.8
Schizophyllum commune	0.05	0.38	0.13	n.a.	5.6
Gloeophyllum trabeum	0.42	0.13	3.23	n.a.	3.4

n.a. = not applicable (species does not produce blastospores); n.d. = not determined.

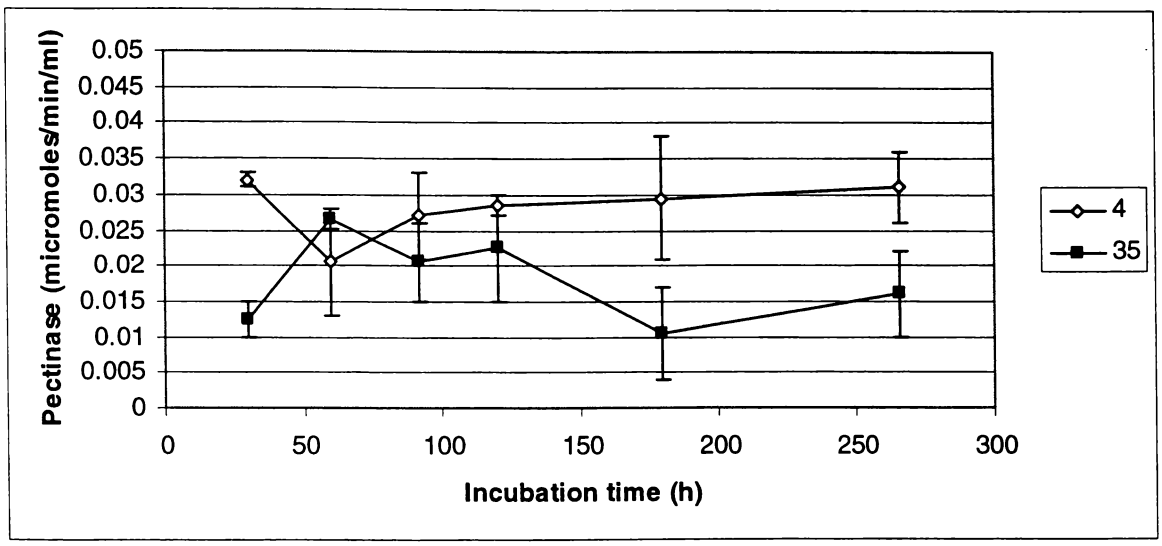


Figure 4.13: Pectinase activity of *S. sapinea* #4 and #35 in growth medium with pectin. Vertical bars represent the range of values obtained for duplicate cultures.

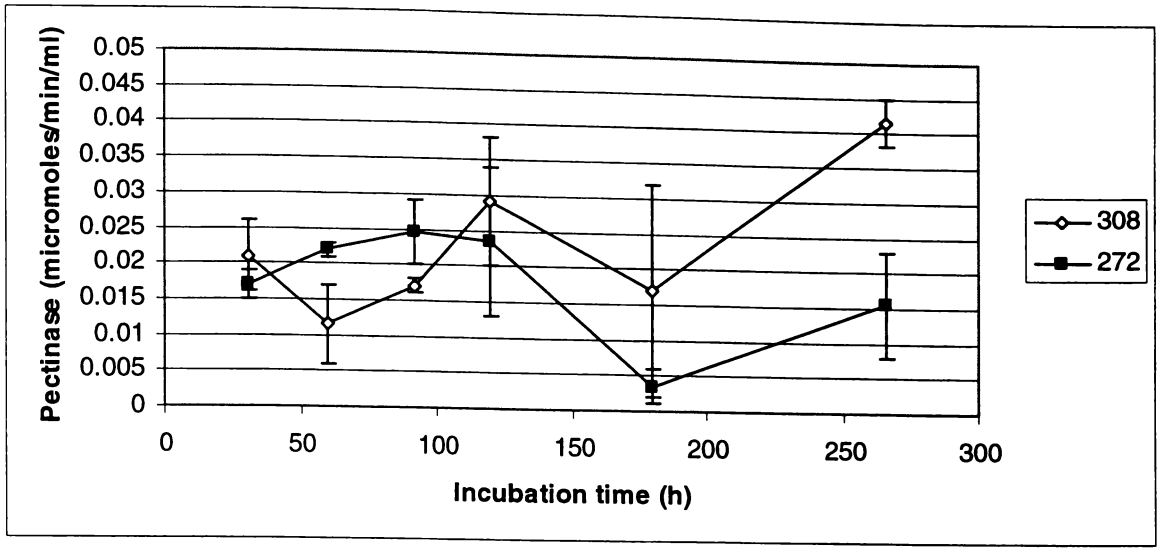


Figure 4.14: Pectinase activity of *O. ips* #308 and *O. piceae* #272 in growth medium with pectin.

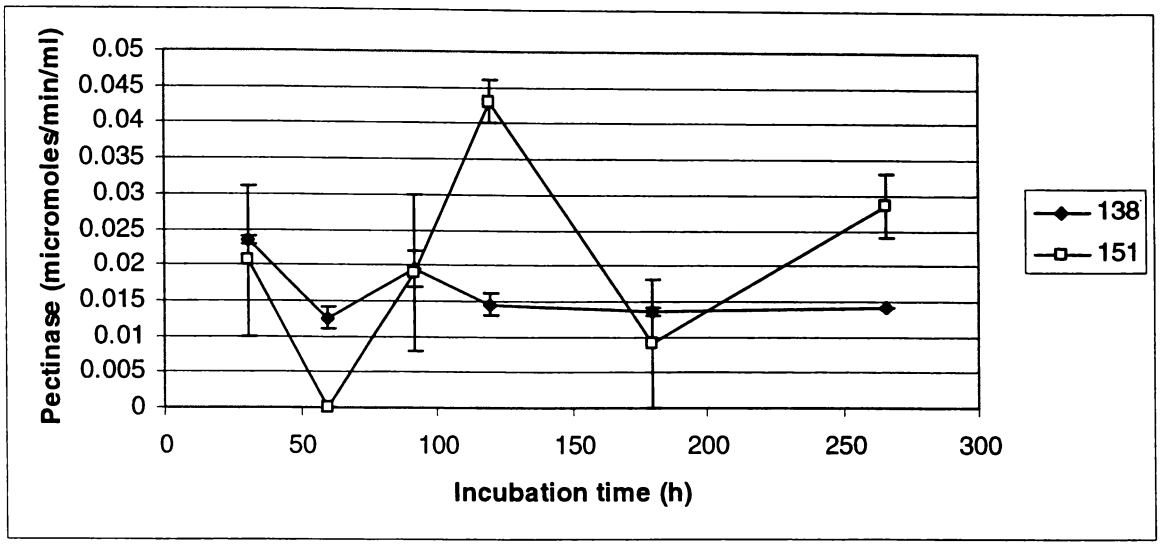


Figure 4.15: Pectinase activity of *O. floccosum* #138 and *O. pluriannulatum* #151 in growth medium with pectin.

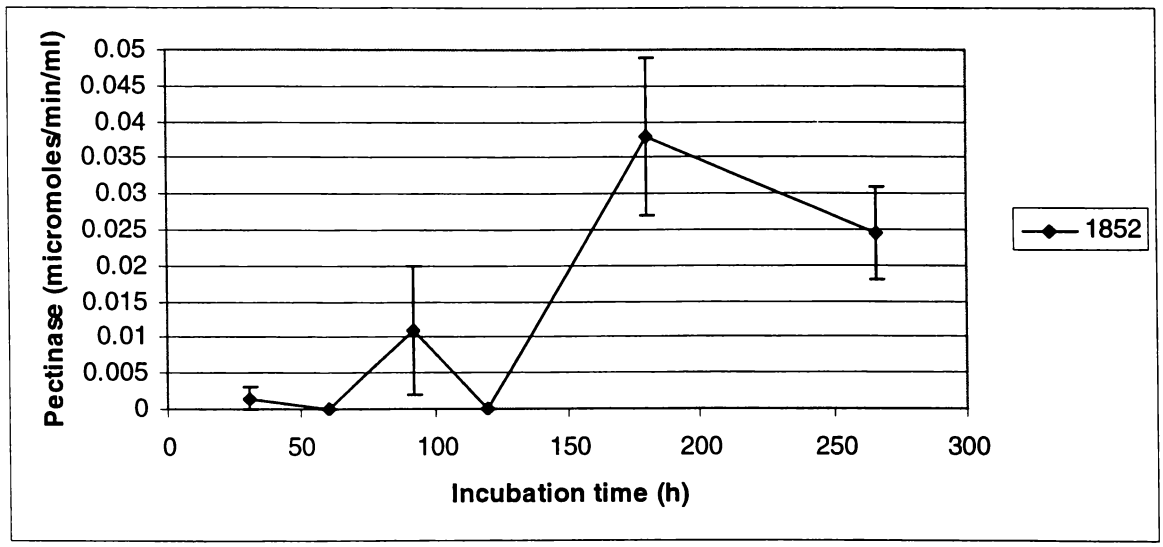


Figure 4.16: Pectinase activity of *Leptographium procerum* #1852 in growth medium with pectin.

Binz (1996a) measured polygalacturonase activities secreted by isolates of the pathogenic sapstaining fungi *O. ulmi* (0.12 µmoles/min/ml) and *O. novo-ulmi* (0.11 µmoles/min/ml) grown on pectin. Results obtained in the present screen indicate that *O. ips* may have the potential to secrete similar amounts of polygalacturonase as *O. ulmi* and *O. novo-ulmi*. According to Harrington (1993), *O. ips* has been reported to be a substantial pathogen (Basham, 1970; Himelick, 1982; Raffa and Smalley, 1988), but it does not appear to be particularly pathogenic in artificial inoculations (Rane and Tattar, 1987; Parmeter et al., 1989). Pectic enzymes play an essential role in phytopathogenesis and can elicit the cascade of defence reactions in plant cells which in favourable cases contain the growth of the pathogen (Jarvis, 1984). The involvement of pectinases in the pathogenesis of the Dutch elm disease was demonstrated by Gagnon (1967) who showed that pectins were the main constituents of the plugging material in the vessels of infected elm trees. The action of pectinases on vessel pits allows the flow of protoplasm from adjoining parenchyma cells into the vessels and contributes to plugging of the vessels.

Schizophyllum commune did not produce significantly higher amounts of pectinase than the sapstaining fungi (0.05 µmoles/min/ml), whereas *G. trabeum* did (0.42 µmoles/min/ml; Figure 4.17). *Gloeophyllum trabeum* produced the least biomass of all strains tested when the growth medium contained pectin. This is not surprising because it has been demonstrated earlier for wood-destroying fungi that a strong enzyme production correlates with a weak mycelial development, i.e. the relationship between enzyme and mycelial production is antagonistic (Lyr, 1959a and 1960).

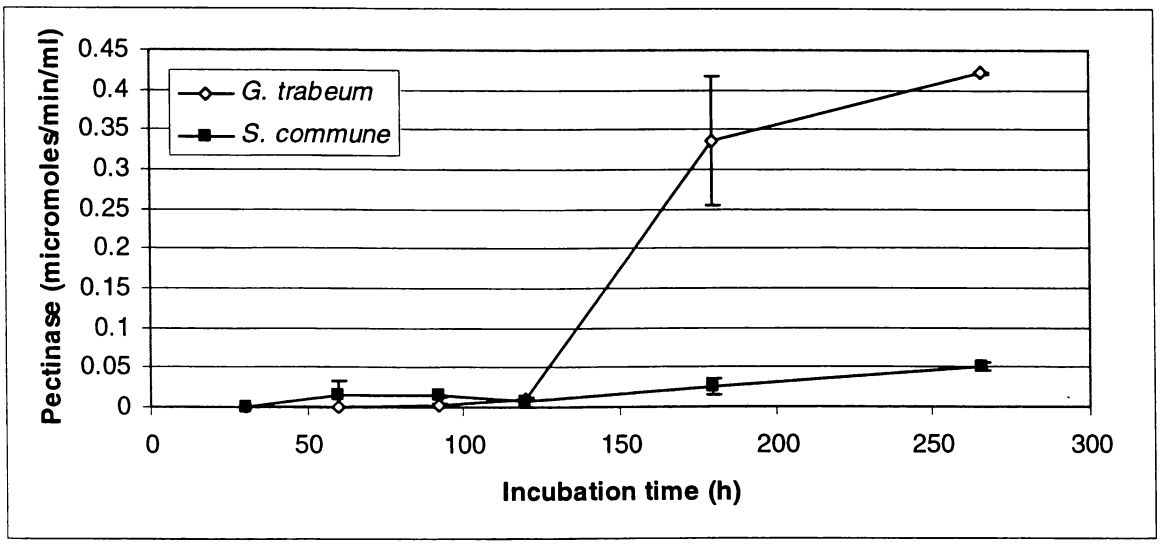


Figure 4.17: Pectinase activity of *S. commune* and *G. trabeum* in growth medium with pectin.

In general, studies of pectin-hydrolyzing enzymes in wood decay fungi are scarce, probably because of the relatively low content (less than 4%) of pectin in wood and because of the primary focus on understanding the degradation of lignified components. However, pectin degradation appears to be a key step in fungal colonization during incipient decay, and consequently, inhibition of pectin degradation could be a target for

new wood preservatives (Green and Clausen, 1999). Lyr (1959a, 1959b, 1960) determined the enzyme-production of ten species of wood-rotting and wood-inhabiting fungi on different media containing pectin, cellulose, amylose, xylan, glucose or a combination of all substrates. Using viscometry, pectinase could always be demonstrated in the culture-fluid, irrespective of the substrate used, but production of pectinase was higher in a medium containing pectin. The brown-rot fungi tested produced by far more pectinase than the white-rot fungi, including an isolate of *S. commune.*

More recently, Green and Clausen (1999) undertook a survey of pectin-degrading enzymes from representative cultures of brown- and white rot fungi. Endopolygalacturonase activity was estimated by cup-plate assay and viscosity reduction of pectin from liquid cultures of fifteen brown-rot and eight white-rot fungi using sodium polypectate as the carbon source. Twelve of the brown-rot (including an isolate of *G. trabeum*) and six of the white-rot fungi tested were positive for at least one of the polygalacturonase test methods.

Polygalacturonase production by *G. trabeum*, *Postia placenta* and *Serpula incrassata* has also been estimated by measuring reducing sugars (Green et al., 1995). Polygalacturonase activity was found to be greatest in *G. trabeum*, was best induced by pectin and exceeded production of xylanase and endoglucanase activity in vitro. Results of the enzyme assays in this thesis also showed that production of pectinase by *G. trabeum* was higher than xylanase activity.

4.4.4.2 Polygalacturonic acid as main carbon source in growth medium

In this experiment, cultures of *O. floccosum* #138, *O. ips* #308, *O. piceae* #272, *S. sapinea* #4 and #35, *Schizophyllum commune* and *Gloeophyllum trabeum* were grown in 10 ml of modified medium II at pH 4.1 using 0.05 g/l yeast extract and 5 g/l polygalacturonic acid as the main carbon source. The objective of this experiment was to determine if higher amounts of pectinase were produced with polygalacturonic acid instead of pectin as a carbon source.

After 49 hours incubation in medium II, the cultures were transferred into 80 ml of medium II in 250 ml-flasks. The pH was again adjusted to 4.1. At this stage, spores were present in all sapstaining cultures except for *O. ips*. All sapstaining isolates tested produced low amounts of pectinase (Table 4.20; Figures 4.18 to 4.20). Pectinase production was most significant at different times after inoculation for the individual cultures. Enzyme activity of *O. piceae* #272 declined after 194 hours incubation, whereas pectinase in the other cultures was still increasing after 266 hours. Among the sapstaining species, pectinase production was most significant for *O. ips* #308.

Table 4.20: Pectinase activity, fungal biomass, blastospore formation and pH of fungal cultures after 266 hours incubation in medium II with polygalacturonic acid as main carbon source. Data represent mean of duplicates (range of values included in Figures 4.18 to 4.20).

Isolate	Pectinase activity (µmoles/min/ml)	Fungal biomass (g)	Pectinase activity (µmoles/min/ml/g)	Blastospores per ml	pH
S. sapinea #35	0.04	0.07	0.57	n.a.	6.5
S. sapinea #4	0.04	0.07	0.57	n.a.	6.5
O. floccosum #138	0.03	0.04	0.75	$1.85 \cdot 10^7$	6.7
O. piceae #272	0.02	0.04	0.5	$3.95 \cdot 10^7$	6.3
O. ips #308	0.11	0.05	2.20	$1.71 \cdot 10^7$	6.2
Schizophyllum commune	0.04	0.10	0.40	n.a.	6.1
Gloeophyllum trabeum	0.11	0.03	3.67	n.a.	5.8

n.a. = not applicable (species does not produce blastospores).

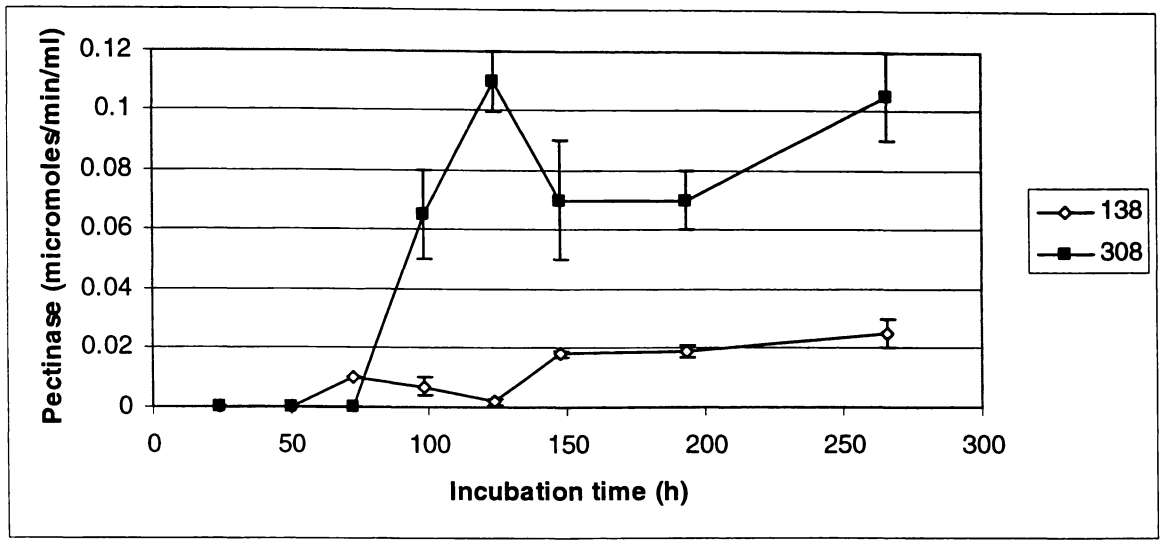


Figure 4.18: Pectinase activity of *O. floccosum* #138 and *O. ips* #308 in growth medium with polygalacturonic acid. Vertical bars represent the range of values obtained for duplicate cultures.

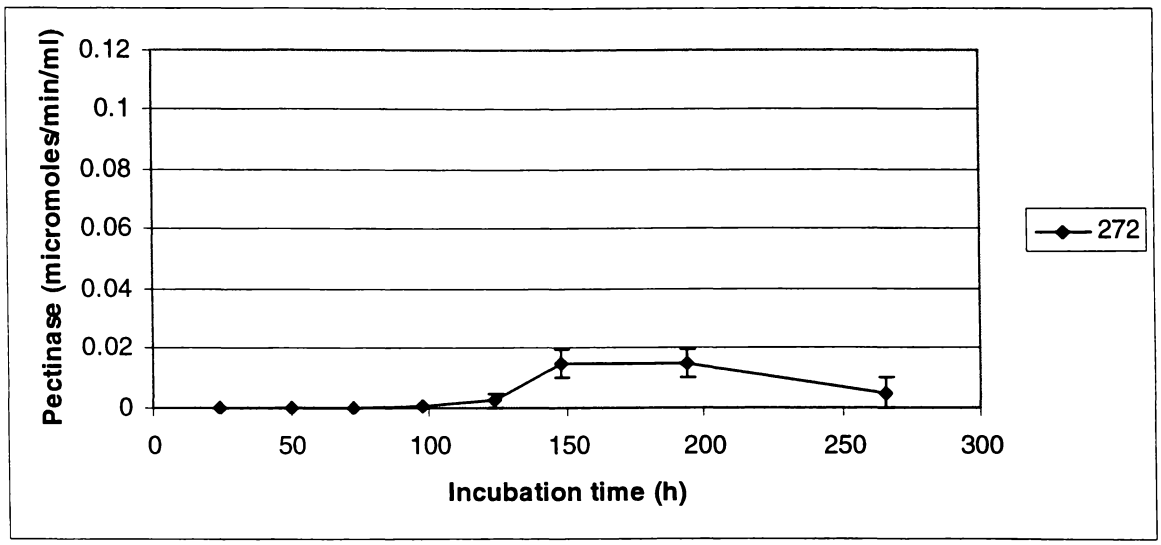


Figure 4.19: Pectinase activity of *O. piceae* #272 in growth medium with polygalacturonic acid.

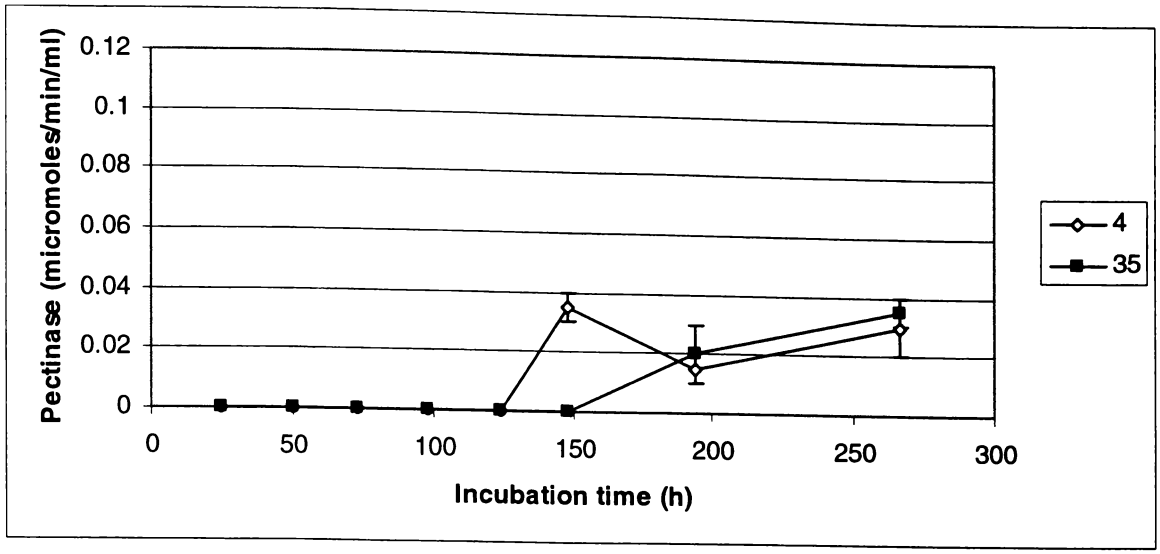


Figure 4.20: Pectinase activity of *S. sapinea* #4 and #35 in growth medium with polygalacturonic acid.

In general, fungal biomass, blastospore numbers and pectinase activity were in the same range when the growth medium was supplemented with polygalacturonic acid or with pectin; however, enzyme activity of *O. ips* #308 was significantly higher when the medium contained polygalacturonic acid as a carbon source. The pH in the growth medium increased significantly during the incubation (from 4.1 to 6 and higher). In the previous screen, the opposite effect had occurred; the initial pH of 5.6 was decreased to around 3 in all cultures except for *Schizophyllum commune*.

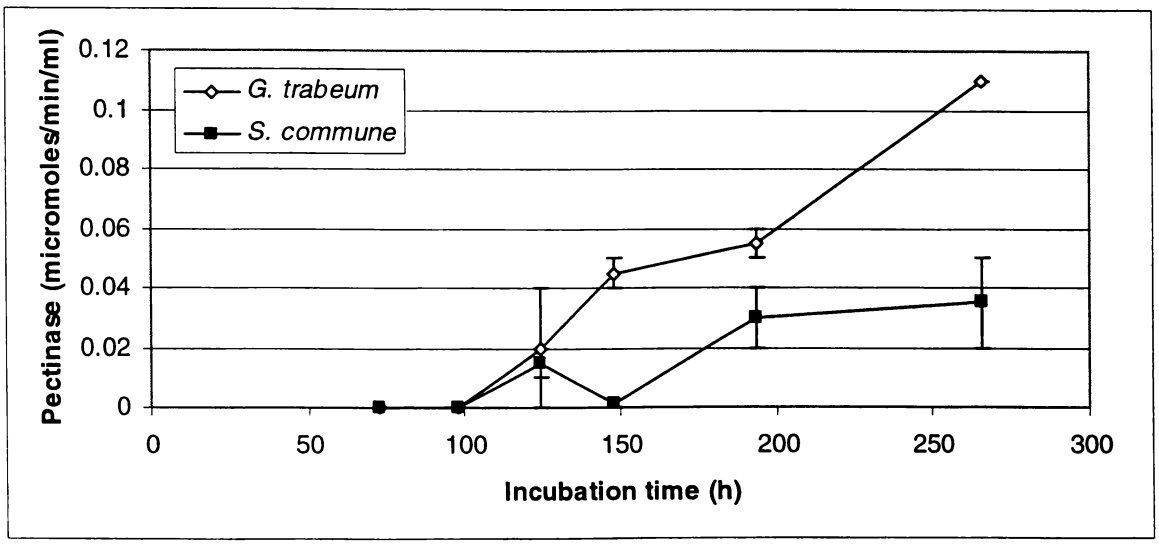


Figure 4.21: Pectinase activity of *S. commune* and *G. trabeum* in growth medium with polygalacturonic acid.

On polygalacturonic acid, pectinase activity was 0.04 µmoles/min/ml for *S. commune* and 0.11 µmoles/min/ml for *G. trabeum* (Figure 4.21). Results from both screenings show that only the brown-rot fungus *G. trabeum* produced significantly higher amounts of pectinase than the sapstaining fungi (0.42 µmoles/min/ml when grown on pectin). *Gloeophyllum trabeum* as well as *S. commune* produced more pectinase when the growth medium contained pectin instead of polygalacturonic acid.

4.4.4.3 Pectinase activity and growth characteristics of *Ophiostoma ips*

It was decided to continue the work on pectinase with *O. ips* because this *Ophiostoma* species has been reported to be a substantial pathogen (Basham, 1970, Himelick, 1982; Raffa and Smalley, 1988), although it does not appear to be particularly pathogenic in artificial inoculations (Rane and Tattar, 1987; Parmeter et al., 1989). Pectinase levels are associated with pathogenicity (Basham, 1970; Himelick, 1982; Raffa and Smalley, 1988).

In the following sections, experiments were described and analysed with regard to pectinase activity and growth characteristics of *O. ips*. Firstly, pectinase activity in relation to fungal growth was investigated. The objective of this experiment was to determine at which stage during incubation enzyme activity was most significant. In the following experiments, the influence of the medium composition on expression of pectinase activity was determined. Since an extracellular hyphal sheath was observed for species of *Ophiostoma* (Gharibian et al., 1996; see also Chapter 6) as well as other sapstaining fungi (Schmid and Liese, 1965) which might serve to transfer enzymes to the substrate, it was also tested if there was cell-bound pectinase activity in *O. ips*.

O ips #308 was grown in medium I (10 ml-universals) for two days. Cultures were then transferred into medium II (80 ml in 250 ml-flasks) containing 0.05 g/l yeast extract and 5 g/l pectin. The medium was buffered with 40 mM 2,2-dimethylsuccinic acid (Sigma) to keep the pH constant and adjusted to pH 3.9 using 1 M NaOH. Supernatants of the cultures were harvested and total fungal biomass was determined at different stages during the incubation (Figure 4.22). In the course of these experiments, a new set of duplicate flasks of the culture were used at each timepoint so that no lines were drawn between individual data points (Figures 4.22 to 4.25).

Pectinase was detected when dimethylsuccinic acid was used in the growth medium but the amounts secreted were low (Figure 4.23). In the samples harvested after 180 hours and 266 hours incubation, the enzyme could not be detected under the parameters of the assay. However, in the supernatants harvested after 334, 454 and 574 hours, pectinase was detectable which suggests that secondary metabolism was occurring. Pectinase activity was most significant after 574 hours incubation (0.03 µmoles/min/ml).

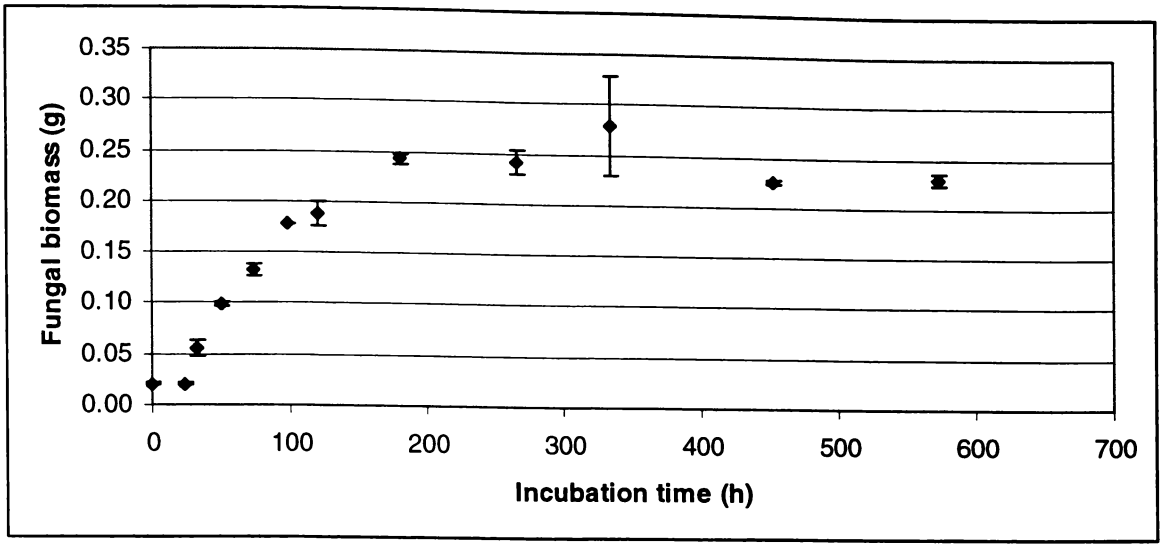


Figure 4.22: Growth of *O. ips* #308 during 574 hours incubation (buffered medium). Vertical bars represent the range of values obtained for duplicate cultures.

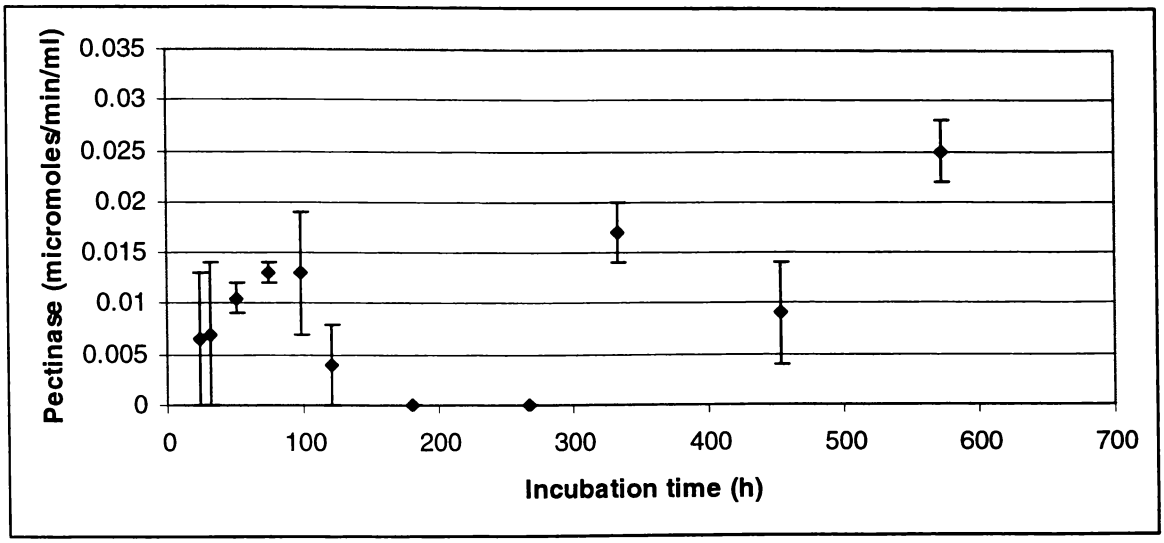


Figure 4.23: Pectinase production by *O. ips* #308 during 574 hours incubation (buffered medium). Vertical bars represent the range of values obtained for duplicate cultures.

The experiment was repeated for a duration of 266 hours without using dimethylsuccinic acid in the growth medium to determine whether the buffer inhibited pectinase production (Figures 4.24 and 4.25). The result was negative although slightly more pectinase was detected when the growth medium did not contain dimethylsuccinic acid. In the unbuffered medium, the initial pH of 6.5 was decreased to 3.4 after 266 hours incubation. After 40 hours incubation, the pH had already decreased to 3.7.

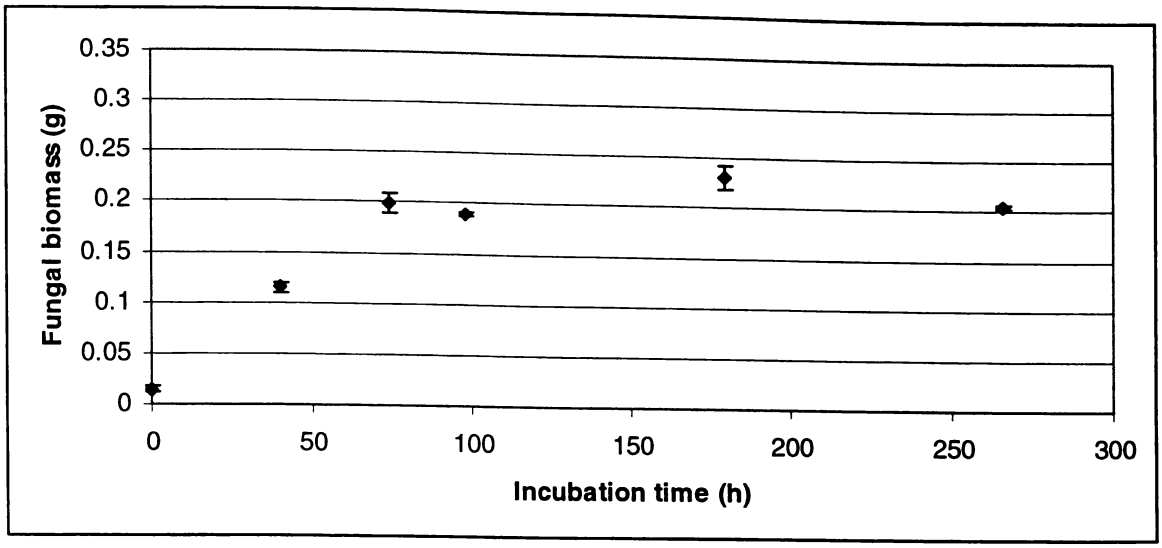


Figure 4.24: Growth of *O. ips* #308 during 266 hours incubation (unbuffered medium). Vertical bars represent the range of values obtained for duplicate cultures.

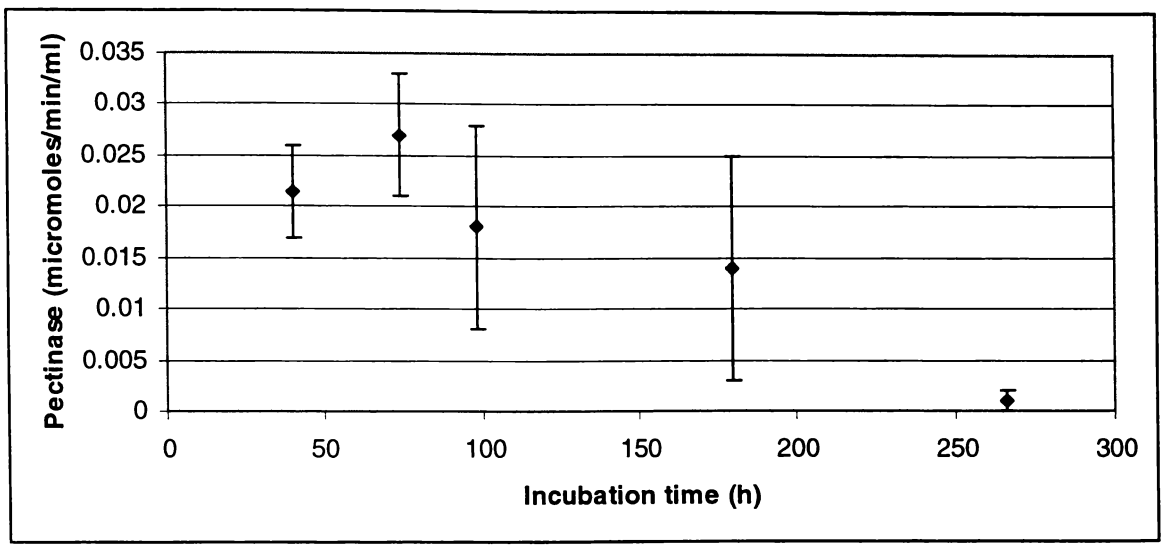


Figure 4.25: Pectinase production by *O. ips* #308 during 266 hours incubation (unbuffered medium). Vertical bars represent the range of values obtained for duplicate cultures.

These experiments confirmed that *O. ips* #308 secretes pectinase, however, in low amounts (maximum of 0.03 µmoles/min/ml). The use of dimethylsuccinic acid as a buffer in the growth medium neither inhibited pectinase production nor fungal growth. However, pectinase activity was slightly more significant when dimethylsuccinic acid was omitted from the growth medium. Pectinase activity was most significant during the early stationery growth phase of the fungus, i.e. after about 74 hours. When the growth medium was buffered, no pectinase activity could be detected between 120 and 266 hours incubation. However, after 334, 454 and 574 hours incubation, pectinase activity was detected in buffered culture which is likely due to autolysis because fungal growth had stopped at that time (Figure 4.22).

4.4.4.4 Influence of medium composition on expression of pectinase activity in *O. ips*

The objectives of the following experiments were to detect and quantify pectinase activity of *O. ips* when the culture was a) initially grown in starter cultures containing less yeast-malt-extract; b) initially grown in regular starter cultures which were subsequently washed in buffer. In addition, it was sought to establish a correlation between enzyme activity and fungal growth under these conditions.

Three different sets of starter cultures (10 ml) with the following compositions were prepared:
#1: medium II with 0.05 g/l yeast extract, 5 g/l polygalacturonic acid and 5.84 g/l (40 mM) dimethylsuccinic acid at pH 3.7;
#2: 2 g/l yeast and 10 g/l malt;
#3: 2 g/l yeast and 5 g/l malt.

After two days, the cultures were transferred into unbuffered medium II (80 ml in 250 ml-flasks) at pH 5.6 containing 5 g/l pectin. Cultures were incubated for 180 hours. Supernatants were harvested and fungal biomass was determined in intervals as described earlier.

In the starter cultures #1, there was no growth. It is likely that the amount of yeast-malt extract in the medium was too low, and it is unlikely that the buffer inhibited fungal growth because in previous experiments, cultures grew well if the medium contained dimethylsuccinic acid. The pH decreased from 5.6 to 3.4 in growth media #2 as well as #3 which confirms previous results. After 74 hours incubation, fungal biomass of the cultures #2 and #3 was almost the same (Figure 4.26), i.e. the initial advantage of a higher amount of malt extract in culture #2 had been lost.

In this experiment, there were no significant differences between pectinase activities when starter cultures #2 and #3 were used, with the exception of supernatants harvested after 40 hours incubation (Figure 4.27). Maximal pectinase activities were between 0.03 and 0.04 µmoles/min/ml which is lower than enzyme activities obtained using the standard starter cultures which contain a higher amount of malt-extract than starter cultures #2 and #3. It seems therefore that the use of a richer medium results in higher pectinase activity.

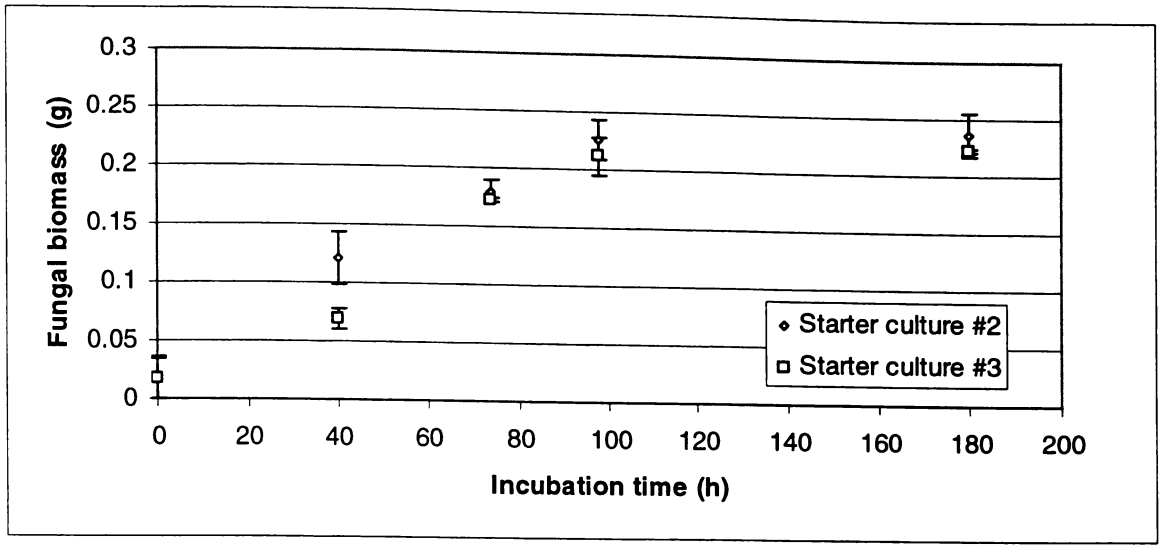


Figure 4.26: Growth of *O. ips* #308 using different amounts of malt extract in starter cultures (#2: 2 g/l yeast and 10 g/l malt; #3: 2 g/l yeast and 5 g/l malt). Vertical bars represent the range of values obtained for duplicate cultures.

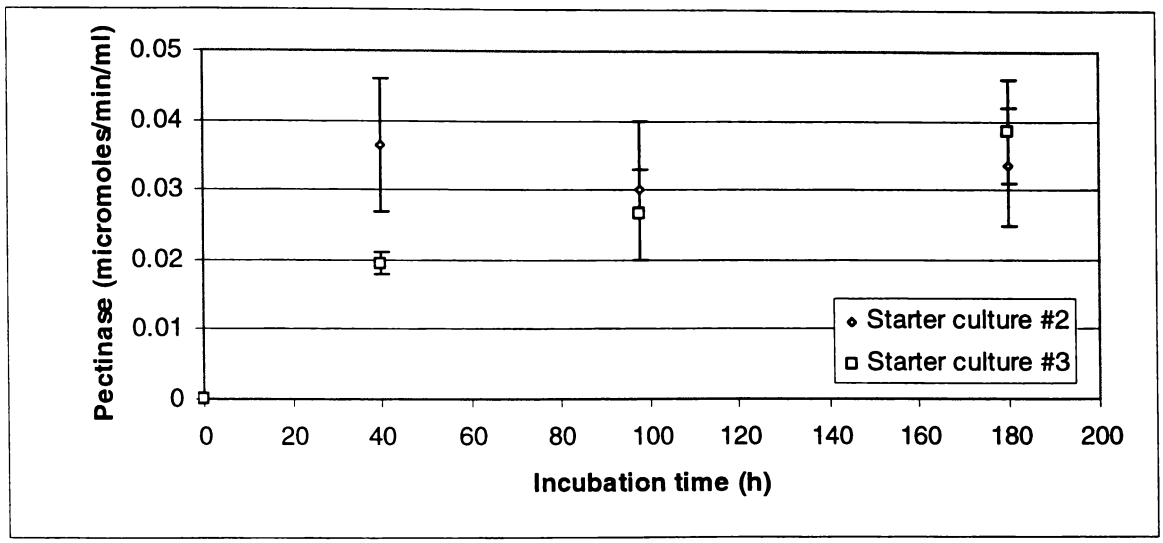


Figure 4.27: Pectinase production of *O. ips* #308 using different amounts of malt-extract in starter cultures. #2: 2 g/l yeast and 10 g/l malt; #3: 2 g/l yeast and 5 g/l malt. Vertical bars represent the range of values obtained for duplicate cultures.

As stated at the beginning of this section, another objective was to determine pectinase activity when *O. ips* was initially grown in regular starter cultures which were subsequently washed in buffer. The aim was to grow up a healthy culture, to wash out the yeast and malt-extract from the starter cultures, and to transfer the culture into a medium containing pectin as the single carbon source in order to determine if *O. ips* was able to grow on this substrate exclusively and demonstrate enzyme activity. For this experiment, starter cultures of *O ips* #308 with medium I were prepared (see Chapter 2). After two days, two starter cultures (10 ml) each were transferred into one sterile centrifuge tube. The tubes were balanced using PBS-buffer (8 g NaCl, 0.2 gKCl, 1.44 gNa_2HPO_4, 0.24 gKH_2PO_4 and one litre of distilled water, adjusted to pH 7.2) and centrifuged for 30 minutes at 20,000 rpm and 4°C. Supernatants were decanted and mycelial pellets

resuspended in PBS-buffer. The cultures were further centrifuged for 30 minutes at 20,000 rpm and 4°C, and the supernatants were decanted. Twenty ml of sterile medium II with pectin as carbon source were added to each tube. The tubes were mixed well. Ten ml each of the spun cultures were transferred into 80 ml of medium II in 250 ml-flasks at an initial pH of 4.6. Cultures were incubated in a rotary shaker at 25°C for 190 hours. Supernatants from three flasks were harvested in intervals, spun down in a bench centrifuge at 13,000 rpm and frozen at -20°C until usage. Fungal biomass was determined in parallel as described earlier. Two sets of controls were included; one set consisted of medium II only (no fungal cultures), and the other set of cultures that were pre-grown using standard medium I prior to transferring into medium II without washing the cultures in buffer. Fungal dry weights of the control flasks were determined only at the end of the experiment.

During the course of this experiment, the pH of the growth medium decreased to 3.8. The pH of the cultures which were not washed prior to inoculation into medium II decreased to 3.6. As observed earlier, melanisation of the cultures started after approximately 74 hours, i.e. during the exponential growth phase of the fungus.

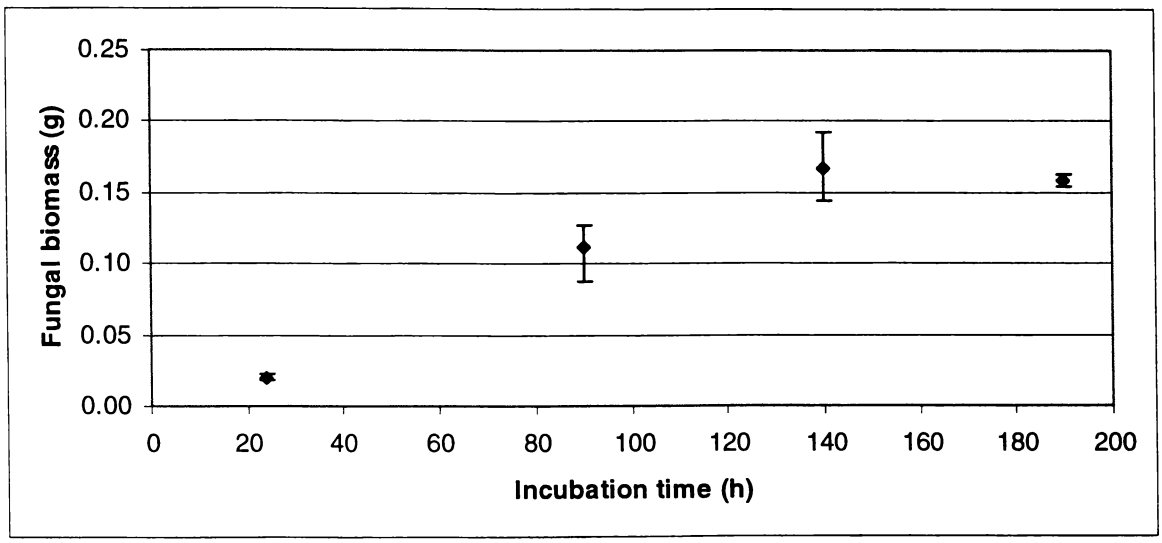


Figure 4.28: Growth of *O. ips* #308 using washed starter cultures. Vertical bars represent the range of values obtained for triplicate cultures.

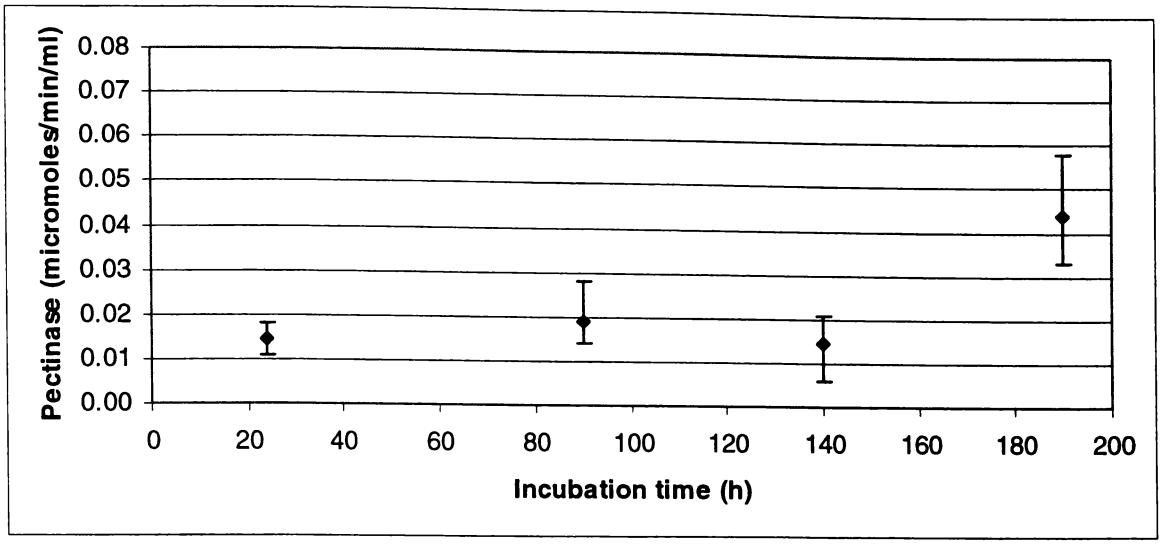


Figure 4.29: Pectinase production of *O. ips* #308 using washed starter cultures. Vertical bars represent the range of values obtained for triplicate cultures.

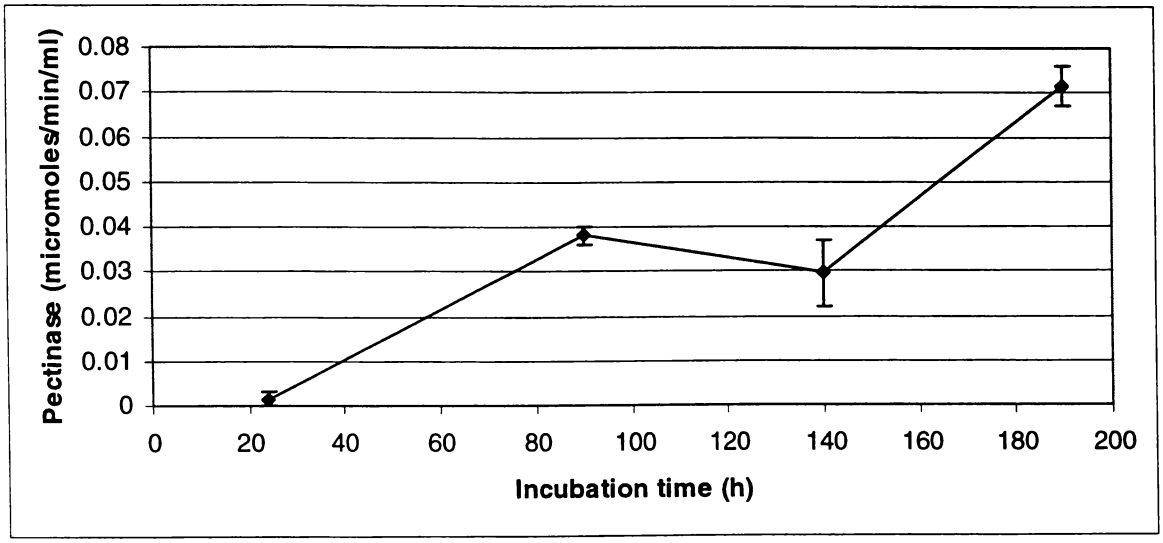


Figure 4.30: Pectinase production of *O. ips* #308 using standard starter cultures (controls). Vertical bars represent the range of values obtained for duplicate cultures.

When the starter cultures were washed in buffer, *O. ips* did not produce as much biomass as in earlier experiments (Figure 4.28) but pectinase activity was about the same as previously, with maximal activities around 0.04 µmoles/min/ml after 190 hours incubation (Figure 4.29). Fungal biomasses of the duplicate cultures which were not washed were 0.15 g and 0.14 g, determined after 190 hours incubation.

Following pectinase activity over incubation time, the same pattern was obtained, independent of whether the starter cultures had been washed in buffer (Figure 4.29) or not (Figure 4.30): enzyme activity increased up to 90 hours after incubation, decreased subsequently and increased again, indicating the occurrence of autolysis in the cultures which was confirmed by microscopic examination. Autolysis in the cultures which had been washed was also indicated by the fact that after 140 hours incubation, pectinase activity was still increasing whereas fungal growth, as measured in biomass, was not.

4.4.4.5 Determination of cell-bound pectinase activity in *O. ips*

The objective of this experiment was to determine if there was cell-bound pectinase activity in *O. ips*, i.e. activity associated with the extracellular fungal sheath. Starter cultures of *O. ips* #294 were grown for 36 hours and transferred into medium II (80 ml in 250 ml flasks) containing pectin as a carbon source. After 3 days and 6 days, 3 ml of culture were harvested from triplicate flasks and frozen immediately for later use in the DNSA-assay. The rest of the cultures was transferred into sterile 250ml-centrifuge tubes and spun down for 15 min at 4°C. The supernatants were then decanted and the mycelial pellets resuspended in assay buffer (McIlvaine citrate phosphate buffer at pH 4). The washed pellets were spun down again in a centrifuge for 30 min. at 4°C, and the procedure was repeated once more. Finally, 3 ml of the supernatants were harvested, and the fungal pellets were resuspended. In summary, the following solutions were used in the DNS-assay:

- the original supernatant;
- the supernatant after washing and spinning;
- the mycelial pellet suspension after grinding with a drill.

In addition, fungal dry weights were determined using two extra flasks after 3 days and 6 days cultivation each. In all assays, the reaction mixture consisted of 50 µl supernatant, 150 µl buffer (McIlvaine citrate phosphate buffer at pH 4) and 300 µl substrate (0.33% polygalacturonic acid in buffer) and was incubated for 60 min. at 30°C.

The results of this experiment were summarized in Table 4.21. Activities of the original supernatant were between 0.17 and 0.21 µmoles/min/ml which is more significant than the values determined for *O. ips* #308. As could be expected for an extracellular enzyme, after washing in buffer, there was no pectinase activity. When the fungal pellet was ground and used in the assay, there was also no or only comparably low activity (maximal 0.04 µmoles/min/ml). It can be concluded that pectinase was present in the extracellular supernatant and not in the hyphal sheath of *O. ips* #294.

Table 4.21: Pectinase activities of *O. ips* #294 determined in original supernatant, in supernatant after washing and in the mycelial pellet (average of triplicate cultures).

	Pectinase activity (µmoles/min/ml)			**Fungal biomass (g) in 90 ml culture**
	Orig. supernatant	Supernatant after washing	Mycelial pellet	
After 3 days	0.21	0	0.04	0.20
After 6 days	0.17	0	0	0.17

4.4.5 Screening for amylase activity

Amylase activity of sapstaining fungi was determined for comparative purposes since amylase is not a cell-wall degrading enzyme. Results of the screen are shown in Figures 4.31-4.35. Amylase activity was highest between 48 and 55 hours of incubation, depending on the fungal isolate. Maximal amylase activities of supernatants harvested during 60 hours incubation were between 0.24 µmoles/min/ml and 0.46 µmoles/min/ml (Table 4.22). There was no correlation between fungal biomass and blastospore formation, as had been found in previous experiments.

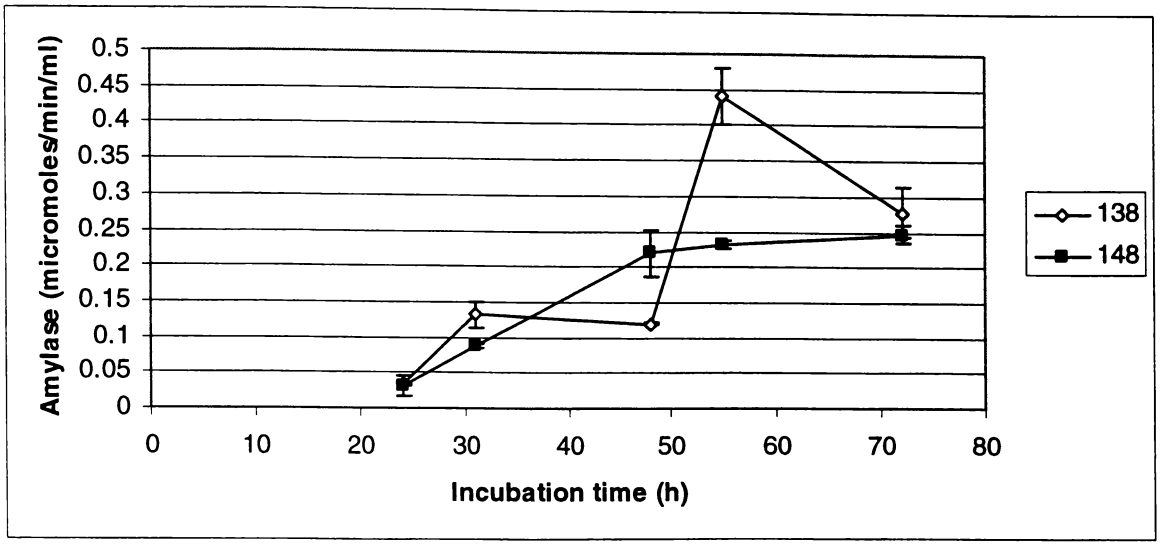


Figure 4.31: Amylase activities of *O. floccosum* #138 and #148. Vertical bars represent the range of values obtained for duplicate cultures.

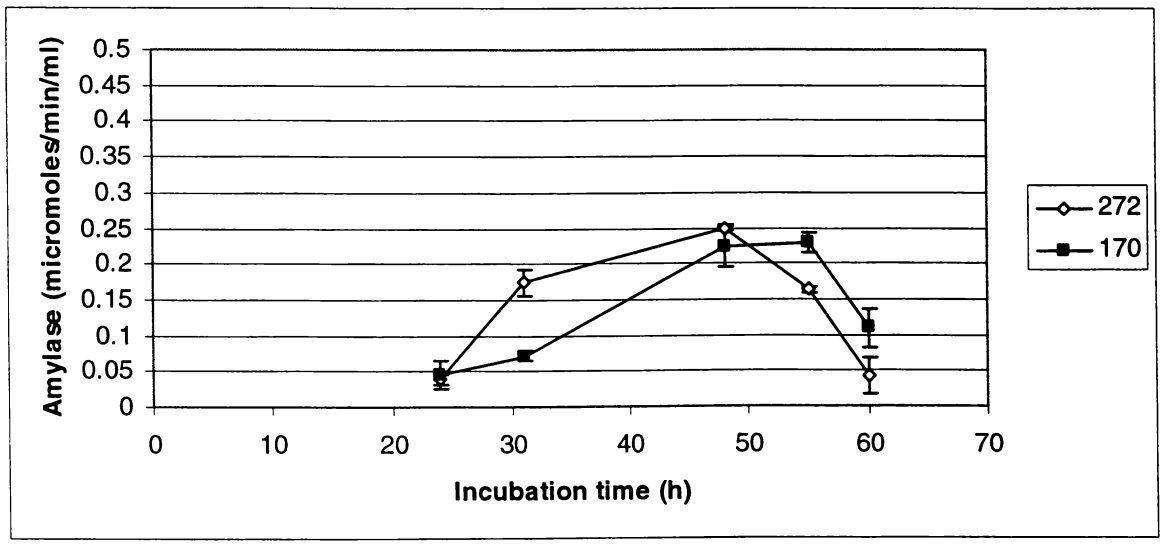


Figure 4.32: Amylase activities of *O. piceae* #272 and #170.

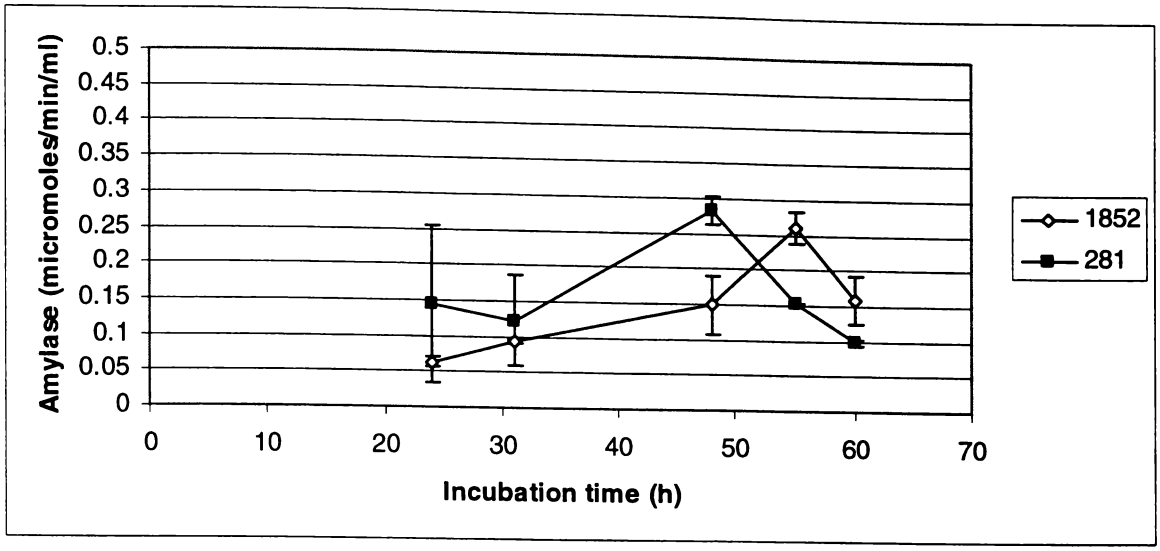


Figure 4.33: Amylase activities of *L. procerum* #1852 and #281.

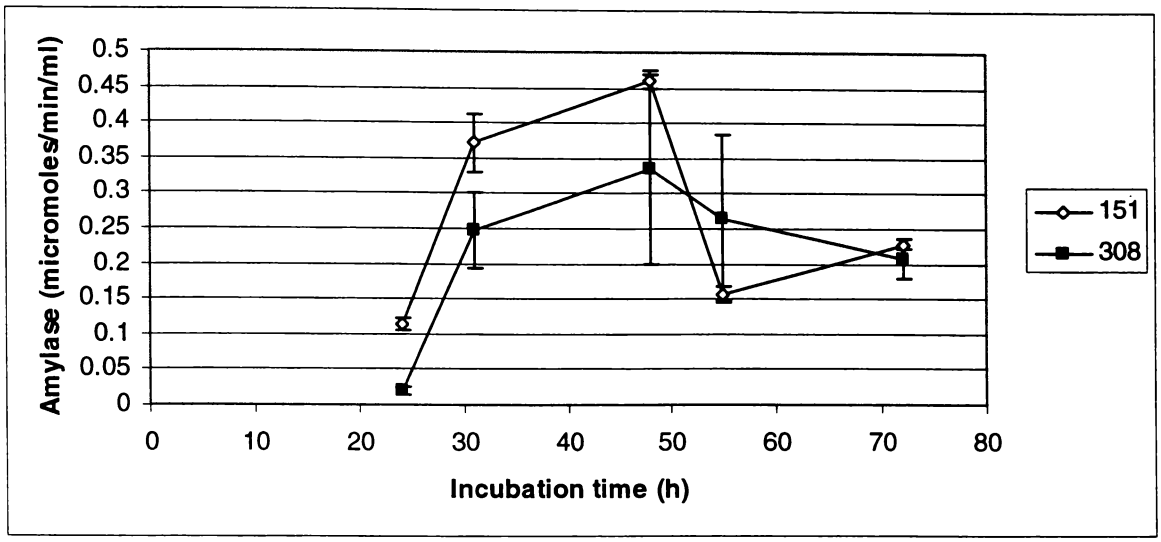


Figure 4.34: Amylase activities of *O. pluriannulatum* #151 and *O. ips* #308.

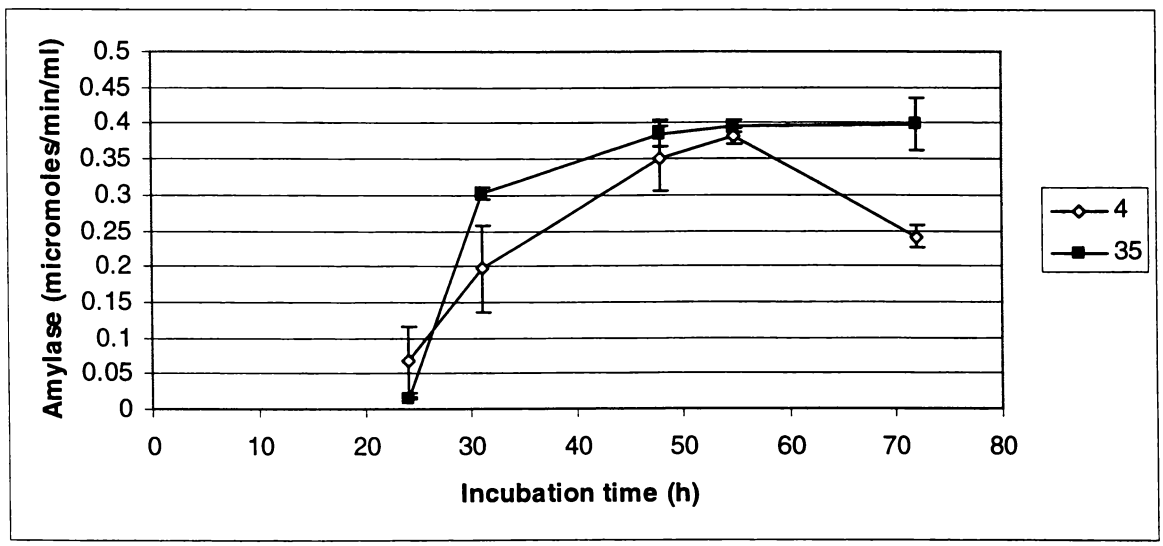


Figure 4.35: Amylase activities of *S. sapinea* #4 and #35.

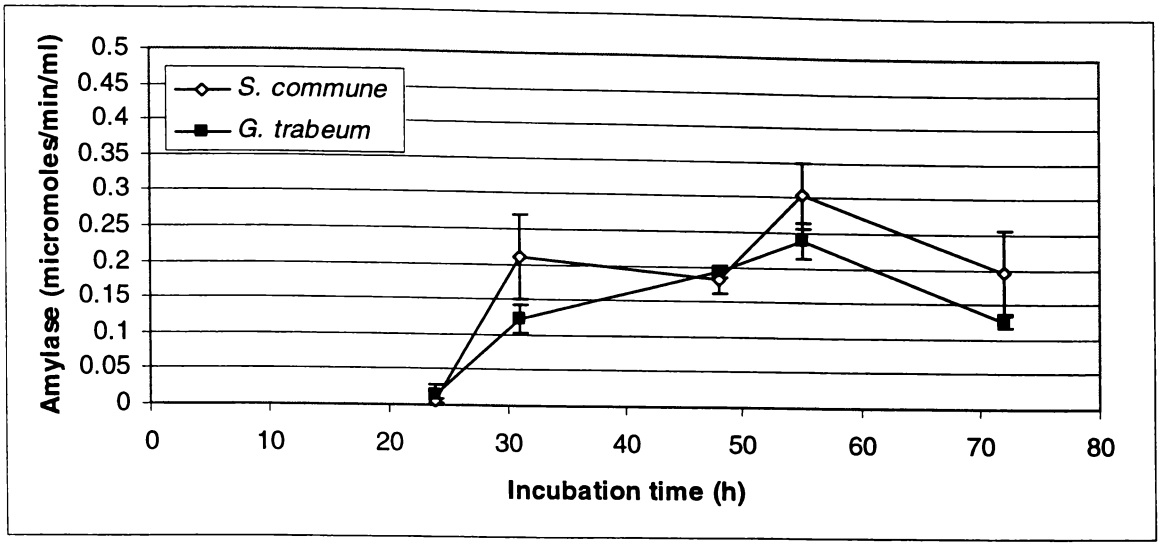


Figure 4.36: Amylase activities of *S. commune* and *G. trabeum*.

Table 4.22: Amylase activities of sapstaining and decay fungi after 72 hours incubation. Values represent the mean of duplicate cultures (for range of values, see Figures X-X).

Isolate	Amylase activity (µmoles/min/ml)	Fungal biomass (g)	Amylase activity (µmoles/min/ml/g)	Blastospores per ml	pH
S. sapinea #35	0.40	0.37	1.24	n.a.	2.7
S. sapinea #4	0.24	0.30	1.30	n.a.	2.9
O. floccosum #138	0.28	0.22	2.00	n.d.	2.7
O. floccosum #148	0.25	0.20	1.20	$1.1 \cdot 10^8$	2.8
O. ips #308	0.21	0.06	7.17	n.d.	5.5
O. pluriannulatum #151	0.23	0.18	2.56	$2.2 \cdot 10^8$	3.0
L. procerum #1852	0.16	0.18	1.44	$0.8 \cdot 10^8$	4.1
L. procerum #281	0.10	0.15	1.87	$3.2 \cdot 10^8$	2.7
O. piceae #170	0.11	0.17	1.47	$0.9 \cdot 10^8$	2.9
O. piceae #272	0.04	0.10	2.50	$3.0 \cdot 10^8$	3.1
S. commune	0.20	0.08	3.88	n.a.	5.2
G. trabeum	0.12	0.06	4.00	n.a.	3.7

n.d. = not determined; n.a. = not applicable (species does not produce blastospores).

Amylase activities of *S. commune* and *G. trabeum* (Figure 4.36) were in the same range as activities of the sapstaining fungi. As had been found for the sapstaining fungi, enzyme activity of the decay fungi was most significant after 55 hours incubation and decreased afterwards. On an individual unit per equivalent biomass basis, the amounts of amylase produced by the sapstaining fungi tested were significantly higher than the amounts of xylanase, mannanase and pectinase. This confirms that sapstaining fungi preferably use nutrients like starch that are easily accessible in wood, as has been demonstrated by King and Eggins (1973), Nilsson (1974), Umezurike (1969), Tabirih and Seehann (1984) and Encinas and Daniel (1999). Most likely, amylase activity and digestion of starch partly

accounts for the small overall weight loss of 1 % which was measured for infected radiata pine samples during 16 weeks incubation (see Chapter 5).

5 Physical and chemical changes caused by sapstaining fungi

5.1 Introduction and Objectives

Strength loss is a more sensitive indicator of fungal deterioration than weight loss (Armstrong and Savory, 1959; Liese and Pechmann, 1959; Hardie, 1980). A disadvantage of weight loss determinations is the long incubation period required to obtain significant values. In addition, the performance of a biologically degraded material in normal service is not always reflected by weight loss determinations.

Among the strength properties of wood, toughness has proven to be the most sensitive indicator of decay (Chapman, 1933; Trendelenburg, 1940; Richards, 1954; Kennedy, 1958; Wilcox, 1978; Seifert, 1993). When weight losses caused by decay fungi reach 5-10%, losses in toughness of at least 60-80% can be expected (Wilcox, 1978). The properties that appear to be the next most sensitive to decay are the measurements of work associated with bending. Here, losses of 50-70% can be expected at weight losses of 5-10%. Modulus of rupture and modulus of elasticity also can be reduced by 60-70%. Most other strength properties may decrease less drastically in early stages of decay, even though all are essentially reduced in the late stages of decay. At 10% weight loss, one should expect losses of about 60% in compression perpendicular to the grain, 40% in compression parallel to the grain, 50-60% in tension parallel to the grain and about 20% in shear and hardness (Wilcox, 1978).

The strength of lumber in service refers to its ability to resist external forces and loads and is influenced by a large number of factors, e.g. (Cown, 1999):

- Regular wood features:
  - Wood density
  - Microfibril angle
  - Cambial age
  - Moisture content
  - Growth rate
- Irregular features (defects):
  - Knots
  - Grain deviation
  - Compression wood
  - Mechanical damage
  - Pith
- Design factors:
  - Member size
  - Load sharing
  - Load duration

In addition to these wood features, the results of strength properties testings and weight loss determinations are strongly influenced by the fungal species tested as well as inoculation and incubation methods and length of exposure time.

It was the principal objective of this chapter to determine the effect of sapstaining fungi on toughness of radiata pine sapwood and to measure weight losses caused by these fungi. It has been shown that weight losses caused by various *Ophiostoma* species on softwoods vary between 1-4 % after three months, but can be as high as 25 % on hardwoods (Seifert, 1993). The specific gravity of stained wood may also be reduced by 1 to 2 %, while compression strength parallel to the grain and modulus of rupture may be reduced by 1 to 5 % (Chapman and Scheffer, 1940). It is generally accepted that staining fungi do not dramatically alter the strength properties of wood, except for toughness (Seifert, 1993). Toughness (impact bending strength) is a measure of the energy required to cause rapid failure in a centrally loaded small wood specimen and is regarded as the most sensitive indicator of fungal decay in wood (Trendelenburg, 1940; Wilcox, 1978). Encinas *et al.* (1998) reported changes in toughness that correlated with weight losses in birch (*Betula verrucosa*), Scots Pine (*Pinus sylvestris*) and Caribbean Pine (*Pinus caribaea var. hondurensis*) after six months´ incubation with the bluestain fungus *Lasiodiplodia theobromae* (syn. *Botryodiplodia theobromae*). This is in agreement with earlier studies (Findlay and Pettifor, 1937; Thunell, 1952; v. Pechmann et al., 1964) showing toughness losses of up to 30 % during four months´ incubation in pine wood. Chapman and Scheffer (1940) reported extraordinarily high reductions in toughness of Southern pine of up to 75 % during an incubation period of only 30 days with *Ceratostomella pilifera*, *C. pini*, *C. ips* and *Graphium rigidum*. In contrast, Tabirih and Seehann (1984) found that compression and impact bending strength of Abachi (*Triplochiton scleroxylon*) did not decrease after 16 weeks´ exposure to *Botryodiplodia theobromae*.

There has only been one report on the effect of sapstain on toughness of *Pinus radiata* (Da Costa, 1955) in which closely matched specimens were inoculated with *Sphaeropsis sapinea* (*Diplodia pinea*) and four other unidentified Australian staining fungi for up to 12 weeks. No significant difference in toughness was detected between inoculated samples and controls.

Except for a recent study by Encinas et al. (1998) involving the tropical sapstaining fungus *L. theobromae*, there are no publications available in which strength losses of wood inoculated with sapstaining fungi have been investigated using modern microscopical techniques. Although microscopical analyses were mentioned in the earlier studies on the effect of sapstain on the properties of timber, e.g. by Findlay and Pettifor (1937, 1939a, 1939b) and Chapman and Scheffer (1940), these investigations were clearly limited due to the absence of the electron microscope. Also, to our knowledge, there are no studies in which the effect of *Ophiostoma* spp. on both strength and chemical properties of radiata pine have been evaluated.

In conclusion, there are contradictory reports in the literature about the influence of sapstaining fungi on toughness of wood. This is because strength properties testings and weight loss determinations are influenced by fungal species, wood species, inoculation and incubation methods. Care has to be taken when general conclusions for sapstaining fungi have been drawn from results which were obtained with one isolate of a particular species only. In addition, it has to be considered that controversy has surrounded the taxonomy of fungi belonging to the genera of *Ceratocystis*, *Ophiostoma* and *Ceratocystiopsis* (Halsted, 1890; Sydow and Sydow, 1919; Upadhyay and Kendrick, 1975). One fungal species may have been refered to differently by various researchers. When fungal species are being referred to in this thesis, their names are quoted as in the original reference.

Another goal of this chapter was to analyse the chemical components of the wood samples which had been inoculated with selected sapstaining and decay fungi and tested for toughness and weight loss. Specifically, infected samples and their corresponding controls were analysed for dichloromethane (DCM) extractives, Klason lignin, acid soluble lignin and carbohydrates (arabinose, galactose, glucose, xylose and mannose) in order to determine if there were significant differences between samples and controls.

5.2 Literature Review: Influence of sapstaining fungi on wood properties

5.2.1 Wood structure (general) and characteristics of radiata pine

The structure and chemical composition of wood have a significant influence on its degradation by microorganisms and the resulting patterns of decay. Cell types, chemical composition and cell wall morphology may all govern the effects of enzymes on the wood substrate. The main wood components are cellulose, hemicelluloses and lignin in various proportions, the chemical structures of which are described in Chapter 4. Large differences in the chemical components exist among various wood species (Fengel and Wegener, 1989) and also within different types of cells within wood (Eriksson et al., 1990) which may explain differences in the degradative potential of fungi.

The lignin content of angiosperms is generally lower than that of gymnosperms. The type of lignin found in hardwoods and softwoods is also different. The basic structural unit of lignin may be substituted in two or three positions. The addition of one methoxyl group to the phenol ring results in a guaiacyl unit, the addition of two methoxyl groups results in a syringyl unit. Hardwoods contain varying ratios of syringyl and guaiacyl types of lignin whereas conifers have primarily guaiacyl lignin (Fengel and Wegener, 1989). The greatest concentration of lignin is found in the middle lamella, however, most (60% to 80%) of the lignin is located in the secondary wall (Fergus et al., 1969; Musha and Goring, 1975) because it constitutes a large proportion of the total cell wall area. The distribution of hemicellulose parallels that of lignin within the wall (Parameswaran and Liese, 1982).

Hemicelluloses surround the cellulose microfibrils and occupy spaces between fibrils. Crystalline and amorphous forms of cellulose occur within the cell wall. Cellulose molecules form microfibrils, and these form fibrils. A model depicting the arrangement of lignin, hemicellulose and cellulose within the cell wall has been proposed by Kerr and Goring (1975). In this model, a matrix of lignin and hemicellulose encrusts the cellulose fibrils (Figure 5.1).

The woody cell wall is composed of various layers. The middle lamella and primary wall make up the compound middle lamella which is located between the secondary walls of adjacent cells (Core et al., 1979). The secondary wall consists of three layers designated S_1, S_2 and S_3 with the S_3 being closest to the cell lumen. The S_2-layer which is usually the largest, is the middle layer. In each layer, the cellulose occurs in different microfibrillar orientations around the cell axis (Figure 5.1).

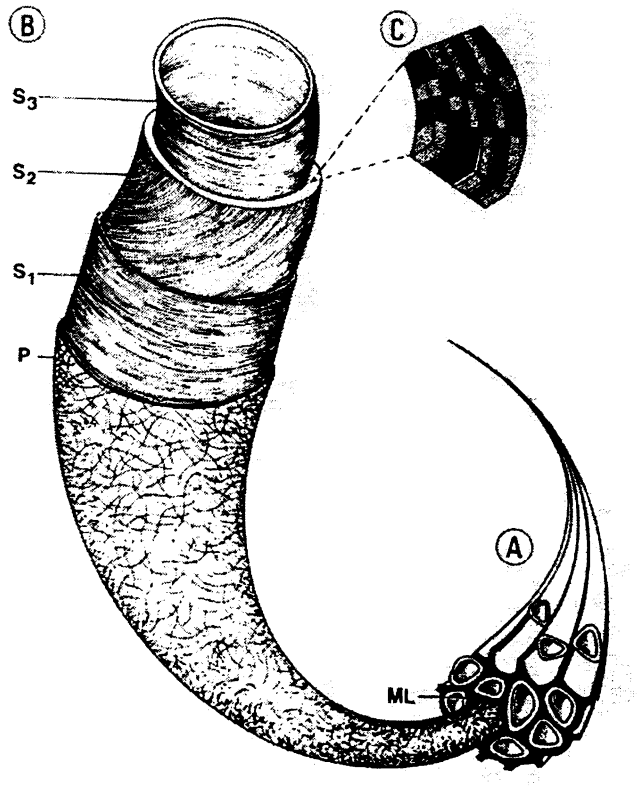


Figure 5.1: Structure of cell wall layers in tracheids and ultrastructural arrangement of lignin and carbohydrates in the secondary wall (Eriksson et al., 1990). (A) Tracheids, (B) Cell wall layers, (C) Ultrastructural arrangement of lignin and carbohydrates in the secondary wall, redrawn from Kerr and Goring, 1975; black: lignin-hemicellulose matrix; white: hemicellulose; stippled: cellulose fibrils.
ML: Middle lamella; P: Primary wall; S_1, S_2, S_3: layers of the secondary wall. Some wood species have an additional warty layer (not shown) over the S_3.

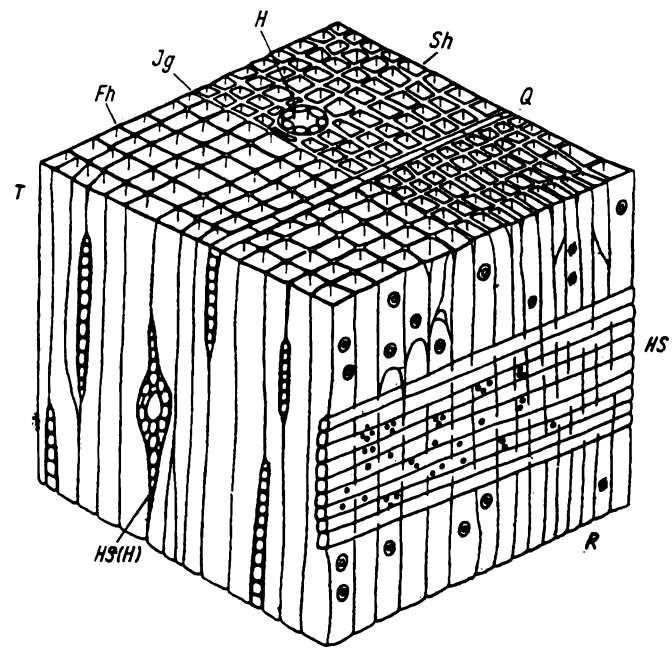


Figure 5.2: Block of sound softwood (Wagenführ, 1989). Q cross-section, T tangential section, R radial section, Fh earlywood tracheids, Sh latewood tracheids, Jg growth ring boundary, HS wood ray, H resin canal, HS resin conducting wood ray.

Gymnosperm wood is composed of tracheids and parenchyma cells that occur in latewood or earlywood regions (Figure 5.2). The axially oriented tracheids account for more than 95% of radiata pine wood by volume, and their length varies from 1 mm near the pith to 4 mm in outer wood (Harris, 1991). Average tracheid properties in juvenile and mature wood are compared in Table 5.1. In radiata pine, juvenile wood can be described as the region surrounding the pith in which tracheids are relatively short with thin walls, wood density is lowest, and microfibril angles and spiral grain highest (Cown, 1999). For practical purposes, it has been commonly defined as ten growth rings from the pith, and is considered to extent upwards in the tree in a cylinder.

Table 5.1: Typical tracheid dimensions in radiata pine. Source: Cown (1999).

Wood type	Property	Earlywood	Latewood
Juvenile	Length (mm)	2.0	2.5
	Diameter (μm)	30.0	20.0
	Wall thickness (μm)	3.0	4.0
Mature	Length (mm)	3.5	4.0
	Diameter (μm)	40.0	30.0
	Wall thickness (μm)	4.0	5.0

Axial parenchyma is sparse in radiata pine (Harris, 1991) so the bulk of parenchyma cells is contained in the wood rays. Wood rays which extent radially through the stem, crossing the axial tracheids at right angles, are uniseriate but multiseriate when resin canals are present. At the margins of a ray, one or more rows of thicker-walled ray tracheids which show dentations (tooth-like thickenings) can often be distinguished.

Axial resin canals are lined with thin-walled epithelium (short, brick-shaped parenchyma cells), normally five to six cells thick, which can be seen in transverse sections. In the living tree, the parenchyma cells secrete resin into these canals.

Pits allow water to pass from cell to cell in the living tree, and with few exceptions appear as pit pairs, i.e., one on either side of the double wall between adjacent cells. Pit pairs between parenchyma cells are simple, comprising a more or less straight-sided hole down through the secondary wall as far as the primary wall which forms an external closing membrane. Together with the membranes on either side of it which contain the cytoplasm of living cells, the pit membrane forms an osmotic system, allowing passage of water and dissolved substances in response to concentration gradients, hydrostatic pressure, and probably metabolic activity (Harris, 1991). However, unlike the membranes around the cytoplasm, the pit membrane is holopermeable, i.e. fully rather than selectively permeable.

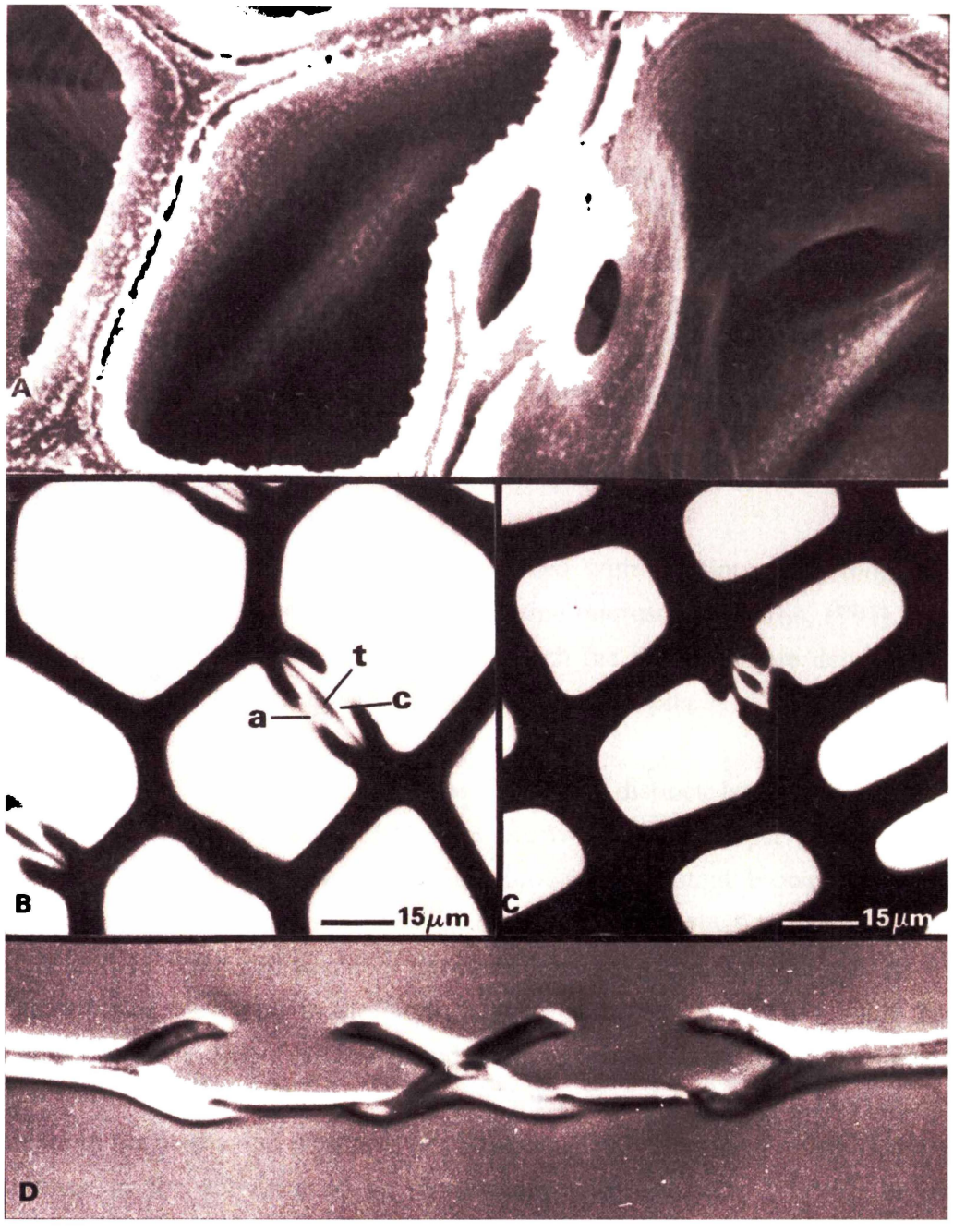


Figure 5.3: (A) Scanning electron micrograph of a bordered pit pair showing the pit aperture and a transverse cut through the pit chamber. (B) Section through an earlywood bordered pit pair; a = pit aperture; t = torus; c = pit chamber. (C) Section through a latewood bordered pit pair. (D) Aspirated earlywood pit pairs with tori closely pressed to the pit apertures. All micrographs from Harris, 1991.

Pit pairs between tracheids are bordered, i.e., the secondary wall develops into a dome-shaped structure over the pit, with a central cavity above the pit chamber thus formed (Figure 5.3). In bordered pits, the structure of the primary wall forming the membrane is also modified, first by the addition of a central thickening of microfibrils in circular arrangement to form a torus, and then by the development of radiating strands, with openings between them which join the torus to the periphery of the pit. When a pit pair is situated between a parenchyma cell and a tracheid, each cell type produces its own characteristic pit structure: a simple pit on the side of the parenchyma cell and a bordered pit on the side of the tracheid. This type of pit pair is called half-bordered, and in the case

of pine, pinoid. Pits between tracheids and parenchyma are variable in size and shape, numbering one to four per cross field.

The membranes of bordered pits in the earlywood of radiata pine are mostly unaspirated in fresh sapwood, but they aspirate during the process of drying and during heartwood formation (Harris, 1991). These practically irreversible changes, first described by Phillips (1933), have important consequences for the permeability of wood and on wood processing. In a latewood tracheid of radiata pine, the relative proportions of the torus, supporting strands, and pit chamber do not provide sufficient flexibility for the torus to move over and block off the pit aperture. Therefore, about half of the pits in latewood tracheids remain unaspirated during drying (Langrish and Walker, 1993).

Anatomically, radiata pine cannot be distinguished with certainty from lodgepole pine (*P. contorta*) or ponderosa pine (*P. ponderosa*) using microscopy (Harris, 1991). Details of value for identification include the degree to which the dentations are developed on the inside of ray tracheids and the appearance of the cross-field pits.

Radiata pine typically has creamy white sapwood with distinct, but not prominent, light-brown latewood bands (Harris, 1991). The heartwood is a deeper cream, sometimes pinkish when first cut, but changes to light brown or chestnut brown when dry. The sapwood zone in radiata pine is typically wide. Heartwood formation begins between the ages of 12 and 14 years and advances outwards from the pith at an average rate of about half a growth ring each year in the North Island and slightly less in the South Island of New Zealand (Cown, 1999). As heartwood is being formed, large amounts of extractives are deposited in the cells. Aliphatic compounds, terpenes and phenolic substances which accumulate in the heartwood (Hillis, 1968; Sjöström, 1981) may exert a significant amount of resistance to the activities of microorganisms (Eriksson et al., 1990). In addition, heartwood formation is characterized by a decrease in moisture content. Lower moisture content in combination with the increase in resin content results in some darkening of the wood colour (Cown, 1999). However, in contrast with many other species, the sapwood and heartwood of radiata pine does not differ greatly in appearance, and in dry wood, the two zones may be hard to distinguish.

Growth rings are relatively large and distinct in radiata pine, with visible differences between earlywood and latewood. The proportion of latewood typically ranges from 10% near the pith to 50% in the outerwood (Cown, 1999). Visual latewood percentage is a rough guide to average wood density (more latewood – higher density). The transition from earlywood to latewood is gradual compared with that found in species such as the Southern pines or Douglas fir, and radiata pine wood is considered even-textured by comparison.

A common feature of radiata pine is compression wood which is usually associated with leaning, swept or distorted stems. Its presence can be detected from what appear to be wider, darker-brown latewood bands (Harris, 1991). In compression wood, the secondary wall layers are reduced to only an S_1 and S_2, and the S_2-layer is very large with helical checks that follow the microfibrillar angle (Timell 1982, 1986). Severe compression wood can be most disadvantageous for a variety of uses. Another common defect encountered in radiata pine is internal checking which has been variously associated with moisture stress, wind and other stresses (Harris, 1991). Fine radial, sometimes diamond-shaped internal checks occur in freshly felled logs, extending from the first-formed earlywood, but each contained within a single annual growth layer.

5.2.2 Determination of toughness (impact bending strength)

Toughness (impact bending strength or shock resistance) is a combined measurement of bending strength and plasticity of a material. Different procedures exist for measuring the toughness of wood. According to British Standard 373 (1957), a load is applied at the centre of the test piece by the impact of a weight of 50 lb falling freely from successive heights increasing by regular increments. The test is continued until either complete failure or a deflection in 6 inches has been obtained. A different test has been developed by the Forest Products Laboratory of the United States Department of Agriculture around 1920 (Gerhards, 1968) which works in the following way: a pendulum which can be equipped with different weights and adjusted to several angles is allowed to drop. As it swings, it pulls a chain attached to the wood specimen to be tested. The chain is attached to a drum which rotates with the pendulum. The impact load is delivered at the midpoint of a 240-mm span. It is necessary to state the face of the specimen to which the force was applied because radial toughness is considerably lower than tangential toughness in softwoods (Gerhards, 1968; Kollmann, 1982). The energy absorbed in fracturing the specimen is calculated from the initial and final angles of swing of the pendulum and the machine constants (Mack, 1979) and measured in inch-pounds or Joules, respectively:

Toughness value = k (cos β - cos α)

with k = machine constant for the position of weight on the pendulum; α = initial angle of pendulum; β = final angle of pendulum.

The DENISON toughness tester (Figure 5.4) which was used in the experiments of this thesis operates basically in the same way as the machine developed by the Forest Products Laboratory of the United States Department of Agriculture. The standard sample size is 280·20·20 mm. However, in the experiments of the present study, a non-standard sample size of 115·15·15 mm was used to facilitate a uniform inoculation and incubation procedure of the samples.

Figure 5.4: DENISON toughness tester in Forest Research Timber Engineering Laboratory, Rotorua.

A testing machine developed in Europe (Amsler Universal wood testing machine), includes an option for toughness testing which also employs the energy of a falling pendulum. However, the Amsler machine is designed in such a way that the specimen is struck directly by the falling pendulum. At present, there is no satisfactory method available to convert data from one toughness test to another (Bodig and Jayne, 1993) although some correction factors have been developed (Gerhards, 1968).

An advantage of the toughness test is that large numbers of samples can be measured rapidly. A disadvantage is that the measuring unit (inch-pounds) cannot be related directly to other strength values. Nevertheless, toughness tests are very useful for a rough approximation of the decay capability by comparing infected samples with matching controls. In structural applications, the ability to sustain loads without excessive deflection and without breakage is critical.

5.2.3 Strength reductions caused by *Ophiostoma* sp. and *S. sapinea*

Among the earlier studies on the effect of sapstain on the strength of wood were those by Rudeloff (1897, 1899), von Schrenck (1903), Münch (1907, 1908) and Weiss and Barnum (1911) (quoted in Chapman and Scheffer, 1940). Included were tests of compression parallel to the grain, bending strength, toughness and hardness. According to Chapman and Scheffer (1940), the results were variable and generally inconclusive, probably due to differences in the original mechanical properties of the wood and the moisture content of the specimens at the time of testing. However, none of the results obtained in these investigations suggested that any practically significant decrease in strength properties resulted from sapstain. In the 1940s, there was a revival of research on the influence of sapstain on the strength of wood (Mayer-Wegelin et al., 1931; Chapman, 1933; Findlay and Pettifor, 1937, 1939; Jalava, 1938; Chapman and Scheffer, 1940). In these studies, more consideration was given to the moisture content of the wood and, with the exception of the study by Mayer-Wegelin et al. (1931), all or a substantial part of the work was done on matched specimens in which stain had been produced by artificial inoculation and laboratory-controlled incubation. It is important to consider that in using naturally stained wood material, there are no matching uninfected controls available, and it is usually unknown which and how many fungi have been contributing to the stain produced. In addition, it is unknown if decay fungi were present, and the early studies would not have recognized the possibility of soft rot which was not fully described until the 1950's by Savory (1954, 1955) and Liese (1959); for the influence of soft rot on strength properties, see Armstrong and Savory (1959); Liese and v.Pechmann (1959); Liese and Ammer (1964).

The results of the investigations from the 1940's generally confirmed the findings of the earlier ones (quoted in Chapman and Scheffer, 1940) but revealed that sapstain may reduce the toughness of wood considerably. Other wood properties, i.e. compression and bending strength, hardness and specific gravity, have been reported to be only slightly or not measurably affected by sapstaining fungi, with the exception of one study by Findlay and Pettifor (1939) involving the tropical wood species Obeche. In the following, investigations on the effect of *Ceratocystis*, *Ophiostoma* and other sapstaining fungi on strength properties of wood will be reviewed in more detail.

Chapman (1933) investigated the effect of *Graphium rigidum* and *Ceratostomella pilifera* on shortleaf pine (*Pinus echinata*), loblolly pine (*P. taeda*) and longleaf pine (*P. palustris*) using end-and sidematching samples. The initial objective of this study was to test the effects of different steam treatments (30 min at atm. pressure or 5 min at 12 pounds´ pressure) on the susceptibility of certain species of Southern pine to sapstaining fungi which was achieved by means of strength tests. Static bending, compression parallel to the grain and toughness (Forest Products Laboratory) tests were performed after an incubation time of 30 days. The sapstaining fungi caused an average reduction of approximately one

fourth in toughness and total work in bending in unheated wood and of nearly one half in steamed wood. The reduction in toughness was slightly greater than in total work. A comparison of the coefficients of variation for unheated wood indicated that inoculation of *P. echinata* or *P. taeda* with staining fungi increased the variability of the toughness values (Table 5.2). However, in heated wood, there was no indication of increased variability after inoculation. In two of the wood species investigated, heat treatment seemed to eliminate the differences in susceptibility of the wood to deterioration by the fungi.

Table 5.2: Effect of treatment on standard deviation and coefficient of variation in toughness tests. Source: Chapman (1933).

Treatment	Wood species	No. of samples tested	Standard deviation	Coefficient of variation
Not steamed, not inoculated	*P. echinata*	16	57.2	20.4
	P. taeda	25	65.1	12.9
	P. palustris	24	77.6	15.1
Not steamed, inoculated	*P. echinata*	16	87.3	42.8
	P. taeda	25	96.2	24.1
	P. palustris	24	87.2	22.3
Steamed*, inoculated	*P. echinata*	16	34.4	18.8
	P. taeda	25	35.6	11.1
	P. palustris	24	77.5	38.0

* Steamed for 30 min. at 100°C.

It was concluded by Chapman (1933) that even relatively mild heat treatments which were used for sterilizing wood may cause changes in the wood that make it a more congenial substrate for sapstaining fungi than unheated wood. The toughness of infected unsteamed wood was about 70% of that of the unstained controls. The toughness of wood which had been steamed prior to inoculation with sapstaining fungi was only 50% of that of the controls. The specific gravity was not perceptibly affected by either of the sapstaining fungi.

Findlay and Pettifor (1937) performed experiments to determine the specific gravity, toughness, compression strength parallel to the grain and hardness of Scots pine sapwood infected with two sapstaining fungi, *Ceratostomella coerulea* and one unidentified isolate. Two sets of tests were carried out; the first was done on matched samples some of which were sterilized by steaming and in which infection was artificially produced, and the second on samples selected from naturally stained lumber. Tests were performed after different periods of incubation, the longest being 26 weeks. It was found that for each of the two staining fungi which had been artificially infected, there was a progressive decrease in toughness with length of incubation. The decrease in toughness reached about 40% in the wood stained by *C. coerulea* and about 25% in the samples less heavily stained by the unidentified isolate (Table 5.3). The effect of the staining fungi on toughness was found to be much greater when the timber had first been sterilized by steaming at 100°C for 90 minutes.

Table 5.3: Results of toughness tests with artificially infected specimens. Source: Findlay and Pettifor (1937).

Treatment	Incubation time (weeks)	Specific gravity	Energy absorbed (inch-pounds)	Appr. average of cross area stained (%)
Control	-	0.408	116	-
Control sterilized	-	0.412	120	-
C. coerulea	3	0.414	109	8
C. coerulea	12	0.413	101	12
C. coerulea	26	0.407	70	88
Unidentified isolate	3	0.412	110	18
Unidentified isolate	12	0.409	109	28
Unidentified isolate	26	0.405	90	29

The sapstaining fungi tested did not affect compression strength parallel to grain. However, a slight decrease in specific gravity of 1-2% was determined to be statistically significant and attributed to depletion of storage material in the wood rays as there was no evidence of cell wall degradation. The results obtained by Findlay and Pettifor (1937) are in general agreement with the results obtained in previous studies. Findlay and Pettfor (1937) recommend that sapstained wood should not be used for applications in which a high degree of resistance to impact is required, for example in the construction of aircraft.

Two years later, Findlay and Pettifor (1939a) published additional work relating to their previous study in which they tested the effect of *C. coerulea* on the compression strength parallel to the grain. Tests were done non-destructively on the same specimens before and after two months' incubation. Samples were divided into two broad groups at the end of incubation, those with very slight or no staining and those with moderate to heavy staining. There was a very small (0.7%) decrease in the modulus of elasticity for the lightly stained material which was not statistically significant. The slightly greater (2.8%) decrease for the more heavily stained timber was found to be significant. From a practical point of view, however, the decrease, even for the more heavily stained timber, was found to be insignificant.

Chapman and Scheffer (1940) performed compression strength tests parallel to the grain, toughness tests (Forest Products Laboratory) and static bending tests with Southern pine inoculated with *Ceratostomella pilifera*, *C. pini*, *C. ips* and *Graphium rigidum* for 30 days. Side- and end-matching pairs were used, and most specimens were steam-heated for 30 min. at 100°C whereas a few were heated for 5 min. at a steam pressure of 12 pounds. Toughness and work to maximum load were both regarded as an index of shock resistance and the only properties that were greatly affected. The greatest significant reduction in toughness was about 75 % and the smallest about 9 %. The greatest significant reduction in work to maximum load was about 41 % and the smallest about 14 %. In six out of the total 20 tests, the stain was accompanied by a significant reduction in specific gravity, the greatest amounting to 3.8 % and the smallest to 1.4 %. The effect of staining on

compression parallel to the grain was quite variable; in many cases an increase in this property was noted. This was probably due to moisture differences between samples and controls; the samples were found to be drier at time of testing than the controls. In five cases, there were significant reductions in compression strength which were between 5 and 10 %. However, these were caused only by one species, *G. rigidum*, and are not necessarily representative for all sapstaining fungi. Individually significant reductions in modulus of rupture, ranging from about 3 to 10 %, occurred in five out of nine tests. It should be stressed that the tests were performed with wood that had been sterilized by heat. Greater strength losses in heated wood can be explained by assuming that the heat causes hydrolysis of certain constituents of the cell wall. The four species of sapstaining fungi did not affect the wood with equal severity, and the same fungus did not have the greatest effect on all properties. Microscopic examination revealed that direct cell-wall penetration was common with *G. rigidum*, less common with all strains of *C. pilifera* and hardly occurring with *C. ips* and *C. pini*. The observed cell-wall penetration of *G. rigidum* hyphae in the tracheids also explained the reduction in specific gravity of the wood by this species which was almost 4 %. According to Findlay and Pettifor (1937), a reduction in specific gravity resulting strictly from a destruction of storage materials in the wood rays would probably not exceed about 2 % in Scots pine. In conclusion, a correlation between the abundance of direct cell-wall penetration by the fungi and reduction in toughness was suggested.

Interestingly, although *C. ips* and *C. pini* produced the darkest stain, they had the least effect on strength properties (Chapman and Scheffer, 1940). The intensity of stain therefore does not seem to be indicative of the influence on strength properties. In contrast, Findlay and Pettifor (1937) demonstrated that toughness loss in naturally infected pine was roughly proportional to the extent of stain (Table 5.4).

Table 5.4: Relation between percentage of stained area over the cross-section and toughness. Source: Findlay and Pettifor (1937).

Percentage area stained	Average specific gravity	Average toughness (inch-pounds)
0	0.399	84.1
0-39	0.392	79.2
40-69	0.388	77.5
70-100	0.400	73.7

In summary, Chapman and Scheffer (1940) suggested that fully developed sapstain in naturally infected pine may reduce specific gravity by 1 to 2%, compression strength parallel to the grain and modulus of rupture 1 to 4 or 5% and toughness 15 to 30%. Exceptions to these conclusions may be found in certain tropical wood species, for example obeche (Findlay and Pettifor, 1939b). Obeche samples stained with *B. theobromae* lost as much as 43% of toughness, 20% of bending strength, 7% in stiffness in unsterilized timber and 13% of specific gravity. With a reduction in specific gravity of

about 2%, the corresponding reduction in strength caused by sapstaining fungi did not appear to be much less than that caused by a decay fungus. The effect of sapstain on the specific gravity and strength of pine artificially inoculated with four species and with different strains of some of them was essentially the same as reported for prior, similarly controlled studies.

Toughness involves compression strength (the initial failure occurs on the compression side of the bent specimen) as well as tensile strength of the timber. Toughness is dependent not only on the load which the specimen can carry but also on the degree to which it will bend without actual fracture, i.e. on the tensile strength of the outer fibres of a specimen. The first tests to determine the effect of *C. coerulea*, *C. pilifera*, *Diplodia natalensis* on the longitudinal tensile strength of timber were done by Pettifor and Findlay (1946). Unsterilized samples were used as well as samples sterilized by autoclaving. After 64 days of exposure, losses in tensile strength due to attack by the two species of *Ceratostomella* and by *D. natalensis* were 5-6% and 17%, respectively; after 94 days, the losses were 11-12% and 14%, respectively.

Thunell (1952) confirmed the earlier investigations by Findlay and Pettifor (1937, 1939a). Sapstain caused by *Ophiostoma pini* produced small but statistically significant decreases in bending and compression strengths (up to 10%) of pine sapwood and a loss of up to 30% in impact bending strength after four months' incubation (Thunell, 1952). It was concluded that these effects had no practical significance in timber to be used for building work in which static strength properties are of major importance.

The only report on the effect of sapstain on strength properties of radiata pine was published by Da Costa (1955). No significant decrease was found in the bending strength and toughness of *Pinus radiata* samples which were inoculated with *S. sapinea* and incubated for up to 12 weeks. In the study by Da Costa (1955), closely matched specimens were used. Tests were done using green wood (moisture content 140-150%) as well as wood after air-drying to 12% moisture content. Additional strength tests were done using four other common unidentified Australian sapstaining fungi. The same result was obtained as with *S. sapinea*.

Von Pechmann et al. (1964) tested toughness of pine wood inoculated with 11 fungal species (Table 5.5), among them some soft-rot isolates, after an incubation time of 12 weeks. Out of the 11 species tested, seven caused a significant decrease in toughness, i.e. *C. piceae*, *C. minor*, *C. coerulescens*, *S. lundbergii*, *Alternaria humicola*, *A. tenuis* and *Aureobasidium pullulans*. The soft-rot fungus *Alternaria humicola* caused the highest toughness loss of all isolates tested (on average 12.1%). Weight loss was determined by the difference between dried samples and controls since the wood had not been equilibrated prior to inoculation. Weight losses were between 0.6% and 4.8% after 12 weeks and did

not correlate to toughness losses. Highest weight losses were caused by *Alternaria tenuis* (4.8%).

Table 5.5: Strength and weight losses caused by staining fungi after 12 weeks incubation.

Fungal species	Number of sets*	Weight loss (%)	Average toughness loss (%)	Level of statistical significance (%)
Ceratocystis pilifera	28	1.7	2.4	-
C. piceae	14	2.5	4.2	0.5
C. minor	14	2.0	3.6	1.0
C. coerulescens	7	1.8	9.3	0.5
C. penicillata	10	0.9	3.7	-
Scopularia lundbergii	13	1.1	3.2	1.0
S. corsicana	14	2.6	2.1	-
Alternaria humicola	12	1.3	12.1	0.5
A. tenuis	14	4.8	4.6	0.5
Aureobasidium pullulans	18	0.6	4.8	0.5
Macrophoma pinea	14	0.9	1.4	-

* One set consists of two samples and two matching controls.

The results confirmed previous work by Findlay and Pettifor (1937) for the same incubation time and by Thunell (1952) considering that the incubation time in this study had been four months. Data obtained by Chapman and Scheffer (1940) for a short incubation period of 30 days, however, could not be confirmed.

The effect of common sapstaining fungi on specific wood properties are summarized in Tables 5.6 and 5.7.

Table 5.6: Review of the effect of sapstaining fungi on strength properties of wood.

Fungal species	Wood species	Strength property	Reduction	Reference
Ceratostomella ips	*Pinus taeda*	Toughness	8-46%	Chapman and Scheffer (1940)
Ceratostomella pilifera	*Pinus taeda*	Toughness	36-71%	Chapman and Scheffer (1940)
Ceratostomella pilifera	*Pinus palustris*	Toughness	41-63%	Chapman (1933)
Ceratostomella pilifera	*Pinus palustris*	Modulus of rupture	3%	Chapman and Scheffer (1940)
Ceratostomella pilifera	*Pinus palustris*	Compression parallel to grain	0-6%	Chapman and Scheffer (1940)
Ceratostomella pilifera	*Pinus palustris*	Work to maximum load	25-42%	Chapman and Scheffer (1940)
Ceratostomella pilifera	*Pinus nigra*	Tensile strength (long.)	11-12%	Pettifor and Findlay (1946)
Ceratocystis piceae	*Pinus sp.*	Toughness	4%	v. Pechmann et al. (1964)
Ceratostomella pini	*Pinus taeda*	Toughness	30%	Chapman and Scheffer (1940)
Ophiostoma pini	*Pinus* sp.	Toughness	up to 30%	Thunell (1952)
Ophiostoma pini	*Pinus* sp.	Compression parallel to grain	up to 10%	Thunell (1952)
Ophiostoma pini	*Pinus* sp.	Bending perp. to grain	up to 10%	Thunell (1952)
Ceratocystis minor	*Pinus* sp.	Toughness	4%	v. Pechmann et al. (1964)
Ceratocystis coerulescens	*Pinus* sp.	Toughness	9%	v. Pechmann et al. (1964)
Ceratostomella coerulea	*Pinus sylvestris*	Toughness	40%	Findlay and Pettifor (1937)
Ceratostomella coerulea	*Pinus sylvestris*	Modulus of elasticiy	up to 3%	Findlay and Pettifor (1939a)
Ceratostomella coerulea	*Pinus sylvestris*	Surface hardness	2-10%	Findlay and Pettifor (1937)
Ceratostomella coerulea	*Pinus nigra*	Tensile strength (long.)	11-12%	Pettifor and Findlay (1946)
Graphium rigidum	*Pinus taeda*	Toughness	6-75%	Chapman and Scheffer (1940)
Graphium rigidum	*Pinus echinata*	Toughness	16-24%	Chapman (1933); Chapman and Scheffer (1940)
Graphium rigidum	*Pinus taeda*	Toughness	23-74%	Chapman (1933); Chapman and Scheffer (1940)
Graphium rigidum	*Pinus palustris*	Toughness	18-67%	Chapman (1933)
Graphium rigidum	*Pinus taeda*	Modulus of rupture	4%	Chapman and Scheffer (1940)
Graphium rigidum	*Pinus echinata*	Modulus of rupture	3-10%	Chapman and Scheffer (1940)
Graphium rigidum	*Pinus echinata*	Work to maximum load	25%	Chapman and Scheffer (1940)
Graphium rigidum	*Pinus taeda*	Work to maximum load	24-28%	Chapman and Scheffer (1940)
Graphium rigidum	*Pinus echinata*	Compression parallel to grain	5-10%	Chapman and Scheffer (1940)
Graphium rigidum	*Pinus taeda*	Compression parallel to grain	7%	Chapman and Scheffer (1940)
Leptographium lundbergii	*Pinus* sp.	Toughness	3%	v. Pechmann et al. (1964)
Diplodia natalensis	*Pinus nigra*	Tensile strength (long.)	17%	Pettifor and Findlay (1946)
Sphaeropsis sapinea	*Pinus radiata*	Bending strength	0%	Da Costa (1955)
Sphaeropsis sapinea	*Pinus radiata*	Toughness	0%	Da Costa (1955)
Botryodiplodia theobromae	*Triplochiton scleroxylon*	Toughness	43%	Findlay and Pettifor (1939b)
Botryodiplodia theobromae	*Triplochiton scleroxylon*	Bending strength	20%	Findlay and Pettifor (1939b)
Botryodiplodia theobromae	*Triplochiton scleroxylon*	Stiffness	7%	Findlay and Pettifor (1939b)
Botryodiplodia theobromae	*Triplochiton scleroxylon*	Toughness	0%	Tabirih and Seehann (1984)
Botryodiplodia theobromae	*Pinus sylvestris*	Toughness	20%	Encinas et al. (1998)
Botryodiplodia theobromae	*Pinus caribaea*	Toughness	25%	Encinas et al. (1998)
Botryodiplodia theobromae	*Betula verrucosa*	Toughness	60%	Encinas et al. (1998)
Botryodiplodia theobromae	*Pouteria duclitan*	Toughness	74%	Arenas et al. (1968)
Botryodiplodia theobromae	*Pouteria duclitan*	Bending strength	23%	Arenas et al. (1968)
Aureobasidium pullulans	*Pinus* sp.	Toughness	5%	v. Pechmann et al. (1964)

Table 5.7: Losses in weight and specific gravity caused by sapstaining fungi.

Fungal species	Wood species	Affected property	Reduction	Reference
Ceratocystis ips	*Nyssa sylvatica*	Weight	4.3%	Eslyn and Davidson (1976)
Ceratocystis ips	*Pinus* sp.	Weight	0%	Eslyn and Davidson (1976)
Ceratostomella ips	*Pinus taeda*	Specific gravity*	0%	Chapman and Scheffer (1940)
Ceratocystis piceae	*Fagus grandifolia*	Weight	11.4%	Eslyn and Davidson (1976)
Ceratocystis piceae	*Acer saccharum*	Weight	17.3%	Eslyn and Davidson (1976)
Ceratocystis piceae	*Pinus* sp.	Weight	0%	Bergman and Nilsson (1968, 1971)
Ceratocystis piceae	*Picea* sp.	Weight	0%	Bergman and Nilsson (1968, 1971)
Ceratocystis piceae	*Pinus radiata*	Weight	3.3%	Greaves (1973)
Ceratocystis piceae	*Pinus* sp.	Weight	2.5%	v. Pechmann et al. (1964)
Ceratostomella coerulea	*Pinus sylvestris*	Specific gravity*	1-2%	Findlay and Pettifor (1937)
Ceratocystis coerulescens	*Pinus* sp.	Weight	1.8%	v. Pechmann et al. (1964)
Ceratostomella pini	*Pinus taeda*	Specific gravity*	1.4%	Chapman and Scheffer (1940)
Ceratocystis pluriannulata	*Nyssa sylvatica*	Weight	3.8%	Eslyn and Davidson (1976)
Ceratocystis pluriannulata	*Pinus* sp.	Weight	0%	Eslyn and Davidson (1976)
Ceratocystis pilifera	*Nyssa sylvatica*	Weight	5.6%	Eslyn and Davidson (1976)
Ceratocystis pilifera	*Pinus* sp.	Weight	0%	Eslyn and Davidson (1976)
Ceratostomella pilifera	*Pinus palustris*	Specific gravity*	2.1-2.6%	Chapman and Scheffer (1940)
Ceratocystis minor	*Pinus* sp.	Weight	2.0%	v.Pechmann et al. (1964)
Ceratocystis stenoceras	*Nyssa sylvatica*	Weight	16.8%	Eslyn and Davidson (1976)
Ceratocystis stenoceras	*Pinus* sp.	Weight	0%	Eslyn and Davidson (1976)
Leptographium sp.	*Nyssa sylvatica*	Weight	5%	Eslyn and Davidson (1976)
Leptographium sp.	*Pinus* sp.	Weight	0%	Eslyn and Davidson (1976)
Leptographium lundbergii	*Pinus* sp.	Weight	1.1%	v. Pechmann et al. (1964)
Sphaeropsis sapinea	*Pinus radiata*	Weight	0.8%	Butcher (1968)
Graphium rigidum	*Pinus taeda*	Specific gravity*	3.0-3.8%	Chapman and Scheffer (1940)
B. theobromae	*Triplochiton scleroxylon*	Specific gravity*	13%	Findlay and Pettifor (1939b)
B. theobromae	*Pinus sylvestris*	Weight	4%	Encinas et al. (1998)
B. theobromae	*Pinus caribaea*	Weight	5%	Encinas et al. (1998)
B. theobromae	*Betula verrucosa*	Weight	9%	Encinas et al. (1998)
Aureobasidium pullulans	*Pinus* sp.	Weight	1.7-2.1%	Seifert (1964)
Aureobasidium pullulans	*Pinus* sp.	Weight	0.6%	v. Pechmann et al. (1964)

* based on ratio of oven-dry weight / volume at time of test.

The toughness of sapstained southern pine salvaged after beetle-attack was investigated by Sinclair et al. (1979). Approximately 1200 small toughness specimens were prepared from green, healthy and beetle-killed southern pine at various times after foliage fade. The latter samples were free of defects with the exception of sapstain; however, the causative agents of sapstain were not identified. Toughness of the wood generally decreased with increased time between foliage fade and harvesting of the beetle-killed timber. The majority of the loss in toughness occurred with the first warm season following the death of a tree regardless of the time of foliage fade. Relatively little loss occurred in subsequent warm periods. Clear wood taken from top logs of dead trees showed a greater reduction of toughness than that from corresponding butt logs. It was unknown if the results could be applied to full-size structural lumber. However, it was suggested that caution should be exercised when lumber sawn from trees which were dead for extended periods of time is used in dynamic stress conditions.

5.2.4 Strength reductions caused by *Botryodiplodia theobromae*

It has been rather generally assumed that all sapstaining fungi derive their nutrients from the reserve food materials in the medullary rays. While this may be true for the majority of fungal species which cause sapstain in coniferous wood, results show that other so-called sapstaining fungi, for example *B. theobromae*, may in fact cause slight incipient decay (Umezurike, 1978; Encinas and Daniel, 1995; Encinas et al., 1998).

Among the earliest mechanical strength tests involving *B. theobromae* were those by Findlay and Pettifor (1939b) on small selected samples of Obeche (*Triplochiton scleroxylon*) which were inoculated with *B. theobromae* for 6 and 12 weeks. Very few cases of direct cell-wall penetration were observed; penetration of the cell walls occurred almost entirely through the pits. However, samples stained with *B. theobromae* lost as much as 43% of toughness, 20% of bending strength, 7% in stiffness (in unsterilized timber) and 13% of specific gravity. Chemical analysis of the heavily stained samples showed that the fungus caused a marked depletion in the content of water soluble components, and that it also attacked the cellulose, pentosans and lignin, behaving like a typical white-rot fungus.

Tabirih and Seehann (1984) did not find a reduction in toughness caused by *B. theobromae* on Abachi wood. However, Encinas et al. (1998) determined toughness losses which correlated with losses in weight found in birch (*Betula verrucosa*), Scots pine (*Pinus sylvestris*) and Caribbean pine (*Pinus caribaea* var. *hondurensis*) over a six months period after inoculation with *B. theobromae*. Toughness and weight losses were highest in birch and reached almost 60% and 9%, respectively, which was about twice as high as for the pine species. Higher strength losses at later stages of incubation were strongly correlated with the degree of degradation of parenchyma cells. Encinas and Daniel (1999) also determined that weight losses of 8% in birch, 3.5% in Scots pine and 4.5% in Caribbean pine caused by *B. theobromae* were correlated with depletion of non-structural components which supported the early, explosive growth of the fungus in the three wood species. When these components became limited, growth in the pine species was suppressed while further development was supported by weak cell wall degradation in birch.

Arenas et al. (1968) observed 74% reduction in toughness and 23% loss in bending strength in the tropical hardwood Duklitan after three months incubation with *B. theobromae*. A decrease in static bending strength caused by *B. theobromae* was also found on poplar wood (Pinheiro, 1971) and on rubber wood (Florence et al., 1998). There was no significant reduction in compression strength due to infection of rubber wood (Florence et al., 1998) and Abachi wood (Tabirih and Seehann, 1984) by *B. theobromae*.

Results from Florence et al. (1998) indicated significant differences in density of rubber wood samples inoculated with *B. theobromae* and controls after four months' incubation.

Tabirih and Seehann (1984) reported a slight reduction in density of *Triplochiton scleroxylon* stained with *B. theobromae* which was in accordance with the consumption of accessory compounds, especially starch, in the parenchymatous tissue. Umezurike (1978) observed that the colonization of wood blocks by *B. theobromae* was similar to that of soft-rot fungi, with cavity formation in the S_2-layer of the secondary wall.

The effect of *B. theobromae* on toughness, bending strength, specific gravity and weight of several wood species was summarized in Tables 5.6 and 5.7.

5.2.5 Permeability characteristics of wood infected with sapstaining fungi

In addition to strength effects, staining fungi increase wood permeability by breaking down ray parenchyma cells, by direct penetration of tracheid walls and by removing pit membranes (Lindgren and Scheffer, 1939; Lindgren, 1952; Liese and Hartmann-Fahnenbrock, 1953; Zabel and Morrell, 1992). Since pits represent the major limiting factor in fluid flow through fibers and tracheids, the removal of the pit membrane makes the wood markedly more receptive to movement of fluids. As a result, wood infected with sapstaining fungi will wet and dry more rapidly than sound wood. These effects are particularly important in the finishing industry, since stained wood will absorb excessive solution and often finish unevenly. This material will also absorb water more quickly in service, increasing the development of checks which provide entry points for decay fungi (Zabel and Morrell, 1992). Increased porosity might also lead to more rapid leaching out of preservatives.

King and Eggins (1973) suggested that the cellulolytic and pectinolytic activities of microfungi considerably influence their ability to enhance the permeability of wood. Two factors were considered important with regard to the ability of microfungi to enhance the permeability of wood: the ability of colonizing organisms to produce wood degrading enzymes and the presence of suitable environmental conditions (nutrients etc.) which are necessary for the production of wood-degrading enzymes. Suolahti and Wallén (1958) had reported earlier that a significant improvement in permeability of pine sapwood could be obtained by treatment with pectinase.

Ray tissue and pit margo microfibrils are particularly susceptible to the action of pectinases and cellulases. Nicholas and Thomas (1968) observed that pectinase degraded pit membranes and ray parenchyma cell walls of loblolly pine whereas hemicellulase altered the pit membrane only to a limited degree. Cellulase removed the margo fibrils and partially degraded the torus.

The increased permeability of fungal colonized wood has been exploited to improve the penetration of preservatives in difficult-to-treat wood species like spruce and Douglas fir (Bauch et al., 1970). Treatments consisted of ponding and commercial enzyme solutions.

Ponding increased the radial permeability of green and air-dry sapwood of spruce considerably but slowly over 16 weeks, whereas in pine, improvement of the penetrability occurred fast. Pre-treatment with a commercial pectinase improved permeability satisfactorily only in green wood. Both ponding and pretreatment with enzymes did not cause any noticeable improvement in permeability of heartwood. Bauch et al. (1970) also determined that impact bending strength of wood subjected to ponding and enzyme treatment was reduced by about 75%. In contrast, Meyer (1974) reported decreased creosote permeability of Douglas fir and white spruce and no influence of enzyme treatments on toughness. However, only mild enzyme treatments were employed. In comparison, creosote permeability of lodgepole pine was substantially increased by enzyme treatments.

5.2.6 Susceptibility of wood infected with sapstaining fungi to decay

The colonization of radiata pine by air-borne fungi has been recorded to proceed successively from sapstaining fungi to moulds to rot fungi (Butcher, 1968a). This succession indicates that early-colonizing sapstaining fungi might render the wood substrate more susceptible to attack by decay fungi. However, the belief, prevalent in lumber markets, that stained wood is more susceptible to subsequent decay has not been adequately proven or disproven (Seifert, 1993). In general, conditions which favour the development of stain may also favour the development of decay. Greater hygroscopicity in stained wood tends to create conditions conducive to fungal growth for longer periods, increasing the risk of fungal decay (Zabel and Morrell, 1992).

Findlay (1939) mentioned an investigation by Johann (1931) in which it was found that timber which contains living sapstaining fungi is more resistant against decay fungi than sound wood. However, when the sapstaining fungi were dead, no difference in resistance against decay could be detected. On the other hand, Findlay (1939) found slightly more decay in stained wood than in sound wood. It was suggested that this was due to greater porosity in sapstained wood which absorbs water more rapidly than clean wood. Findlay (1939) concluded, however, that slight differences in decay resistance are without practical significance.

Von Pechmann et al. (1964) inoculated pine wood samples with *Aureobasidium pullulans*, *Alternaria tenuis*, *Ceratocystis minor* and *Ceratocystis pilifera* and incubated these for 40 days. The samples were subsequently inoculated with several brown rot fungi, *Poria vaporaria*, *Polyporus stipticus* and *Coniophora puteana*, and incubated for further 40 days. Toughness and weight loss experiments were used to determine the effect of the pre-infection on the decay capacity of the brown-rot fungi. The decay capacity of the brown-rot fungi alone was determined by comparison with untreated samples. Von Pechmann et al. (1964) found that there was higher weight and toughness loss in the sound wood samples than in the pre-infected samples. The pre-infection lead to a milder and slower

decay but in general, did not inhibit decay. The decay fungi were less inhibited by *Alternaria tenuis* than by the two species of *Ceratocystis*. It was also found that the additional infection with decay fungi did not significantly change the appearance of the wood infected with sapstaining fungi which is important for practical purposes. Decay may be present, although not obvious, in stained wood.

5.3 Material and Methods

5.3.1 Inoculation and incubation of wood samples

For experiments I and II, freshly cut log bolts (600 mm long, 350 mm in diameter) were selected from three different radiata pine trees. The 24-year-old trees originated from Tokoroa, Central North Island of New Zealand. For experiments III and IV, flat-sawn radiata pine boards (obtained from Putaruru sawmill, Carter Holt Harvey, North Island) were used. Side-matching specimens (110 mm long, 15·15 mm; non-standard size) were prepared from the sapwood (Figure 5.5). Samples with visible anatomical defects, knots, cross and spiral grain, were excluded. Eight specimens each were put into a resealable plastic bag and the corresponding side-matching controls into a different bag. Specimens were sterilised by gamma-irradiation (27.6 kGy) at Mallinckrodt Veterinary Ltd., Lower Hutt, prior to inoculation.

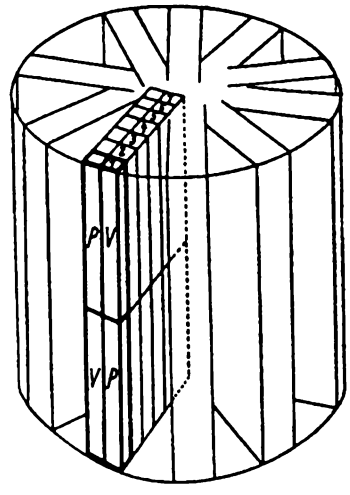


Figure 5.5: Preparation of side-matching samples from log bolts. P = sample, V = control.

Five ml of the starter cultures were added to 80 ml of medium I in 250 ml flasks and incubated at 120 rpm and 25°C in a rotary shaker for two days (sapstaining fungi; Table 5.8) or 10 days (*G. trabeum*, *S. commune* and *P. gigantea*). Prior to inoculation, blastospore concentrations and fungal dry weights of the cultures were determined (Table 5.9). Fungal biomass of *S. sapinea* and the decay fungi was determined using three additional flasks each. Cultures were filtered through pre-dried and weighed filter-papers which were subsequently dried in an oven at 80°C until constant weight was obtained. Fungal biomass of *S. sapinea*, *G. trabeum* and *S. commune* (means of duplicate cultures)

was 0.20g, 0.04g and 0.16g, respectively. Prior to inoculation, wood samples used for inoculation with the decay fungi were slightly dried out for 15 hours by opening the zip-locks of the plastic bags in a laminar flow cabinet. This was done to create conditions more favourable for colonization of the wood by the decay fungi.

Table 5.8: List of sapstaining fungi used in toughness and weight loss tests.

Species	Isolate #	Origin	Date isolated
Ophiostoma floccosum	138	Riverhead Forest, North Island	29-1-1997
O. floccosum	F13	Albino strain	-
O. floccosum	F40	Albino strain	-
O. piceae	272	Rotorua, North Island	21-4-1997
O. pluriannulatum	151	Abel Tasman National Park, South Island	30-1-1997
O. pluriannulatum	5040	Albino strain	-
O. pluriannulatum	F3410	Albino strain	-
O. ips	308	Waipa sawmill, Rotorua, North Island	9-4-1997
O. ips	294	Nelson, South Island	1-5-1997
O. ips	1191	Maharangi Forest, North Island	28-8-1997
O. ips	P36	Kinleith Forest, North Island	12-1-2000
Leptographium procerum	1852	Whitford Forest, North Island	29-1-1998
Sphaeropsis sapinea	4	Dome State Forest, North Island (pine cone)	Spring 1996
S. sapinea	35	Kinleith Forest, North Island (live tree)	13-12-96

Table 5.9: Inoculum determinations in toughness and weight loss experiments.

Isolate	Experiment	Blastospores per ml	Fungal dry weight (g)
O. floccosum #138	I	$18.90 \cdot 10^8$	n.d.
O. pluriannulatum #151	I	$74.50 \cdot 10^8$	n.d.
O. pluriannulatum #5040	I	$188.0 \cdot 10^8$	n.d.
S. sapinea #4	I	n.a.	0.20
O. ips #308	II	$1.08 \cdot 10^8$	n.d.
O. piceae #272	II	$2.25 \cdot 10^8$	n.d.
L. procerum #1852	II	$1.15 \cdot 10^8$	n.d.
G. trabeum	II	n.a.	0.04
S. commune	II	n.a.	0.16
O. ips #308	III	0 (mycelia only)	0.19
O. ips #294	III	$0.03 \cdot 10^8$	0.19
O. ips #1191	III	$3.60 \cdot 10^8$	0.26
O. ips #P36	III	$2.80 \cdot 10^8$	0.20
O. floccosum #F13	III	$0.81 \cdot 10^8$	0.35
O. foccosum #F40	III	$2.30 \cdot 10^8$	0.19
O. pluriannulatum #F3410	III	$2.60 \cdot 10^8$	0.21
S. sapinea #35	III	n.a.	0.30
G. trabeum	III	n.a.	0.20
P. gigantea	III	n.a.	0.24
O. ips #308	IV	$6.30 \cdot 10^8$	0.22
O. ips #294	IV	$4.50 \cdot 10^8$	0.20
S. sapinea #4	IV	n.a.	0.57
G. trabeum	IV	n.a.	0.37

n.d. = not determined; n.a. = not applicable (species does not produce blastospores).

Inoculation with the sapstaining fungi was performed by pipetting six ml of liquid culture onto eight wood samples in each bag and subsequent shaking of the bags. The decay fungi were inoculated by adding mycelia to the wood after decanting the growth medium. Controls were treated with six ml of sterile medium I. Four bags (32 samples; experiments I and II) or five bags (40 samples; experiments III and IV), were prepared per fungus and incubation period. Samples and controls were incubated at 25°C for 8 and 16 weeks (experiment I) or 16 weeks only (experiments II, III and IV).

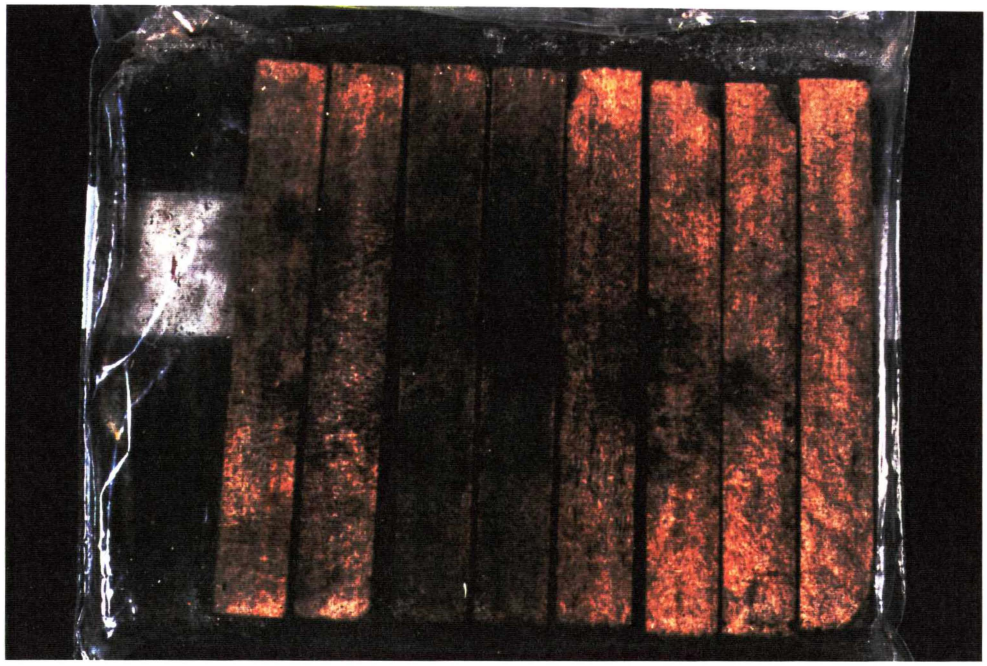


Figure 5.6: Incubation of samples used in toughness experiments using resealable plastic bags.

In experiment IV, for the determination of toughness and weight loss caused by *O. ips* #308, *S. sapinea* #4 and *G. trabeum*, an additional incubation method was used in order to achieve a more even discolouration of the wood samples. Pickle-jars were filled with 60 ml each of a medium containing 20 g malt-extract and 10 g agar per litre distilled water. Plastic lids were lightly screwed on the jars which were autoclaved for 20 minutes at 120°C. The lids were then tightened before placing each jar carefully on one of its sides. After the media had set, 24 jars each were inoculated with a mycelial plug of *O. ips* #308, *S. sapinea* #4 and *G. trabeum*. The mycelial plugs were placed in the centre of the agar. The same amount of jars were left uninoculated to serve as controls. The jars were sealed with lids and parafilm and incubated at 25°C for three weeks in an incubation chamber. The wood samples were conditioned to a moisture content of less than 130% since it had been found in experiments I, II and III that the moisture content had been too high for optimum fungal development. Six bags containing eight wood samples each and six bags with the corresponding eight controls were used for each fungus. Two wood samples were added to each jar, with one tangential side of the sample facing the top of the jar. The jars used for *G. trabeum* were equipped with two small sterile supports so the wood was not in

direct contact with the mycelium. The jars were sealed with lids and parafilm and were incubated at 25°C for 16 weeks.

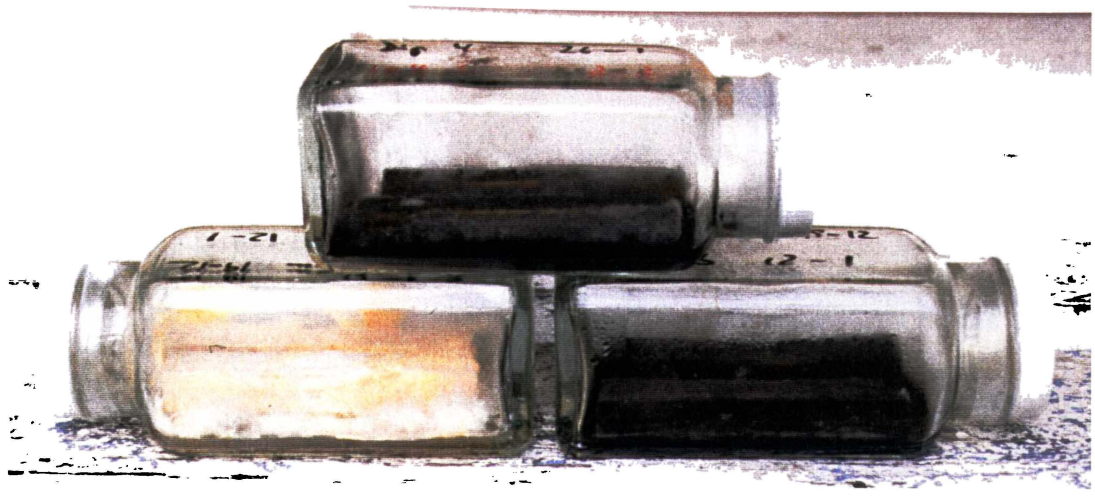


Figure 5.7: Incubation of samples used in toughness experiments using pickle-jars.

In summary, in experiment IV, *O. ips* #308, *S. sapinea* #4 and *G. trabeum* were inoculated onto wood samples in pickle-jars and resealable plastic bags, and *O. ips* #294 was inoculated onto wood samples in plastic-bags only. All samples in experiment IV were incubated for 16 weeks.

5.3.2 Toughness and weight loss testing

At the end of each incubation period, the samples were removed from the bags, visually assessed for colour and intensity of stain, conditioned to 14 % equilibrium wood moisture content and weighed. The wood moisture content (%) of a sample was determined by using the following equation:

(wet weight minus dry weight / dry weight)·100.

Toughness was determined using a pendulum-type machine (DENISON, Leeds, U.K.; Figure 5.4) set at an initial angle of 60° and weight position five (machine constant 80.9 Joules). In each case, the impact occurred on the radial face of the sample, i.e. parallel to the growth rings (British Standard 373, 1957). After toughness testing, samples were oven dried and dry weights of the specimens were obtained. In experiment III, one set (bag) of samples of each treatment was not dried but kept at 12% wood moisture content for microscopical analysis (Chapter 6). Weight loss was regarded as the difference in dry weights between samples and controls.

5.3.3 Statistical analysis

The data were analysed on a bag-to-bag basis and not on a sample-to-sample basis. Weight loss was defined as the difference between dry weights of inoculated samples and controls. Results from experiments I to IV were analysed independently. Data were subjected to

analysis of variance (experiment I only) and residual maximum likelihood using Genstat software (Patterson and Thompson, 1971; Anon., 1993).

5.3.4 Chemical analysis of samples used in toughness and weight loss tests

After toughness testing, radiata pine samples which had been inoculated with *O. floccosum* #138, *O. pluriannulatum* #151, *S. sapinea* #4 (experiment I), *O. ips* #308 and *G. trabeum* (experiments II and III) and incubated for 16 weeks were analysed for extractives, Klason lignin, acid soluble lignin and carbohydrates (arabinose, galactose, glucose, xylose and mannose). Eight samples of one set and eight controls of the corresponding set were air-dried and ground prior to extraction with dichloromethane (samples from toughness and weight loss experiment I) or *t*-butyl methyl ether (samples from experiments II and III) in a Soxtec apparatus. The extracts were concentrated and weighed to determine the level of extractives. Extractive-free wood samples (250 mg) were analysed after sulfuric acid hydrolysis according to TAPPI Method T 249 cm-85. Klason lignin (acid insoluble lignin) was gravimetrically determined, while acid soluble lignin was determined by absorbance at 205 nm according to TAPPI Useful Method UM 250. Wood carbohydrates (expressed as anhydro-monosaccharide residues) were determined by high performance anion-exchange chromatography using a Dionex CarboPac PA-1 column (Pettersen and Schwandt, 1991). Each sample was analysed in duplicate, and the reported results are an average of the duplicates.

5.4 Results and discussion

5.4.1 Physical changes caused by sapstaining and decay fungi

Stain colour, judged visually after 8 and 16 weeks incubation, was most intense for the samples inoculated with the wild-type isolates of *S. sapinea*, *L. procerum* and *O. ips* whereas *Ophiostoma floccosum*, *O. piceae* and *O. pluriannulatum* caused a light, grey-brownish discolouration (Figures 5.8 and 5.9). The 16-weeks-samples were slightly darker than the 8-weeks-samples. In comparison, the samples inoculated with the albino-strains were significantly lighter. In the present study, no apparent difference in the fracture pattern between the samples inoculated with wildtype- or albino-strains and their controls was observed. Some of the samples infected with *G. trabeum* were extremely brittle due to severe decay and excluded from subsequent tests. Also, samples showing signs of mould contamination were discarded.

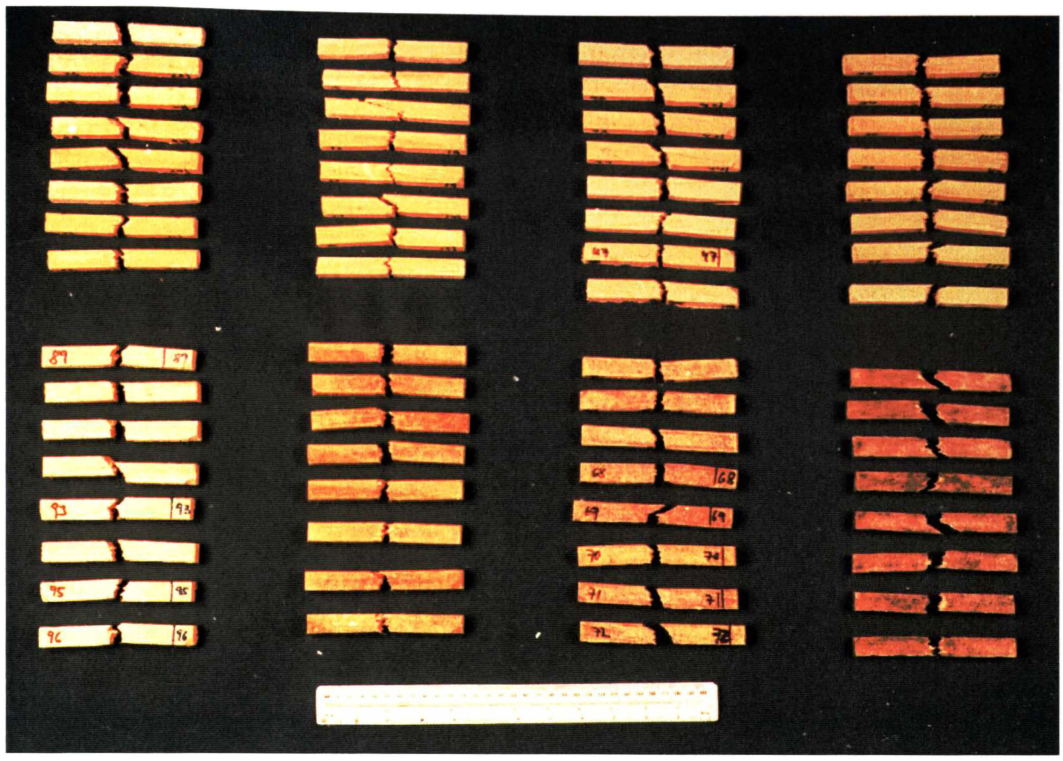


Figure 5.8: Radiata pine samples after toughness test (experiment I). Top row: controls; bottom row: matching samples, from left to right: *O. pluriannulatum* #5040 (albino-strain), *O. floccosum* #138, *O. pluriannulatum* #151, *S. sapinea* #4.

Figure 5.9: Radiata pine samples after toughness test (experiment II). Top row: controls; bottom row: matching samples, from left to right: *O. piceae* #272, *L. procerum* #1852, *O. ips* #308, *S. commune* and *G. trabeum* (moderately decayed).

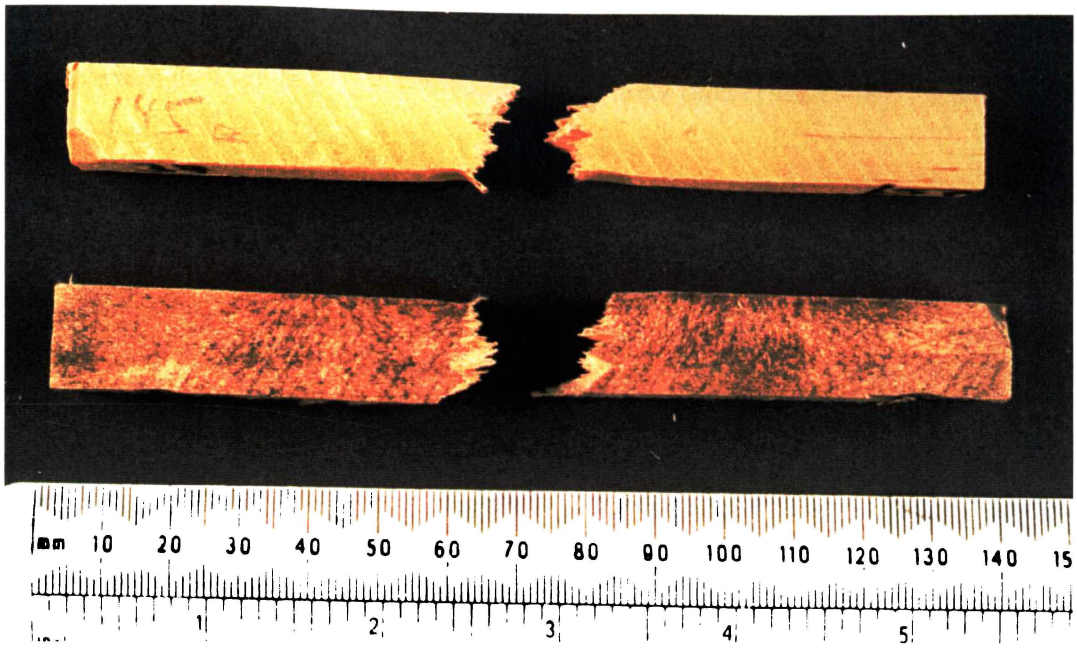


Figure 5.10: Radiata pine inoculated with *S. sapinea* #4 and matching control after toughness test (experiment I).

Mean toughness showed large variability on a bag-to-bag basis (Tables 5.10 to 5.14) because of the range of wood densities included and because toughness is very sensitive to small localized grain deviations (Fagan and McLain, 1983). However, variability of toughness between samples and controls of each set was low due to the use of side-matching specimens. The complete data from all toughness and weight loss experiments can be found in Appendix 3. Statistical analysis of the four experiments showed that there was no significant ($p<0.05$) difference in toughness between samples inoculated with sapstaining fungi and controls, except for *O. ips* #308 which caused 18% toughness loss without weight loss in experiment II.

In experiment I, overall toughness was reduced by 0.86 Joules (standard error of difference = 0.82) and overall dry weight by 0.10 g (standard error of difference = 0.05). The overall weight loss of inoculated samples in experiment I is slight, but statistically significant ($p < 0.05$). However, the reduction in weight is so small that it has to be considered insignificant from a practical point of view. Furthermore, there was no statistically significant difference in toughness and dry weights when comparing the two different incubation times.

In the statistical analysis of experiments II, III and IV the within set and between set information was combined to obtain one mean for the control. Residual maximum likelihood revealed that in terms of dry weight, only the samples inoculated with *G. trabeum* were significantly different ($p<0.05$) from the controls. It should be stressed that in this study, weight loss is defined as the difference in final dry weights between samples and controls. Usually, weight loss is expressed as a percentage of the original

weight of a sample. A direct relationship between the initial weight of a wood block and absolute weight loss has been established for radiata pine (Hedley and Armstrong, 1975).

Table 5.10: Mean toughness and weight loss values observed in inoculated radiata pine after eight and 16 weeks of incubation (experiment I).

Treatment	Replicates (no. of bags*)		Toughness (Joules)		Dry weight (g)	
Weeks	8	16	8	16	8	16
O. floccosum #138	3	3	23.42	24.29	12.37	13.00
Control	3	3	23.98	25.64	12.35	13.16
Sed**			2.46	2.46	0.14	0.14
S. sapinea #4	2	4	14.61	20.19	9.84	10.44
Control	2	4	13.08	20.77	10.02	10.51
Sed**			3.02	2.13	0.17	0.12
O. pluriannulatum #151	4	4	14.32	15.17	9.43	9.40
Control	4	4	14.63	18.63	9.53	9.51
Sed**			2.13	2.13	0.12	0.12
O. pluriannulatum #5040	4	3	15.14	25.01	9.37	10.35
Control	4	3	14.71	26.71	9.36	10.61
Sed**			2.13	2.46	0.12	0.14

*One bag contains 8 samples or controls.

**Sed = Standard error of difference.

Table 5.11: Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation (experiment II).

Treatment	Replicates (no. of bags*)	Toughness (Joules)	Dry weight (g)
L. procerum #1852	4	20.86	9.44
Control	4	18.67	9.51
O. piceae #272	4	28.81	9.65
Control	4	26.40	9.89
O. ips #308	4	17.70	10.21
Control	4	21.55	10.20
S. commune	4	24.04	12.00
Control	4	35.19	12.07
G. trabeum	4	10.63	9.15
Control	4	27.22	9.98
Sed**		1.55	0.26

*One bag contains 8 samples or controls.

**Sed = Standard error of difference between any fungus and control.

Table 5.12: Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation (experiment III).

Treatment	Replicates (no. of bags*)	Toughness (Joules)	Dry weight (g)
O. ips #294	5	21.39	10.43
Control	5	21.11	10.47
O. ips #308	5	20.61	10.41
Control	5	20.32	10.57
O. ips #1191	5	23.20	10.99
Control	5	22.47	10.75
O. ips #P36	5	20.18	10.96
Control	5	18.73	10.92
O. floccosum #F40	5	20.27	10.34
Control	5	21.62	10.78
O. floccosum #F13	5	21.45	10.42
Control	5	20.73	10.21
O. pluriannulatum #F3410	5	17.36	11.41
Control	5	18.15	11.23
S. sapinea #35	5	24.05	11.36
Control	5	19.80	10.98
G. trabeum	5	13.32	10.68
Control	5	18.76	10.80
P. gigantea	5	11.70	10.15
Control	5	13.91	10.47
Sed**	5	1.33	0.26

*For weight loss determination, 4 replicates (bags) were used per fungus. One bag contains 8 samples or controls. **Sed = Standard error of difference between any fungus and control.

Table 5.13: Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation in plastic bags (experiment IV).

Treatment	Replicates (no. of bags*)	Toughness (Joules)	Dry weight (g)
O. ips #308	5	22.32	11.02
Control	5	20.66	10.85
O. ips #294	5	21.17	10.95
Control	5	18.08	8.39
S. sapinea #4	5	21.80	10.76
Control	5	18.78	10.77
G. trabeum	5	17.05	10.38
Control	5	20.55	11.03
Sed**		1.18	0.22

*One bag contains 8 samples or controls. **Sed = Standard error of difference between any fungus and control.

Table 5.14: Mean toughness and weight loss values observed in inoculated radiata pine after 16 weeks of incubation in pickle-jars (experiment IV).

Treatment	Replicates (no. of bags*)	Toughness (Joules)	Dry weight (g)
O. ips #308	6	20.40	10.88
Control	6	15.05	11.03
S. sapinea #4	6	21.22	10.96
Control	6	21.78	10.77
G. trabeum	6	10.70	9.27
Control	6	19.91	10.76
Sed**		1.18	0.18

*One bag contains 8 samples or controls; 2 samples or controls each used per jar.

**Sed = Standard error of difference between any fungus and control.

Gloeophyllum trabeum caused a weight loss of 8 % in experiment II but has been shown to cause weight losses of up to 43% in Sitka spruce (Kirk and Highley, 1973) and up to 18% in Douglas-fir heartwood (Winandy and Morrell, 1993). Kreber and van der Waals (1999) determined weight losses caused by several strains of *Phlebiopsis gigantea* in a soil-block test ranging from 0.3% to 26.5% on radiata pine and 0.1% to 52.2% on European beech (*Fagus sylvatica*). *Phlebiopsis gigantea* #104 which was also used in this thesis research, caused 27% weight loss on radiata pine and 50% weight loss on European beech after 10 weeks of incubation (Kreber and van der Waals, 1999); in the same study, *G. trabeum* caused a weight loss of 71% on radiata pine.

In another recent study on the decay potential of selected New Zealand decay fungi, four isolates of *P. gigantea* caused dry weight mass losses in radiata pine between 4.5% and 7% after 18 weeks incubation using a method which simulated an above-ground test situation (Ah Chee et al., 1998). Butcher and Howard (1968) reported 17.5% weight loss caused by *P. gigantea* on radiata pine after 8 weeks using a soil-block method.

The weight loss caused by *G. trabeum* in the present study is comparatively low but may be attributed to the fact that green wood was used. The samples which were used for inoculation with the decay fungi were only slightly dried prior to inoculation. Sapstaining fungi are early colonisers of wood whereas Basidiomycete fungi usually require lower wood moisture contents for successful colonization (Butcher, 1968a). In addition, the same incubation conditions were employed for the decay fungi as for the sapstaining fungi. It is not surprising that under these conditions, *G. trabeum* and *P. gigantea* did not reach their optimum decay potential. Higher weight losses could be expected if a soil-block or Kolle-flask test had been used and the wood moisture content had been adjusted to its optimum for fungal growth prior to inoculation. An optimum wood moisture range of 40-60% and a maximum range of 80-210% have been reported for *Gloeophyllum* spp. (Schmidt, 1994).

In the present study, wood moisture levels of radiata pine were in the upper maximum range for this species due to the use of green wood. Initial wood moisture content of the samples used for inoculation with *P. gigantea* was between 163% and 185% which is above the optimum of 50-75% reported for this species (Butcher and Drysdale, 1991).

With regard to incubation temperatures, the decay fungi tested prefer temperatures above 25°C as was used in the present study. Optimum temperatures for mycelial growth are 35°C for *G. trabeum*, 28-35°C for *P. gigantea* and 30-36°C for *S. commune* (Schmidt, 1994).

In experiment III, neither *G. trabeum* nor *P. gigantea* caused any weight loss although the physiological conditions for fungal development were the same as in experiment II, and there were no significant differences in the initial wood moisture content of the samples used in experiments II and III (Table 5.15). However, since the basidiomycete cultures were grown in liquid culture as were the sapstaining fungi, it is possible that more liquid growth medium was added to the samples used in experiment III. Most of the growth medium was decanted before adding the the inoculum but some of it was used to transfer the fungal mycelium to the wood. Consequently, the samples in experiment III might have become wetter than the ones used in experiment II.

Table 5.15: Initial wood moisture content (%) of samples used for inoculation with *G. trabeum* in experiments II and III.

Treatment	Set	Initial wood moisture content (%) of samples in experiment II (61% toughness loss)	in experiment III (29% toughness loss)
G. trabeum	1	236	174
Controls	1	203	163
G. trabeum	2	114	170
Controls	2	108	169
G. trabeum	3	244	140
Controls	3	191	140
G. trabeum	4	153	166
Controls	4	114	160

Although overall density of the samples used in experiment II was lower than in experiment III (Table 5.16), it in unlikely that differences in the density of the samples have an effect on weight loss over an incubation period of four months. Hedley and Armstrong (1975) examined the decay capability of *G. trabeum* in the laboratory on 20-mm cubes of radiata pine. They found that rates of decay were faster in low-density wood, however, both low and high-density wood was decayed to maximum capacity (70% weight loss) after three months.

Table 5.16: Nominal density (oven-dry weight / volume at test) of samples used for inoculation with *G. trabeum* in experiments II and III.

Treatment	Set	Nominal density (kg/m^3)	
		in experiment II (61% toughness loss)	in experiment III (29% toughness loss)
G. trabeum	1	325	509
Control	1	345	502
G. trabeum	2	538	429
Control	2	549	428
G. trabeum	3	325	427
Control	3	335	440
G. trabeum	4	277	446
Control	4	334	451

In addition, the amount of inocula used cannot explain either why *G. trabeum* caused higher toughness and weight losses in experiment II than in III. In experiment II, the mycelial dry weight of the inoculum was 0.04 g compared to 0.20 g in experiment III. In conclusion, the most likely explanation for the higher decay potential of *G. trabeum* in experiment II is that the wood material used was, for genetic reasons, more susceptible to decay than the samples used in experiment III which were from a different source.

In experiment IV, *G. trabeum* caused slight, but statistically significant ($p<0.05$) weight loss when the wood samples were incubated in pickle-jars, but not when they were incubated in plastic-bags (Tables 5.13 and 5.14). At the same time, however, *G. trabeum* caused significant toughness loss which amounted to 46% (pickle-jars) and 17% (plastic-bags). Obviously, the lower wood moisture content of the samples which were incubated in pickle-jars promoted more severe decay by *G. trabeum* which resulted in higher toughness losses. With regard to the two sapstaining fungi incubated in pickle-jars in experiment IV, *O. ips* #308 and *S. sapinea* #4, the amount and distribution of the discolouration was the same as in the previous experiments, i.e. the slightly lower wood moisture content of the wood samples in experiment IV did not have an influence on the melanisation pattern of the samples.

According to Butcher and Drysdale (1991), *S. commune* failed to cause decay of radiata pine sapwood in laboratory tests with pure cultures which is in accordance with the results obtained in experiment II. Schmidt and Liese (1980) also reported that *S. commune* is a weak wood-destroying fungus. In a Kolle-flask-test, using 12 isolates of *S. commune* and fresh Scots pine as a substrate, a weight loss of only 0.5% on average after 3 months was determined (Schmidt and Liese, 1980). Higher weight losses may be expected when *S. commune* is growing in hardwoods since white-rot fungi preferably attack hardwoods (Schmidt, 1994).

All three decay fungi tested caused significant toughness loss without weight loss or without significant weight loss. *Gloeophyllum trabeum* reduced toughness by up to 61%, *S. commune* by 32% and *P. gigantea* by 16%. These results confirm that the toughness test is a more sensitive indicator of decay than weight loss determination (Hardie, 1980). Winandy and Morrell (1993) showed that for brown-rot decay caused by *G. trabeum* and *Postia placenta*, strength loss occurs faster than weight loss. They found a significant linear relationship between strength loss and weight loss in Douglas-fir heartwood, even during the earliest stages of incipient brown-rot decay. A significant portion of the initial effect of brown-rot fungi on wood strength occurred as a direct result of hemicellulose decomposition; strength losses approached 60 to 70% as the glucomannans and xylan main chains of the hemicelluloses were degraded (Winandy and Morrell, 1993).

In experiment II, *O. ips* #308 caused 18% toughness whereas in experiment III and IV, no reduction in toughness was determined. Differences in the wood moisture content of the samples prior to inoculation cannot explain why this strain caused toughness loss in one experiment only (Table 5.17). Wood moisture content of the samples inoculated with *O. ips* #308 that did cause a toughness loss was between 99 % and 211 % while moisture content of the samples in experiment III that did not cause a significant toughness loss was between 151 % and 195 %.

Table 5.17: Initial wood moisture content (%) of samples used for inoculation with *O. ips* #308 in experiments II and III.

Treatment	Set	Wood moisture content (%) of samples in experiment II (18% toughness loss)	in experiment III (no toughness loss)
O. ips #308	1	107	160
Controls	1	108	151
O. ips #308	2	99	155
Controls	2	100	158
O. ips #308	3	166	195
Controls	3	202	187
O. ips #308	4	211	158
Controls	4	201	151

Although overall density of the samples used in experiment II was lower than in experiment III (Table 5.18), it is unlikely that differences in density of the samples have an effect on weight loss over an incubation period of four months (Hedley and Armstrong, 1975; compare earlier discussion on results for *G. trabeum*).

Table 5.18: Nominal density (oven-dry weight / volume at test) of samples used for inoculation with *O. ips* #308 in experiments II and III.

Treatment	Set	Nominal density (kg/m^3)of samples	
		in experiment II (18% toughness loss)	in experiment III (no toughness loss)
O. ips #308	1	565	377
Controls	1	564	388
O. ips #308	2	375	458
Controls	2	324	476
O. ips #308	3	324	416
Controls	3	327	449
O. ips #308	4	328	446
Controls	4	342	460

In conclusion, the samples used in experiment II might have been genetically more susceptible to fungal attack by *O. ips* than the samples used in experiment III and IV which were from a different source. This is essentially the same explanation as for the results obtained with *G. trabeum* (see discussion earlier in this section).

In experiment III, it seems paradoxical that inoculation of the samples with *S. sapinea* #35 increased toughness significantly. However, the same kind of phenomenon has been reported by Richards (1954) who observed an increase in toughness of samples inoculated with *G. trabeum* compared to controls which was attributed to the chance association of several unusually tough specimens. There is a possibility that a weaker material, i.e. a material with lower maximum bending strength, may actually have a higher toughness as it holds on longer after reaching maximum load and therefore absorbs more work. In general, toughness is one of the most variable strength properties of wood (Trendelenburg, 1940; Waterman and Hansbrough, 1957; Forest Products Laboratory, 1987). The coefficient of variability of toughness values can be as high as 34% when sound standard wood samples are assayed (Forest Products Laboratory, 1987). This is mainly because toughness is very sensitive to small localized grain deviations and the fact that truly straight-grained toughness samples are rare (Fagan and McLain, 1983). A deviation in the S_1-layer of redwood of only 5 degrees resulted in reductions of 30 and 16% for radial and tangential toughness, respectively (Fagan and McLain, 1983). A similar effect may be expected in fast-growing softwoods like radiata pine. It has been demonstrated that the microfibril angle which relates to the winding angle of the cellulose microfibrils in the S_2-layer of softwood tracheids has two major effects on wood properties of radiata pine (Cave and Walker, 1994; Walker and Butterfield, 1995). Firstly, the stiffness of the cell wall increases five-fold from pith to cambium as the microfibril angle decreases from 40° to 10°; secondly, longitudinal shrinkage increases with microfibril angle but in a highly non-linear manner and is responsible for some degrade on drying, especially crook (Walker and Butterfield, 1995).

Since toughness is correlated to density (Kollmann, 1951; Niemz, 1993), densities of samples inoculated with *S. sapinea* #35 and their controls were compared (Table 5.19), and it is obvious that there were no significant differences between samples and controls. Another possible explanation for the increased toughness of the inoculated samples could be differences in the wood moisture content between samples and controls at the time of testing. Toughness, like other strength properties, is correlated to the wood moisture content (Kollmann, 1951; Niemz, 1993). Toughness of green wood is higher than toughness of comparable air-dried wood (Gerhards, 1968) because it requires more energy to break a wet sample than a dry sample (toughness is defined as the energy needed to fully fracture a specimen under a high loading rate). However, due to the equilibration of samples and controls after incubation, no differences in wood moisture content at the time of testing could be expected. Finally, a further explanation could be a possible weight gain of the inoculated samples due to the added inoculum which made the samples heavier and tougher.

Table 5.19: Nominal density (oven-dry weight / volume at test) and moisture content at test of samples inoculated with *S. sapinea* #35 in experiment III.

Treatment	Set	Nominal density (kg/m^3)	Wood moisture content at test (%)
S. sapinea #35	1	441	15.2
Controls	1	430	14.7
S. sapinea #35	2	492	15.2
Controls	2	492	14.9
S. sapinea #35	3	479	15.5
Controls	3	468	15.0
S. sapinea #35	4	500	15.1
Controls	4	462	15.2

In general, it was observed that the stain in most of the samples that had been inoculated with (wildtype) staining fungi was restricted to the outer portion of the wood. However, microscopical analysis of the samples (Chapter 6) confirmed that fungi were present in the central part of the samples although they were not melanised. Differences in the melanisation of the sapstaining fungi could be attributed to the moisture gradient present in the wood samples. The inner part of the samples was likely to be higher than the outer part which had the opportunity to slightly dry over time, even though the wood was contained in incubation bags. The moisture level in the central portion of the samples was probably too high for promoting melanisation of the fungi. Scattered colouration on the inside of inoculated wood blocks and darker stain on the outside has been noted by other researchers (Breuil et al., 1988; Gharibian et al., 1996). Kreber et al. (1999) report that higher wood moisture levels in radiata pine increment cores promoted deeper discolouration by sapstaining fungi than lower levels. It should be stressed that even if the inside of some samples used in this study was not stained, this does not have a consequence for the

toughness test because the outer skin of a sample is the most highly stressed part during this type of test (Findlay and Pettifor, 1937).

5.4.2 Chemical changes caused by sapstaining and decay fungi

When comparing the amounts of structural wood components present in radiata pine, as determined in the chemical analysis, it has to be taken into account that samples and controls are compared and not samples prior to inoculation and after inoculation with fungal isolates. In the present analysis, a higher amount of a particular carbohydrate in a sample as compared to in a control can therefore be explained by the fact that in the sample, a higher amount of a particular carbohydrate was initially present.

None of the sapstaining fungi tested caused significant reductions of lignin or structural carbohydrates (arabinose, galactose, glucose, xylose and mannose) in radiata pine sapwood after 16 weeks' incubation, but all fungal strains, with the exception of *O. pluriannulatum*, reduced the amount of extractives significantly (Tables 5.20 to 5.23). Of the isolates tested, *O. floccosum* degraded extractives most effectively (53.6% reduction). *Sphaeropsis sapinea* and *O. ips* reduced extractives by 14.6% and 18.1% (experiment II), respectively.

In toughness and weight loss experiment II, *G. trabeum* caused 8% weight loss, whereas in experiment III, no weight loss was determined. Chemical analysis of the samples infected with *G. trabeum* may explain these results. Although there was some reduction in Klason lignin by *G. trabeum* in experiment III, it appears that a more significant degradation of the carbohydrates as observed in experiment II outweighs the effect of lignin reduction, resulting in a weight loss of 8%. In experiment II, *G. trabeum* did not reduce the lignin content of the wood (the amount of acid soluble lignin was actually "increased" in the samples as compared to the controls) but caused higher losses in carbohydrates, particularly in arabinose (46.7% reduction) and galactose (44.4% reduction). The "increase" of acid soluble lignin, amounting to 50%, observed in the samples infected with *G. trabeum* in experiment II, is probably due to the presence of acid-soluble fungal material.

It was observed in experiments II and III that there were more extractives present in the samples infected with *G. trabeum* than in the respective controls (Table 5.21) which is also likely due to extracted fungal material.

Table 5.20: Extractives and lignin (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks' incubation (experiment I). Two replicate sets of analyses done in duplicate.

Treatment	Extractives (g/100g)	Klason lignin* (g/100g)
O. floccosum #138	0.13	29.1
Control	0.28	29.3
O. pluriannulatum #151	0.47	27.5
Control	0.44	27.3
S. sapinea #4	0.35	27.3
Control	0.41	27.3

* Values for acid soluble lignin of samples and controls were between 0.50 and 0.59 g/100g.

Table 5.21: Extractives and lignin (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks' incubation (experiments II and III).

Treatment	Extractives (g/100g)	Klason lignin* (g/100g)
O. ips #308 (Exp. II)	0.23	26.0
Control	0.32	26.4
O. ips #308 (Exp. III)	0.32	26.8
Control	0.37	26.5
G. trabeum (Exp. II)	0.63	29.7
Control	0.48	28.0
G. trabeum (Exp. III)	0.47	26.4
Control	0.37	28.0
Estimated accuracy**	-	1.2

* Values for acid soluble lignin of samples and controls were between 0.43 and 0.51 g/100g, except for *G. trabeum* (exp. II); see text.

** Expected standard deviation of four determinations (two replicate sets of analyses done in duplicate).

Table 5.22: Carbohydrates composition (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks' incubation (experiment I). Two replicate sets of analyses done in duplicate.

Treatment	Arabinose (g/100g)	Galactose (g/100g)	Glucose (g/100g)	Xylose (g/100g)	Mannose (g/100g)
O. floccosum #138	1.2	4.6	41.7	4.4	9.7
Control	1.3	4.5	42.4	4.4	9.7
O. pluriannulatum #151	1.3	1.6	43.8	4.6	10.4
Control	1.4	1.9	43.0	4.9	10.3
S. sapinea #4	1.4	1.8	43.5	4.9	10.7
Control	1.5	1.9	43.5	4.9	10.7

Table 5.23: Carbohydrates composition (g/100g of oven dried wood) determined in inoculated samples and controls after 16 weeks' incubation (experiments II and III).

Treatment	Arabinose (g/100g)	Galactose (g/100g)	Glucose (g/100g)	Xylose (g/100g)	Mannose (g/100g)
O. ips #308 (Exp. II)	1.4	1.3	47.5	6.1	15.2
Control	1.3	1.5	46.2	5.7	15.7
O. ips #308 (Exp. III)	1.1	2.1	44.9	6.0	11.7
Control	1.3	1.4	47.4	6.2	12.1
G. trabeum (Exp. II)	0.8	1.5	42.5	5.3	11.4
Control	1.5	2.7	44.6	6.4	13.0
G. trabeum (Exp. III)	1.4	1.8	48.5	5.8	14.2
Control	1.2	1.6	50.1	6.4	14.2
Estimated accuracy*	0.2	0.2	2.0	0.6	1.0

* Expected standard deviation of four determinations (two replicate sets of analyses done in duplicate).

In the enzyme screenings (Chapter 4) it was found that *G. trabeum* produced low amounts of cellulase which is in accordance with independent studies involving this isolate (Ritschkoff et al., 1992; Kreber et al., 2000). This result is reflected in the minor loss of glucose of the samples infected with *G. trabeum* determined in the present analysis (4.7% in experiment II and 3.2% in experiment III).

Kirk and Highley (1973) report total weight losses of up to 43% in Sitka spruce caused by *G. trabeum* in soil- and agar-block tests. Maximal losses of individual cell wall components were 11% for lignin, 55% for glucan, 80% for mannan and 65% for xylan. Glucomannans were removed faster than xylose, and both types of hemicelluloses were removed faster than cellulose. Even higher losses in lignin and wood sugars in pine after 12 weeks of decay by *G. trabeum* were reported by Eriksson et al. (1990). The total weight loss amounted to 57.2%. Lignin was reduced by 27.3%, glucose by 73.9%, xylose by 90.7% and mannose by 91.8%. In the present thesis, the losses of structural compounds were not as high as measured by Eriksson et al. (1990), and this discrepancy may be explained by differences between fungal strains, wood species and methods of measuring decay.

Winandy and Morrell (1993) found that *G. trabeum* removed 20% of arabinose and 10% of galactose and xylose in Douglas-fir heartwood during the first 34 days of an incubation period of 177 days. They did not observe any additional decreases in the relative percentages of arabinose upon prolonged incubation. Galactose, mannose, xylose and glucose were removed to varying degrees, but there was no decrease in Klason lignin. Alkali solubility of wood colonized by *G. trabeum* increased from 8.7 to 24.2% during the 177-day incubation period, suggesting a high rate of carbohydrate decomposition.

Winandy and Morrell (1993) concluded that *G. trabeum* aggressively degraded hemicellulose sidechains in Douglas-fir heartwood and, later in the incipient decay process, the glucomannan main chain. Glucose concentration, as a measure of cellulose utilisation, was reduced only slightly over the incubation period, which is in accordance with the results obtained in this thesis.

The main difference between sapstaining fungi and *G. trabeum* with regard to changes caused in cell wall composition is that sapstaining fungi do not degrade lignin or significant amounts of carbohydrates but do reduce extractives. Consumption of extractives by several New Zealand isolates has been measured in independent experiments (Farrell et al., 1998; Table 5.24). Two isolates of *S. sapinea* and four isolates of *Ophiostoma* were tested for pitch reduction after inoculation onto previously sterilized radiata pine wood chips. *Ophiostoma piceae* #40 and *S. sapinea* #35 caused significant pitch reduction of 32% and 28%, respectively. The other strains of *Ophiostoma* reduced DCM extractives by 15-20%. The fact that there was significant extractive consumption complements the observation of the sapstaining fungi growing in the resin canals of radiata pine (see Chapter 6).

Table 5.24: Pitch reduction in radiata pine by New Zealand sapstaining fungi. Source: Farrell (1998).

Isolate	DCM extractives (g/100g wood)	Extractives decrease in relation to control (%)
Control	0.75	
Control	0.72	
Ophiostoma piceae #40	0.50	32
Pesotum cupullatum #67	0.60	19
Ophiostoma querci #19	0.61	18
Ophiostoma querci #27	0.63	15
Sphaeropsis sapinea #35	0.53	28
Sphaeropsis sapinea #4	0.59	20

Recent results by Martínez-Inigo et al. (1999) showed that two other sapstaining fungi, *Ophiostoma ainoae* and *Ceratocystis allantospora*, have the ability to degrade triglycerides and long chain fatty acids in Scots pine sapwood. However, sterols and resin acids in sapwood as well in heartwood were not or poorly removed by these fungi. The removal of total extractives was higher in sapwood than in heartwood. The highly concentrated extractive fraction in pine heartwood mainly consists of resin acids which is inhibitory to fungal growth. Remarkably, *O. ainoae* caused moderate weight losses in extractive-free sapwood and heartwood of 6% and 2.9%, respectively, which is equivalent to a degradation of 6.1% of the holocellulose content in sapwood and of 3.8% in heartwood. *Ophiostoma ainoae* did not cause any significant alteration of the lignin, however.

Seifert (1964) measured changes in wood components caused by the sapstaining fungus *A. pullulans* and observed a reduction of approximately 7 % of cellulose and 3-4 % of the

pentosans (hemicelluloses) in pine wood, but no lignin degradation, after 10 weeks incubation.

Depletion of non-structural soluble sugars, starch, lipids and nitrogen from kiln-dried Caribbean pine, Scots pine and birch sapwood blocks over a five months incubation period with *B. theobromae* was studied by Encinas and Daniel (1999). Consumption of non-structural compounds was correlated with fungal growth and total dry weight losses. After 30 days incubation, almost all starch, glucose and fructose had been removed in birch which corresponded to a weight loss of about 2%. In Scots and Caribbean pine however, starch, sucrose and fructose were reduced to about 10% of their original values after 30 days when 2% weight loss was reached. In the two pine species, glucose was not depleted to the same extent as the other sugars.

The results of the experiments from the present thesis are in agreement with findings by Tabirih and Seehann (1984) who investigated the effect of the tropical sapstaining fungus *B. theobromae* on Abachi wood. They found that the structural wood components remained unchanged after four months incubation with the fungus whereas readily accessible sugars were fully metabolised. The determined weight loss of Abachi wood was caused by losses in cell contents. However, *B. theobromae* has been described as capable of a soft-rot-like attack of wood by other researchers (Findlay and Pettifor, 1939; Umezurike, 1969; Encinas and Daniel, 1996).

In conclusion of the present quantitative analysis, there was no significant difference in the lignin and carbohydrate composition when comparing wood samples inoculated with *O. floccosum* #138, *O. pluriannulatum* #151, *S. sapinea* #4, *O. ips* #308 and their corresponding controls. The fact that there were no major differences with regard to the composition of the cell wall components of sapstained wood and controls reflects the results obtained in the weight loss experiments. None of the sapstaining fungi caused significant weight loss.

6 Morphological aspects of sapstain fungal growth in radiata pine

6.1 Introduction

For microscopy, *Pinus radiata* (D. Don) wood samples from the toughness and weight loss experiments II and III were used. The objectives were to determine:

- the spatial distribution of sapstaining fungi in wood and possible morphological differences between the fungal species;
- if unmelanised fungal hyphae were also present in the central unstained part of some wood blocks;
- mode of fungal movement between cells, i.e. by direct cell-wall penetration (formation of bore-holes and appressoria) and/or perforation of the pit-membranes;
- the extent of degradation of the non-lignified ray parenchyma cells which might explain the small but statistically significant overall weight loss caused by sapstaining fungi in experiment I of the toughness and weight loss tests.

6.2 Literature Review

6.2.1 Ultrastructural aspects of sapstain development in wood

As early as 1907, Münch demonstrated that hyphae of *Ophiostoma* species were concentrated in the ray parenchyma and resin ducts of infected wood. From the tendency of sapstaining fungi to penetrate wood along the rays, the stained areas appear wedge-shaped in the cross-section with the apex of the wedge towards the centre of the tree. The preferential early growth of hyphae along the ray parenchyma is thought to be related to the availability of starch and sugars in these cells. Fewer hyphae are usually observed in ray tracheids which are considered to hold less freely available carbohydrates (Eaton and Hale, 1993). However, sapstain hyphae colonize wood in tangential and longitudinal directions as well. The growth rate in the lumina of axial cells is considerably faster than in the radial and tangential directions where cell wall and pit penetration occurs (Eaton and Hale, 1993).

The most comprehensive investigations into the growth of *Ophiostoma* species in naturally stained Scots pine (*Pinus sylvestris*) and spruce (*Picea abies*) sapwood as well as in artificially infected pine sapwood were undertaken by Liese and Schmid (1961, 1962, 1964). They found that hyphae growing on the inner cell wall of tracheids do not show any enzymatic alteration of the wall structure. Hyphae of *O. coeruleum* and *O. piceae* pass from one tracheid to the next via bordered pits by dislodging the torus and breaking through the margo. For the passage through the pits, the hyphae penetrate the torus in its full width of about 2-3 µm without constrictions, apparently by the aid of mechanical pressure. In addition, the cell wall itself can be penetrated by a special hyphal apex which

is called transpressorium. The transpressorium consists of a stalk and a head part with a pointed tip (Liese and Schmid, 1964). During penetration, small canals of 0.2-0.6 µm diameter are formed in the cell wall (Liese, 1970b). Possibly, enzymes might be produced at the hyphal tip to prepare the way for the transpressorium through the cell wall layers. The mechanism of growth is being described as passive: the tip of the transpressorium is pushed into the cell wall through intercalary growth at the joint of the mother cell and by its hydrostatic pressure (Liese, 1970a). When the hypha emerges into the lumen of an adjacent tracheid, it reverts to its original diameter. Then it continues to grow directly across the lumen until it reaches the opposite cell wall surface where a new transpressorium forms, and the process of cell wall penetration is repeated.

Hyphae have been shown to penetrate even thin silver or aluminium foils which supports the hypothesis that sapstaining fungi progress through the cell wall mechanically (Liese, 1970b). Some evidence of enzymatic changes of the cell wall structure was observed on the pit fields of pine (Liese and Schmid, 1961). However, no occurrence of cell wall deterioration with complete lysis was detected, but it is unknown if partial lysis might occur (Liese, 1970a; Table 6.1). It was demonstrated that under suitable growth conditions, staining fungi can be induced to release C_x (endocellulase) components of the cellulase enzyme complex (Rösch et al., 1969). However, the presence of the C_1-component necessary for the enzymatic degradation of native cellulose has not been conclusively demonstrated. Thus, it is very likely that cell wall penetration is achieved by a combination of localized enzymatic action at the tip of the transpressorium and mechanical pressure for pushing the hyphae through the wall.

Table 6.1: Action of fungi and bacteria during wood deterioration. Source: Liese (1970a).

Organism	Penetration of		Cell wall deterioration with	
	pits	cell walls (hyphal type)	partial lysis	complete lysis
Bacteria	++	-	++	*
Blue stain fungi	++	+ transpressoria	*	-
Soft rot fungi	+	++ perforation hyphae	++	*
Brown rot fungi	+	++ perforation hyphae	++	-
White rot fungi	+	++ perforation hyphae	+	++
Simultaneous rot fungi	+	++ perforation hyphae and hyphae	-	++

* unknown; - no occurrence; + sparse occurrence; ++ regular occurrence.

In contrast to other reports on *Ophiostoma* species, Krapivina (1960) found histochemical evidence of enzymatic action during bore-hole formation in *O. pini* and *Discula pinicola*, classifying these fungi which produced cavities in the secondary layer of the cell wall of tracheids in pine as soft-rot fungi.

Ballard et al. (1984) confirmed the proposed mechanical nature of the host cell wall penetration by sapstaining fungi. They studied the growth of staining fungi in naturally infected lodgepole pine (*Pinus contorta* var. *latifolia*) sapwood. The dominant causative pathogens were *Hyalorhinocladiella*, *Verticicladiella* and *Leptographium* (Ballard et al., 1984, unpublished observations). These fungi were inoculated into pine trees by mountain pine beetles (*Dendroctonus ponderosae*) resulting in eventual tree death. The fungal hyphae seemed to utilize penetration pegs that originated from an appressorium-like structure. According to Ballard et al. (1984), a mechanical penetration process was indicated by the deformation of cell wall layers, but secondary enzymatic activity was also suggested.

Based on microscopic analyses, Sachs et al. (1970) and Gagnon (1967) suggested enzyme activity of the pathogenic sapstaining fungi *Ceratocystis fagacearum* and *C. ulmi*. *Ceratocystis fagacearum* had the capability to degrade the walls of infected sapwood cells, including the middle lamella (Sachs et al., 1970). Lysis progressed from the lumen outward, much like the thinning action of white rot fungi, and both cellulose and lignin were affected. Hyphal penetration within the cell wall lead to cavities of varying size, shape and direction. Nilsson (1973) found no cavities produced by sapstaining fungi, with the exception of *Ceratocystis piceae* which produced a few cavities in pine after 29 weeks of incubation. However, in birch, numerous cavities were already found after four weeks of incubation.

Histochemical tests showed that *C. ulmi* can alter lignin and pectic substances in white elm (Gagnon, 1966). The action of pectic enzymes on vessel pits may allow the flow of protoplasm from adjoining parenchyma cells into the vessels and contribute to plugging of the vessels which affects the movement of water. Thus these enzymes not only provide nutrients for the fungus but also have a direct role in the pathogenesis of the elm disease. Histological changes and barrier zone formation as a defense reaction against radial colonization of the xylem by *Ophiostoma ulmi* were investigated in detail by Rioux and Ouellette (1989, 1991a, 1991b).

Appressoria have been found in many plant pathogenic fungi, such as the cereal pathogens *Colletotrichum graminicola* and *Magnaporthe grisea*. Appressoria were suggested to produce enormous turgor pressure, but it was demonstrated for the first time only recently that appressoria can exert sufficient pressure to enable mechanical infection of plants (Talbot, 1999). By allowing appressoria to form on an optical waveguide composed of a polydimethylsiloxane membrane sandwiched between two thin films of aluminium, the

forces exerted by the penetration pegs of *C. graminicola* could be visualized and quantified (Bechinger et al., 1999). It was shown that there is no absolute requirement for enzymatic activity and that *C. graminicola* can physically break through cuticles and epidermal cell walls of mono- and dicotyledonous plants. The high pressure of mature appressoria from plant pathogenic fungi is partly due to the melanin layer that forms an inner cell wall layer. Mutants of *Magnaporthe* and *Colletotrichum* species that fail to synthesize melanin are non-pathogenic and do not generate turgor (Talbot, 1999).

In contrast to sapstaining fungi which occur predominantly in temperate climates, the tropical sapstaining fungus *Botryodiplodia theobromae* has been reported to cause significant cell wall degradation in aspen, rubberwood, birch, Caribbean and Scots pine (Encinas and Daniel, 1995, 1996, 1997; Encinas et al., 1998) and rubberwood (Wong and Singh, 1997, 1998). The amount of degradation which was determined using weight loss in wood substance and by optical and electron microscopy observations leads to the conclusion that *B. theobromae* behaves like a typical soft-rot fungus and is not representative of the majority of sapstaining fungi. Staining fungi use the wood substance primarily as a habitat, drawing most of their nutrients from stored materials in wood parenchyma (Wilcox, 1973). The deterioration caused by stain and mould fungi does not proceed beyond the stage of fine bore-hole formation whereas in soft-rot, a significant portion of the wood substance is decomposed. *B. theobromae* has even been reported to produce cavities which appear to lie at the same angle as the spiralling microfibrils in the S_2-layer of the tracheid wall (Umezurike, 1978). This is indicative of a typical soft-rot type attack (Khalili et al., 2001).

6.2.2 Ultrastructural aspects of brown rot decay in wood

Like most rot fungi, brown rot hyphae normally first colonize softwoods via rays and axial resin canals and from there grow into axial tracheids via penetration of cross-pit field membranes. The hyphae grow inside the cell lumina in contact with the cell wall into which the secreted enzymes were once thought to diffuse freely (Keilich et al., 1970). More recent studies (Hirano et al., 1997; Kim et al., 2001) suggest that in the initial stages of decay, wood cell walls are more likely to be penetrated by low molecular weight substances. Fungal enzymes enter the cell walls after it is somewhat modified, becoming more porous. The carbohydrate components of the cell walls are hydrolysed and the products are taken up by the hyphae where they are metabolized. In contrast with white rot fungi, brown rot fungi normally cause a rapid depolymerisation of cellulose in wood which leads to considerable strength reduction even at low weight losses (Zabel and Morrell, 1992). In the last stages of decay, often a skeleton of predominantly lignin material remains. The resulting wood has a brown colour and when dry, it may break easily into cubical pieces. In the final stages of brown rot, the decayed wood is converted into a powdery mass.

The penetration of the hyphae of wood-destroying fungi into wood cell walls is mostly a chemical rather than a mechanical action. It is accomplished by the secretion of enzymes which are capable of converting insoluble wood substances into soluble forms, in advance of the actual penetration of hyphae. The enzymatic action produces minute openings (boreholes) in cell walls through which the hyphae pass. Pit apertures are sufficiently large to permit easy penetration of fungal enzymes whereas these enzymes are too large (molecular weight around 40,000 daltons) to diffuse freely into the capillaries of wood cell walls in either the water saturated or dry state (Keilich et al., 1970). A pre-cellulolytic stage in which the hemicellulases might play a role in increasing the accessibility of the cellulose has been suggested but the results by Keilich et al. (1970) indicate that these enzymes, too, are probably too large to diffuse into the so-called transient capillaries. It was suggested that smaller cellulase components of molecular weights in the range of 10,000 daltons may increase the accessibility of the larger cellulase. In addition, the possibility of a non-enzymatic pre-hydrolytic stage has also been suggested. This was later confirmed when the involvement of at least four non-enzymatic agents (oxalic acid, siderophores, Fentons reagent leading to hydroxyl radical production and glycopeptides) in brown rot decay was shown (summarized in Daniel, 1994, and references therein). How these agents may function with enzymes *in situ* to degrade amorphous and crystalline cellulose is not understood, however. TEM observations with regard to brown rot decay can be summarized as follows (Daniel, 1994):

- close contact between the hyphae and wood cell walls is not necessary for fungal activity and cellulose depolymerisation; the agent(s) responsible are able to diffuse through the S3-layer into the S2-layer where morphological changes are first apparent;
- the involvement of an unknown low molecular weight agent (probably an oxidising agent) in the initial phases of degradation is probable as the speed of the attack process indicates;
- demethylation of lignin is indicated by progressive production of dihydroxyphenolic substances in brown rotted cell walls by reaction of osmium tetroxide with demethylated phenolic units;
- the degradation and solubilisation of lignin is shown by the removal of substances from middle lamella regions, particularly cell corner regions;
- extracellular sheaths of brown rot hyphae are involved in wood cell wall decay.

6.3 Material and Methods

Radiata pine sapwood infected with the following fungi was examined by microscopy: *Ophiostoma ips* #308, *Sphaeropsis sapinea* #4, *Leptographium procerum* #1852 and *Gloeophyllum trabeum*. The wood samples were cut into small pieces and soaked in distilled water under vacuum for 24 hours. Using a microtome, 20 µm thin cross, radial and tangential sections were cut from different parts of the blocks. The samples inoculated with *G. trabeum* which were selected for microscopy work were moderately decayed. Heavily degraded samples were extremely brittle and not used for microtomy. Instead,

handsections of heavily decayed blocks were taken using a sharp razor blade and analysed under polarized light for loss of birefringence. Sections of sound wood were prepared for comparison.

Initially, sections were stained with toluidine blue to enhance the contrast of fungal hyphae but it was subsequently discovered that the fungal hyphae were easily recognizable without any staining. In addition, the melanisation produced by the different fungal species was easier to compare in unstained sections. The microtomed sections were analysed using a Zeiss Photomicroscope II. For scanning electron microscopy, the sections were fixed in osmium tetroxide for 48 hours, washed four times (every 30 minutes) with distilled water, dehydrated in an acetone series (once in 50% and 95% acetone for 20 min. each and twice in 100% acetone for 20 min. each) and air-dried. The samples were then mounted onto aluminium stubs with a double-sided carbon tape and gold-coated in a sputter coater. The specimens were examined with a Cambridge Stereoscan 240 electron microscope.

6.4 Results and Discussion

Since there were no obvious differences in the spatial distribution of the various sapstaining fungal species within the wood, the results were described in general for all sapstaining fungi investigated. Figures 6.1, 6.2 and 6.3 show the spatial distribution of hyphae of *S. sapinea* in radiata pine sapwood in cross, radial and tangential sections.

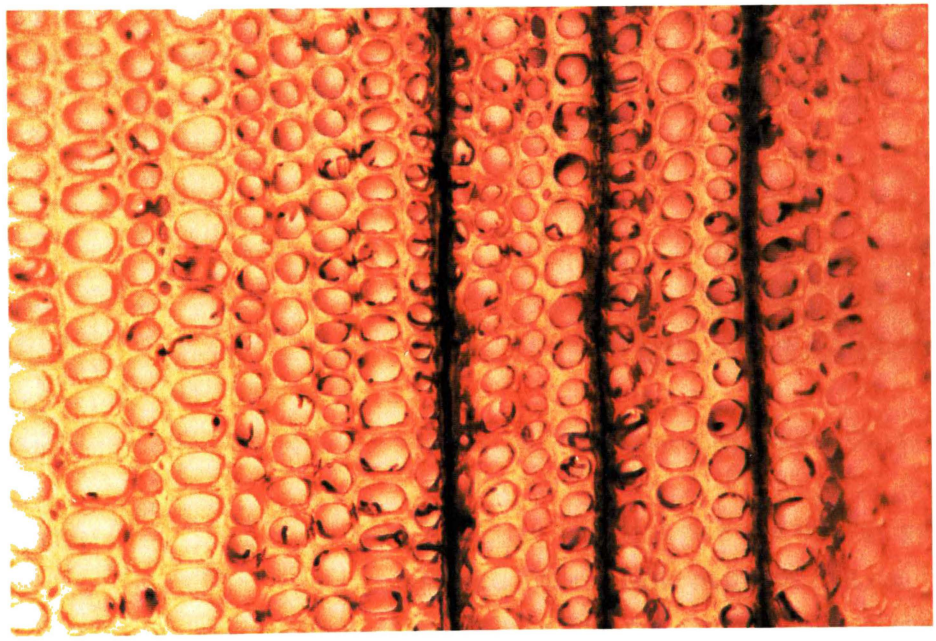


Figure 6.1: Spatial distribution of *S. sapinea* growing in radiata pine (cross section, 200 x).

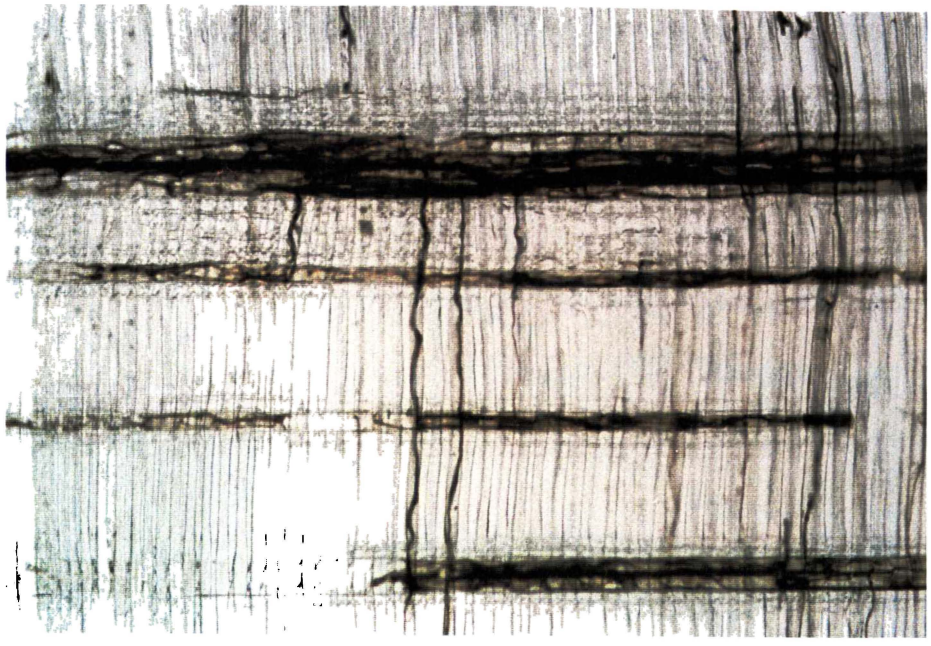


Figure 6.2: Spatial distribution of *S. sapinea* growing in radiata pine (radial section, 100 x).

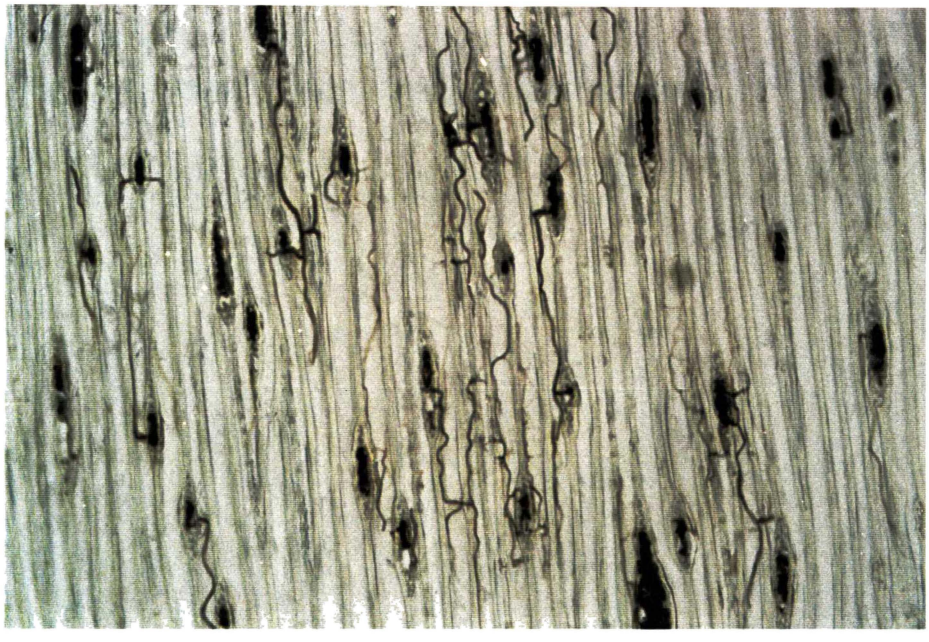


Figure 6.3: Spatial distribution of *S. sapinea* growing in radiata pine (tangential section, 100 x).

Hyphae of sapstaining fungi initially colonize the woody tissue via the ray parenchyma cells (Figure 6.4, 6.5 and 6.6) which contain readily available nutrients that can be quickly metabolized by the fungus (Eaton and Hale, 1993). The ray parenchyma cell walls appear to be degraded which might be due to fungal enzymatic activity. Since ray parenchyma cells in radiata pine are not lignified (Bamber and Davies, 1969), they are likely to be more susceptible to fungal degradation. Ballard et al. (1982) showed complete destruction of ray parenchyma cells after extensive colonization of lodgepole pine with several sapstaining fungi. Cross and tangential sections revealed large concentrations of hyphae also in the resin canals, surrounding epithelial cells and adjacent tracheids. Like the ray parenchyma

cell walls, the walls of the resin canal tissue of radiata pine are unlignified in the sapwood (Bamber, 1972).

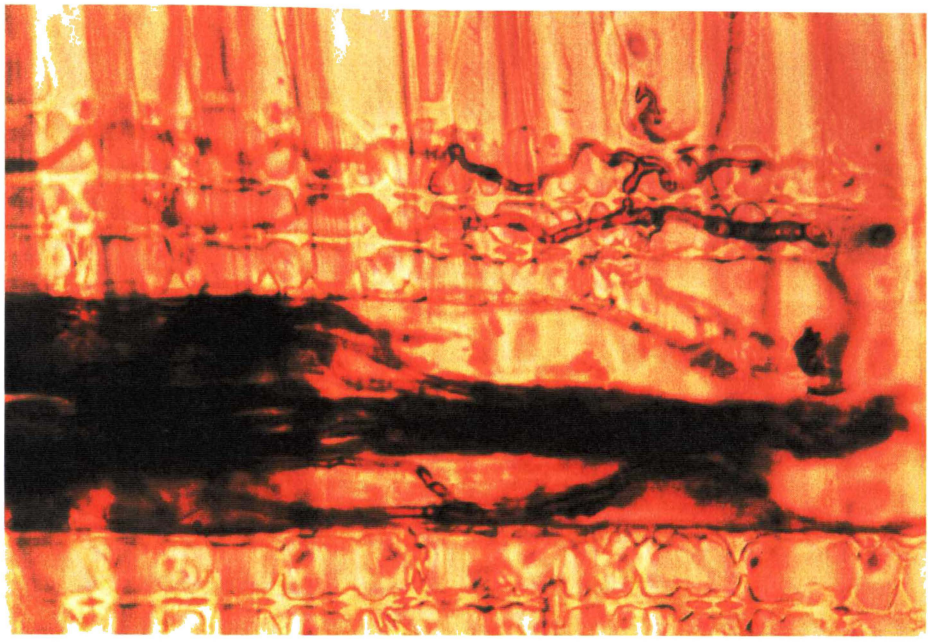


Figure 6.4: *Sphaeropsis sapinea* growing in ray cells of radiata pine (500 x).

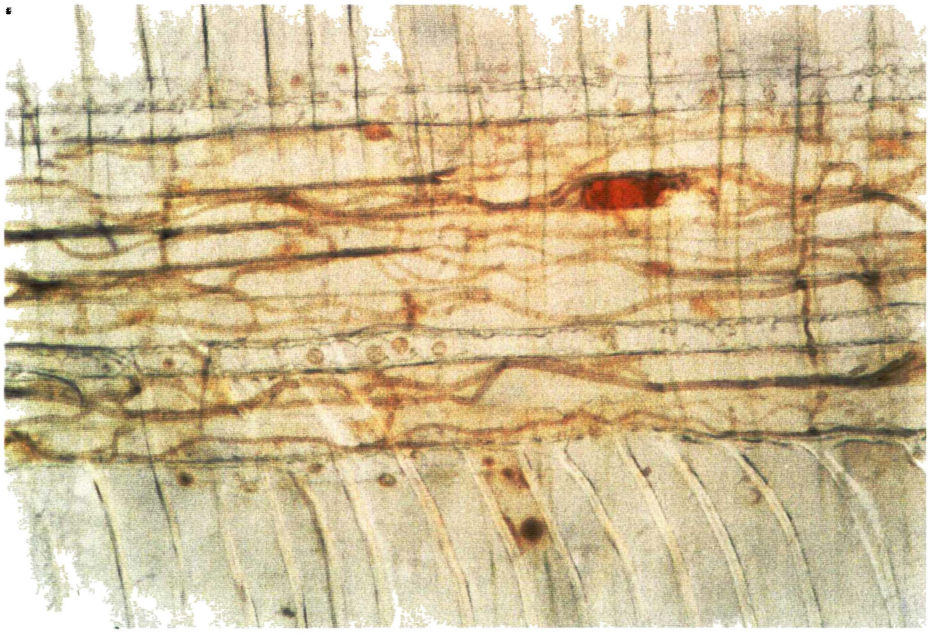


Figure 6.5: *Leptographium procerum* growing in ray parenchyma cells and, to a lesser extent, in ray tracheids of radiata pine (1000 x).

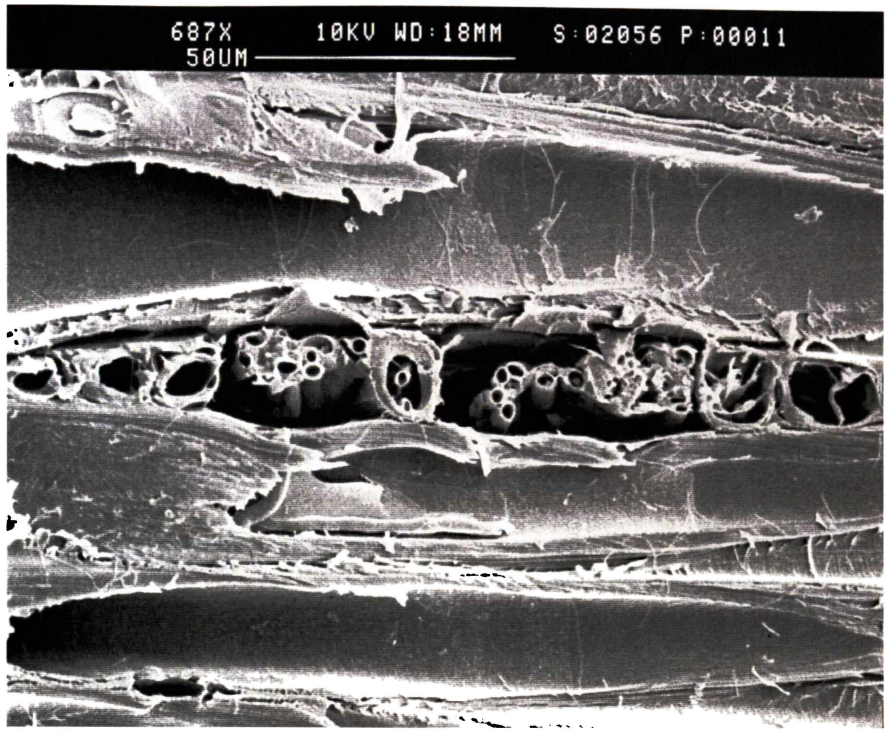


Figure 6.6: *Sphaeropsis sapinea* growing in ray cells of radiata pine.

Generally, the ray tracheids in radiata pine were less colonized than the parenchyma cells (Figure 6.4 and 6.5). Hyphae grew from ray tracheid to ray tracheid via bordered pits. They entered the longitudinal tracheids by penetrating the membrane of half-bordered pits which connect the ray parenchyma cells and the tracheids in cross-field pit regions (Figure 6.7). Hyphae grew from longitudinal tracheid to tracheid via bordered pits (Figure 6.7, 6.8, 6.9, 6.10).

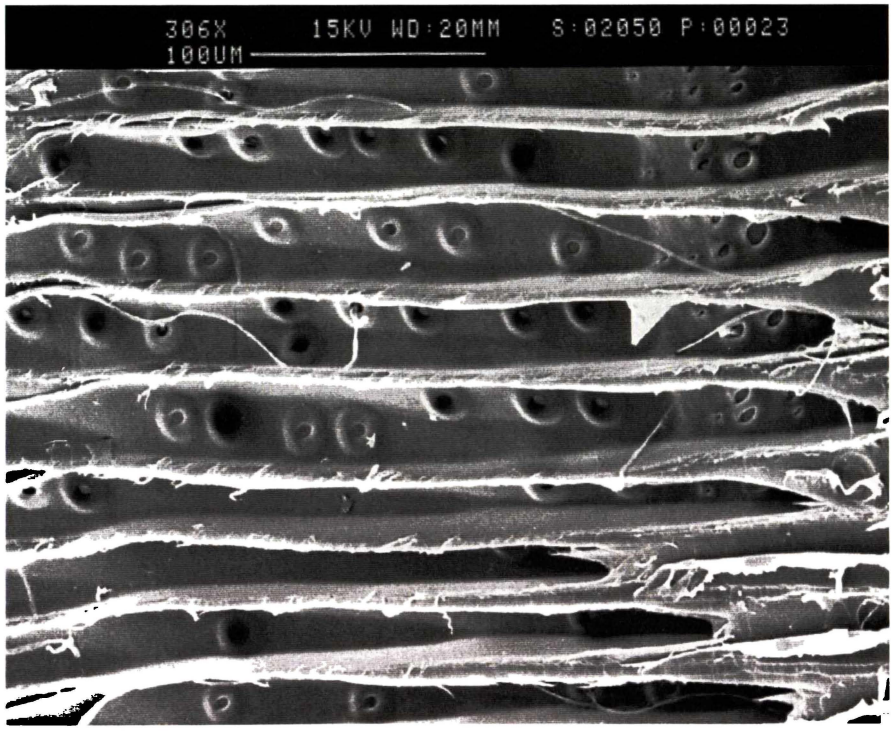


Figure 6.7: *Leptographium procerum* penetrating bordered pits and pinoid pits (cross-field pits) of radiata pine.

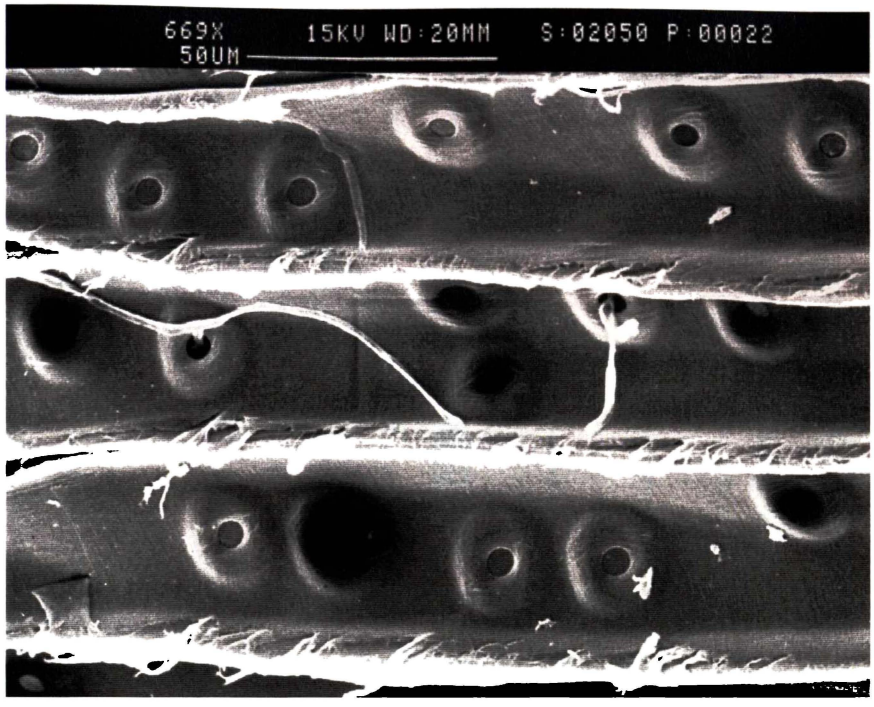


Figure 6.8: *Leptographium procerum* penetrating bordered pits of radiata pine.

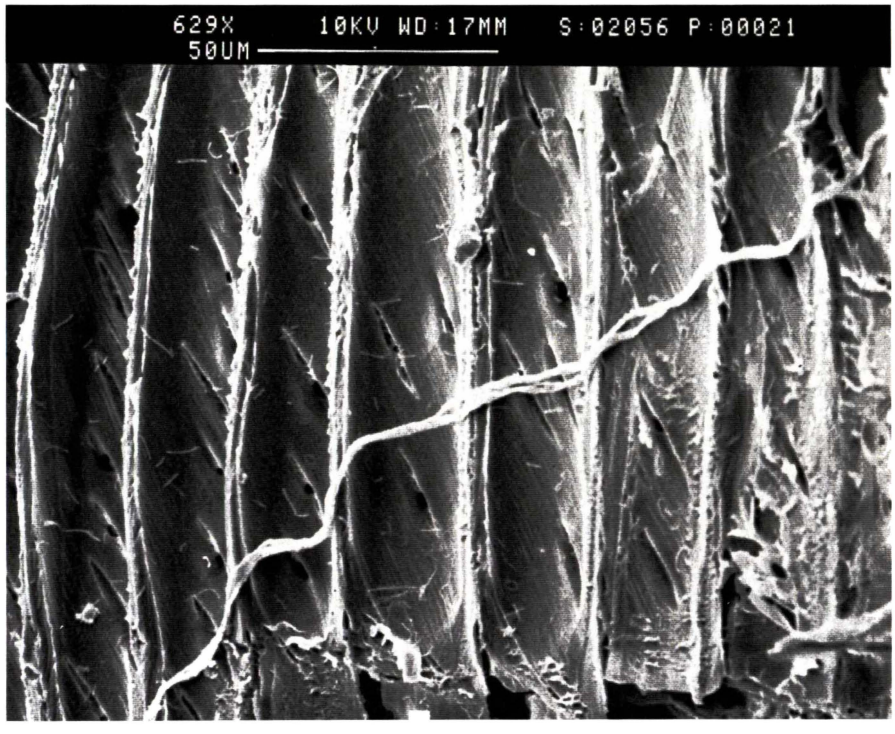


Figure 6.9: *Ophiostoma ips* crossing from cell to cell via pits.

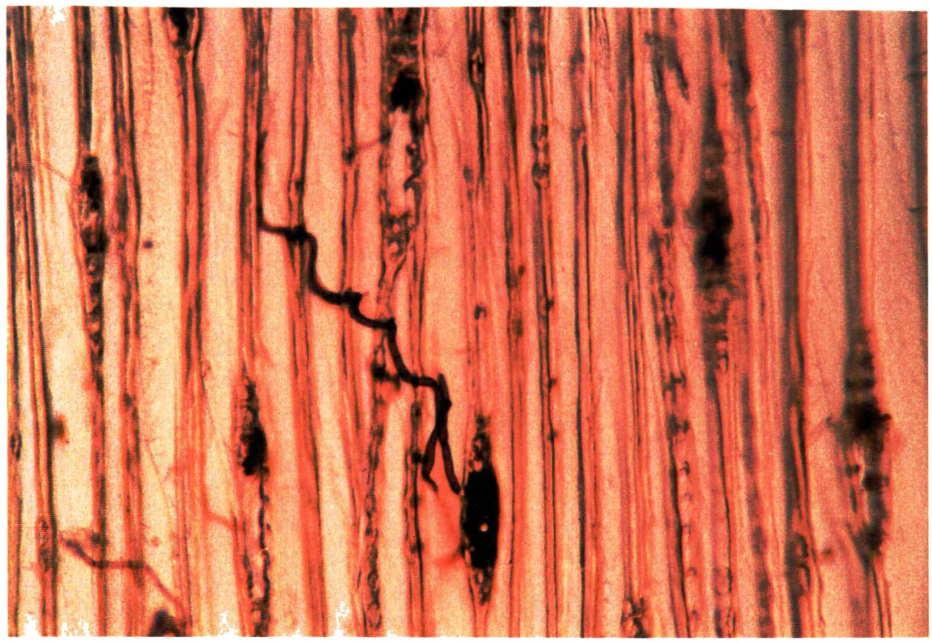


Figure 6.10: *Sphaeropsis sapinea* crossing from cell to cell through pits (200 x).

Fungal hyphae were present in the lumen of longtitudinal tracheids but there was no evidence of cell wall degradation (Figure 6.11 and 6.12).

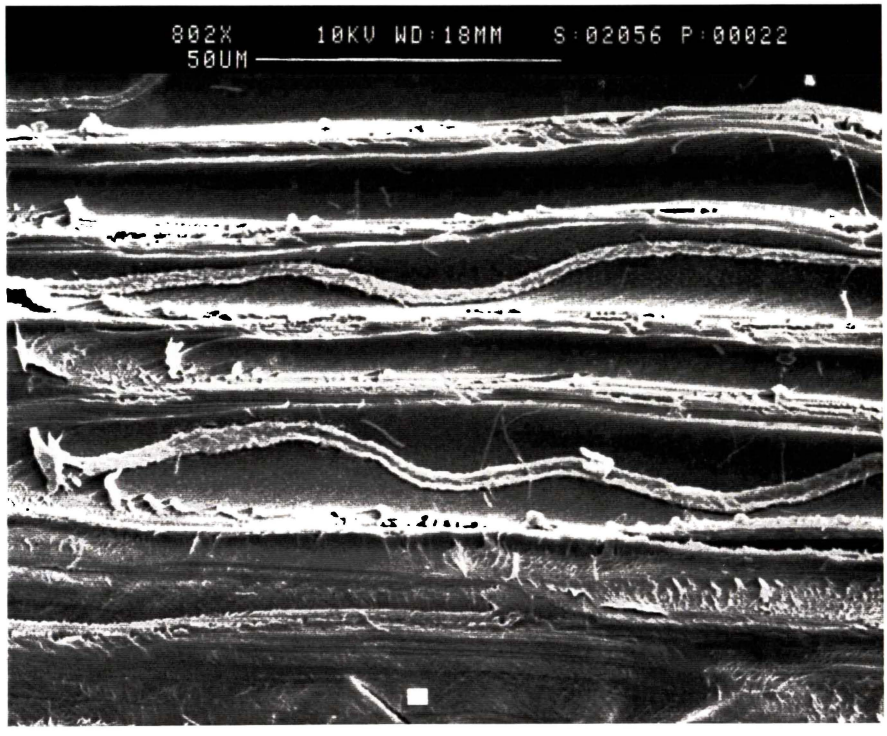


Figure 6.11: *Ophiostoma ips* growing on tracheid walls without causing degradation.

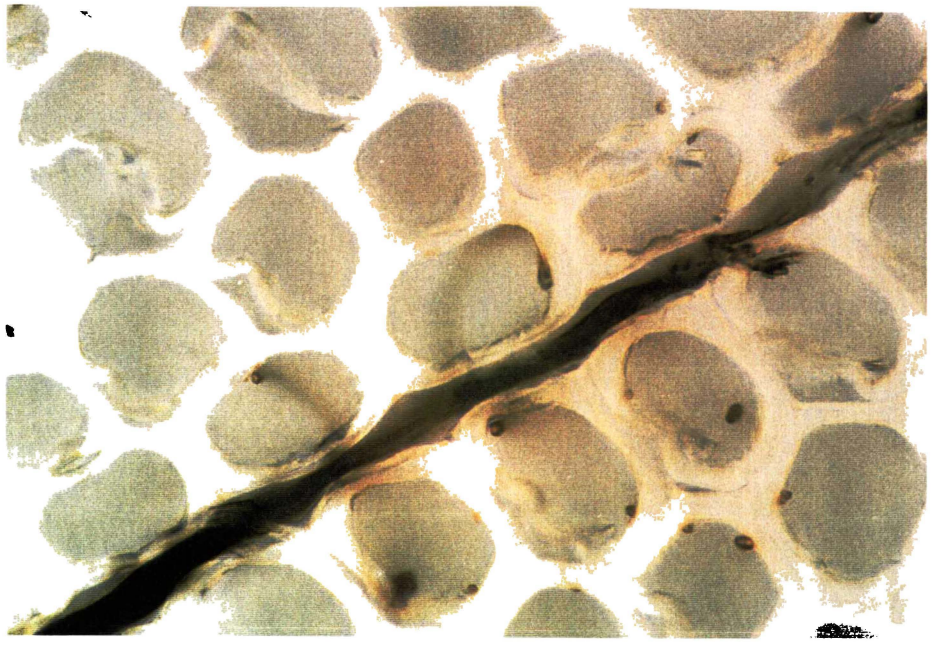


Figure 6.12: *Ophiostoma ips* growing in ray and crossing from ray to tracheid without causing degradation (640 x).

According to Liese and Schmid (1961), there are two general ways the hyphae of *O. coeruleum* and *O. piceae* can grow between cells of Scots pine (*Pinus sylvestris*) and spruce (*Picea abies*): i) penetration of bordered pits (between tracheids) and window pits (between axial tracheids and horizontal ray parenchyma cells) without formation of appressoria or bore hyphae; ii) direct penetration of the cell wall via formation of an appressorium from which a fine bore hypha is mechanically pressed through the wall by intercalar growth; the latter way is less frequently observed than the first. In this thesis research, fungal penetration of pits was commonly observed but appressoria or bore hyphae were not present although *O. ips* may be capable of penetrating cell walls directly (Figure 6.13, 6.14 and 6.15). Interestingly, this is the fungal isolate which caused a toughness loss of 18% in experiment II.

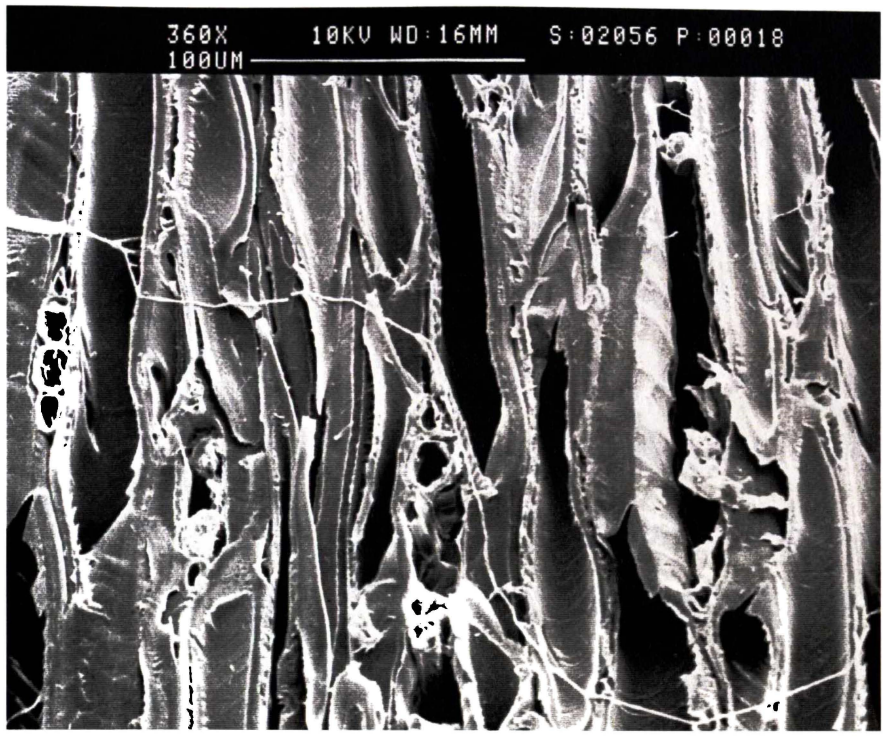


Figure 6.13: Extensive hypha of *Ophiostoma ips* growing through cells of radiata pine.

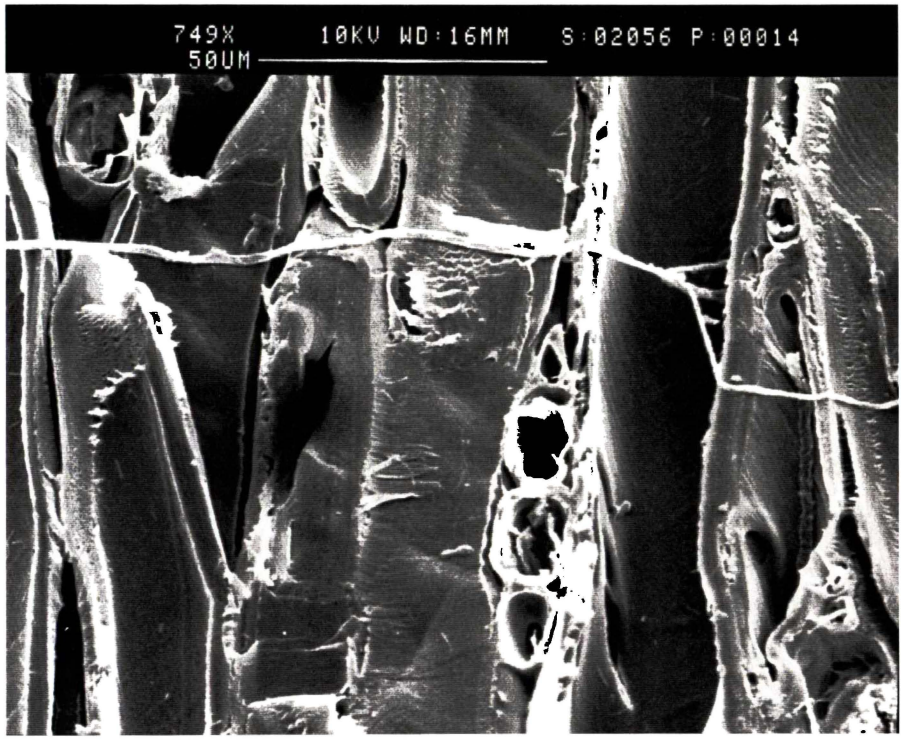


Figure 6.14: Detail of Figure 6.13 (left margin) in higher magnification.

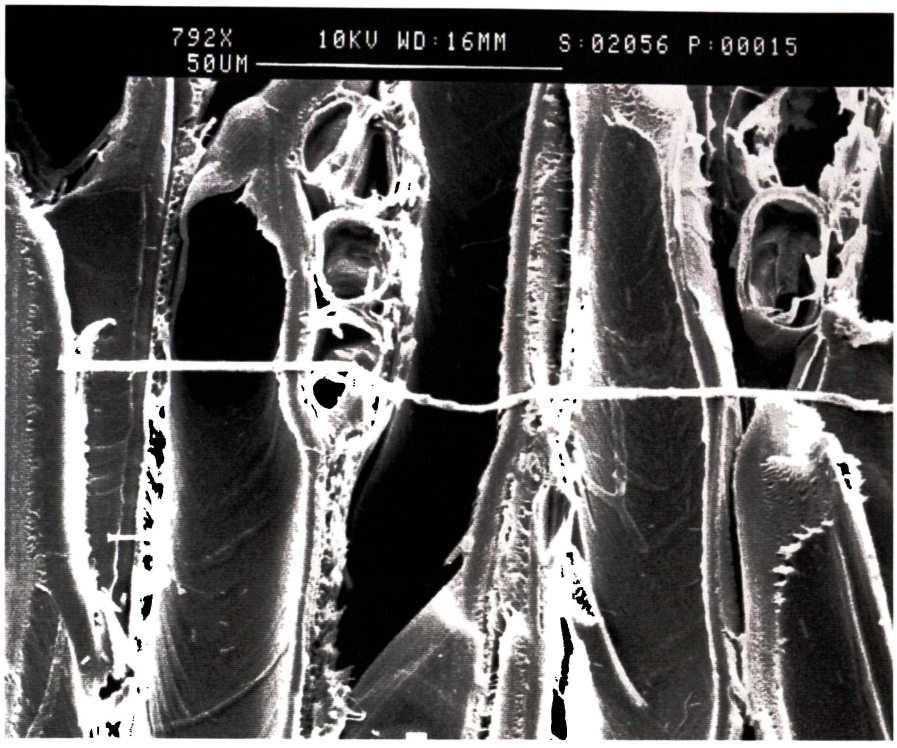


Figure 6.15: *Ophiostoma ips* crossing tracheid lumina.

The formation of bore holes in tracheid walls was also not commonly observed in another *Ophiostoma* species, *O. piliferum* (Blanchette et al., 1992). *Ophiostoma ulmi* does not seem to penetrate the cell walls of elm wood directly (Pomerleau, 1970; King et al., 1974; Miller and Elgersma, 1976; Scheffer and Elgersma, 1982). However, this method of fungal colonization and spread cannot be excluded (Ouellette, 1962; MacDonald and McNabb, 1970; Krause and Wilson, 1972).

While in this study, there was no evidence of wood cell wall degradation by *S. sapinea*, (Figure 6.16), Foster and Marks (1968) reported that fungal hyphae of the same species penetrated the wood tissue of radiata pine by lysing the middle lamellae and occasionally causing some erosion of cell walls. An increase in densely staining material in the ray cell walls was observed which may indicate removal of the cellulosic fraction. Foster and Marks (1968) therefore suggested that *S. sapinea* is a soft rot type pathogen. However, it was acknowledged that the wood used for analysis had been taken from a growing stem so the tracheids might not have been fully lignified at the time of attack and may have been particularly susceptible to a soft rot type of infection. Birch (1936) did not find any evidence of destruction of lignified tissues by *S. sapinea.*

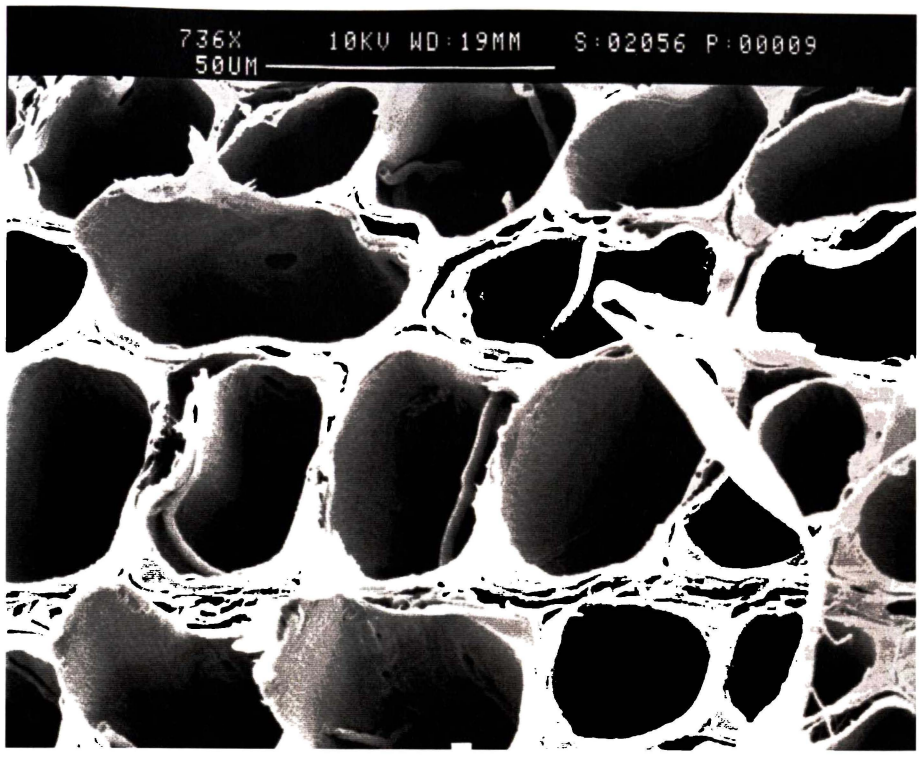


Figure 6.16: Thick hyphae of *Sphaeropsis sapinea* growing from cell to cell by penetrating bordered pits. No signs of cell wall damage.

In an early investigation on the growth of *Scopularia phycomyces* in Scots pine, Liese and Hartmann-Fahnenbrock (1953) occasionally observed fungal growth in the middle lamellae. This would explain a decrease in strength of the cell wall, and likely loss in toughness of the wood. However, these observations could not be confirmed later (Liese and Schmid, 1961), and it was concluded that hyphal growth in this layer had evolved after sample preparation.

It is likely that in vivo, small amounts of enzymes are produced by the sapstaining fungi investigated which can facilitate penetration of pit membranes. Some evidence of enzymatic modification of the membranes of cross-field pits of Scots pine was provided by Liese and Schmid (1961). It has been shown for other sapstaining fungi, e.g. *Aureobasidium pullulans* and *Sclerophoma pityophila*, that they are able to rapidly penetrate membrane filters of regenerated cellulose of very small dimensions (i.e. 0.2 µm diameter). This suggests that these species are secreting cellulolytic enzymes which facilitate cell wall penetration (Bardage and Daniel, 1997). Earlier investigations by Liese and Schmid (1964), on the other hand, have shown that *Ceratocystis pilifera* and *Ceratocystis piceae* are able to grow through aluminium and silver foils which indicates that these fungi can grow through cell walls exclusively by mechanic force. They also found that *Aureobasidium pullulans* was unable to grow through these foils.

It is also likely the small amounts of enzyme detected help facilitate attachment of the fungal hyphae to wood cell walls. The fungi are surrounded with an easily recognizable slime sheath which could retain and transport these enzymes (Figure 6.17 and 6.18).

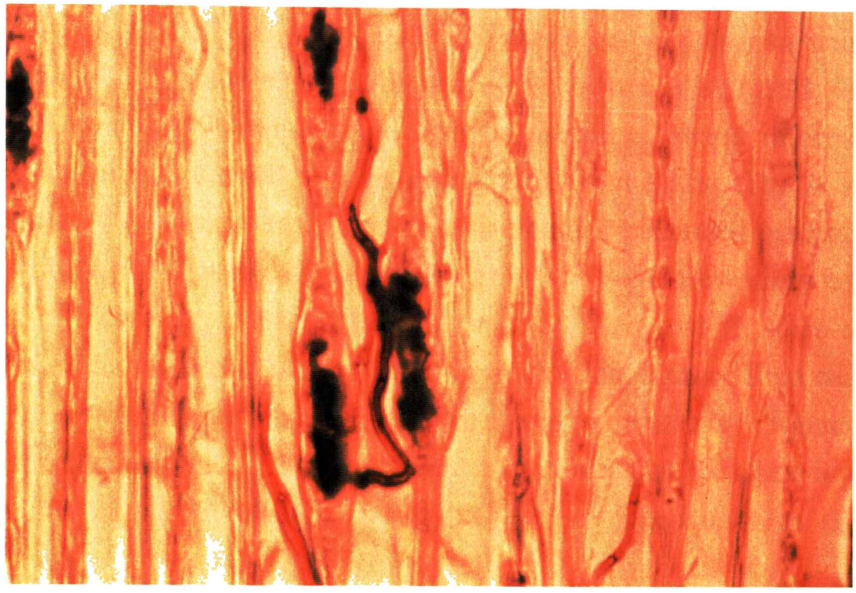


Figure 6.17: Slime sheath surrounding hyphae of *S. sapinea* (313 x).

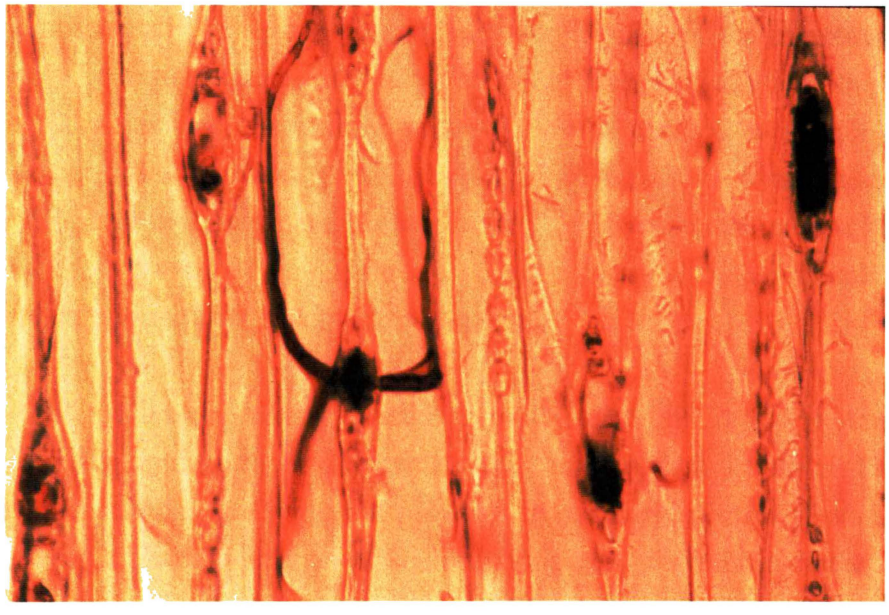


Figure 6.18: Slime sheath surrounding hyphae of *S. sapinea* (313 x).

An extracellular sheath was also observed around *O. piceae* growing in lodgepole pine and aspen (Gharibian et al., 1996). Schmid and Liese (1965) observed secretion of slime substances by hyphae of *Aureobasidium pullulans*, *Ceratocystis pilifera* and *Phialocephala phycomyces*. A possible function of the hyphal slime envelope might be the transfer of extracellular enzymes to the substrate. The hyphal sheath might also protect the enzymes from inactivation and retain them close to the microorganism. Optimal pH levels of 7 to 9 for xylanases and pectinases have been reported so the extracellular sheath may also serve

to maintain an environment favourable to enzyme activity since wood is acidic, with a typical pH of 4-5 (Gharibian et al., 1996). Using immunolabelling techniques, it was demonstrated for the brown-rot fungus *Postia placenta*, that extracellular enzymes are weakly bound to the linear mycofibrillar elements of the hyphal sheath (Green et al., 1992). Immunolabelling has been widely used to localise enzymes (Benhamou et al., 1987; Blanchette et al., 1989; Daniel et al., 1989 and 1996; Boher et al., 1995; Hoffert et al., 1995; Gharibian et al., 1996) and would also be useful to obtain information on the localization of enzymes produced by sapstaining fungi in the host. The *in-situ* detection and localization of these enzymes by means of antibodies generated against the purified proteins could provide evidence of their possible role in vivo. Observations by Ouellette et al. (1995) indicate that the extracellular sheath in *O. ulmi* and *O. novo-ulmi* may be implicated in the breakdown of host cell walls some distance away from the fungal cell. The sheath in *O. ulmi* and *O. novo-ulmi* has been found to contain ß-1,3-glucans as one of its components (Chamberland, 1994). Similarly, it is known that the slime layer of hyphae of *Aureobasidium pullulans* contains α-glucan which is called pullulan (Bender et al., 1959).

The question that arises from the observation that sapstaining fungi penetrate the pit membranes is whether the hyphae are growing through the margo (the perforated outer part of the pit membrane) or the torus (the thickened central part of the membrane). Hyphal growth can only be expected in tracheids which are at least partially air-filled so the pits are aspirated. Consequently, the margofibrils are closely attached to the pit wall so the fungal hyphae penetrate the torus as the location which offers the least resistance as has been shown by Liese and Hartmann-Fahnenbrock (1953) and Liese and Schmid (1961). They never found any hyphae growing through the margo and also no evidence of cell wall dissolving enzymes in the bore holes of bordered pits.

The margo contains less cellulose than the torus (Fengel, 1972) and in theory, would therefore by itself be more prone to enzymatic degradation than the torus. However, the torus in sapwood contains no lignin but polyoses, particularly pectins (Bauch, Liese and Scholz, 1968) which would enable sapstaining fungi to grow through the torus using pectinolytic enzymes. Using a commercial pectinase solution, the degradation of the torus in *Pinus densiflora* has been shown by Imamura et al (1974). More recently, Daniel et al. (1996) observed that pectinase caused swelling of half-bordered and bordered pit membranes of *Pinus sylvestris*, removal of electron dense materials and pronounced delamination of half-bordered pit membranes after prolonged incubation. Imamura et al. (1974) also demonstrated that a commercial hemicellulase degraded only the embedding substances of the margo. It is not known if the sapstaining fungi investigated in the present study have the capability to grow through the margo as well as the torus. The observed production of xylanase by all sapstaining fungi tested would allow for successful fungal growth also through the margo.

The higher permeability of sapstained wood in comparison to sound wood can be explained as a result of the holes through the tori caused by the fungal hyphae (Liese and Hartmann-Fahnenbrock, 1953). These holes are nearly as large as the hyphae themselves. When wood is seasoned below the fiber saturation point, the hyphae also dry and shrink, leaving part of the passage free. Consequently, fluids are able to pass more easily through the pits.

According to Liese and Schmid (1961), the bordered pits neither influence the direction of growth nor stimulate the hyphae to enter the pits. However, in this study it was observed that the hyphae in the tracheid lumen often suddenly branch off in a 90°-angle towards a pit membrane (Figure 6.18). This indicates that there may be a mechanism in place which signals the hyphae the direction towards a pit membrane.

Hyphae of *G. trabeum* grew inside the cell lumina in contact with the cell walls and seemed to penetrate the cell walls exclusively through the pits without formation of boreholes. A similar observation was made for the brown-rot fungus *Poria monticola* which however, in the later stages of decay, enlarged pit canals to such an extent that they could not be distinguished from true boreholes (Wilcox, 1968, quoted in Panshin and de Zeeuw, 1980). Transmission electron microscopy would be required to determine the possibility of direct cell wall penetration by *G. trabeum*. In any case, *G. trabeum* was shown to cause significant toughness losses in radiata pine. A large proportion of reduction in toughness has been attributed to fungal growth within the ray cells (Chapman and Scheffer, 1940) which indicates that the formation of boreholes is not essential to cause toughness loss.

The depolymerization of crystalline cellulose by *G. trabeum* was observed by polarized light microscopy. A loss of birefringence is associated with early stages of brown-rot. The cell walls of decayed wood appear matt (Figure 6.19) in contrast to sound cell walls which look shiny (Figure 6.20).

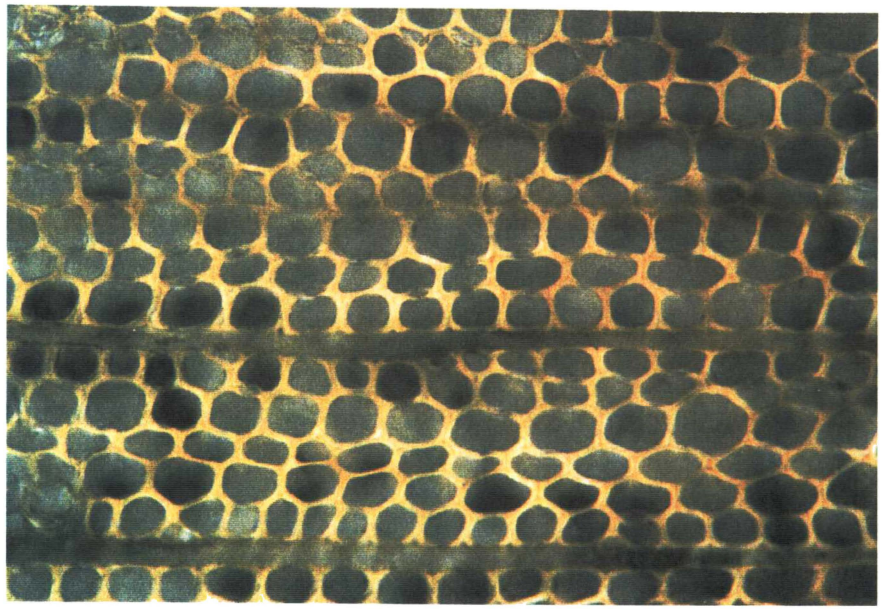


Figure 6.19: Degradation of radiata pine caused by *G. trabeum* (polarized light, 200 x). A loss of birefringence is associated with early stages of brown-rot.

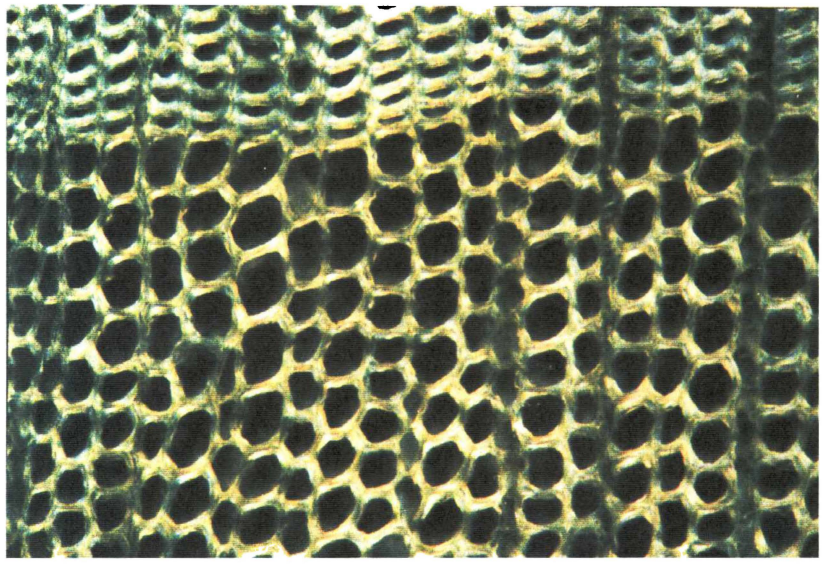


Figure 6.20: Sound radiata pine (polarized light, 200 x).

During the early stages of decay, some cells were more severely degraded than others (Figure 6.19), and this may be the reason for the variable birefringence of cell walls observed. The cells appeared to maintain their usual form but would shatter and collapse under mechanical pressure because they lack the strength cellulose provides the cell wall. As decay progressed, all cells were uniformly attacked and had a buckled appearance resulting in loss of wood integrity. Since the cellulose fraction of the cell walls was depleted, the samples were also more prone to cutting artefacts. According to Blanchette et al. (1990), the loss of polysaccharides is first seen in the S_2-layer which progresses to the S_1- and S_3-layers of the secondary walls. The cell walls appear porous and do not readily absorb some histological stains. The middle lamella is intact but is no longer rigid.

Ultrastructural observations reveal that secondary wall layers are not discernible (Blanchette et al., 1990).

In summary, sapstaining fungal hyphae were abundantly present in the ray parenchyma cells, in tracheid lumina and resin canals of radiata pine sapwood. There were no differences in the spatial distribution of the sapstaining fungi in the wood with regard to the fungal species. Microscopy revealed that unmelanised fungal hyphae were present in the central unstained part of some wood samples inoculated with sapstaining fungi (some weakly melanised hyphae can be seen in Figure 6.5). This information was helpful to evaluate the results from the toughness and weight loss tests. Thorough fungal colonization of the wood blocks was regarded as a prerequisite for these tests, however, the outer skin of a wood sample is the most stressed part during the toughness test (see Chapter 5). Growth of sapstaining fungi between wood cells was exclusively via penetration of pit membranes although microscopy showed that *Ophiostoma ips* might have the capability to grow directly through cell walls. *Gloeophyllum trabeum* also seemed to penetrate the cell walls exclusively through the pits. The non-lignified ray parenchyma cell walls of the wood samples inoculated with sapstaining fungi appeared to be degraded. The ray tracheids in radiata pine were colonized by the sapstaining fungi to a much lesser extent than the parenchyma cells which confirms earlier observations by Liese and Schmid (1961, 1962, 1964).

7 Summary, Conclusions and Suggestions for Future Work

Except for some investigations on *Ophiostoma ulmi* and *O. novo-ulmi* (Svaldi and Elgersma, 1982; Binz, 1996), the causative agents of Dutch elm disease, there is little quantitative information available on cell-wall degrading enzymes of *Ophiostoma* species. In order to determine the effect of sapstain on wood strength, physiological and biochemical processes that are involved in growth of sapstaining fungi need to be identified, especially enzymes participating in possible cell-wall degradation. Some sapstaining fungi have been reported to exhibit cellulolytic, hemicellulolytic or pectinolytic activity in artificial media (Seifert, 1964; Rösch et al., 1969; Umezurike, 1969; King and Eggins, 1973; Nilsson, 1973; Binz, 1996a, 1996b), but they seem to lack a complete enzyme system for the degradation of lignin (Liese, 1970b).

The sapstaining fungi tested in the present thesis research did not show any cellulase activity under various test conditions whereas two decay fungi, *G. trabeum* and *S. commune*, did (Table 7.1 and 7.2). However, xylanase, mannanase and pectinase were detected in the extracellular supernatants in liquid cultures of sapstaining fungi (Table 7.1). Of these enzymes, xylanase was secreted in highest amounts. The results of the screen for xylanase and mannanase suggest that, although the galactoglucomannans are the major hemicelluloses in softwoods (Fengel and Wegener, 1989), the arabino-4-methylglucurononxylans are preferably used by sapstaining as well as decay fungi when growing in softwoods. This may be attributed to the accessibility of the xylans in the S_3-layer of the cell wall.

Table 7.1: Summary of extracellular enzyme activities of sapstaining and decay fungi in liquid culture.

Isolate	Maximal activity (µmoles/min/ml)*					
	Cellulase	Xylanase	Mannanase	Pectinase[1]	Pectinase[2]	Amylase
O. floccosum #138	0	0.28	0	0.02	0.03	0.44
O. floccosum #148	0	0.37	0	n.d.	n.d.	0.24
O. piceae #272	0	0.08	0.04	0.03	0.02	0.25
O. piceae #170	0	0.17	0.29	n.d.	n.d.	0.25
O. ips #308	0	0.04	0	0.04	0.11	0.43
O. pluriannulatum #151	0	0.51	0	0.04	n.d.	0.46
L. procerum #1852	0	0.06	0	0.04	n.d.	0.26
S. sapinea #4	0	1.59	0.06	0.03	0.04	0.39
S. sapinea #35	0	1.64	0.01	0.03	0.04	0.46
S. commune	1.31	0.31	0.19	0.05	0.04	0.31
G. trabeum	0.95	0.10	0.02	0.42	0.11	0.24

[1] on pectin; [2] on polygalacturonic acid; n.d. = not determined.

*Activities per g of fungal biomass were calculated for the individual enzymes (see Chapter 4) but cannot be compared between different enzymes because biomass was determined after different incubation times for the individual enzymes.

Table 7.2: Summary of extracellular enzyme activities of *G. trabeum* and *S. commune* in liquid culture.

Enzyme	Activity (μmoles/min/ml)		Activity (μmoles/min/ml/g)*	
	S. commune	*G. trabeum*	*S. commune*	*G. trabeum*
Cellulase	1.31	0.95	14.56	23.75
Xylanase	0.31	0.10	2.07	1.25
Mannanase	0.19	0.02	0.73	0.17
Pectinase (on pectin)	0.05	0.42	0.13	3.23
Pectinase (on polygalacturonic acid)	0.04	0.11	0.40	3.67
Amylase	0.31	0.24	3.88	4.00

*Activities of the two fungal species can be compared for each enzyme but not between different enzymes because biomass was determined after different incubation times for the individual enzymes.

Ophiostoma species seem to be exceptional since practically all cellulase-producing microorganisms also produce xylanases and vice versa (Eriksson et al., 1990). The information in the literature whether or not xylanolytic and cellulolytic systems are under separate or common regulatory control in fungi varies. For example, it has been proposed that in the white-rot fungus *Polyporus adustus*, cellulases, mannanases and xylanases are under the control of a single common, regulatory gene (Eriksson and Goodell 1974). In contrast, it has been suggested that in *Trichoderma reesei*, the syntheses of cellulases and xylanases are under separate control (Eriksson et al., 1990, and references therein).

Amylase activity of the fungal species tested was more significant than xylanase, mannanase and pectinase activities which confirms that sapstaining fungi preferably metabolize easily accessible, non-structural wood components like starch (Brush et al., 1994; Gao et al., 1994).

An important conclusion from the enzyme screenings is that extracellular supernatants for enzyme determinations of sapstaining fungi should only be harvested during the exponential growth phase of the cultures because otherwise, there will be interference with autolytic fungal enzymes. During the late stationary growth phase, autolysis was observed for *Ophiostoma* species, i.e. the fungi were digesting their own cell walls which have been shown to contain cellulose (Rosinski and Campana, 1964; Jewell, 1974; Benhamou, 1988) and polygalacturonic acid (Benhamou, 1988).

Further insight into the biochemical and physiological characteristics of sapstaining fungi can be expected by studying the enzyme secretion of these fungi on various other substrates, for example arabinose, galactose or complex hemicelluloses isolated from wood. Binz (1996b) determined that xylanase production of *O. ulmi* and *O. novo-ulmi* doubled in the presence of arabinose and when the organisms were grown in the mycelial form.

Several of the sapstaining isolates tested in this thesis demonstrated more significant xylanase activity than the decay fungi *G. trabeum* and *S. commune*. This may be attributed to the fact that the culture conditions for the sapstaining and decay fungi tested were identical and that a considerable amount of variation can be expected when comparing enzyme activities of different strains by a single fungal species. It is difficult to produce culture and assay conditions in a screen that accommodate sapstaining as well as decay fungi since both have very different physiological requirements. It also has to be taken into account that in vivo, the synergistic action of various cellulolytic and hemicellulolytic enzymes contribute to loss of structural wood integrity by decay fungi.

With regard to xylanase activity of *S. commune*, it can be expected that enzyme activity is more significant when a different substrate is used. Haltrich et al. (1996) demonstrated that for the induction of xylan-degrading enzymes by *S. commune*, cellulose or low-molecular-mass compounds derived from cellulose, such as cellobiose or sophorose, had to be present. Xylan or xylobiose were ineffective as inducers, resulting only in constitutive levels of xylanase activity formed (Haltrich et al., 1996).

If sapstaining fungi produce cell-wall degrading enzymes, even if in small amounts, what are their possible functions? A possible function of these enzymes may be the facilitation of the colonization of wood cells, specifically, pectinase may support the fungal penetration of pit membranes. It is likely that the extracellular hyphal sheath which has been observed for *S. sapinea* (see Figures 6.17 and 6.18 in Chapter 6) as well as previously for *Ophiostoma piceae* (Gharibian et al., 1996), *A. pullulans* (Bender et al., 1959; Schmid and Liese, 1965) and for decay fungi (Eriksson et al., 1990), contain these enzymes. The hyphal sheaths are known to contain proteinaceous materials and various polysaccharides (Bender et al., 1959; Eriksson et al., 1990, and references therein). They coat the growing tip and also occur along the length of the hypha (Figures 6.17 and 6.18 in Chapter 6). Although the role of the slime sheath is uncertain, it does appear to be an important source of support and hyphal contact with the cell wall (Schmid and Liese, 1965; Eriksson et al., 1990). The slime appears to house and transport depolymerization agents for cell wall attack by decay fungi or, in the case of sapstaining fungi, to utilize the cell contents of ray parenchyma cells (Eriksson et al., 1990). As has been suggested for other fungi in wood, the sheath might also protect enzymes from inactivation and retain extracellular enzymes close to the microorganism, offering a nutritional competitive advantage to the fungus in vivo (Kalisz et al., 1987; Green III et al., 1992). Since wood is acidic, with a typical pH of 4-5, the extracellular sheath may also serve to maintain an environment favourable to enzyme activity (Gharibian et al., 1996).

In addition, pectinase and xylanase could be involved in the pathogenesis of certain sapstaining fungi. The presence of genes encoding pectolytic enzymes and their synthesis and secretion have been demonstrated in a range of pathogenic fungi (Hardham and

Mitchell, 1998). The involvement of pectinases in the pathogenesis of Dutch elm disease has been demonstrated (Gagnon, 1967). The action of pectinases on vessel pits allows a flow of protoplasm from adjoining parenchyma cells into the vessels and contributes to plugging of the vessels. With regard to xylanase, the presence of one or more xylanase in culture filtrates has been reported for many pathogenic as well as non-pathogenic fungi (Binz, 1996b). It remains to be resolved if xylanases are involved in the pathogenesis of certain sapstaining fungi. Binz (1996b) suggested that xylanases of *O. ulmi* and *O. novo-ulmi* are involved in the pathogenesis of Dutch elm disease. He found that *O. novo-ulmi* and *O. ulmi* both secreted similar amounts of xylanase into the culture medium. However, it has been reported that in vivo, *O. novo-ulmi* has a stronger cell-wall degrading ability and shows more active penetration of vessel walls through pit membranes than *O. ulmi* (Miller and Elgersma, 1976). Obviously, the observation that both species produce equal quantities of extracellular xylanases in vitro does not necessarily reflect the situation in vivo during the infection process (Binz, 1996b). It is still possible that enzyme secretion during the development of Dutch elm disease may be regulated differently than in vitro (Binz, 1996b). According to Binz (1996b), the production of specific antibodies against the two xylanases and the subsequent immunohistochemical localization in the host should resolve this question. Another possible function of the xylanases demonstrated for *O. ulmi* and *O. novo-ulmi* could be the elicitation of defense responses in the host, as has been reported for the xylanase of *Trichoderma viride* which is an elicitor of plant defense responses in *Nicotiana tabacum* (Binz, 1996b, and references therein).

The involvement of mannanase in the pathogenesis of certain sapstaining fungi may also be possible but remains purely speculative at the present state of knowledge. *Ophiostoma piceae* which is saprophytic but has also been described as weakly parasitic (Brasier and Kirk, 1993), was the only fungal species in the present thesis research which showed mannanase activity. The fact that none of the other sapstaining species tested showed mannanase activity, does not exclude the possibility that these species do secrete mannanase. In future work, it would be important to verify if *Ophiostoma* species other than *O. piceae* produce mannanase. Mannanases are usually secreted into the medium in which the microorganism is cultivated (Eriksson et al., 1990) so it is very unlikely that mannanase of sapstaining fungi is cell-bound. The possibility that cell-wall degrading enzymes of sapstaining fungi are cell-bound was tested in this thesis research for one enzyme, pectinase (see Chapter 4).

In general, with regard to a possible correlation between cell-wall degrading enzymes and pathogenesis, it remains uncertain if enzyme activities in vitro are comparable to the effect of these enzymes in vivo. Additional experiments are required to determine the involvement of specific enzymes in pathogenicity. The in-situ detection and localization of cell-wall degrading enzymes by means of antibodies generated against the purified proteins could provide evidence of their distribution and possible role in vivo. Another approach would be the cloning of the structural gene encoding a particular enzyme, subsequent

deletion by means of targeted gene disruption and testing of the mutant for altered pathogenicity, as has been suggested by Binz (1996a).
In general, molecular genetic approaches, in particular gene knock-out techniques, have so far failed to provide evidence of an essential requirement for specific enzymes in pathogenesis of fungi (Hardham and Mitchell, 1998). However, studies incorporating inhibitors of enzyme activity, including antibodies, have demonstrated a role for a number of enzymes in the infection process (Hardham and Mitchell, 1998). Antibodies have also been used to determine the sites of enzyme secretion and degradative activity during colonization. Monoclonal antibodies to wood-derived xylanase of *Postia placenta* have been successfully applied to detect incipient wood decay (Clausen et al., 1993).

Results from the enzyme assays were complemented with findings from four toughness and weight loss experiments which revealed that none of the sapstaining and albino isolates tested caused toughness and weight loss in radiata pine, with the exception of *O. ips* #308 which reduced toughness by 18%, however, in one test only. In contrast, three decay fungi, *G. trabeum*, *S. commune* and *P. gigantea*, reduced toughness significantly, however, without or with slight weight losses. Toughness losses were as high as 61% for *G. trabeum*, 32% for *S. commune* and 16% for *P. gigantea* which confirms that toughness loss is a more sensitive indicator of decay than weight loss (Armstrong and Savory, 1959; Liese and Pechmann, 1959; Hardie, 1980) since toughness loss indicates the destruction of the submicroscopic wood structure rather than decomposition of the structural wood components (Trendelenburg, 1940).

It can be concluded from the toughness tests that a careful selection and preparation of wood samples is an absolute requirement in order to reduce inter-sample variability to a level at which small strength losses become significant. By using side-matched samples and controls and excluding material with defects like knots and spiral grain, variability within sets of samples and controls was minimized in the toughness experiments of this thesis.

The use of green wood for inoculation with the test fungi in the toughness and weight loss experiments was advantageous because it allowed for rapid processing of a large number of samples, for minimising the risk of contamination and for reflecting naturally occuring conditions for sapstain development. However, in return, it had to be accepted that a high variation in wood moisture content resulted in some uneven growth and colonization of the wood by sapstaining fungi. The use of green wood also did not provide the decay fungi with optimum conditions for colonisation which resulted in minimal or no weight losses, as discussed earlier.

An important conclusion from the toughness and weight loss experiments in the present thesis is that toughness loss may occur without weight loss as has been determined for

O. ips #308 in experiment II and *G. trabeum* and *P. gigantea* in experiments III and IV. In order to explain this conclusion, it is necessary to evaluate the possible causes of toughness loss by a fungal microorganism. Reductions in toughness of wood due to fungal colonization can be caused by:

- Degradation of lignified cell wall material, i.e. mainly the tracheids in softwoods;
- Degradation of non-lignified parenchyma cells;
- Direct cell wall penetration by the fungi.

Although in-vitro cellulase activity of *G. trabeum* was low (see Chapter 4), microscopical analysis of the samples inoculated with *G. trabeum* revealed depolymerisation of crystalline cellulose in tracheid walls (Chapter 6) which presumably lead to a loss in wood integrity and toughness. Degradation and solubilisation of lignin is not characteristic of the action of brown rot fungi but has been reported (Daniel, 1994, and references therein). *Ophiostoma ips* did not degrade lignified cell wall material. Direct cell wall penetration was neither observed for *G. trabeum* nor *O. ips* #308 (Chapter 6). Consequently, toughness loss of the samples inoculated with *O. ips* #308 in experiment II can only be explained with the extent of degradation of non-lignified parenchyma cells, starch and extractives which is in accordance with the results obtained in the chemical analysis and in the amylase screening.

A correlation between the occurrence of cell wall perforations by fungi and toughness of the wood was suggested earlier by Chapman and Scheffer (1940). They found that direct cell-wall penetration in Southern pine was common with *G. rigidum*, less common with all strains of *C. pilifera* tested and hardly occurring with *C. ips* and *C. pini*. At the same time, *G. rigidum* caused the highest reductions in toughness. However, Chapman and Scheffer (1940) state that "a large proportion of the reduction in toughness in all cases was accomplished by the typical operations of the fungi strictly within the ray cells, as was essentially the case with *C. ips* and *C. pini*. Further evidence for the correctness of this assumption is offered by the fact that certain decay fungi may markedly affect the toughness of wood without penetrating the walls of the wood fibers or tracheids." The latter was confirmed in the present study; *G. trabeum* caused significant toughness loss without direct cell wall penetration.

Eusebio (1964) also tried to correlate the extent of direct cell-wall penetration and reduction in toughness. He suggested that direct cell wall penetration by *C. coerulea*, *C. ips* as well as *Alternaria tenuis* indicated a reduction in toughness of *Pinus strobus*. On the other hand, hyphal penetration by *Leptographium lundbergii* and *Cytospora pini* was exclusively through the pit apertures and should not cause a reduction in toughness. In order to verify his hypothesis, Eusebio (1964) correlated the reductions in toughness found in an independent study by Crossley (1956), with the microscopic observations from his own study. The following results were obtained (Eusebio, 1964):

a) Fungi which penetrated the cell walls directly:

Fungus:	Reduction in toughness (%) according to Crossley (1956):
Ceratocystis coerulea	29.7
C. ips	10.6
Alternaria tenuis	42.9

b) Fungi which penetrated only the pit apertures:

Fungus:	Reduction in toughness (%) according to Crossley (1956):
Leptographium lundbergii	1.46
Cytospora pini	0.46

It should be mentioned that the microscopic analysis in the study by Eusebio (1964) was performed on samples which had been incubated under optimum conditions for only 14 days whereas the values obtained by Crossley (1956) were based on test beams of the same wood species which had been incubated with the same fungi for four weeks. Consequently, the physical changes in wood which had been incubated twice as long can expected to be more significant. Possible differences in sample size and moisture content of the samples which are not mentioned in the study by Eusebio (1964) also have to be taken into account. In summary, the investigation by Eusebio (1964) confirms the earlier hypothesis by Chapman and Scheffer (1940) on the correlation between fungal cell wall penetration and toughness loss but does not provide original data which correlate microscopical observations and toughness.

The results obtained in the present thesis confirm the only report on the effect of sapstain on toughness of radiata pine (DaCosta, 1955). DaCosta (1955) used Australian isolates of *S. sapinea* as well as four other unidentified sapstaining fungi. Strength tests were performed with wood in the green state (wood moisture content 140-150%) as well as after air-drying to 12 % moisture content. No significant decrease was found in the bending strength and toughness of *Pinus radiata* samples after 12 weeks incubation with *S. sapinea*. Additional strength tests using the other common but unidentified Australian sapstaining fungi provided the same results.

However, in other studies on impact bending strength of Scots pine infected with *Ophiostoma* and *Ceratocystis* species (Findlay and Pettifor, 1937; Thunell, 1952; v. Pechmann, 1964), toughness losses of up to 30 % during four months´ incubation have been reported. According to v. Pechmann et al. (1964), weight losses of pine inoculated with *Ceratocystis pilifera*, *C. minor*, *C. piceae*, *C. coerulescens*, *C. penicillata* and *Aureobasidium pullulans* were between 0.6 and 2.5% after 12 weeks which may be attributed to degradation of extractives and starch. These weight losses were determined in the same way as in the present thesis, i.e. by comparison of final dry weights between inoculated samples and controls.

Chapman and Scheffer (1940) measured extraordinarily high reductions in toughness of Southern pine of up to 75 % during an incubation period of only 30 days, caused by *Ceratostomella pilifera*, *C. pini*, *C. ips* and *Graphium rigidum*. Considering the amount of toughness loss caused by these fungi in a very short period, it might be questionable if the species were correctly identified or if decay fungi were also present in the wood samples tested.

In summary, the following objectives were identified as possible milestones for future sapstain research, fundamental and applied (order does not represent a ranking in terms of importance):

- Clarify mode of growth of sapstaining fungi, specifically of *O. ips*, between wood cells, using confocal laser scanning and/or environmental scanning electron microscopy;
- Immunolocalization of cell-wall degrading enzymes of sapstaining fungi;
- Identify occurrence of mannanase activity in sapstaining species other than *O. piceae;*
- Determine composition and function of the extracellular hyphal sheath in sapstaining fungi;
- Investigate chemotaxis of growth patterns of sapstaining fungi within wood cells, i.e. determine what regulates the branching-off of the microorganism (it was observed that the hyphae in the tracheid lumen often suddenly branch off in a 90°-angle towards a pit membrane which indicates that there may be a mechanism in place which signals a hypha the direction towards a pit membrane; compare Microscopy Chapter);
- Determine the susceptibility of sapstained wood to subsequent decay;
- Investigate possible correlation between cell-wall degrading enzymes and pathogenicity of sapstaining fungi.

Results from the present thesis which were obtained using four complementary methods suggest that the major wood components lignin, cellulose and hemicelluloses are not the main nutrient sources for several sapstaining fungi isolated in New Zealand. This confirms previous findings that generally, sapstaining fungi do not degrade lignified cell walls but derive their nutrition from contents of dead cells (Liese and Schmid, 1961, 1962, 1964; Liese, 1970b) which distinguishes them from other wood-inhabiting fungi (soft rot and decay fungi). In order to colonize wood, sapstaining fungi use the non-structural components in wood as carbon and energy sources, and consequently produce hydrolytic enzymes (proteases, lipases and amylases) to metabolize macromolecules such as proteins, glycerides and starch. These enzymes are extracellular because the mode of fungal growth and nutrient acquisition requires that filamentous fungi secrete enzymes into their

environment in order to break down macromolecules into units which are suitable for uptake into the cell.

Under certain physiological conditions, some sapstaining fungi may be able to develop a soft rot-type decay capacity, depending on the host species, as has been reported for *S. sapinea* (Foster and Marks, 1968), *B. theobromae* (Umezurike, 1978; Encinas and Daniel, 1995; Encinas et al., 1998) and *A. pullulans* (Seifert, 1964; Crane et al., 1996), but this was not observed in the present thesis research. However, the fact that in one out of three experiments, *O. ips* was able to cause 18% toughness loss alerts to the fact that care has to be taken when general conclusions are drawn from investigations involving microorganisms. In addition, wood tissue disintegration is not only determined by the type of enzymes produced and environmental conditions but also by the fine structure and physicochemical properties of the wood cell wall, for example the lignin content and distribution as well as the polysaccharide composition, which may explain why some sapstaining fungi are capable of decay some host species, but not in others.

Hyphae of sapstaining fungi pass from one cell to another by growing through the pit membranes. Using light and scanning electron microscopy, direct cell wall penetration was not observed in this thesis research although it cannot be excluded that *Ophiostoma ips* might have the capability to grow directly through cell walls. *Gloeophyllum trabeum* also seemed to penetrate the cell walls exclusively through the pits. The non-lignified ray parenchyma cell walls of the wood samples inoculated with sapstaining fungi appeared to be degraded. The ray tracheids in radiata pine were colonized by the sapstaining fungi to a much lesser extent than the parenchyma cells which confirms earlier observations by Liese and Schmid (1961, 1962, 1964). The amount of degradation of the non-lignified ray parenchyma cells may explain the small but statistically significant overall weight loss in toughness and weight loss experiment I. Lignin has an important function for structural wood integrity in the sense that it represents an effective protective barrier against attack by sapstaining fungi.

Even though small amounts of hemicellulolytic enzymes and pectinase were detected in the present research, the effect of sapstaining fungi on wood quality of radiata pine has to be considered cosmetic and non-degradative which confirms the evaluation of sapstain according to the New Zealand Timber Grading Rules (NZS 3631, 1988). In general, sapstaining fungi do not affect the structural wood integrity of radiata pine. If severe sapstain occurs which obscures the grain, there is a possibility that decay fungi may also be present, and since decay fungi do affect the strength of timber, it seems to be a wise precaution not to allow for the use of severely sapstained timber for structural purposes, as recommended in NZS 3631 (1988). The use of selected albino-strains of the naturally occurring sapstain population in New Zealand as biocontrol agents on radiata pine logs and

timber can therefore be recommended since these fungi were not found to decrease toughness nor to cause weight loss in radiata pine.

REFERENCES

Abraham, L. D., Roth, A., Saddler, J. N., Breuil, C. (1993): Growth, nutrition, and proteolytic activity of the sap-staining fungus *Ophiostoma piceae*. Canadian Journal of Botany 71: 1224-1230.

Abraham, L. D., Breuil, C. (1996): Isolation and characterization of a subtilisin-like serine proteinase secreted by the sap-staining fungus *Ophiostoma piceae*. Enzyme and Microbial Technology 18: 133-140.

Abraham, L. D., Breuil, C., Bradshaw, D. E., Morris, P. I., Byrne, T. (1997): Proteinases as potential targets for new generation anti-sapstain chemicals. Forest Products Journal 47(9): 57-62.

Abraham, L., Hoffman, B., Gao, Y., Breuil, C. (1998): Action of *Ophiostoma piceae* proteinase and lipase on wood nutrients. Canadian Journal of Microbiology 44: 698-701.

Ah Chee, A., Farrell, R. L., Stewart, A., Hill, R. A. (1998): Decay potential of Basidiomycete fungi from *Pinus radiata*. Procedings of the 51st New Zealand Plant Protection Conference, Hamilton, New Zealand: 235-240.

Ah Chee, A. (2001): Basidiomycete wood decay fungi from *Pinus radiata*; biology and biological control. PhD thesis, Lincoln University, New Zealand.

Akamatsu, Y., Takasashi, M., Shimada, M. (1991): Enzymatic formation of oxalate from oxaloacetate with cell-free extracts of the brown-rot fungus *Tyromyces palustris* in relation to biodegradation of cellulose. Proc. 6th Internat. Symp. on Wood and Pulping Cellulose: 257-261.

Alexander, S. A., Horner, W. E., Lewis, K. J. (1988): *Leptographium procerum* as a pathogen of pines. Pp. 97-112 in Harrington, T. C. and Cobb, F. W. (Eds.): *Leptographium* root diseases on conifers. APS Press, St. Paul, Minnesota.

Alexopoulos, C. J., Mims, C. W., Blackwell, M. (1996): Introductory Mycology. Fourth Edition. John Wiley and Sons. 869 pp.

Allen, L. H. (1980): Mechanism and control of pitch deposition in newsprint mill. Tappi Journal 63(2): 81-87.

Anon. (1993): Genstat 5 release 3 reference manual. Clarendon Press, Oxford, U.K. 796 pp.

Arenas, C. V., Mendoza, E. M., Banatin, C. C. (1968): The effect of the staining fungus *Diplodia theobromae* (Pat.) Nowell on the toughness and bending strength properties of Duklitan (*Pouteria duclitan* (Blanco) Baehni). Wood Preservation Report 3(2), University of the Philippines, Forest Products Research Institute, Laguna, Philippines.

Armstrong, F. H., Savory, J. G. (1959): The influence of fungal decay on the properties of timber. Holzforschung 13: 84-89.

Arx, J. A. von (1952): Über die Ascomycetengattungen *Ceratostomella* Sacc., *Ophiostoma* Syd. und *Rostrella* Zimmerman. Antonie van Leeuwenhoek 18: 201-213.

Ayer, W. A., Browne, L. M., Feng, M. C., Orszanska, H., Saeed I-Ghomi, H. (1986a): The chemistry of the blue stain fungi. Part 1: Some metabolites of *Ceratocystis* species associated with mountain pine beetle infected Lodgepole pine. Canadian Journal of Chemistry 64: 904-909.

Ayer, W. A., Kratochvil, H., Allen, E., Browne, L. M., Dufresne, C., Figueroa, D., Szenthe, A. (1986b): The chemistry of the blue stain fungi. Part 2: Some essential metal levels of diseased and healthy Lodgepole Pine. Canadian Journal of Chemistry 64: 910-913.

Ayer, W. A., Attah-Poku, S. K., Browne, L. M., Orszanska, H. (1987): The chemistry of the blue stain fungi. Part 3: Some metabolites of *Ceratocystis minor* (Hedgcock) Hunt. Canadian Journal of Chemistry 65: 765-769.

Bailey, M. J., Biely, P., Poutanen, K. (1992): Interlaboratory testing of methods for assay of xylanase activity. Journal of Biotechnology 23: 257-270.

Bakshi, B. K. (1951): Studies on four species of *Ceratocystis*, with a discussion on fungi causing sap-stain in Britain. Mycological Papers 35: 1-16.

Ballard, R. G., Walsh, M. A., Cole, W. E. (1982): Blue-stain fungi in xylem of lodgepole pine: a light-microscope study on extent of hyphal distribution. Canadian Journal of Botany 60: 2334-2341.

Ballard, R. G., Walsh, M. A., Cole, W. E. (1984): The penetration and growth of blue-stain fungi in the sapwood of lodgepole pine attacked by mountain pine beetle. Canadian Journal of Botany 62: 1724-1729.

Bamber, R. K., Davies, G. W. (1969): Lignification of ray parenchyma cell walls in the wood of *Pinus radiata* D. Don. Holzforschung 23(3): 83-84.

Bamber, R. K. (1972): Properties of the cell walls of the resin canal tissue of the sapwood and heartwood of *Pinus lambertiana* and *Pinus radiata*. Journal of the Institute of Wood Science 31: 32-35.

Banerjee, S., Breuil, C., Brown, D. L. (1995): Aminopeptidase activity of staining fungi grown in artificial media. Enzyme and Microbial Technology 17: 347-352.

Bardage, S. L., Daniel, G. (1997): The ability of fungi to penetrate micropores: implications for wood surface coatings. Material und Organismen 31(4): 233-245.

Bartnicki-Garcia, S. (1968): Cell wall chemistry, morphogenesis and taxonomy of fungi. Annual Review of Microbiology 22: 87-108.

Bartnicki-Garcia, S. (1994): The Oomycetes are bona fide fungi. Abstracts of the 1994 meeting of the American Phytopathological Society, Albuquerque, New Mexico, August 6-10, 1994.

Basham, H. G. (1970): Wilt of loblolly pine inoculated with blue-stain fungi of the genus *Ceratocystis*. Phytopathology 60: 750-754.

Bauch, J., Liese, W., Scholz, F. (1968): Über die Entwicklung und stoffliche Zusammensetzung der Hoftüpfelmembranen von Längstracheiden in Coniferen. Holzforschung 22: 145-153.

Bauch, J., Liese, W., Berndt, H. (1970): Biological investigations for the improvement of the permeability of softwoods. Holzforschung 24(6): 199-205.

Bavendamm, W. (1954): Holzschädlingstafel: Bläuepilze. Holz als Roh- und Werkstoff 12 (5): 205-208.

Bechinger, C., Giebel, K.-F., Schnell, M., Leiderer, P., Deising, H. B., Bastmeyer, M. (1999): Optical measurements of invasive forces exerted by appressoria of a plant pathogenic fungus. Science 285: 1896-1899.

Beckman, C. H. (1956): Production of pectinase, cellulases, and growth-promoting substance by *Ceratostomella ulmi*. Phytopathology 46: 605-609.

Behrendt, C. J., Blanchette, R. A., Farrell, R. L. (1995a): Biological control of blue-stain fungi in wood. Phytopathology 85: 92-97.

Behrendt, C. J., Blanchette, R. A., Farrell, R. L. (1995b): An integrated approach, using biological and chemical control, to prevent blue stain in pine logs. Canadian Journal of Botany 73: 613-619.

Bell, A. A., Wheeler, M. H. (1986): Biosynthesis and functions of fungal melanins. Annual Review of Phytopathology 24: 411-451.

Benade, E., Wingfield, M. J., van Wyk, P. S. (1995): Conidium development in the *Hyalorhinocladiella* anamorph of *Ophiostoma ips*. Mycologia 87(3): 298-303.

Bender, H., Lehmann, J., Wallenfels, K. (1959): Pullulan, ein extracelluläres Glucan von *Pullularia pullulans*. Biochimica et Biophysica Acta 36: 309-316.

Benhamou, N., Ouellette, G. B. (1986): Use of pectinases complexed to colloidal gold for the ultrastructural localization of polygalacturonic acids in the cell walls of the fungus Ascolyx abietina

Benhamou, N., Chamberland, H., Ouellette, G. B., Pauze, F. J. (1987): Ultrastructural localization of β-(1→4)-D-glucans in two pathogenic fungi and in their host tissues by means of an exoglucanase-gold complex. Canadian Journal of Microbiology 33: 405-417.

Benhamou, N. (1988): Ultrastructural localization of carbohydrates in the cell walls of two pathogenic fungi: a comparative study. Mycologia 80(3): 324-337.

Benko, R. (1987): Antagonistic effect of some mycorrhizal fungi as biological control of sapstain. The International Research Group on Wood Preservation. Document IRG/WP/1314. Stockholm, Sweden.

Berbee, M. L., Taylor, J. W. (1992): Convergence in ascospore discharge mechanism among pyrenomycete fungi based on 18S ribosomal RNA gene sequence. Molecular Phylogenetics and Evolution 1: 59-71.

Bergman, O., Nilsson, T. (1968): On outside storage of birch chips at Mörrum's Sulphate Mill. Department of Forest Products, Royal College of Forestry, Stockholm. Research Notes No. R 60. 56 pp.

Bergman, O., Nilsson, T. (1971): Studies on outside storage of sawmill chips. Department of Forest Products, Royal College of Forestry, Stockholm. Research Notes No. R 71. 54 pp.

Berndt, H., Liese, W. (1971): Untersuchungen über die Enzyme von Bläuepilzen. III. Pectintranseliminase und Mannanase bei *Aureobasidium pullulans*. Archiv für Mikrobiologie 79: 140-146.

Biehn, W. L., Dimond, A. E. (1971): Effect of pectin source and sugars on polygalacturonase production by *Ceratocystis ulmi*. Phytopathology 61: 745-746.

Biely, P., Heinrichová, K., Kruziková, M. (1996): Induction and inducers of the pectolytic system in *Aureobasidium pullulans*. Current Microbiology 33: 6-10.

Binz, T. (1996a): Vergleichende Untersuchung des zellwandabbauenden Enzymkomplexes der beiden Erreger der holländischen Ulmenkrankheit *Ophiostoma ulmi* (Buism.) Nannf. und *O. novo-ulmi* Brasier und Isolation und Charakterisierung von zwei extrazellulären Xylanasen und einer Laccase aus *Ophiostoma novo-ulmi*. PhD-thesis, University of Freiburg, Switzerland.

Binz, T., Canevascini, G. (1996b): Xylanases from the Dutch elm disease pathogens *Ophiostoma ulmi* and *Ophiostoma novo-ulmi*. Physiological and Molecular Plant Pathology 49:
159-175.

Binz, T., Canevascini, G. (1997): Purification and partial characterization of the extracellular laccase from *Ophiostoma novo-ulmi*. Current Microbiology 35(5): 278-281.

Binz, T., Gremaud, C., Canevascini, G. (1997): Production and purification of an extracellular ß-galactosidase from the Dutch elm disease fungus *Ophiostoma novo-ulmi*. Canadian Journal of Microbiology 43: 1011-1016.

Birch, T. T. C. (1936): *Diplodia pinea* in New Zealand. New Zealand State Forest Service Bulletin No. 8, Wellington, New Zealand.

Björkman, E. (1946): On the conditions for the occurrence of timber-yard blue-stain and methods for its control. Medd. SkogsforsknInst., Stockholm, 35 (7).

Blanchette, R. A., Abad, A. R., Farrell, R. L., Leathers, T. D. (1989): Detection of lignin peroxidase and xylanase by immunocytochemical labeling in wood decayed by Basidiomycetes. Applied and Environmental Microbiology 55(6): 1457-1465.

Blanchette, R. A., Nilsson, T., Daniel, G. F., Abad, A. (1990): Biological degradation of wood. In: Rowell, R. M., Barbour, R. J. (eds.): Archaeological Wood: Properties, Chemistry and Preservation. Advances in Chemistry Series No. 225. American Chemical Society, Washington, D.C.: 141-174.

Blanchette, R. A., Farrell, R. L., Burnes, T. A., Wendler, P. A., Zimmerman, W., Brush, T. S., Snyder, R. A. (1992): Biological control of pitch in pulp and paper production by *Ophiostoma piliferum*. Tappi Journal 75: 102-106.

Blanco, P., Sieiro, C., Villa, T. G. (1999): Production of pectic enzymes in yeasts. FEMS Microbiology Letters 175: 1-9.

Blodgett, J. T., Stanosz, G. R. (1997): *Sphaeropsis sapinea* morphotypes differ in aggressiveness, but both infect nonwounded red or jack pines. Plant Disease 81: 143-147.

Bloomfield, B. J., Alexander, M. (1967): Melanins and resistance of fungi to lysis. Journal of Bacteriology 93: 1276-1280.

Bodig, J., Jayne, B. A. (1993): Mechanics of Wood and Wood Composites. Krieger Publishing Company. Malabar, Florida. 712 pp.

Boher, B., Kpemoua, K., Nicole, M., Luisetti, J., Geiger, J. P. (1995): Ultrastructure of interactions between cassava and *Xanthomonas campestris* pv. *manihotis*: Cytochemistry of cellulose and pectin degradation in a susceptible cultivar. Phytopathology 85: 777-788.

Brada, D., Schekman, R. (1988): Coincident localization of secretory and plasma membrane proteins in organelles of the yeast secretory pathway. Journal of Bacteriology 170: 2775-2783.

Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Brasier, C. M. (1978): Mites and reproduction in *Ceratocystis ulmi* and other fungi. Transcriptions of the British Mycological Society 70: 81-89.

Brasier, C. M. (1986): The population biology of Dutch elm disease: its principal features and some implications for other host-pathogen systems. Advances in Plant Pathology 5: 55-118.

Brasier, C. M. (1991): *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. Mycopathologia 115: 151-161.

Brasier, C. M., Kirk, S. A. (1993): Sibling species within *Ophiostoma piceae*. Mycological Research 97: 811-816.

Brasier, C. M., Mehrotra, M. D. (1995): *Ophiostoma himal-ulmi* sp. nov., a new species of Dutch elm disease fungus endemic to the Himalayas. Mycological Research 99: 205-215.

Bravery, A. F. (1968): Microbiological breakdown of cellulose in the presence of alternative carbon sources. Journal of Science and Food Agriculture 19: 133-135.

Breuil, C., Seifert, K. A., Yamada, J., Rossignol, L., Saddler, J. N. (1988): Quantitative estimation of fungal colonization of wood using an enzyme-linked immunosorbent assay. Canadian Journal of Forest Research 18: 374-377.

Breuil, C., Huang, J. (1994): Activities and properties of extracellular proteinases produced by staining fungi grown in protein-supplemented liquid media. Enzyme and Microbial Technology 16: 602-607.

Breuil, C., Yagodnik, C., Abraham, L. (1995): Staining fungi growing in softwood produce proteinases and aminopeptidases. Material und Organismen 29: 15-25.

Breuil, C. (1998): Wood as a nutritional resource for staining fungi. In Morrell, J. J. (Ed.): Biology and Prevention of Sapstain. Conference proceedings from Whistler, B.C., Canada, May 1997. Forest Products Society publication no. 7273, Madison, U.S.A.: 1-5.

Brisson, A., Gharibian, S., Eagen, R., Leclerc, D. F., Breuil, C. (1996): Localization and characterisation of the melanin granules produced by the sap-staining fungus *Ophiostoma piceae*. Material und Organismen 30(1): 23-32.

British Standards Institution (1957): British Standard 373: Methods of testing small clear specimens of timber. London.

Brookhouser, L. W., Peterson, G. W. (1971): Infection of Austrian, Scots and ponderosa pines by *Diplodia pinea*. Phytopathology 61: 409-414.

Brush, T. S., Farrell, R. L., Ho, C. (1994): Biodegradation of wood extractives from southern yellow pine by *Ophiostoma piliferum*. Tappi Journal 77 (1): 155-159.

Butcher, J. A. (1968a): The ecology of fungi infecting untreated sapwood of *Pinus radiata*. Canadian Journal of Botany 46: 1577-1589.

Butcher, J. A. (1968b): The causes of sapstain in red beech. New Zealand Journal of Botany 6: 376-385.

Butcher, J. A., Howard, M. (1968c): Outside storage of *Pinus radiata* wood chips in New Zealand. Tappi 51(4): 118A-122A.

Butcher, J. A., Drysdale, J. A. (1991): Biodeterioration and natural durability. Chapter 9 in Kininmonth, J. A., Whitehouse, L. J. (Ed.): Properties and Uses of New Zealand Radiata Pine. Volume One: Wood Properties. New Zealand Ministry of Forestry and Forest Research Institute, Rotorua, New Zealand.

Butler, M. J., Day, A. W. (1998): Fungal melanins: a review. Canadian Journal of Microbiology 44: 1115-1136.

Cave, I. D., Walker, J. C. F. (1994): Stiffness of wood in fast-grown plantation softwoods: the influence of microfibril angle. Forest Products Journal 44(5): 43-48.

Chamberland, H. (1994): Gold labelling methods for the ultrastructural localization of host wall and pathogen components. In: Petrini, O., Ouellette, G. B. (Eds.): Host wall alterations by parasitic fungi. A.P.S. Press, St. Paul., Minnesota: 1-11.

Chapman, A. D. (1933): Effect of steam sterilization on susceptibility of wood to blue-staining and wood-destroying fungi. Journal of Agricultural Research 47(6): 369-374.

Chapman, A. D., Scheffer, T. C. (1940): Effect of blue stain on specific gravity and strength of Southern pine. Journal of Agricultural Research 61(2): 125-133.

Chen, T., Wang, Z., Gao, Y., Breuil, C., Hatton, J. V. (1994): Wood extractives and pitch problems: analysis and partial removal by biological treatment. Appita Journal 47: 463-466.

Chou, C. K. S. (1982): Susceptibility of *Pinus radiata* seedlings to infection by *Diplodia pinea* as affected by pre-inoculation conditions. New Zealand Journal of Forestry Science 12: 438-441.

Chou, C. K. S. (1987): Crown wilt of *Pinus radiata* associated with *Diplodia* of woody stems. European Journal of Forest Pathology 17: 398-411.

Clausen, C. A., Green, F., Highley, T. L. (1993): Characterization of monoclonal antibodies to wood-derived ß-1,4-xylanase of *Postia placenta* and their application to detection of incipient decay. Wood Science and Technology 27(3): 219-228.

Colley, R. H., Rumbold, C. T. (1930): Relation between moisture content of the wood and blue stain in Loblolly Pine. Journal of Agricultural Research 41: 389-399.

Cooper, P., Downs, S., Farrell, R. (2000): Validation of the Sapstain Danger Index. Paper presented at the 31st annual meeting of the International Research Group on Wood Preservation, Kona, Hawaii, 14-19 May 2000. Document No. IRG/WP00-10333.

Core, H. A., Côté, W. A., Day, A. C. (1979): Wood structure and identification. Syracuse University Press, Syracuse. 182 pp.

Cown, D. J. (1999): New Zealand Pine and Douglas-Fir - Suitability for Processing. Forest Research Bulletin 216. Rotorua, New Zealand. 72 pp.

Crane, P. E., Chakravarty, P., Hutchison, L., Hiratsuka, Y. (1996): Wood-degrading capabilities of microfungi isolated from *Populus tremuloides*. Material und Organismen 30(1):
33-44.

Croan, S. C., Highley, T. L. (1996): Fungal removal of wood sapstain caused by *Ceratocystis coerulescens*. Material und Organismen 30(1): 45-56.

Crossley, R. D. (1956): The effects of five sap stain fungi on the toughness of eastern white pine. State University College of Forestry, Syracuse, N.Y. (Unpublished thesis).

Curtis, K. M. (1930): A dieback of *Pinus radiata* and *P. muricata* caused by the fungus *Botryodiplodia pinea* (Desm.) Petr. Transcriptions of the New Zealand Institute 56: 52-57.

Da Costa, E. W. B. (1955): Effect of blue stain on the strength of *Pinus radiata*. C.S.I.R.O. Forest Products Newsletter no. 209, Melbourne, Australia: 1-2.

Daniel, G., Nilsson, T., Pettersson, B. (1989): Intra- and extracellular localization of lignin peroxidase during the degradation of solid wood and wood fragments by *Phanerochaete chrysosporium* by using transmission electron microscopy and immuno-gold labeling. Applied and Environmental Microbiology 55(4): 871-881.

Daniel, G. (1994): Use of electron microscopy for aiding our understanding of wood biodegradation. FEMS Microbiology Reviews 13: 199-233.

Daniel, G., Singh, A., Nilsson, T. (1996): Ultrastructural and immunocytochemical studies on the window and bordered pit membranes of *Pinus sylvestris* L. In: Donaldson, L. A., Singh, A. P., Butterfield, B. G., Whitehouse, L. J. (eds.): Recent advances in wood anatomy. Conference proceedings from the Third Pacific Regional Wood Anatomy Conference, held at the New Zealand Forest Research Institute, Rotorua, November 20-24, 1994.

De Hoog, G. S., Scheffer, R. J. (1984): *Ceratocystis* versus *Ophiostoma*: a reappraisal. Mycologia 76: 292-299.

Deising, H., Heiler, S., Rauscher, M., Xu, H., Mendgen, K. (1996): Cellular aspects of rust infection structure differentiation. In: Nicole, M., Gianinazzi-Pearson (Eds): Histology, Ultrastructure and Molecular Cytology of Plant-Microorganism Interactions. Kluwer, Dordrecht. Pp. 135-156.

Dekker, R. F. H., Richards, G. N. (1975a): Purification, properties, and mode of action of hemicellulase II produced by *Ceratocystis paradoxa*. Carbohydrate Research 42: 107-123.

Dekker, R. F. H., Richards, G. N. (1975b): Structures of the oligosaccharides from the enzymic hydrolysis of hemicellulose by a hemicellulase of *Ceratocystis paradoxa*. Carbohydrate Research 43: 335-344.

Dekker, R. F. H., Richards, G. N. (1976): Hemicellulases: their occurrence, purification, properties and mode of action. Advances in Carbohydrate Chemistry and Biochemistry 32: 277-352.

Del Sorbo, G., Scala, F., Parrella, G., Lorito, M., Comparini, C., Ruocco, M., Scala, A. (2000): Functional expression of the gene *cu*, encoding the phytotoxic hydrophobin cerato-ulmin, enables *Ophiostoma quercus*, a nonpathogen on elm, to cause symptoms of Dutch Elm disease. Molecular Plant-Microbe Interactions 13 (1): 43-53.

Deshpande, M. S., Rale, V. B., Lynch, J. M. (1992): *Aureobasidium pullulans* in applied microbiology: A status report. Enzyme and Microbial Technology 14: 514-527.

Dowding, P. (1969): The dispersal and survival of spores of fungi causing blue stain in pine. Trans. Br. Mycol. Soc. 52: 125-137.

Dowding, P. (1970): Colonisation of freshly bared pine sapwood surfaces by staining fungi. Transcriptions of the British Mycological Society 55: 399-412.

Duncan, C. G., Eslyn, W. E. (1966): Wood-decaying Ascomycetes and Fungi imperfecti. Mycologia 58(4): 642-645.

Eagen, R., Brisson, A., Breuil, C. (1997): The sap-staining fungus *Ophiostoma piceae* synthesizes different types of melanin in different growth media. Canadian Journal of Microbiology 43: 592-595.

Eagen, R., Kim, S. H., Kronstad, J. W., Breuil, C. (2001): An hydroxynaphtalene reductase gene from the wood-staining fungus *Ophiostoma floccosum* complements the buff phenotype in *Magnaporthe grisea*. Mycological Research 105(4): 461-469.

Eaton, R. A., Hale, M. D. C. (1993): Wood: decay, pests and protection.. First edition. Chapman and Hall, London. Chapter 5: Staining fungi and moulds: 130-145.

Elgersma, D. M. (1976): Production of pectic and cellulolytic enzymes by aggressive and non-aggressive strains of *Ophiostoma ulmi*. Netherland Journal of Plant Pathology 82: 161-172.

Encinas, O., Daniel, G. (1995): Wood cell wall biodegradation by the blue stain fungus *Botryodiplodia theobromae* Pat. Material und Organismen 29(4): 255-272.

Encinas, O., Daniel, G. (1996): Decay capacity of different strains of the blue stain fungus *Lasiodiplodia theobromae* on various wood species. Material und Organismen 30(4): 239-258.

Encinas, O., Daniel, G. (1997): Degradation of the gelatinous layer in aspen and rubberwood by the blue stain fungus *Lasiodiplodia theobromae*. IAWA Journal 18(2): 107-115.

Encinas, O., Henningsson, B., Daniel, G. (1998): Changes in toughness and fracture characteristics of wood attacked by the blue stain fungus *Lasiodiplodia theobromae*. Holzforschung 52: 82-88.

Encinas, O., Daniel, G. (1999): Depletion of non-structural compounds during attack of pine and birch wood by the blue stain fungus *Lasiodiplodia theobromae*. Journal of Tropical Forest Products 5(2): 184-196.

Eriksson, K.-E., Goodell, E. W. (1974): Pleiotropic mutants of the wood-rotting fungus *Polyporus adustus* lacking cellulase, mannanase and xylanase. Canadian Journal of Microbiology 20: 371-378.

Eriksson, K.-E., Blanchette, R. A., Ander, P. (1990): Microbial and Enzymatic Degradation of Wood and Wood Components. Springer-Verlag Berlin Heidelberg. 407 pp.

Eslyn, W., Davidson, R. W. (1976): Some wood-staining fungi from pulpwood chips. Memoirs of the New York Botanical Garden 28(1): 50-57.

Eusebio, M. A. (1964): Growth of five staining fungi and stain development in pine sapwood. The Philippine Journal of Forestry 20(1-4): 69-91.

Fagan, G. B., McLain, T. E. (1983): The influence of small grain angle variation on toughness. Wood and Fiber Science 15(2): 94-101.

Farkás, V. (1979): Biosynthesis of cell walls in fungi. Microbiology Review 43: 117-144.

Farrell, R. L., Blanchette, R. A., Brush, T. S., Hadar, Y., Iverson, S., Krisa, K., Wendler, P. A., Zimmerman, W. (1993): Cartapip: A biopulping product for control of pitch and resin acid problems in pulp mills. Journal of Biotechnology 30: 115-122.

Farrell, R. L., Duncan, S. M., Ram, A. P., Kay, S. J., Hadar, E., Hadar, Y., Blanchette, R. A., Harrington, T. C., McNew, D. (1997): Causes of sapstain in New Zealand. Pp. 25-29 in Kreber, B. (Ed.): Strategies for improving protection of logs and lumber. Proceedings of a symposium at the Forest Research Institute, Rotorua, New Zealand. FRI Bulletin No. 204.

Farrell, R. L., Hadar, E., Kay, S. J., Blanchette, R. A., Harrington, T. C. (1998): Survey of Sapstain Organisms in New Zealand and Albino Anti-Sapstain Fungi. In Morrell, J. J. (Ed.): Biology and Prevention of Sapstain. Conference proceedings from Whistler, B.C., Canada, May 1997. Forest Products Society publication no. 7273, Madison, U.S.A.: 57-62.

Farrell, R. L. (1999): Final report to Forestry Consortium. University of Waikato Albino Research Project Relating to Control of Sapstain in *Pinus radiata*. Report no. X199.036.

Farrell, R. L., Mulcahy, J., Nobbs, R., Rose, T., Richardson, D., Ram, A., Thwaites, J., Haryati, T., Held, B., McNew, D., Blanchette, R. A., Harrington, T. C. (2000): Research in progress: resin degradation and brightness increase of radiata pine with fungal treatment in lab and mill trials. Paper presented at the International Symposium on Environmentally Friendly and Emerging Technologies for a Sustainable Pulp and Paper Industry, April 25-29 in Taipei, Taiwan. Conference proceedings published by Taiwan Forestry Research Institute, TFRI Extension Series No. 115.

Farrell, R. L.; Thwaites, J. M. (2001): Future Directions for Biological Control & BioActivity®. The International Research Group on Wood Preservation. Document IRG/WP 01-10416. Stockholm, Sweden.

Fengel, D. (1972): Structure and function of the membrane in softwood bordered pits. Holzforschung 26(1): 1-9.

Fengel, D., Wegener, G. (1989): Wood: Chemistry, Ultrastructure, Reactions. Walter de Gruyter, Berlin, New York. pp. 106-131.

Fergus, B. J., Procter, A. R., Scott, J. A. N., Goring, D. A. I. (1969): The distribution of lignin in sprucewood as determined by ultraviolet microscopy. Wood Science and Technology 3: 117-138.

Findlay, W. P. K., Pettifor, C. B. (1937): The effect of sap-stain on the properties of timber. I. Effect of sap-stain on the strength of Scots pine sapwood. Forestry 11: 40-52.

Findlay, W. P. K. (1939): The effect of sap-stain on the properties of timber. II. Effect of sap-stain on the decay resistance of pine sapwood. Forestry 13: 59-67.

Findlay, W. P. K., Pettifor, C. B. (1939a): The effect of sap-stain on the properties of timber. III. Effect of sap-stain on the modulus of elasticity of Scots pine sapwood. Forestry 13: 146-147.

Findlay, W. P. K., Pettifor, C. B. (1939b): Effect of blue stain on the strength of Obeche (*Triplochiton scleroxylon*). Empire Forestry Journal 18: 259-267.

Findlay, W. P. K. (1959a): Sap-stain of timber (Part I). Forestry Abstracts 20 (1): 1-7.

Findlay, W. P. K. (1959b): Sap-stain of timber (Part II). Forestry Abstracts 20 (2): 167-174.

Fischer, K., Akhtar, M., Blanchette, R. A., Burnes, T. A., Messner, K., Kirk, T. K. (1994): Reduction of resin content in wood chips during experimental biological pulping processes. Holzforschung 48(4): 285-290.

Florence, M., Gnanaharan, R., Sharma, J. K. (1998): Changes in strength properties of rubber wood stained by *Botryodiplodia theobromae*. Material und Organismen 32(4): 315-319.

Fogarty, W. M., Kelly, C. T. (1983): Pectic Enzymes. In: Fogarty, W. M. (Ed.): Microbial Enzymes and Biotechnology. Applied Science Publishers, London: 131-182.

Forest Products Laboratory (1987): Wood Handbook: Wood as an engineering material. Agric. Handb. 72. Washington, D.C., USA. p. 466.

Foster, R. C., Marks, G. C. (1968): Fine structure of the host-parasite relationship of *Diplodia pinea* on *Pinus radiata*. Australian Forestry 32: 211-225.

Fraedrich, S. W., Miller, T. (1995): Mycoflora associated with slach-pine seeds from cones collected at seed orchards and cone-processing facilities in the south-eastern USA. European Journal of Forest Pathology 25: 73-82.

Gagnon, C. (1961): Growth of *Ceratocystis ulmi* (Buisman) C. Moreau in liquid culture media. Canadian Journal of Botany 39: 1087-1093.

Gagnon, C. (1967): Histochemical studies on the alteration of lignin and pectic substances in white elm infected by *Ceratocystis ulmi*. Canadian Journal of Botany 45: 1619-1623.

Gao, Y., Breuil, C., Chen, T. (1994): Utilization of triglycerides, fatty acids and resin acids in lodgepole pine wood by a sapstaining fungus *Ophiostoma piceae*. Material und Organismen 28: 105-117.

Gao, Y., Breuil, C. (1995): Extracellular lipase production by a sapwood-staining fungus, *Ophiostoma piceae*. World Journal of Microbiology and Biotechnology 11: 638-642.

Gao, Y., Chen, T., Breuil, C. (1995): Identification and quantification of nonvolatile lipophilic substances in fresh sapwood and heartwood of lodgepole pine (*Pinus contorta* Dougl.). Holzforschung 49: 20-28.

Gao, Y., Breuil, C. (1998): Properties and substrate specificities of an extracellular lipase purified from *Ophiostoma piceae*. World Journal of Microbiology and Biotechnology 14: 421-429.

Gerhards, C. C. (1968): Effects of type of testing equipment and specimen size on toughness of wood. U.S.D.A. Forest Service Research Paper FPL 97. Madison, Wisconsin. 12 pp.

Gharibian, S., Hoffert, C., Abraham, L. D., Breuil, C. (1996): Localizing an *Ophiostoma piceae* proteinase in sapwood of four tree species using polyclonal antibodies. New Phytologist 133: 673-679.

Gibbs, J. N., Inman, A. (1991): The pine shoot beetle *Tomicus piniperda* as a vector of blue stain fungi to windblown pine. Forestry 64: 239-249.

Gibbs, J. N. (1993): The biology of ophiostomatoid fungi causing sapstain in trees and freshly cut logs. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. Wingfield, M.J., Seifert, K.A., Webber, J.J. (eds.) American Phytopathological Society, Minneapolis: 153-160.

Gooday, G. W., Gow, N. A. R. (1990): Enzymology of tip growth in fungi. Pp. 31-58 in Heath, I. B. (Ed.): Tip growth in plant and fungal cells. Academic Press, San Diego.

Greaves, H., Savory, J. G. (1965): Studies on the microfungi attacking preservative-treated timber with particular reference to their methods of isolation. Journal of the Institute of Wood Science 15: 45-50.

Greaves, H. (1973): Outside storage of tropical hardwood chips. III. Microbial ecology of chip piles after two and four months storage. Appita 27(1): 25-30.

Green, F. III, Clausen, C. A., Larsen, M. J., Highley, T. L: (1992): Immuno-scanning electron microscopic localization of extracellular wood-degrading enzymes within the fibrillar sheath of the brown-rot fungus *Postia placenta*. Canadian Journal of Microbiology 38: 898-904.

Green, F. III, Clausen, C. A., Kuster, T. A., Highley, T. L. (1995): Induction of polygalacturonase and the formation of oxalic acid by pectin in brown-rot fungi. World Journal of Microbiology and Biotechnology 11: 519-524.

Green, F. III, Clausen, C. A. (1999): Production of polygalacturonase and increase of longitudinal gas permeability in Southern pine by brown-rot und white-rot fungi. Holzforschung 53: 563-568.

Grossmann, H. (1930): Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. Zeitschrift für Parasitenkunde (Berlin) 3: 56-102.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C., Pegler, D. N. (1996): Ainsworth & Bisby's Dictionary of the Fungi. Eighth Edition. University Press, Cambridge, UK. 616 pp.

Halsted, B. D. (1890): Some fungus diseases of sweet potato. New Jersey Agricultural College Experiment Station Bulletin 76: 3-32.

Haltrich, D., Nidetzky, B., Kulbe, K. D., Steiner, W., Zupancic, S. (1996): Production of fungal xylanases. Bioresource Technology 58: 137-161.

Hardham, A. R., Mitchell, H. J. (1998): Use of molecular cytology to study the structure and biology of phytopathogenic and mycorrhizal fungi. Fungal Genetics and Biology 24: 252-284.

Hardie, K. (1980): A review of strength testing as a measure of biodeterioration of wood and wood based materials. International Biodeterioration Bulletin 16(1): 1-8.

Harrington, T. C. (1981): Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. Mycologia 73: 1123-1129.

Harrington, T. C. (1987): New combinations in *Ophiostoma* of *Ceratocystis* species with *Leptographium* anamorphs. Mycotaxon 28: 39-43.

Harrington, T. C. (1993): Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. Wingfield, M.J., Seifert, K.A., Webber, J.J. (eds.) American Phytopathological Society, Minneapolis: 141-151.

Harrington, T. C., Wingfield, M. J. (1998): Diseases and the ecology of indigenous and exotic pines. In: D. M. Richardson (ed.): Ecology and Biogeography of Pinus. Cambridge University Press, Cambridge.

Harrington, T. C., McNew, D. L., Steimel, J. P., Hofstra, D., Farrell, R. L. (2001): Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia 93(1): 111-136.

Harris, J. M. (1961): Water conduction in the stems of certain conifers. Nature 189 (4765): 678-679.

Harris, J. M. (1991): Structure of wood and bark. Chapter 2 in Kininmonth, J. A., Whitehouse, L. J. (Ed.): Properties and Uses of New Zealand Radiata Pine. Volume One: Wood Properties. New Zealand Ministry of Forestry and Forest Research Institute, Rotorua, New Zealand.

Hartig, R. (1878): Die Zersetzungserscheinungen des Holzes, der Nadelbäume und der Eiche in forstlicher, botanischer und chemischer Richtung. Julius Springer Verlag, Berlin.151 pp.

Hausner, G., Reid, J., Klassen, G. R. (1993a): On the subdivision of *Ceratocystis s.l.*, based on partial ribosomal DNA sequences. Canadian Journal of Botany 71: 52-63.

Hausner, G., Reid, J., Klassen, G. R. (1993b): *Ceratocystiopsis*: a reappraisal based on molecular criteria. Mycological Research 97: 625-633.

Hedley, M. E., Armstrong, W. (1975): Copper-chrome-arsenate preservative effectiveness in relation to wood density. Material und Organismen 10(1): 67-80.

Henningsson, B., Lundström, H. (1974): Insect-borne blue-stain. Growth and effects caused by water immersion of the wood. Some laboratory tests. Royal Coll. For., Dept. For. Prod. Report R 92.

Hillis, W. E. (1968): Chemical aspects of heartwood formation. Wood Science and Technology 2: 241-259.

Himelick, E. B. (1982): Pine blue-stain associated with the pine wilt syndrome. Journal of Arboriculture 8: 212-216.

Hirano, T., Tanaka, H., Eneki, A. (1997): Relationship between production of hydroxyl radicals and degradation of wood by the brown-rot fungus, *Tyromyces palustris*. Holzforschung 51: 389-395.

Hiratsuka, Y., Chakravarty, P., Miao, S., Ayer, W. A. (1993): Potential for biological protection against blue stain in *Populus tremuloides* with a hyphomycetous fungus, *Stachybotrys cylindrospora*. Canadian Journal of Forest Research 24: 174-179.

Hiratsuka, Y.; Chakravarty, P. (1999): Role of *Phialemonium curvatum* as a potential biological control agent against a blue stain fungus on aspen. European Journal of Forest Pathology 29: 305-310.

Hoffert, C., Gharibian, S., Brown, D. L., Breuil, C. (1995): Immunolocalization of a proteinase of the sap-staining fungus *Ophiostoma piceae* using antibodies to proteinase K. Canadian Journal of Botany 73: 1604-1610.

Hofsten, B. v. (1956): Growth and metabolism of *Ophiostoma multiannulatum* on different sources of carbon. Physiologia Plantarum 9: 624-639.

Howard, R. J., Ferrari, M. A. (1989): Role of melanin in appressorium function. Experimental Mycology 13: 403-418.

Huang, J. W., Kuhlman, E. G. (1990): Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Disease 74: 27-30.

Hudson, H. J. (1986): Fungal Biology. Edward Arnold, London; Baltimore, USA. 298 pp.

Hutchison, L. J., Reid, J. (1988a): Taxonomy of some potential wood-staining fungi from New Zealand. 1. *Ophiostomataceae*. New Zealand Journal of Botany 26: 63-81.

Hutchison, L. J., Reid, J. (1988b): Taxonomy of some potential wood-staining fungi from New Zealand. 2. Pyrenomycetes, Coelomycetes and Hyphomycetes. New Zealand Journal of Botany 26: 83-98.

Imamura, Y., Harada, H., Saiki, H. (1974): Embedding substances of pit membranes in softwood tracheids and their degradation by enzymes. Wood Science and Technology 8: 243-254.

Jacobs, K., Wingfield, M. J., Uzunovic, A., Frisullo, S. (2001): Three new species of *Leptographium* from pine. Mycological Research 105(4): 490-499.

Jalava, M. (1938): Effect of blue stain on the strength of pine timber. Helsinki, Teknillisiä Tiedonantoja (Finland Found. for Forest Prod. Res.), No. 8, 24 pp. (in Finnish, abstract in English in: Chem. Abs. 33: 6018, 1939).

Jarvis, M. C. (1984): Structure and properties of pectin gels in plant cell walls. Plant, Cell and Environment 7: 153-164.

Jewell, T. R. (1974): A qualitative study of cellulose distribution in *Ceratocystis* and *Europhium*. Mycologia 66: 139-146.

Johann, F. F. (1931): Untersuchungen über die Dauerhaftigkeit verblauten Kiefernholzes. Mitteilungen aus Forstwirtschaft und Forstwissenschaft 2: 209-221.

Juzlova, P., Martinkova, L., Kren, V. (1996): Secondary metabolites of the fungus *Monascus*: a review. Journal of Industrial Microbiology 16: 163-170.

Käärik, A. (1960): Growth and sporulation of *Ophiostoma* and some other blueing fungi on synthetic media. Symbolae Botanicae Upsalienses 16. 168 pp.

Käärik, A. (1980): Fungi causing sap stain in wood. The Swedish University of Agricultural Sciences, Department of Forest Products Report No. 114. 112 pp.

Kalisz, H. M., Wood, D. A., Moore, D. (1987): Production, regulation and release of extracellular proteinase activity in Basidiomycete fungi. Transactions of the British Mycological Society 88: 221-227.

Karadzic, D., Vujanovic, V. (1992): A contribution to the study of pathogenic mycoflora of Aleppo pine (*Pinus halepensis* Mill.) in the Mediterranean part of Montenegro. Sumarstvo 74: 31-41.

Kay, S. J. (1997): Biological control of sapstain in New Zealand. Pp. 75-79 in Kreber, B. (Ed.): Strategies for improving protection of logs and lumber. Proceedings of a symposium at the Forest Research Institute, Rotorua, New Zealand. FRI Bulletin No. 204.

Keilich, G., Bailey, P., Liese, W. (1970): Enzymatic degradation of cellulose, cellulose derivatives and hemicelluloses in relation to the fungal decay of wood. Wood Science and Technology 4: 273-283.

Kendrick, W. B. (1962): The *Leptographium* complex. *Verticicladiella* Hughes. Canadian Journal of Botany 40: 771-797.

Kennedy, R. W. (1958): Strength retention in wood decayed to small weight losses. Forest Products Journal 8: 308-314.

Kerr, A. J., Goring, D. A. I. (1975): The ultrastructural arrangement of the wood cell wall. Cellulose Chemistry and Technology 9: 563-573.

Khalili, S., Nilsson, T., Daniel, G. (2001): The use of soft rot fungi for determining the microfibrillar orientation in the S2 layer of pine tracheids. Holz als Roh- und Werkstoff 58: 439-447.

Kile, G. A. (1993): Plant diseases caused by species of *Ceratocystis sensu stricto* and *Chalara*. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. Wingfield, M.J., Seifert, K.A., Webber, J.J. (eds.) American Phytopathological Society, Minneapolis: 173-183.

Kim, G. H., Morrell, J. J. (1998): Biological protection against fungal discoloration: spatial distribution of the bacterial bioprotectant and target fungi on ponderosa pine sapwood. Material und Organismen 32(1): 17-27.

Kim, Y. S., Wi, S. G., Lee, K. H., Singh, A. P. (2001): Cytochemical localisation of hydrogen peroxide production during wood decay by brown-rot fungi *Tyromyces palustris* and *Coniophora puteana*. Holzforschung. In press.

King, B., Eggins, H. O. W. (1973): Decay mechanisms of microfungi which might produce an enhanced permeability in wood. International Biodeterioration Bulletin 9 (1-2): 35-43.

King, E. J., Kutscha, N. P., Campana, R. J. (1974): Preliminary scanning electron microscopy of *Ceratocystis ulmi* in elm wood. Proceedings of the American Phytopathological Society, Abs. 1: 137.

Kirk, T. K., Highley, T. L. (1973): Quantitative changes in structural components of conifer woods during decay by white- and brown-rot fungi. Phytopathology 63: 1338-1342.

Kocková-Kratochvílová, A., Slávikova, E., Zemek, J., Kadleciková, B., Kuniak, L. (1983): Utilization of the hydrolytic activity in taxonomy of hyphal yeast *Ascomycetes*. Folia Microbiologica 28: 301-308.

Koenings, J. W. (1974): Hydrogen peroxide and iron: A proposed system for decomposition of wood by brown-rot Basidiomycetes. Wood and Fiber 62: 66-79.

Kollmann, F. (1951): Technologie des Holzes und der Holzwerkstoffe. Erster Band: Anatomie und Pathologie, Chemie, Physik, Elastizität und Festigkeit. Zweite Auflage, Reprint 1982. Springer-Verlag Berlin, Heidelberg, New York. 1050 pp.

Krapivina, I. G. (1960): Destruction of the secondary layer of the cell wall by blue stain fungi. Lesnoi Zhurnal 3 (1): 130-133. Translation no. 5329 in CSIRO abstracts vol. 8 (1960).

Krause, C. R., Wilson, C. L. (1972): Fine structure of *Ceratocystis ulmi* in elm wood. Phytopathology 62: 1254-1256.

Kreber, B., Morrell, J. J. (1993): Ability of selected bacterial and fungal bioprotectants to limit fungal stain in Ponderosa pine sapwood. Wood and Fiber Science 25(1): 23-34.

Kreber, B., van der Waals, J. (1999): Decay potential of various New Zealand *Phlebiopsis gigantea* strains using a soil block method. The International Research Group on Wood Preservation. Document IRG/WP99-10309. Stockholm, Sweden.

Kreber, B., Eden, D., Chittenden, C., van der Waals, J., Wakeling, R., Carpenter, B. (1999): What triggers fungal melanisation in wood? Pp. 133-137 in Kreber, B. (Ed.): Strategies for improving protection of logs and lumber. Proceedings of a symposium at the Forest Research Institute, Rotorua, New Zealand. FRI Bulletin No. 204.

Kremnický, L., Sláviková, E., Mislovicová, D., Biely, P. (1996): Production of Extracellular ß-Mannanases by Yeasts and Yeast-like Microorganisms. Folia Microbiologica 41 (1): 43-47.

Kremnický, L., Biely, P. (1997): ß-mannanolytic system of *Aureobasidium pullulans*. Archives of Microbiology 167: 350-355.

Kühne, H., Leukens, U., Sell, J., Wälchli, O. (1970): Untersuchungen an bewitterten Holzoberflächen - Erste Mitteilung: Raster-elektronenmikroskopische Beobachtungen an Vergrauungspilzen. (Investigations on weathered wood surfaces - Part I: Scanning electron-microscope observations on mold-fungi causing grey stain.) Holz als Roh- und Werkstoff 28: 223-229.

Lagerberg, T., Lundberg, G., Melin, E. (1927): Biological and practical researches into bluing in Pine and Spruce. Svenska SkogsvFören. Tidskr. 25: 145-272, 561-691.

Langrish, T. A. G., Walker, J. C. F. (1993): Transport processes in wood. Pp. 121-152 in Walker, J. C. F. (Ed.): Primary Wood Processing. Principles and Practice. Chapman and Hall, London.

Leathers, T. D., Kurtzman, C. P., Detroy, R. W. (1984): Overproduction and regulation of xylanase in *Aureobasidium pullulans* and *Cryptococcus albidus*. Biotechnology and Bioengineering Symposium 14: 225-240.

Leathers, T. D. (1986): Color variants of *Aureobasidium pullulans* overproduce xylanase with extremely high specific activity. Applied and Environmental Microbiology 52(5): 1026-1030.

Leathers, T. D., Detroy, R. W., Bothast, R. J. (1986): Induction and glucose repression of xylanase from a color variant strain of *Aureobasidium pullulans*. Biotechnology Letters 8(12): 867-872.

Leone, R., Breuil, C. (1998): Filamentous fungi can degrade aspen steryl esters and waxes. International Biodeterioration and Biodegradation 41: 133-137.

Lever, M. (1973): Colorimetric and fluorometric carbohydrate determination with p-hydroxybenzoic acid hydrazide. Biochemical Medicine 7: 274-281.

Lévieux, J., Cassier, P., Guillaumin, D., Roques, A. (1991): Structures implicated in the transportation of pathogenic fungi by the European bark beetle *Ips sexdentatus* Boerner: ultrastructure of a mycangium. Canadian Entomologist 123: 245-254.

Liese, W., Hartmann-Fahnenbrock, M. (1953): Elektronenmikroskopische Untersuchungen an verblautem Kiefernholz. Holzforschung 7(4): 97-102.

Liese, W. (1959): Die Moderfäule, eine neue Krankheit des Holzes. Naturwissenschaftliche Rundschau 12: 419-425.

Liese, W., Pechmann, H. v. (1959): Untersuchungen über den Einfluß von Moderfäulepilzen auf die Holzfestigkeit. Forstwissenschaftliches Centralblatt 78 (9/10): 271-279.

Liese, W., Schmid, R. (1961): Licht- und elektronenmikroskopische Untersuchungen über das Wachstum von Bläuepilzen in Kiefern- und Fichtenholz. Holz als Roh- und Werkstoff 19: 329-337.

Liese, W., Schmid, R. (1962): Elektronenmikroskopische Untersuchungen über den Abbau des Holzes durch Pilze. Angewandte Botanik 36 (6): 291-298.

Liese, W., Ammer, U. (1964): Über den Einfluß von Moderfäulepilzen auf die Schlagbiegefestigkeit von Buchenholz. (On the Influence of Soft-Rot Fungi on the Impact Bending Strength of Beech Wood.) Holz als Roh- und Werkstoff 22(12): 455-459.

Liese, W., Schmid, R. (1964): Über das Wachstum von Bläuepilzen durch verholzte Zellwände. Phytopathologische Zeitschrift 51 (4): 385-393.

Liese, W. (1970a): The action of fungi and bacteria during wood deterioration. Proceedings of the B.W.P.A. annual convention.

Liese, W. (1970b): Ultrastructural aspects of woody tissue disintegration. Annual Review of Phytopathology 8: 231-258.

Lindgren, R. M., Scheffer, T. C. (1939): Effect of blue stain on the penetration of liquids into air-dry Southern pine wood. Proceedings of the American Wood Preservers´ Association 35: 325-337.

Lindgren, R. M. (1942): Temperature, moisture and penetration studies of wood-staining *Ceratostomellae* in relation to their control. Tech. Bull. U.S. Dep. Agric. No. 807.

Lindgren, R. M. (1952): Permeability of Southern pine as affected by mold and other fungus infection. Proceedings of the American Wood Preservers´ Association 48: 158-174.

Luley, C. J., Gleason, M. L. (1988): *Diplodia* canker (*Sphaeropsis sapinea*) of *Abies concolor* in Iowa. Plant Disease 72: 79.

Lyr, H. (1959a): Die Bildung von Ektoenzymen durch holzzerstörende und holzbewohnende Pilze auf verschiedenen Nährböden. I. Mitteilung: Pectin als C-Quelle. Archiv für Mikrobiologie 33: 266-282.

Lyr, H. (1959b): Die Bildung von Ektoenzymen durch holzzerstörende und holzbewohnende Pilze auf verschiedenen Nährböden. II. Mitteilung: Cellulose als C-Quelle. Archiv für Mikrobiologie 34: 189-203.

Lyr, H. (1960): Die Bildung von Ektoenzymen durch holzzerstörende und holzbewohnende Pilze auf verschiedenen Nährböden. V. Mitteilung: Ein komplexes Medium als C-Quelle. Archiv für Mikrobiologie 35: 258-278.

MacDonald, W. L., McNabb, H. S. jr. (1970): Fine structure observations of the growth of *Ceratocystis ulmi* in elm xylem tissue. BioScience 20: 1060-1061.

Mack, J. J. (1979): Australian methods for mechanically testing small clear specimens of timber. Division of Building Research Technical Paper (second series) no. 31. Commonwealth Scientific and Industrial Research Organization, Australia. 19 pp.

Manacini, P. L., Parini, C., Fortina, M. G. (1988): Pectic enzymes from *Aureobasidium pullulans* LV 10. Enzyme and Microbial Technology 10: 682-685.

Mansfield, S. D., Saddler, J. N., Gübitz, G. M. (1998): Characterization of endoglucanases from the brown rot fungi *Gloeophyllum sepiarium* and *Gloeophyllum trabeum.* Enzyme and Microbial Technology 23: 133-140.

Marcilla, A., Elorza, M. V., Mormeneo, S., Rico, H., Sentandreu, R. (1991): *Candida albicans* mycelial wall structure. Supramolecular complexes released by zymolyase, chitinase and beta-mercaptoethanol. Archives of Microbiology 155: 312-319.

Marks, G. C., Minko, G. (1969): The virulence of *Diplodia pinea* to *Pinus radiata* D. Don. Australian Journal of Botany 17: 1-12.

Martínez-Inigo, M. J., Immerzeel, P., Gutierrez, A., Carlos del Rio, J., Sierra-Alvarez, R. (1999): Biodegradability of extractives in sapwood and heartwood from Scots pine by sapstain and white rot fungi. Holzforschung 53: 247-252.

Mathiesen-Käärik, A. (1953): Eine Übersicht über die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze Schwedens und einige für Schweden neue Bläuepilze. Meddn. St. Skogsforsk. Inst. 43: 3-74.

Mathiesen-Käärik, A. (1955): Einige Untersuchungen über den Sporengehalt der Luft in einigen Bretterhöfen in Stockholm. Svensk. bot. Tidskr. 49: 437-459.

Mayer-Wegelin, H., Brunn, G., Loos, W. (1931): Zur Frage der Bewertung stammblauen Kiefernholzes. Mitt. aus Forstw. u. Forstwiss.: 573-588.

McCall, K. A., Merrill, W. (1980): Selective medium for *Verticicladiella procera.* Plant Disease 64: 277-278.

McNaughton, C. L. (1997): The purification and characterisation of an extracellular lipase from *Ophiostoma piliferum.* Master of Science thesis, Biological Sciences, University of Waikato, Hamilton, New Zealand.

Mendgen, K., Deising, H. (1993): Infection structures of fungal plant pathogens - a cytological and physiological evaluation. New Phytologist 124: 193-213.

Meyer, R. W. (1974): Effect of enzyme treatment on bordered-pit ultrastructure, permeability and toughness of the sapwood of three Western conifers. Wood Science 6(3):: 220-230.

Miller, D. J., Goodell, B. (1981): Blue staining in Ponderosa pine sapwood at moderate and low temperatures. Forest Products Journal 31: 54-59.

Miller, G. L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31: 426-428.

Miller, H. J., Elgersma, D. M. (1976): The growth of aggressive and non-aggressive strains of *Ophiostoma ulmi* in susceptible and resistant elms, a scanning electron microscopical study. Netherlands Journal of Plant Pathology 82: 51-65.

Minter, D. W., Kirk, P. M., Sutton, B. C. (1983): Thallic phialides. Transcriptions of the British Mycological Society 80: 39-66.

Morrell, J. (1999): Global perspective on future opportunities for alternatives to conventional antisapstain treatments. Pp. 7-12 in Kreber, B. (Ed.): Strategies for improving protection of logs and lumber. Proceedings of a symposium at the Forest Research Institute, Rotorua, New Zealand. FRI Bulletin No. 204.

Münch, E. (1907-1908): Die Blaufäule des Nadelholzes. Naturwissenschaftliche Zeitschrift für Forst- und Landwirtschaft 5: 531-573, 6: 32-47, 297-323.

Musha, Y., Goring, D. A. I. (1975): Distribution of syringyl and guaiacyl moieties in hardwoods as indicated by ultraviolet microscopy. Wood Science and Technology 9: 45-58.

New Zealand Ministry of Forestry (1995): A guide to the New Zealand and Australian Timber Grading Rules. 67 pp.

Nicholas, D. D., Thomas, R. J. (1968): The influence of enzymes on the structure and permeability of loblolly pine. Proceedings of the American Wood Preservers' Association 58: 70-76.

Niemz, P. (1993): Physik des Holzes und der Holzwerkstoffe. DRW-Verlag, Leinfelden-Echterdingen, Germany. 243 pp.

Nilsson, T. (1973): Studies on wood degradation and cellulolytic activity of microfungi. Studia Forestalia Suecica No. 104. Royal College of Forestry, Stockholm, Sweden. 40pp.

Nilsson, T. (1974): The degradation of cellulose and the production of cellulase, xylanase, mannanase and amylase by wood-attacking microfungi. Studia Forestalia Suecica No. 114. Royal College of Forestry, Stockholm, Sweden. 61pp.

Ouellette, G. B. (1962): Studies in the infection process of *Ceratocystis ulmi* (Buisman) C. Moreau in American elm. Canadian Journal of Botany 40: 1567-1575.

Ouellette, G. B., Méthot, N., Chamberland, H., Côté, C., Lafontaine, J.-G. (1995): Cytology of irregular growth forms of *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* growing through millipore filter membranes and sterilized elm wood sections. Canadian Journal of Microbiology 41: 1095-1110.

Ouellette, G. B., Chamberland, H., Goulet, A., Lachapelle, M., Lafontaine, J.-G. (1999): Fine structure of the extracellular sheath and cell walls in *Ophiostoma novo-ulmi* growing on various substrates. Canadian Journal of Microbiology 45: 582-597.

Palmer, M. A., Nicholls, T. H. (1985): Shoot blight and collar rot of *Pinus resinosa* caused by *Sphaeropsis sapinea* in forest tree nurseries. Plant Disease 69: 739-740.

Palmer, M. A., McRoberts, R. E., Nicolls, T. H. (1988): Sources of inoculum of *Sphaeropsis sapinea* in forest tree nurseries. Phytopathology 78: 831-835.

Panshin, A. J., de Zeeuw, C. (1980): Textbook of Wood Technology. Structure, Identification, Properties, and Uses of the Commercial Woods of the United States and Canada. Chapter 10: Natural Durability of Wood, Wood Deterioration and Stains: 350-404. Fourth Ed. McGraw-Hill.

Parameswaran, N., Liese, W. (1982): Ultrastructural localization of wall components in wood cells. Holz als Roh- und Werkstoff 40: 145-155.

Parmeter, J. R., Slaughter, G. W., Chen, M.-M., Wood, D. L., Stubbs, H. A. (1989): Single and mixed inoculations of ponderosa pine with fungal associates of *Dendroctonus* spp. Phytopathology 79: 768-772.

Patterson, H. D., Thompson, R. (1971): Recovery of inter-block information when block sizes are unequal. Biometrika 58: 545-554.

Payne, C., Bruce, A. (1999): Screening of bacteria, yeasts and *Trichoderma* isolates for antagonism toward stain and mould fungi on agar media and wood. The International Research Group on Wood Preservation. Document IRG/WP99-20159. Stockholm, Sweden.

Pechmann, H. von, Graessle, E., Wutz, A. (1964): Untersuchungen über Bläuepilze an Kiefernholz. Forstwissenschaftliches Centralblatt 83: 290-314.

Pechmann, H. von (1965): Der Einfluss der Temperatur auf das Wachstum von Bläuepilzen. Material und Organismen Supplement 1: 237-250.

Pearce, R. B. (1996): Antimicrobial defences in the wood of living trees. New Phytologist 132: 203-233.

Peterson, G. W. (1977): Infection, epidemiology and control of Diplodia blight of Austrian, Ponderosa and Scots pines. Phytopathology 67: 511-514.

Pettersen, R. C., Schwandt, V. H. (1991): Wood sugar analysis by anion chromatography. Journal of Wood Chemistry and Technology 11 (4): 495-501.

Pettifor, C. B., Findlay, W. P. K. (1946): Effect of sap-stain on the tensile strength of Corsican pine sapwood (*Pinus nigra* var *calabrica* Schneid.). Forestry 20: 57-61.

Phillips, E. W. J. (1933): Movement of the pit membrane in coniferous woods with special reference to preservative treatment. Forestry 7(2): 109-120.

Pinheiro, A. C. A. (1971): Blue stain in poplar wood. Material und Organismen 6: 93-100.

Pomerleau, R. (1970): Pathological anatomy of the Dutch elm disease. Distribution and development of *Ceratocystis ulmi* in elm tissue. Canadian Journal of Botany 48: 2043-2057.

Poutanen, K., Tenkanen, M., Korte, H., Puls, J. (1991): Accessory enzymes involved in the hydrolysis of xylans. In: Leatham, G. F., Himmel, M. E.: Enzymes in Biomass Conversion. ACS Symp. Series Vol. 460. American Chemical Society, Washington: 426-436.

Powell, M. A., Eaton, R. A., Webber, J. F. (1995): The role of micro-arthropods in the defacement of sawn lumber by sapstain and mould fungi. Canadian Journal of Forest Research 25: 1148-1156.

Preston, A. F. (1999): Global perspective on future trends for the antisapstain industry. Pp. 3-5 in Kreber, B. (Ed.): The 2nd New Zealand Sapstain Symposium. Proceedings of Symposium, Rotorua, New Zealand, 18-19 November. Forest Rsearch Bulletin No. 215.

Puls, J., Schuseil, J. (1993): Chemistry of hemicelluloses: relationship between hemicellulose structure and enzymes required for hydrolysis. In: Coughlan, M. P., Hazlewood, G. P.: Hemicellulose and Hemicellulases. Portland Press, London: 1-27.

Punithalingam, E., Waterston, J. M. (1970): *Diplodia pinea*. In: Descriptions of Pathogenic Fungi and Bacteria, No. 273. Commonwealth Mycological Institute, Kew, Surrey, England.

Raffa, K. F., Smalley, E. B. (1988): Response of red and jack pines to inoculation with microbial associates of the pine engraver, *Ips pini* (Coleoptera: Scolytidae). Canadian Journal of Forestry Research 18: 581-586.

Rane, K. K., Tattar, T. A. (1987): Pathogenicity of blue-stain fungi associated with *Dendroctonus terebrans*. Plant Disease 71: 879-883.

Rautela, G. S., Cowling, E. B. (1966): Simple cultural test for relative cellulolytic activity of fungi. Applied Microbiology 14 (6): 892-898.

Rees, A. A., Webber, J. F. (1988): Pathogenicity of *Sphaeropsis sapinea* to seed, seedlings and saplings of some central American pines. Transcriptions of the British Mycological Society 91: 273-277.

Reese, E. T., Siu, R. G. H., Levinson, H. S. (1950): The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis. Journal of Bacteriology 59: 485-497.

Reese, E. T., Levinson, H. S. (1952): A comparative study of the breakdown of cellulose by microorganisms. Physiologia Plantarum 5: 345-366.

Rennerfelt, E. (1945): The influence of the phenolic substances of Pine heartwood on the growth of certain bluing fungi with an attempt at quantitative measurement of the intensity of bluing. Medd. Skogsförsöksanst. Stockholm 34: 391-416.

Rennerfelt, E. (1951): Über den Zusammenhang zwischen dem Verblauen des Holzes und den Insekten. Oikos 2: 120-137.

Richards, D. B. (1954): Physical changes in decaying wood. Journal of Forestry 52: 260-265.

Reynolds, P. E., Smith, W. H., Jensen, K. F. (1972): Effect of constant and fluctuating temperatures on the *in vitro* growth of *Ceratocystis* species. Transcriptions of the British Mycological Society 59: 1-9.

Riou, C., Freyssinet, G., Fevre, M. (1991): Production of Cell Wall-Degrading Enzymes by the Phytopathogenic Fungus *Sclerotinia sclerotiorum*. Applied and Environmental Microbiology 57 (5): 1478-1484.

Rioux, D., Ouellette, G. B. (1989): Light microscope observations of histological changes induced by *Ophiostoma ulmi* in various nonhost trees and shrubs. Canadian Journal of Botany 67: 2335-2351.

Rioux, D., Ouellette, G. B. (1991a): Barrier zone formation in host and nonhost trees inoculated with *Ophiostoma ulmi*. I. Anatomy and histochemistry. Canadian Journal of Botany 69: 2055-2073.

Rioux, D., Ouellette, G. B. (1991b): Barrier zone formation in host and nonhost trees inoculated with *Ophiostoma ulmi*. II. Ultrastructure. Canadian Journal of Botany 69: 2074-2083.

Rioux, D., Nicole, M., Simard, M., Ouellette, G. B. (1998): Immunocytochemical evidence that secretion of pectin occurs during gel (gum) and tylosis formation in trees. Phytopathology 88: 494-505.

Ritschkoff, A.-C., Viikari, L. (1991): The production of extracellular hydrogen peroxide by brown-rot fungi. Material und Organismen 26: 157-167.

Ritschkoff, A.-C., Buchert, J., Viikari, L. (1992): Identification of carbohydrate degrading enzymes from the brown-rot fungus, *Gloeophyllum trabeum*. Material und Organismen 27: 19-29.

Ritschkoff, A.-C., Buchert, J., Viikari, L. (1994): Purification and characterization of a thermophilic xylanase from the brown-rot fungus *Gloeophyllum trabeum*. Journal of Biotechnology 32: 67-74.

Rösch, R., Liese, W., Berndt, H. (1969): Untersuchungen über die Enzyme von Bläuepilzen. I. Cellulase-, Polygalakturonase-, Pektinesterase- and Laccase-Aktivität. Archiv für Mikrobiologie 67: 28-50.

Rösch, R., Liese, W. (1970): Ringschalen-Test mit holzzerstörenden Pilzen. I. Prüfung von Substraten für den Nachweis von Phenoloxidasen. Archiv für Mikrobiologie 73: 281-292.

Rösch, R., Liese, W. (1971): Untersuchungen über die Enzyme von Bläuepilzen. II. Phenoloxidasen-Aktivität. Archiv für Mikrobiologie 76: 212-218.

Rosinski, M. A., Campana, R. J. (1964): Chemical analysis of the cell wall of *Ceratocystis ulmi*. Mycologia 56: 738-744.

Rudeloff, M. (1897, 1899): Untersuchungen über den Einfluss des Blauwerdens auf die Festigkeit von Kiefernholz. Teil 1-2. Mitt. aus den K. Tech. Versuchsanst. 15: 1-46, 1897, 16: 209-239, 1899.

Sachs, I. B., Nair, V. M. G., Kuntz, J. E. (1970): Penetration and degradation of cell walls in oaks infected with *Ceratocystis fagacearum*. Phytopathology 60: 1399-1404.

Savory, J. G. (1954): Breakdown of timber by Ascomycetes and Fungi imperfecti. Annals of Applied Biology 41: 336-347.

Savory, J. G. (1955): Role of microfungi in the decomposition of wood. Records of the Annual Convention of the British Wood Preservers Association: 3-19.

Scala, A., Tegli, S., Del Sorbo, G., Scala, F. (1994): Role of cerato-ulmin in *Ophiostoma piceae O. ulmi sensu lato* system. Petria 4: 320-321.

Scheffer, R. J., Elgersma, D. M. (1982): A scanning electron microscope study of cell wall degradation in elm wood by aggressive and non-aggressive isolates of *Ophiostoma ulmi*. European Journal of Forest Pathology 12: 25-28.

Scheffer, T. C. (1986): O_2 requirements for growth and survival of wood-decaying and sapwood-staining fungi. Canadian Journal of Botany 64: 1957-1963.

Schmid, R., Liese, W. (1965): Zur Außenstruktur der Hyphen von Bläuepilzen. Phytopathologische Zeitschrift 54 (3): 275-284.

Schmidt, O., Liese, W. (1980): Variability of Wood Degrading Enzymes of *Schizophyllum commune*. Holzforschung 34 (2): 67-72.

Schmidt, O. (1994): Holz- und Baumpilze - Biologie, Schäden, Schutz, Nutzen. Springer-Verlag Berlin Heidelberg: 246 pp.

Schmidt, O., Müller, J. (1996): Praxisversuche zum biologischen Schutz von Kiefernholz vor Schimmel und Schnittholzbläue. Holzforschung und Holzverwertung 48(5): 81-84.

Schrenck, H. von (1903): The bluing and the red rot of the Western Yellow Pine, with special reference to the Black Hills Forest Reserve. Bull. U.S. Bur. Plant Ind. No. 36. 40 pp.

Seehann, G. (1965): Über die Wirkung einer Trocknung und Erwärmung von Nadelholz auf das Wachstum von Bläuepilzen. Holz als Roh- und Werkstoff 23: 341-347.

Seehann, G., Tabirih, P. K. (1983): Micromorphological and physiological studies in *Botryodiplodia theobromae* Pat. Material und Organismen 18(1): 7-25.

Seifert, K. A., Breuil, C., Rossignol, L., Best, M., Saddler, J. N. (1988): Screening for microorganisms with the potential for biological control of sapstain on unseasoned lumber. Material und Organismen 23: 81-96.

Seifert, K. A. (1993): Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. Wingfield, M.J., Seifert, K.A., Webber, J.J. (eds.) American Phytopathological Society, Minneapolis: 141-151.

Seifert, K. (1964): Die Veränderung der chemischen Holzzusammensetzung durch den Bläuepilz *Pullularia pullulans* (de Bary) Berkhout (= *Aureobasidium pullulans* (de Bary) Arnaud). Holz als Roh- und Werkstoff 22(11): 405-409.

Sell, J. (1968): Untersuchungen über die Besiedlung von unbehandeltem und angestrichenem Holz durch Bläuepilze. (Investigations on the infestation of untreated and surface treated wood by blue stain fungi.) Holz als Roh- und Werkstoff 26: 215-222.

Shaw, C. G. III and Dick, M. (1980): Verticicladiella root disease of *Pinus strobus* in New Zealand. Plant Disease 64(1): 96-98.

Shimada, M., Takahashi, M. (1991): Biodegradation of cellulosic materials. In: Wood and Cellulosic Chemistry. Hon, D. N.-S., Shiraishi, N. (eds). Marcel Dekker, Inc., New York and Basel: 621-663.

Siemaszko, W. (1939): Fungi associated with bark beetles in Poland. Planta polonica, Warszawa, 7.

Sietsma, J. H., Wösten, H. A. B., Wessels, J. G. H. (1995): Cell wall growth and protein secretion in fungi. Canadian Journal of Botany 73 (Suppl.1): S388-S395.

Sinclair, S. A., McLain, T. E., Ifju, G. (1979): Toughness of sap-stained Southern pine salvaged after beetle-attack. Wood and Fiber 11(1): 66-72.

Sjöström, E. (1981): Wood chemistry, fundamentals and applications. Academic Press. New York, London, Toronto, Sydney, San Francisco.

Smalley, E. B., Guries, R. P. (1993): Breeding elms for resistance to Dutch elm disease. Annual Review of Phytopathology 31: 325-352.

Smelstorius, J. A. (1974): Chemical composition of Australian-grown *Pinus radiata* D. Don. II. Polysaccharides. Holzforschung 28(2): 67-73.

Spatafora, J. W., Blackwell, M. (1994): The polyphyletic origins of ophiostomatoid fungi. Mycological Research 98: 1-9.

Standards Association of New Zealand (1988): NZS 3631. New Zealand Timber Grading Rules.

Stranks, D. W., Bieniada, J. (1971): A rapid test for cellulolytic activity. International Biodeterioration Bulletin 7(3): 109-111.

Subramanian, C. V. (1983): Hyphomycetes: taxonomy and biology. Academic Press, London. 502 pp.

Suolahti, O., Wallén, A. (1958): Der Einfluß der Naßlagerung auf das Wasseraufnahmevermögen des Kiefernsplintholzes (The influence of water storage on the waterabsorption capacity of pine sapwood). Holz als Roh- und Werkstoff 16(1): 8-17.

Sutherland, M. L., Pearson, S., Brasier, C. M. (1997): The influence of temperature and light on defoliation levels of elm by Dutch elm disease. Phytopathology 87: 576-581.

Svaldi, R., Elgersma, D. M. (1982): Further studies on the activity of cell wall degrading enzymes of aggressive and non-aggressive isolates of *Ophiostoma ulmi*. European Journal of Forest Pathology 12: 29-36.

Swart, W. J., Knox-Davies, P. S., Wingfield, M. J. (1985): *Sphaeropsis sapinea*, with special reference to its occurrence on *Pinus* spp. in South Africa. South African Forestry Journal 35: 1-8.

Swart, W. J., Wingfield, M. J., Knox-Davies, P. S. (1987): Conidial dispersal of *Sphaeropsis sapinea* in three climatic regions of South America. Plant Disease 71: 1038-1040.

Swart, W. J., Wingfield, M. J. (1991): Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. Plant Disease 75(8): 761-766.

Swart, W. J., Wingfield, M. J., Palmer, M. A., Blanchette, R. A. (1991): Variation among South African isolates of *Sphaeropsis sapinea*. Phytopathology 81: 489-493.

Swart, W. J., Wingfield, M. J., van Wyk, P. (1993): Variation in conidial morphology among geographic isolates of *Sphaeropsis sapinea*. Mycological Research 97: 832-838.

Sydow, H., Sydow, P. (1919): Mykologische Mitteilungen. Annales Mycologici 17: 2-47.

Tabirih, P. K., Seehann, G. (1984): Effects of *Botryodiplodia theobromae* attack on Abachi Wood (*Triplochiton sceroxylon* K. Schum.). Material und Organismen 19: 9-21.

Talbot, N. J. (1999): Forcible Entry. Science 285: 1860-1861.

Tegli, S., Scala, A. (1996): Isolation and characterization of non cerato-ulmin producing laboratory induced mutants of *Ophiostoma novo-ulmi*. Mycological Research 100: 661-668.

Temple, B., Horgen, P A. (2000): Biological roles for cerato-ulmin, a hydrophobin secreted by the elm pathogens, *Ophiostoma ulmi* and *O. novo-ulmi*. Mycologia 92(1): 1-9.

Tenkanen, M., Puls, J., Rättö, M., Viikari, L. (1993): Enzymatic deacetylation of galactoglucomannans. Applied and Microbiological Biotechnology 39: 159-165.

Thunell, B. (1952): Einwirkung der Bläue auf die Festigkeitseigenschaften der Kiefer. Holz als Roh- und Werkstoff 10(9): 362-365.

Thwaites, J. M., Farrell, R. L. (2000): Colonisation and detection of New Zealand sapstain fungi. The International Research Group on Wood Preservation. Document IRG/WP00-10329. Stockholm, Sweden.

Timell, T. E. (1967): Recent progress in the chemistry of wood hemicellulose. Wood Science and Technology 1: 45-70.

Timell, T. E. (1982): Recent progress in the chemistry and topochemistry of compression wood. Wood Science and Technology 16: 83-122.

Timell, T. E. (1986): Compression wood in gymnosperms. Springer, Berlin, Heidelberg, New York. 2150 pp.

Trendelenburg, R. (1940): Über die Abkürzung der Zeitdauer von Pilzversuchen an Holz mit Hilfe der Schlagbiegeprüfung. Holz als Roh- und Werkstoff 3 (12): 397-407.

Umezurike, G. M. (1969): Cellulolytic activities of *Botryodiplodia theobromae* Pat. Annals of Botany 33(131): 451-462.

Umezurike, G. M. (1970a): Cellulase and ß-glucosidase components in culture filtrates from *Botryodiplodia theobromae* Pat. Journal of Experimental Botany 21(68): 639-650.

Umezurike, G. M. (1970b): Production of cellulolytic enzymes by *Botryodiplodia theobromae* Pat. Annals of Botany 34(134): 217-227.

Umezurike, G. M. (1971): The purification and properties of extracellular ß-glucosidase from *Botryodiplodia theobromae* Pat. Biochimica et Biophysica Acta 227: 419-428.

Umezurike, G. M. (1978): Staining and degradation of wood blocks and agar media by *Botryodiplodia theobromae*. Transcriptions of the British Mycological Society 71(2): 328-332.

Upadhyay, H. P., Kendrick, W. B. (1975): Prodromus for the revision of *Ceratocystis* (Microascales, Ascomycetes) and its conidial states. Mycologia 67: 798-805.

Upadhyay, H. P. (1981): A monograph of *Ceratocystis* and *Ceratocystiopsis*: University of Georgia Press. Athens, Georgia.

Upadhyay, H. P. (1993): Classification of the ophiostomatoid fungi. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. Wingfield, M.J., Seifert, K.A., Webber, J.J. (eds.) American Phytopathological Society, Minneapolis: 7-13.

Uprichard, J. M. (1991): Chemistry of Wood and Bark. Chapter 4 in Kininmonth, J. A., Whitehouse, L. J. (Ed.): Properties and Uses of New Zealand Radiata Pine. Volume One: Wood Properties. New Zealand Ministry of Forestry and Forest Research Institute, Rotorua, New Zealand.

Uzunovic, A., Webber, J. F. (1998): Comparison of bluestain fungi grown *in vitro* and in freshly cut pine billets. European Journal of Forest Pathology 28: 323-334.

Uzunovic, A., Webber, J. F., Peace, A. J., Dickinson, D. J. (1999a): The role of mechanized harvesting in the development of bluestain in pine. Canadian Journal of Forest Research 29: 242-251.

Uzunovic, A., Yang, D.-Q., Gagné, P., Breuil, C., Bernier, L., Byrne, A., Gignac, M., Kim, S. H. (1999b): Fungi that cause sapstain in Canadian softwoods. Canadian Journal of Microbiology 45: 914-922.

Uzunovic, A., Seifert, K. A., Kim, S. H., Breuil, C. (2000): *Ophiostoma setosum*, a common sapwood staining fungus from western North America, a new species of the *Ophiostoma piceae* complex. Mycological Research 104(4): 486-494.

Valentin, E., Herrero, W., Pastor, J. F. I., Sentandreu, R. (1984): Solubilization and analysis of mannoprotein molecules from the cell wall of *Saccharomyces cerevisiae*. Journal of General Microbiology 130: 1419-1428.

Viljoen, C. D., Wingfield, B. D., Wingfield, M. J. (2000): Computer aided systematic evaluation of morphological characters of the ophiostomatoid fungi. Mycotaxon 74(1): 217-239.

Wagenführ, R. (1989): Anatomie des Holzes. 4th Edition, VEB Fachbuchverlag Leipzig. 334 p.

Wakeling, R. N. (1996): Protection of export logs from fungal degrade. Wood Processing Newsletter Issue No. 19 (May). Forest Research Institute, Rotorua, New Zealand.

Wakeling, R. N: (2000): It´s that time of year again! Wood Processing Newsletter Issue No. 28 (December). Forest Research Institute, Rotorua, New Zealand.

Walker, J. C. F., Butterfield, B. G. (1995): The importance of microfibril angle for the processing industries. N.Z. Forestry 40(4): 34-40.

Wang, Z., Leone, R., Breuil, C. (1997): Why fungal growth is less effective in aspen heartwood than in aspen sapwood. Material und Organismen 31 (2): 145-155.

Waterman, A. M. (1943): *Diplodia pinea*, the cause of disease of hard pines. Phytopathology 33: 1018-1031.

Waterman, A. M., Hansbrough, J. R. (1957): Microscopical rating of decay in Sitka spruce and its relation to toughness. Forest Products Journal 7(2): 77-84.

Weiss, H. F., Barnum, C. T. (1911): The prevention of sap stain in lumber. U.S. Forest Serv. Cir. 192. 19 pp.

White, W. L., Darby, R. T., Stechert, G. M., Sanderson, K. (1948): Assay of cellulolytic activity of molds isolated from fabrics and related items exposed in the tropics. Mycologia 40: 34-84.

Wilcox, W. W. (1968): Changes in wood microstructure through progressive stages of decay. U.S.D.A. Forest Service Research Paper FPL 70.

Wilcox, W. W. (1973): Degradation in Relation to Wood Structure. In: Wood Deterioration and Its Prevention by Preservative Treatments. Vol. I: Degradation and Protection of Wood. D. D. Nicholas (ed.). Syracuse University Press, Syracuse, New York: 380 pp.

Wilcox, W. W. (1978): Review of literature on the effects of early stages of decay on wood strength. Wood and Fiber 9 (4): 252-257.

Winandy, J. E., Morrell, J. J. (1993): Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. Wood and Fiber Science 25(3): 278-288.

Wingfield, M. J., Knox-Davies, P. S. (1980): Association of *Diplodia pinea* with a root disease of pines in South Africa. Plant Disease 64: 221-223.

Wingfield, M. J., Capretti, P., MacKenzie, M. (1988): *Leptographium* spp. as root pathogens of conifers. An international perspective. Pp. 113-128 in Harrington, T. C., Cobb, F. W. (Eds.): *Leptographium* root diseases on conifers. APS Press, St. Paul, Minnesota.

Wong, A. H. H., Singh, A. P. (1997): Micrographic evidence for degradation of the normal fibre walls in rubberwood (*Hevea brasiliensis*) by the common blue-stain fungus *Botryodiplodia theobromae*. Pp 296-300 in Razak, A. R. et al. (Eds.): Proceedings of the First ASEAN Microscopy Conference, Colorcom Grafik System Sdn. Bhd., Kuala Lumpur, Malaysia.

Wong, A. H. H., Singh, A. P. (1998): Degradation of the normal fibre walls of rubberwood (*Hevea brasiliensis*) by the tropic sapstain fungus *Botryodiplodia theobromae*. The International Research Group on Wood Preservation. Document IRG/WP/98-10286. Stockholm, Sweden.

Yang, D.-Q. (1999): Staining ability of various sapstaining fungi on agar plates and on wood wafers. Forest Products Journal 49(11/12): 78-90.

Zabel, R. A., Morrell, J. J. (1992): Wood Microbiology: Decay and its prevention. Academic Press Inc., San Diego, California. 476 pp.

Zheng, Y., Ruddick, J. N. R., Breuil, C. (1994): Factors affecting the growth of *Ophiostoma piceae* on lodgepole pine heartwood. Material und Organismen 29 (2): 105-117.

Zimmerman, W. C., Blanchette, R. A., Burnes, T. A., Farrell, R. L. (1995): Melanins and perithecial development in *Ophiostoma piliferum*. Mycologia 87(6): 857-863.

Zimmermann, G., Butin, H. (1973): Untersuchungen über Hitze- und Trockenresistenz holzbewohnender Pilze. Flora 162: 393-419.

Zink, P., Fengel, D. (1988): Studies on the colouring matter of blue-stain fungi. Part 1: General characterization and the associated compounds. Holzforschung 42: 217-220.

Zink, P., Fengel, D. (1989): Studies on the colouring matter of blue-stain fungi. Part 2: Electron microscopic observations of the hyphae walls. Holzforschung 43: 371-374.

Zink, P., Fengel, D. (1990): Studies on the colouring matter of blue-stain fungi. Part 3: Spectroscopic studies on fungal and synthetic melanins. Holzforschung 44: 163-168.

APPENDIX 1 – GROWTH CURVES OF SAPSTAINING FUNGI IN LIQUID MEDIA BASED ON BLASTOSPORE COUNTS AT DIFFERENT TEMPERATURES.

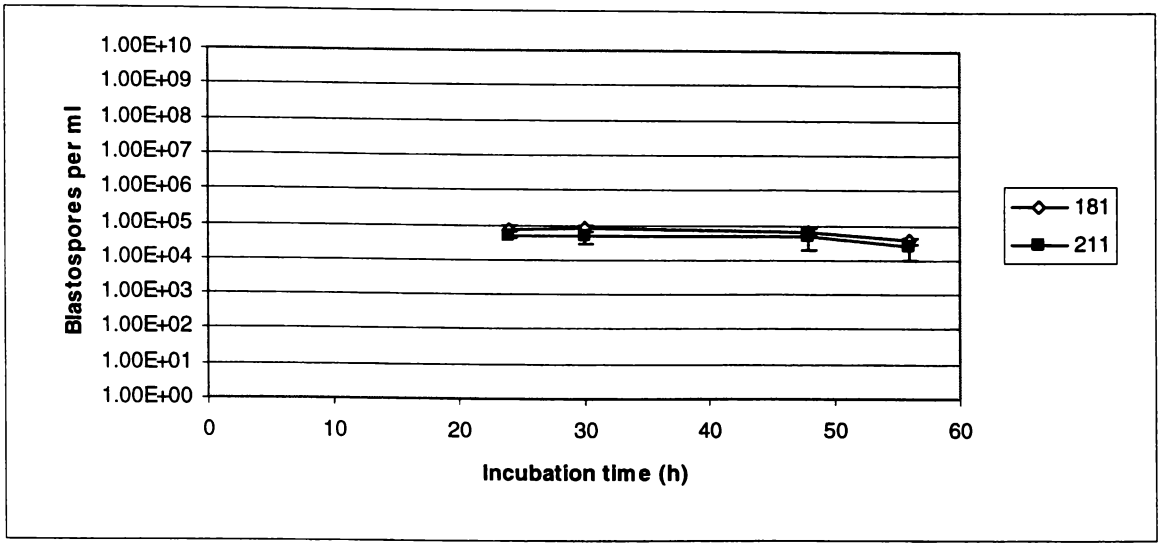


Figure A1.1: Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 4°C.

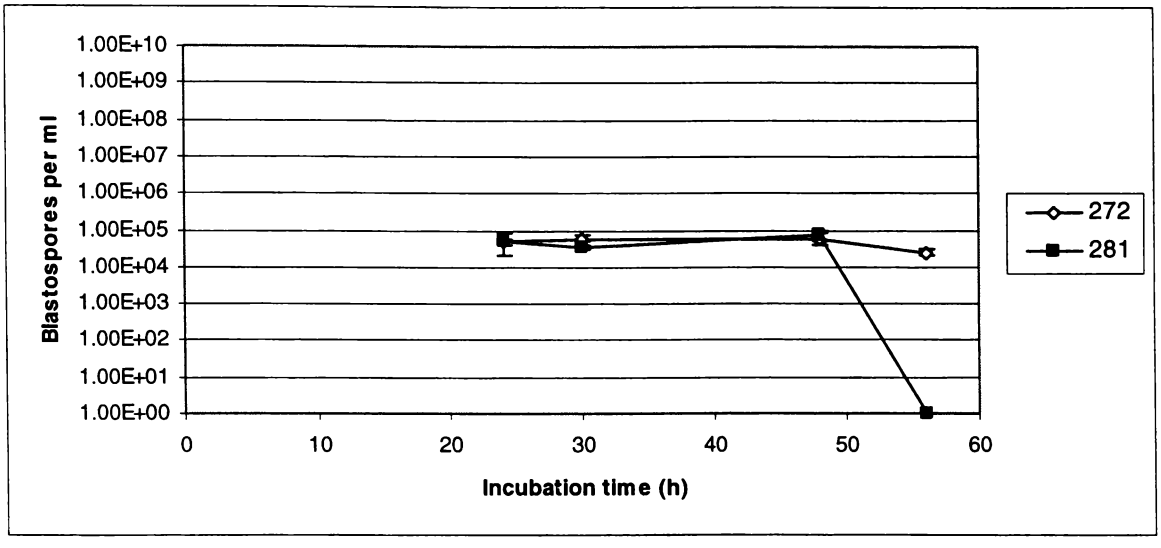


Figure A1.2: Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 4°C.

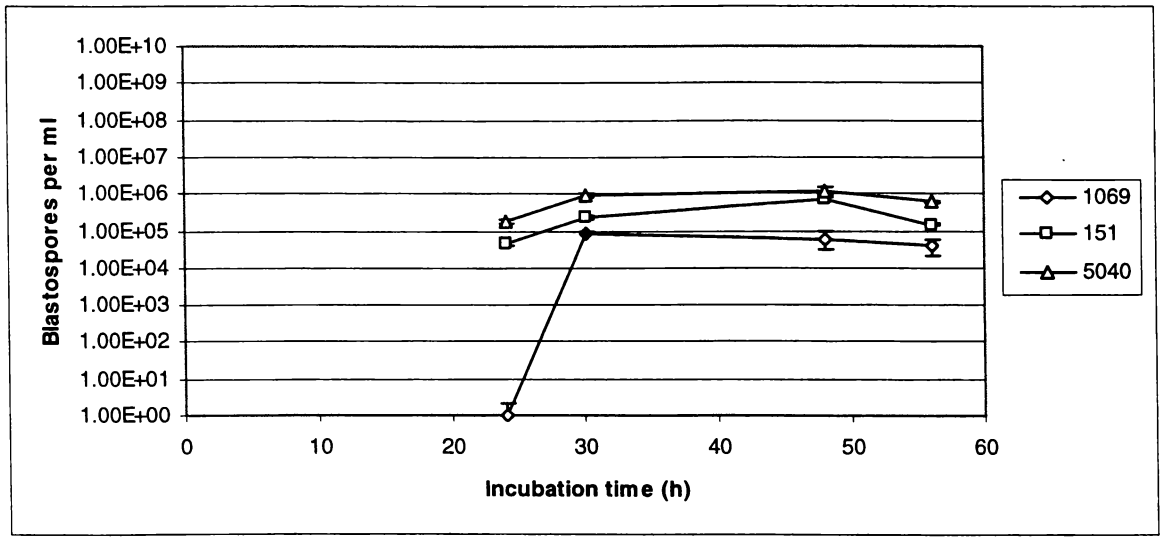


Figure A1.3: Blastospore counts for *O. querci* #1069 and *O. pluriannulatum* #151 and #5040 in liquid media at 4°C.

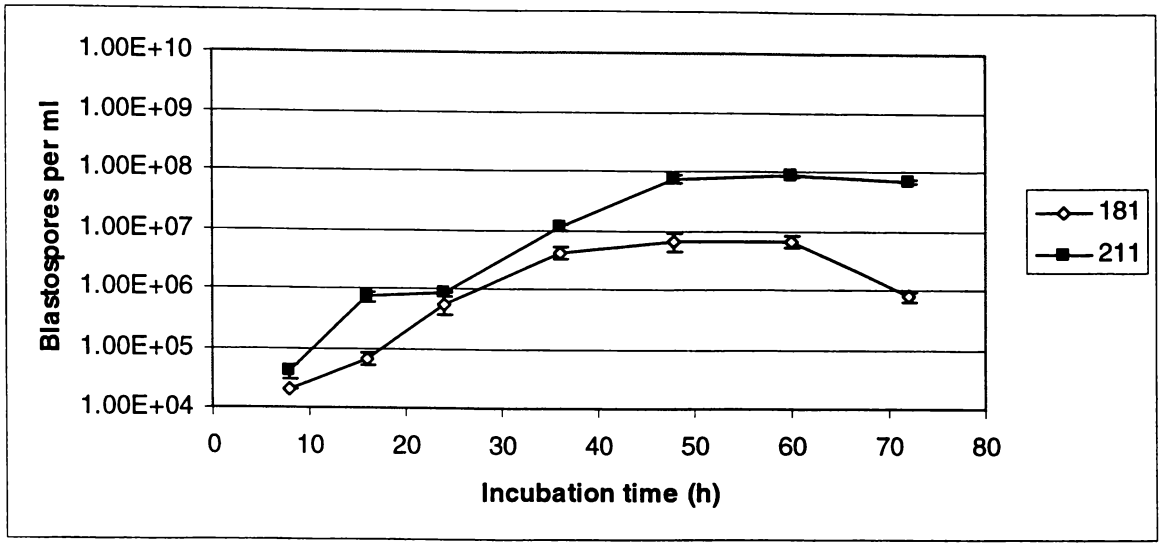


Figure A1.4: Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 10°C.

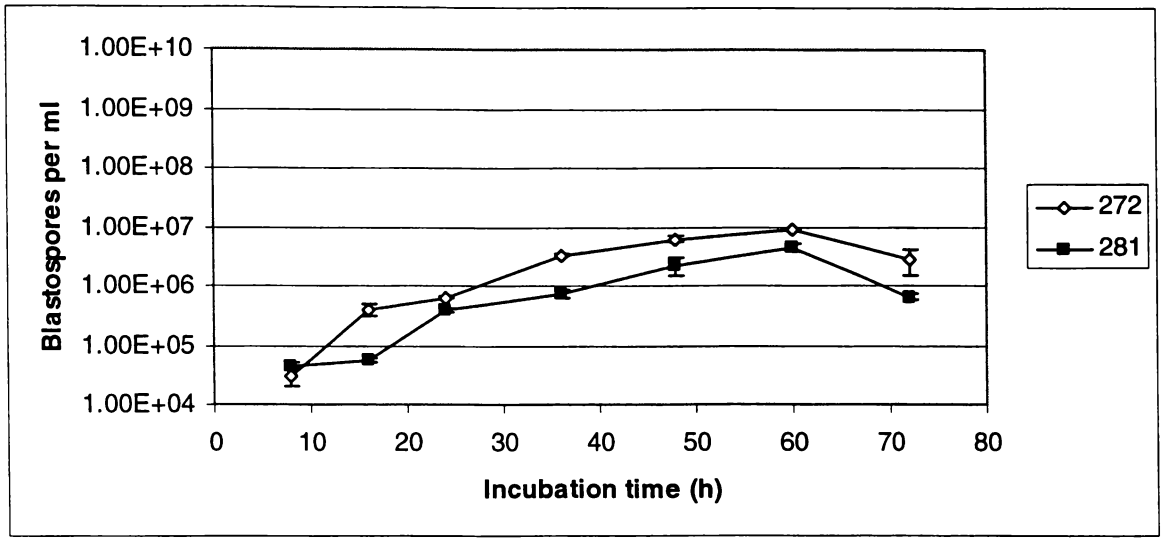


Figure A1.5: Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 10°C.

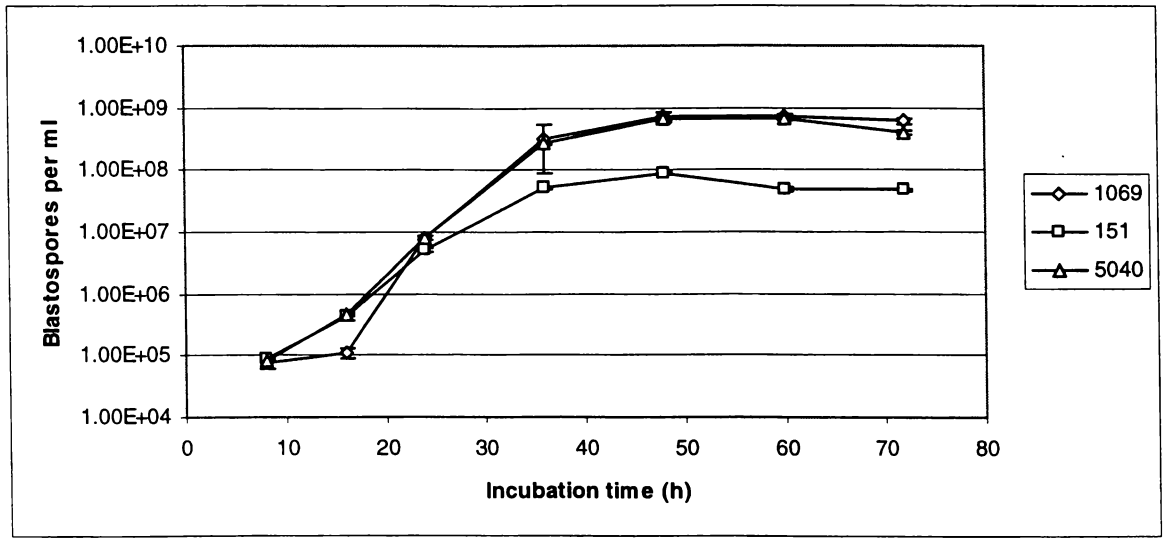


Figure A1.6: Blastospore counts for *O. querci* #1069 and *O. pluriannulatum* #151 and #5040 in liquid media at 10°C.

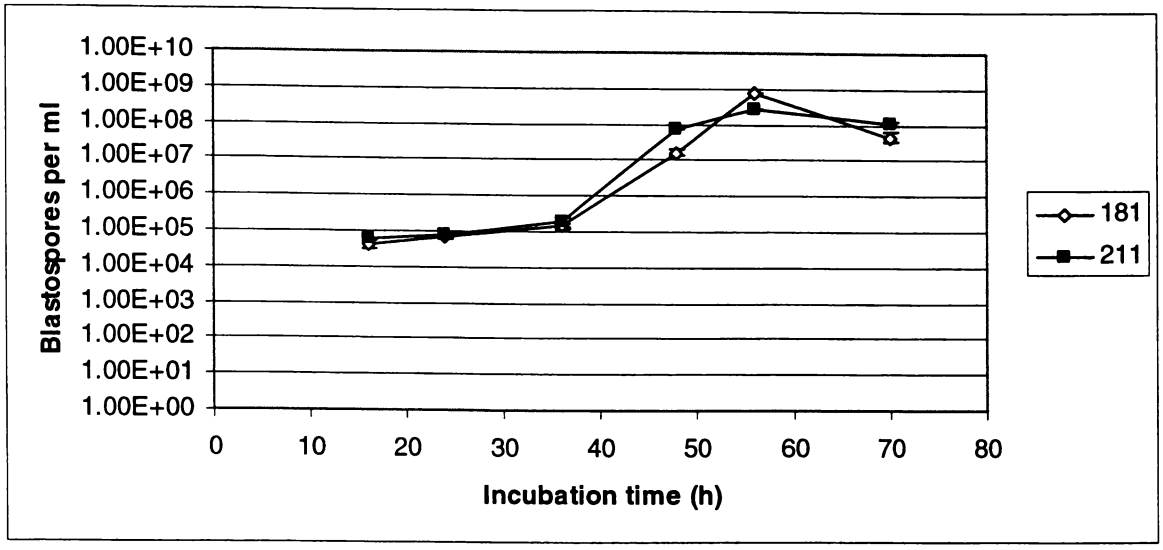


Figure A1.7: Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 15°C.

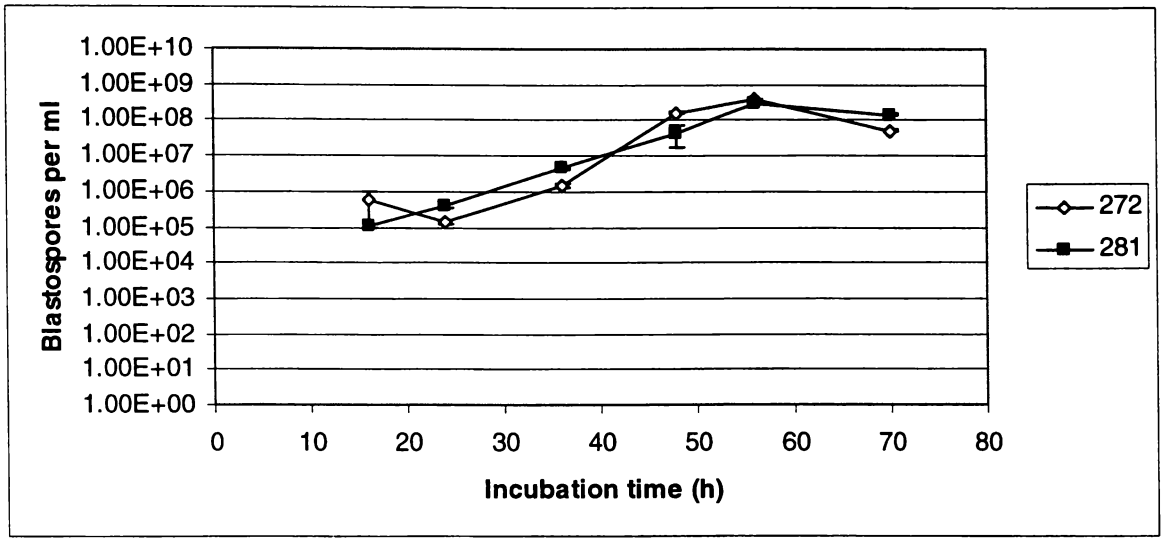


Figure A1.8: Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 15°C.

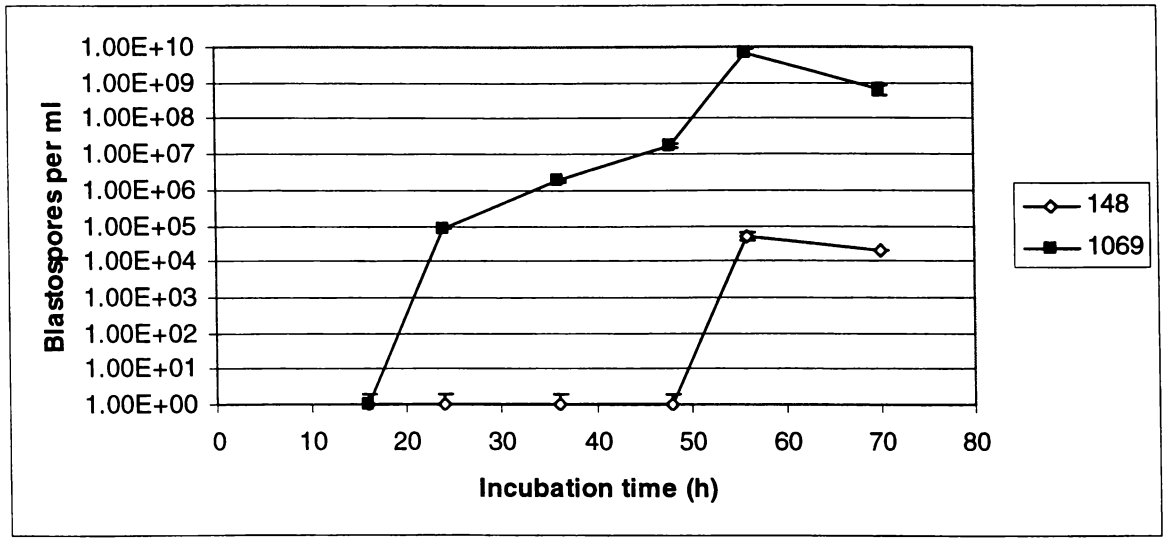


Figure A1.9: Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 15°C.

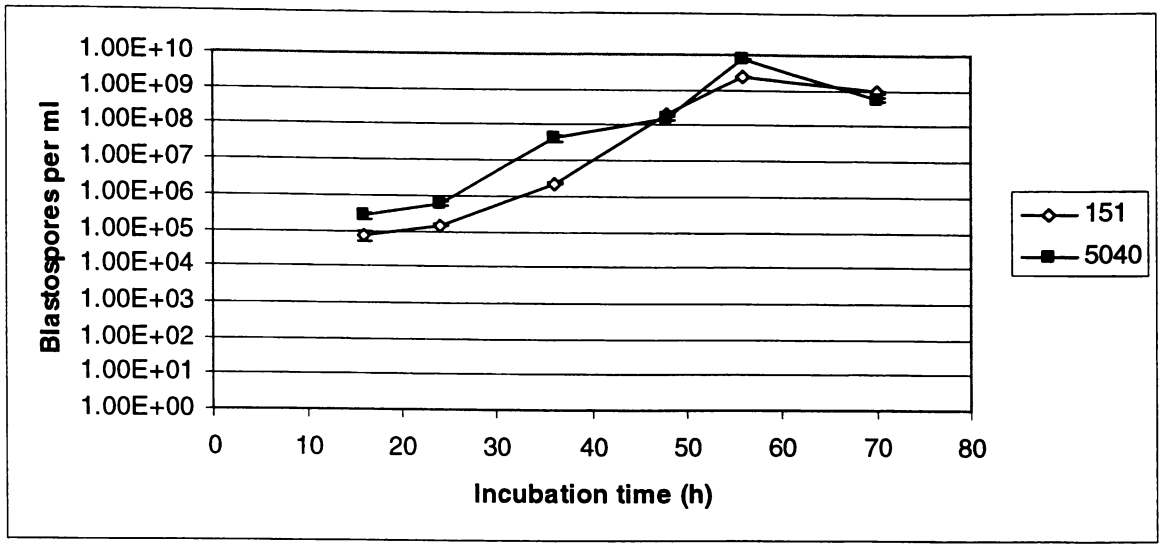


Figure A1.10: Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 15°C.

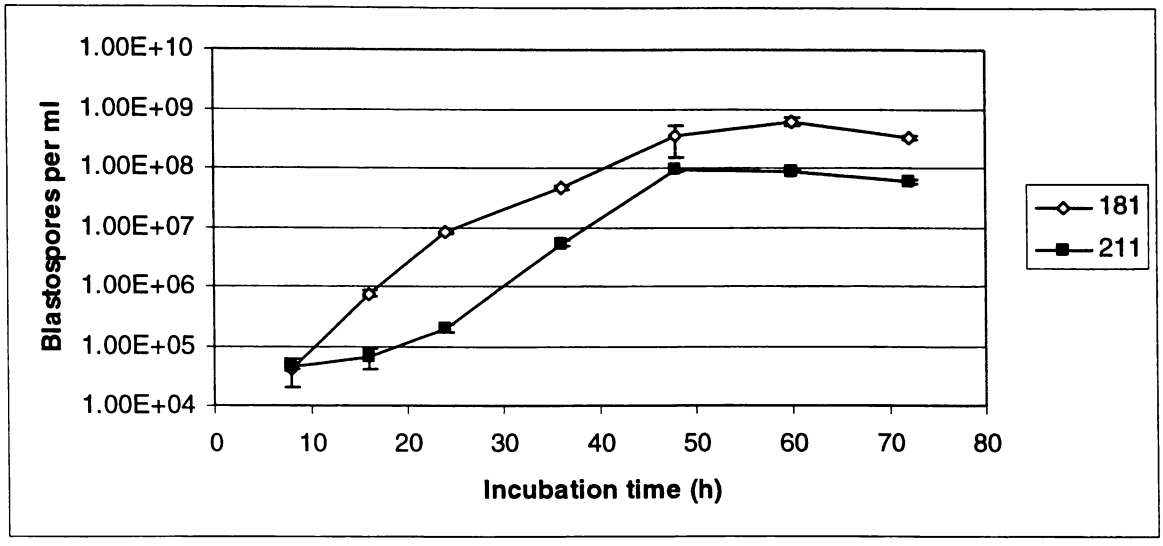


Figure A1.11: Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 20°C.

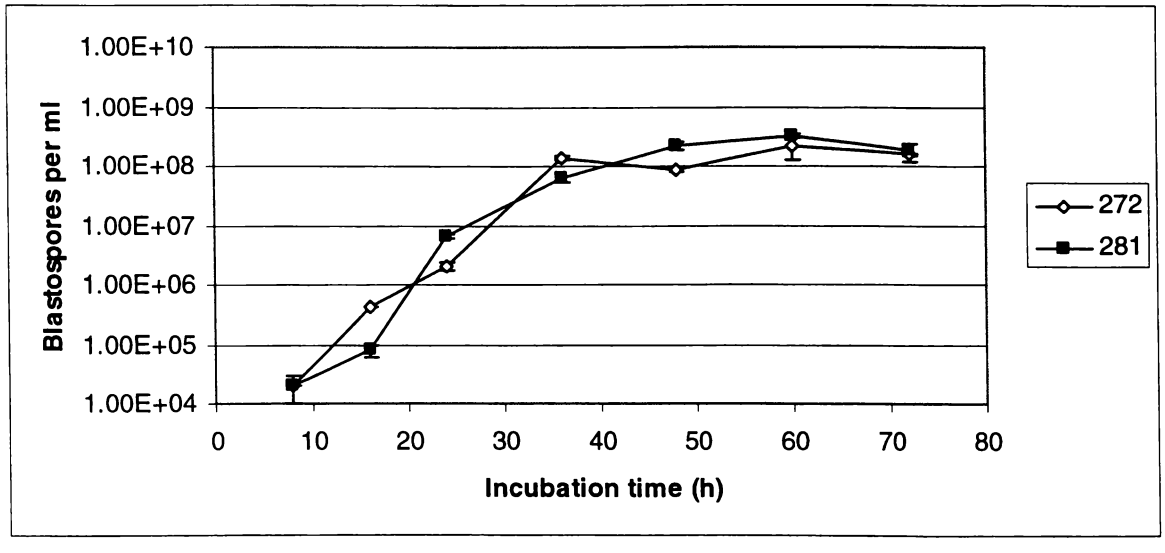


Figure A1.12: Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 20°C.

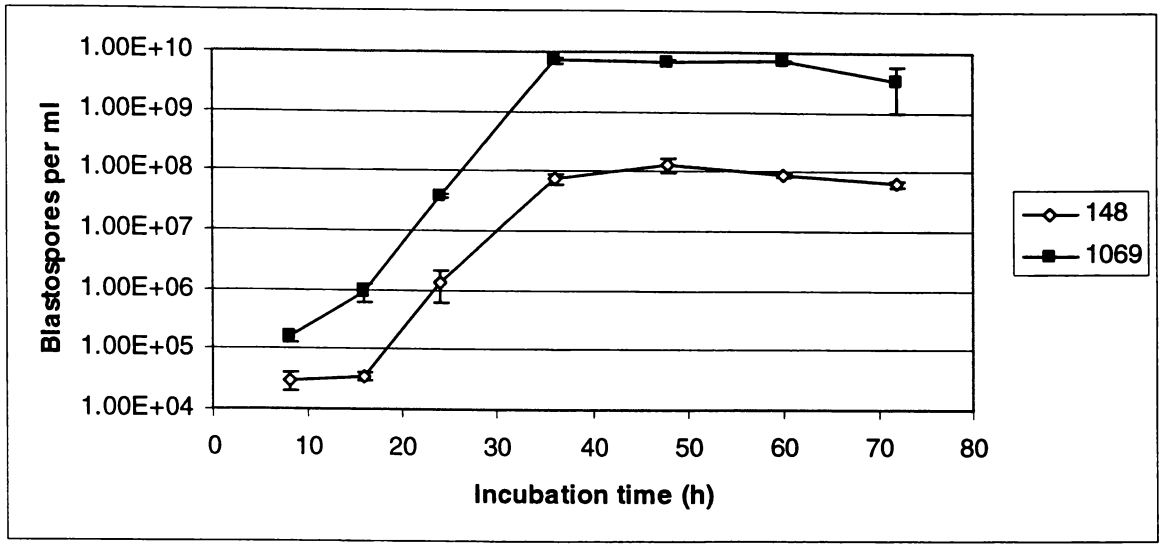


Figure A1.13: Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 20°C.

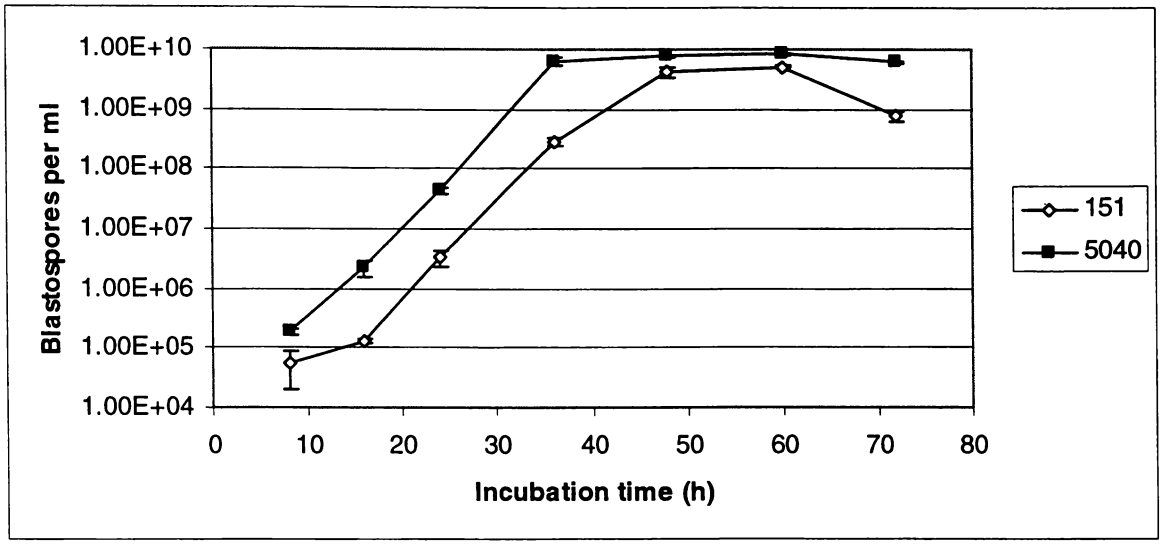


Figure A1.14: Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 20°C.

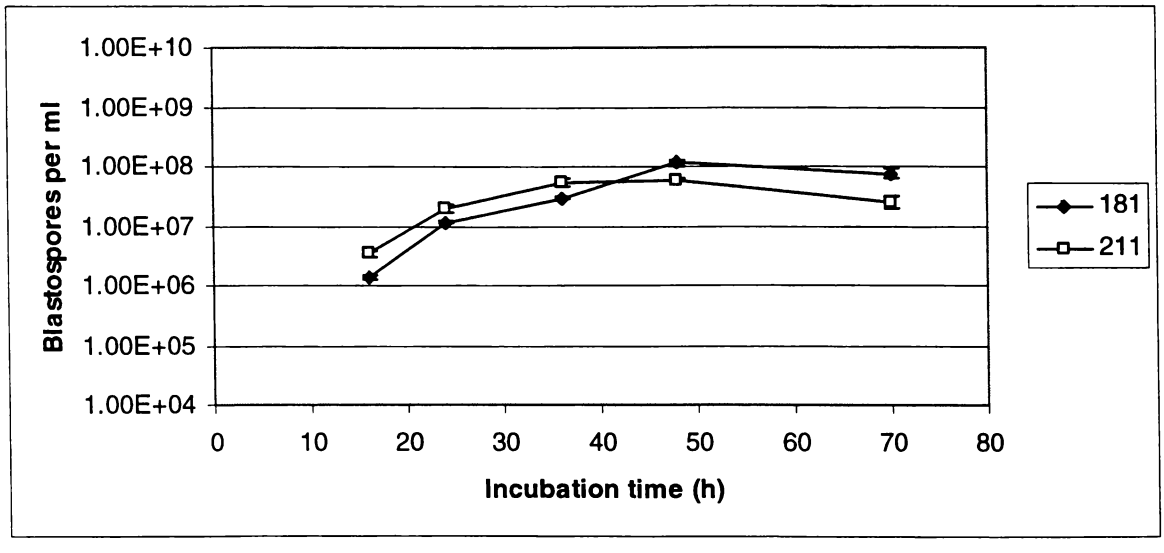


Figure A1.15: Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 30°C.

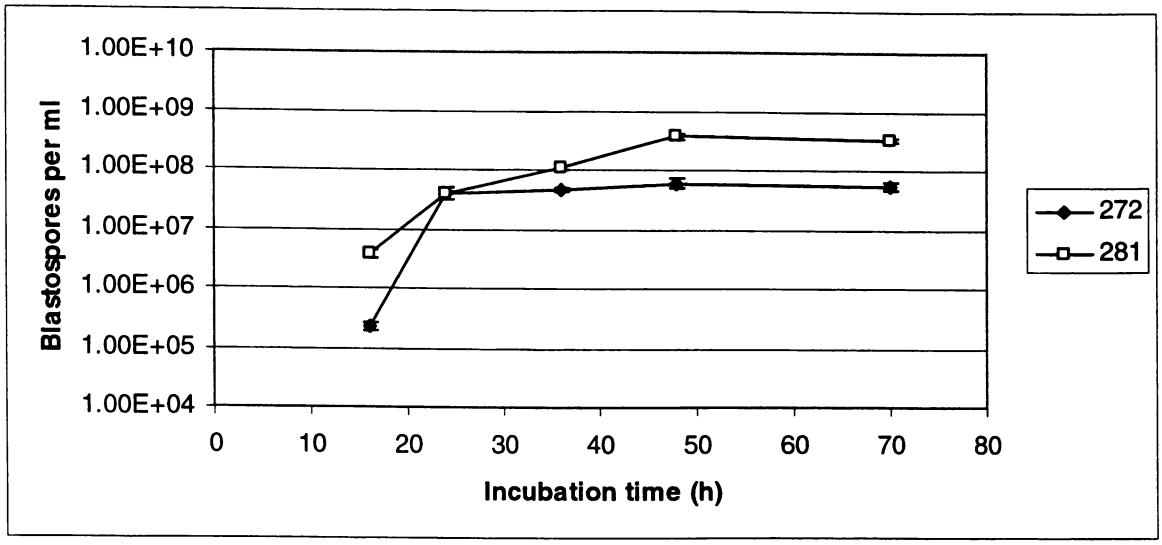


Figure A1.16: Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 30°C.

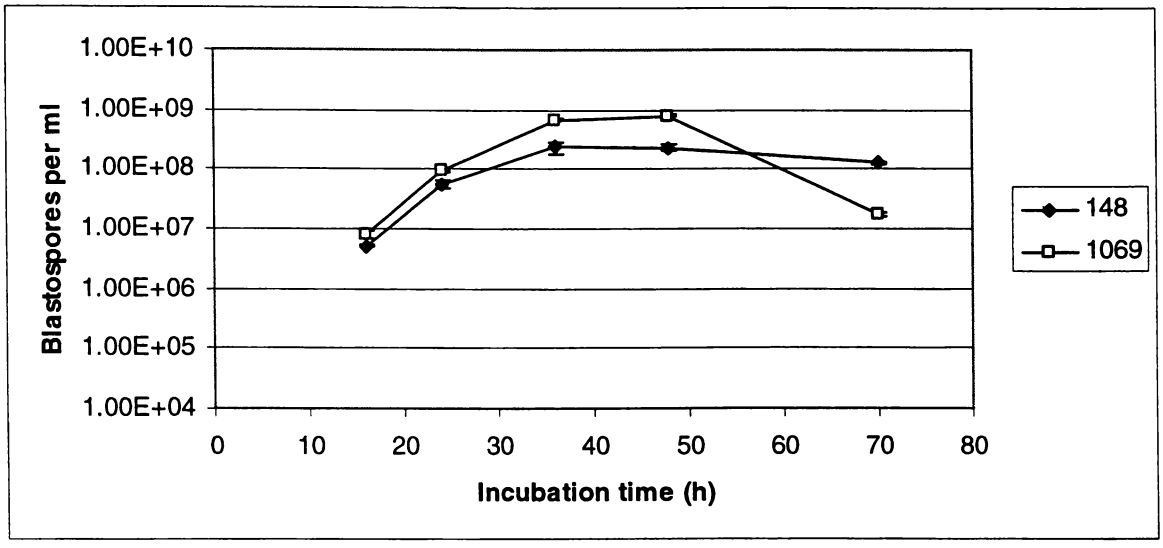


Figure A1.17: Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 30°C.

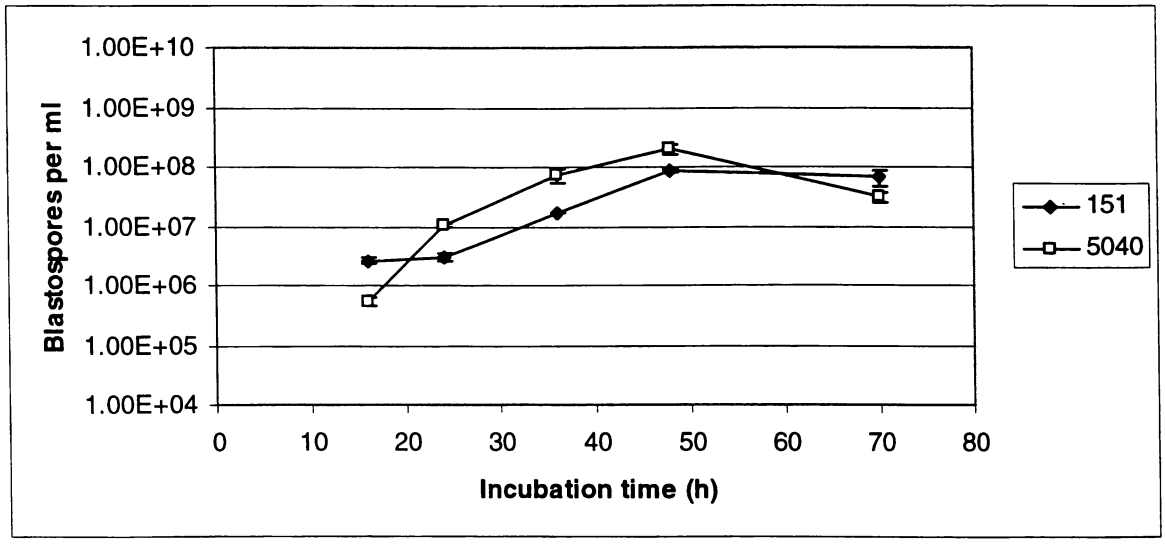


Figure A1.18: Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 30°C.

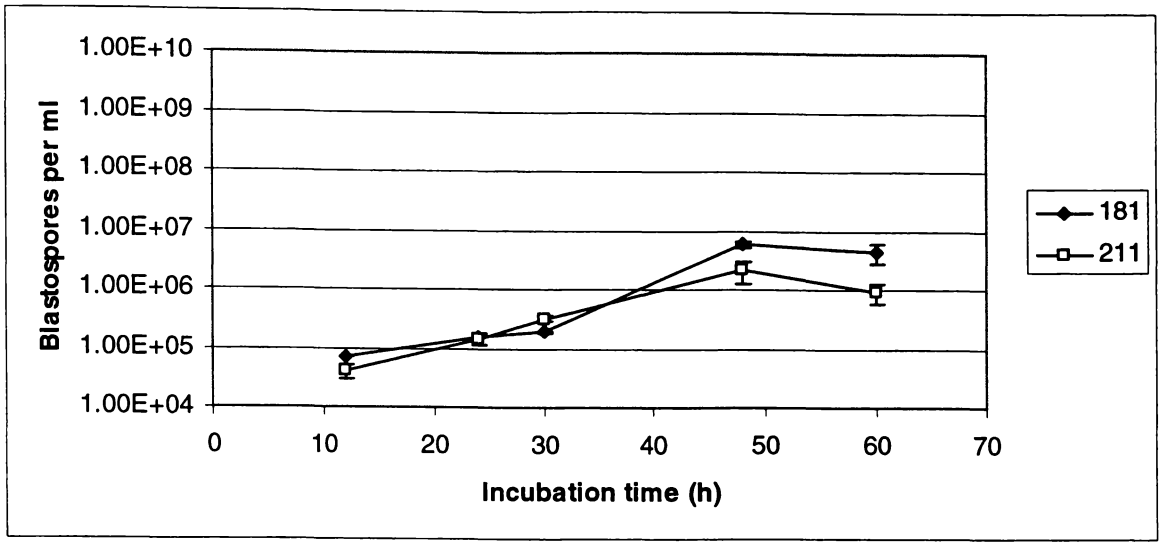


Figure A1.19: Blastospore counts for *O. ips* #181 and *P. cupulatum* #211 in liquid media at 35°C.

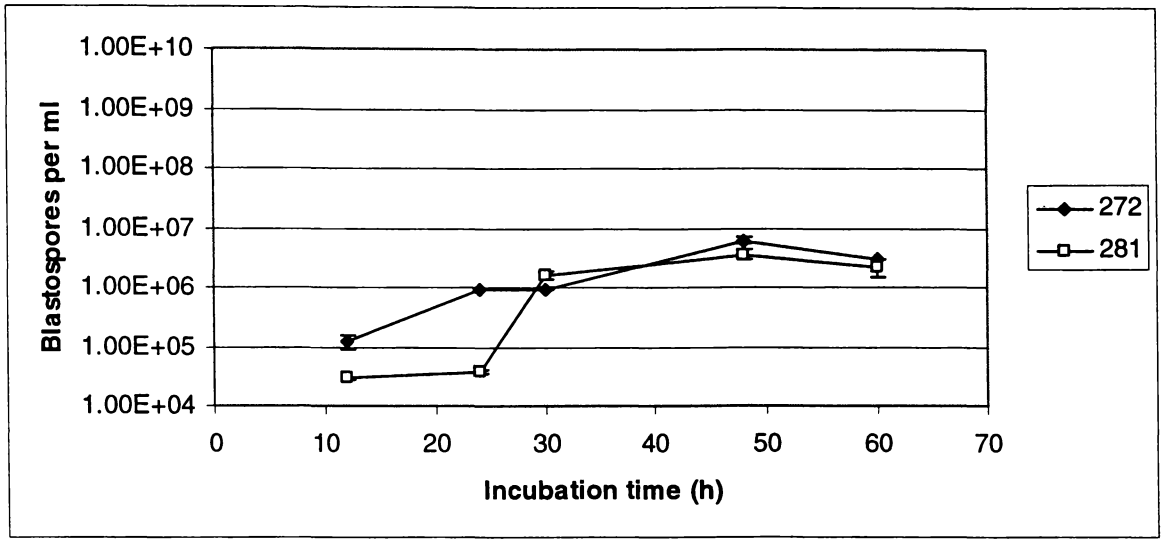


Figure A1.20: Blastospore counts for *O. piceae* #272 and *L. procerum* #281 in liquid media at 35°C.

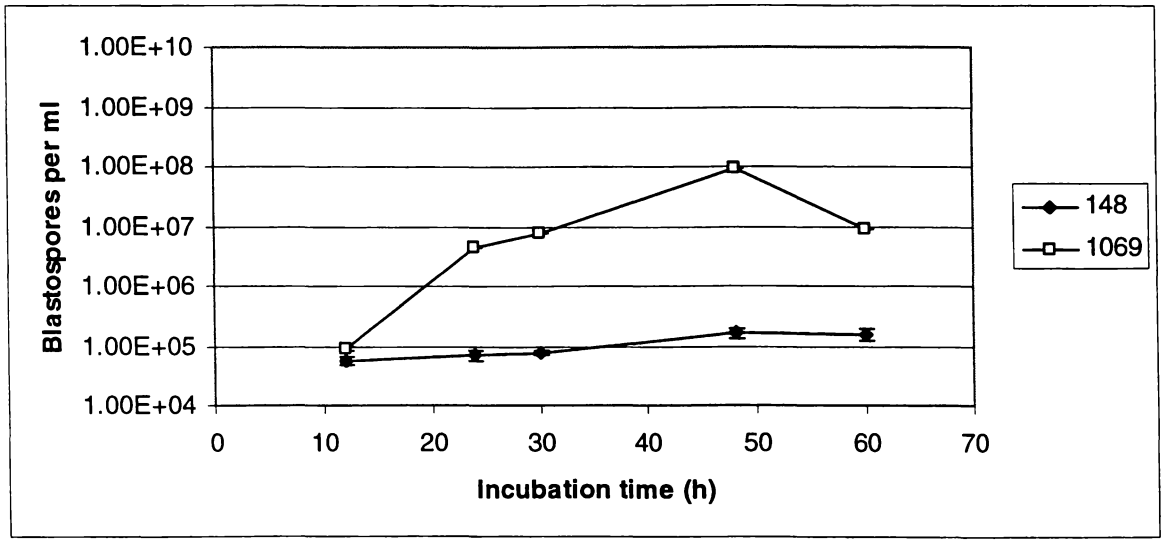


Figure A1.21: Blastospore counts for *O. floccosum* #148 and *O. querci* #1069 in liquid media at 35°C.

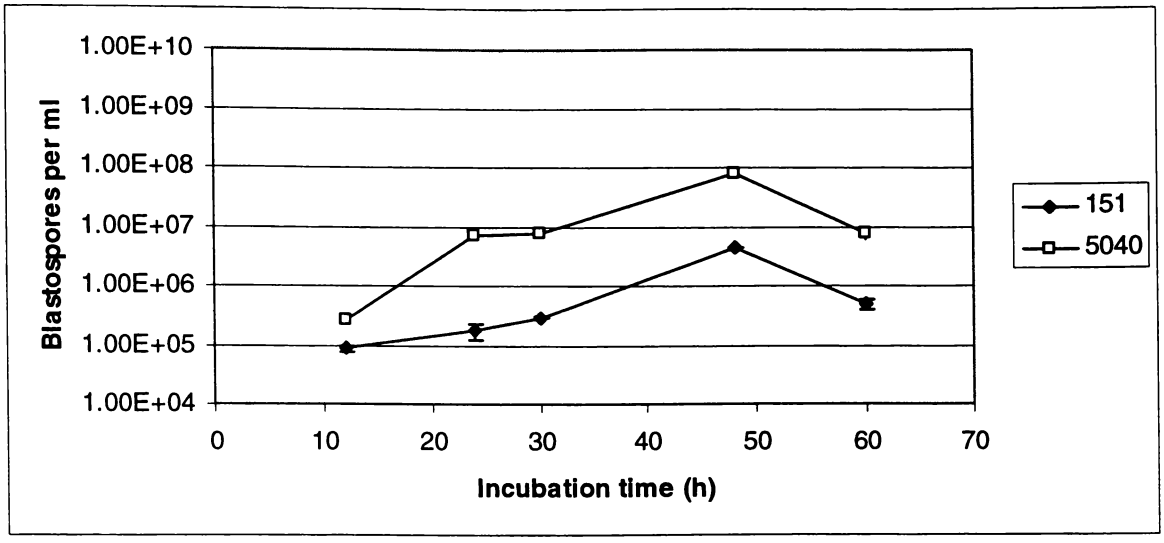


Figure A1.22: Blastospore counts for *O. pluriannulatum* #151 and #5040 in liquid media at 35°C.

APPENDIX 2 – GROWTH CURVES OF SAPSTAINING FUNGI IN LIQUID MEDIA BASED ON FUNGAL BIOMASS DETERMINATIONS AT DIFFERENT TEMPERATURES.

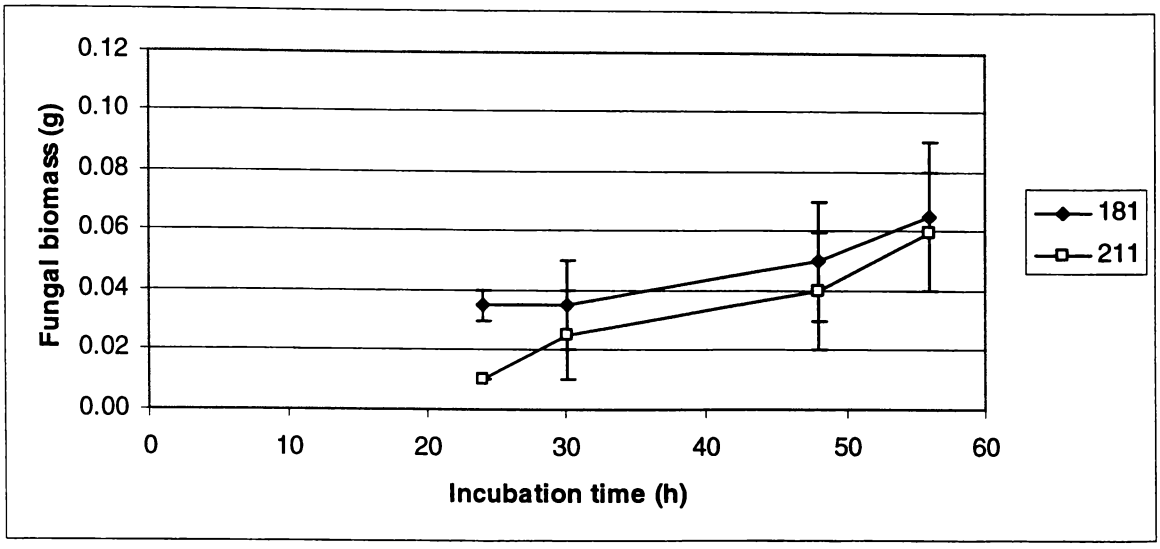


Figure A2.1: Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 4°C.

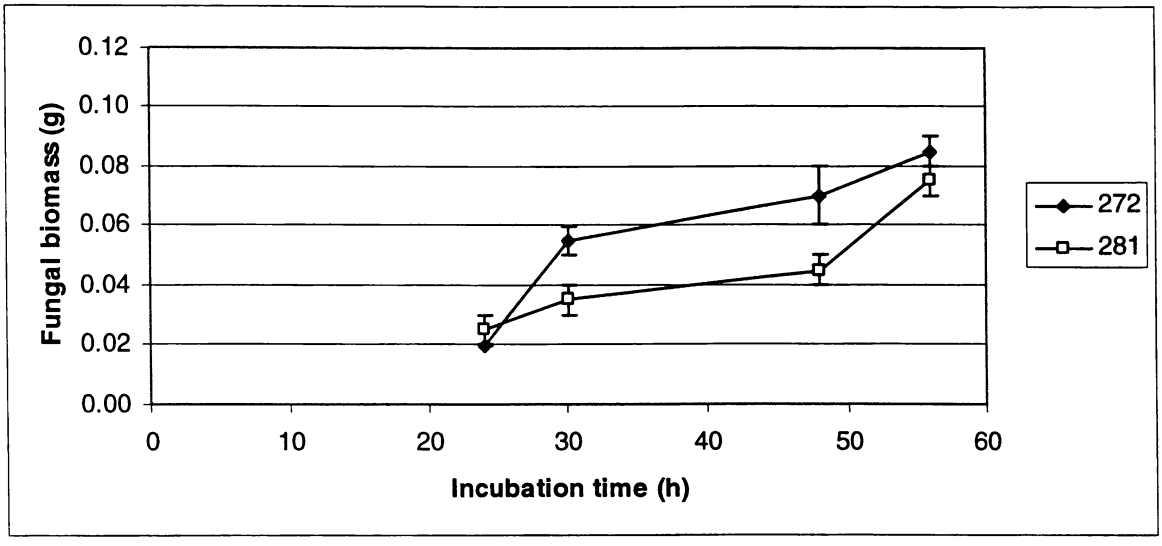


Figure A2.2: Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 4°C.

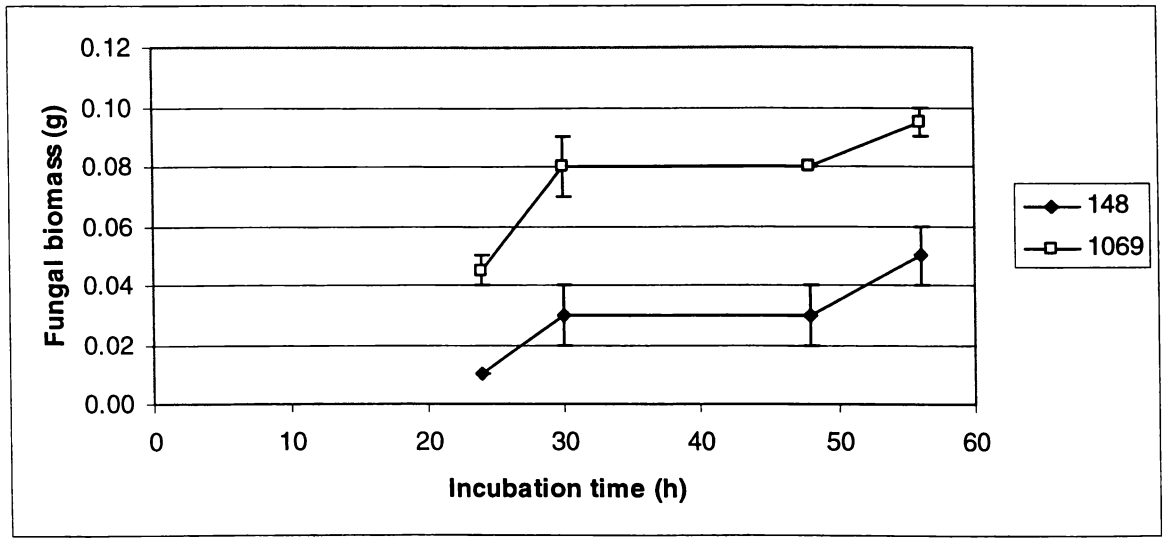


Figure A2.3: Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 4°C.

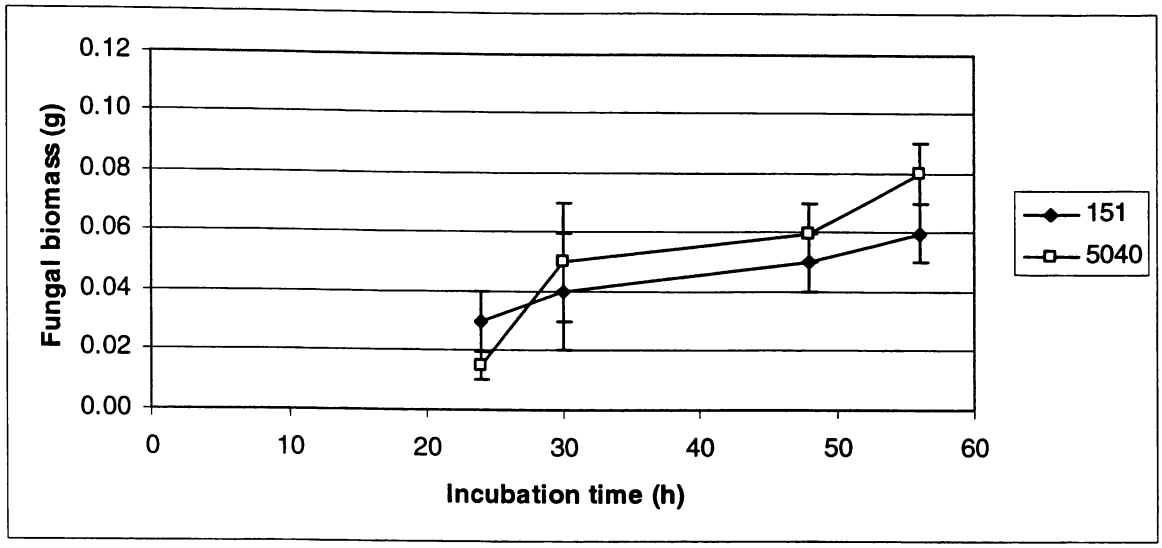


Figure A2.4: Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 4°C.

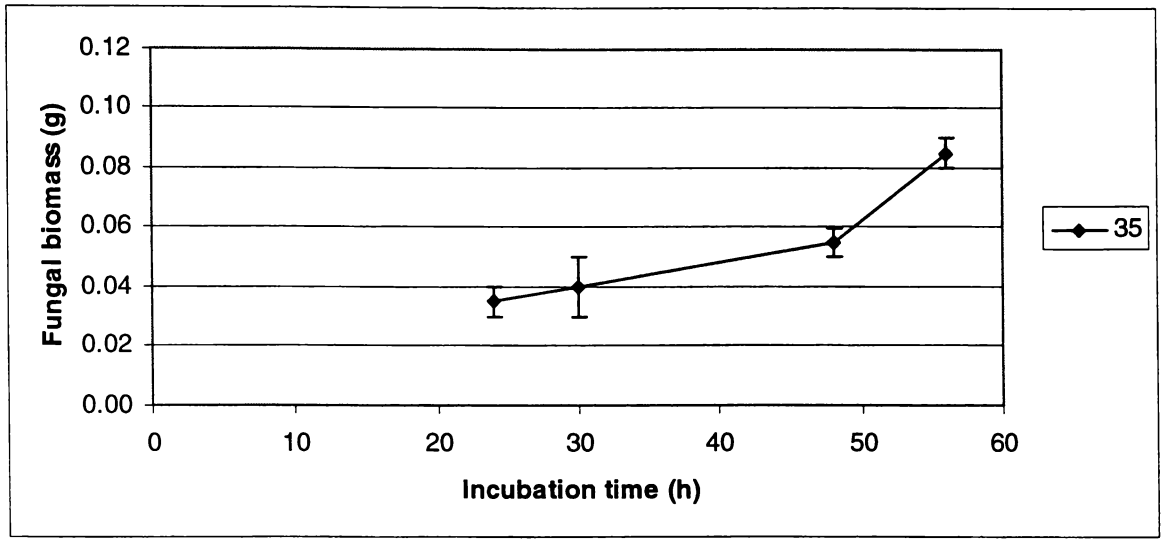


Figure A2.5: Biomass accumulation of *S. sapinea* #35 in liquid media at 4°C.

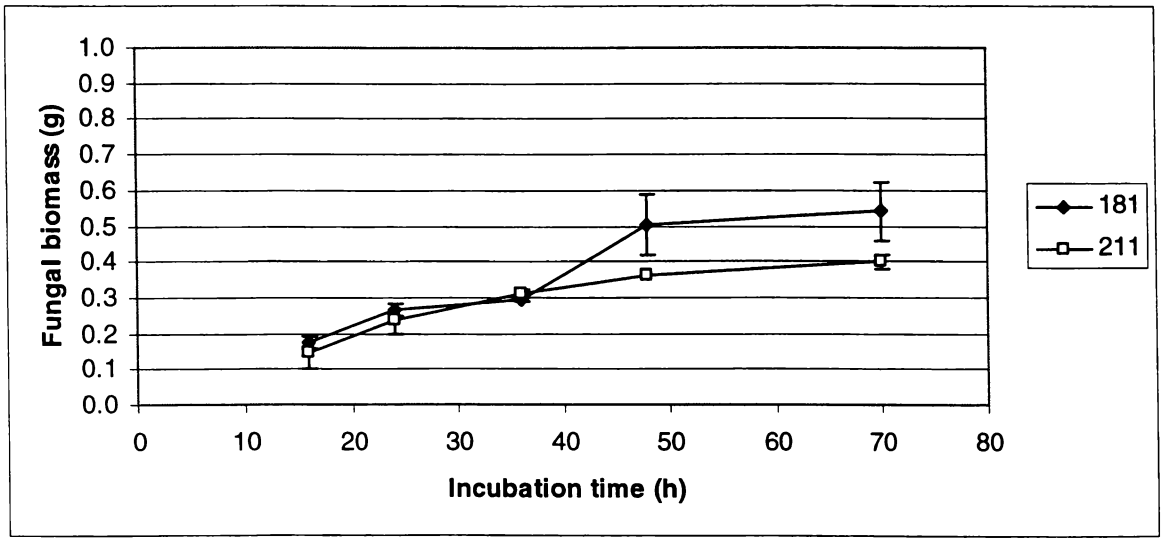


Figure A2.6: Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 30°C.

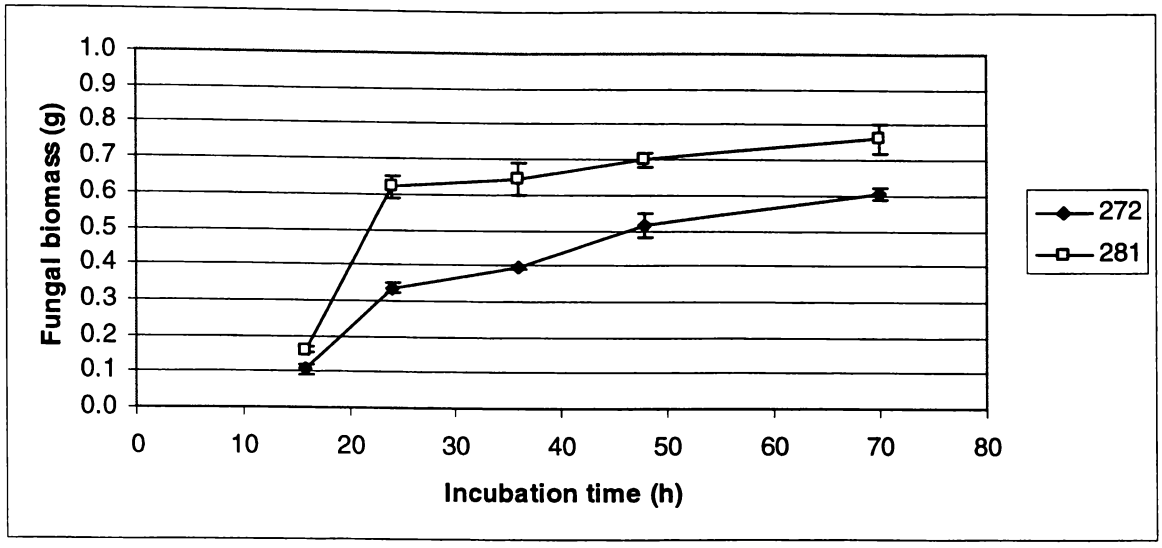


Figure A2.7: Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 30°C.

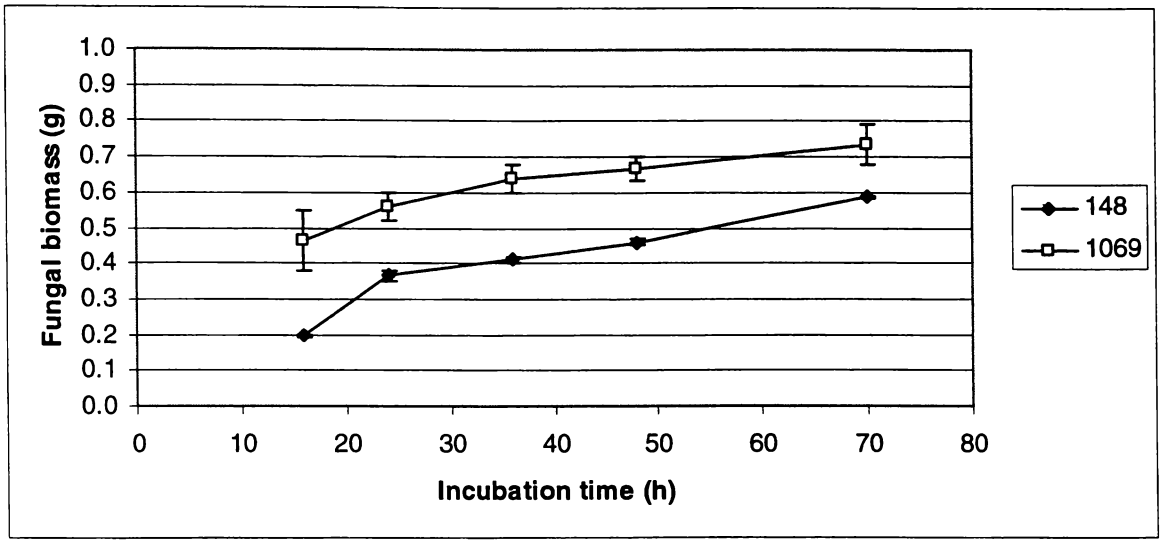


Figure A2.8: Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 30°C.

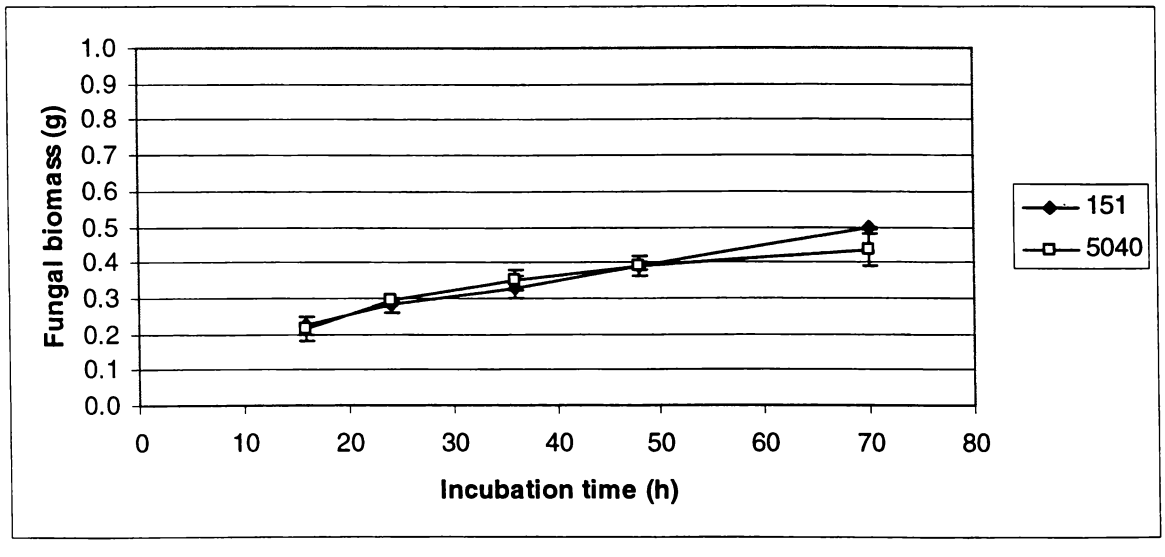


Figure A2.9: Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 30°C.

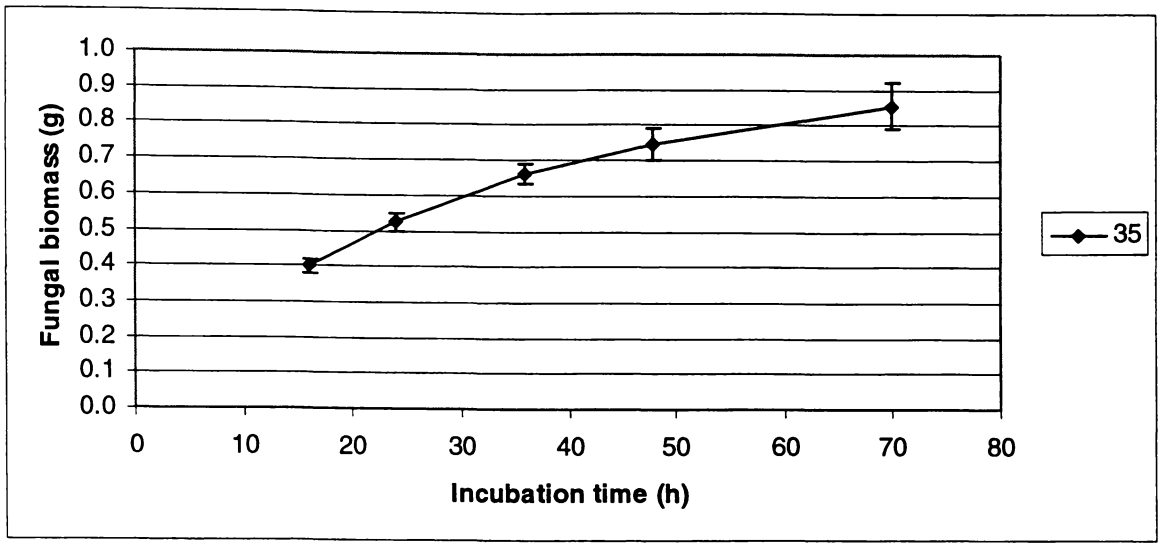


Figure A2.10: Biomass accumulation of *S. sapinea* #35 in liquid media at 30°C.

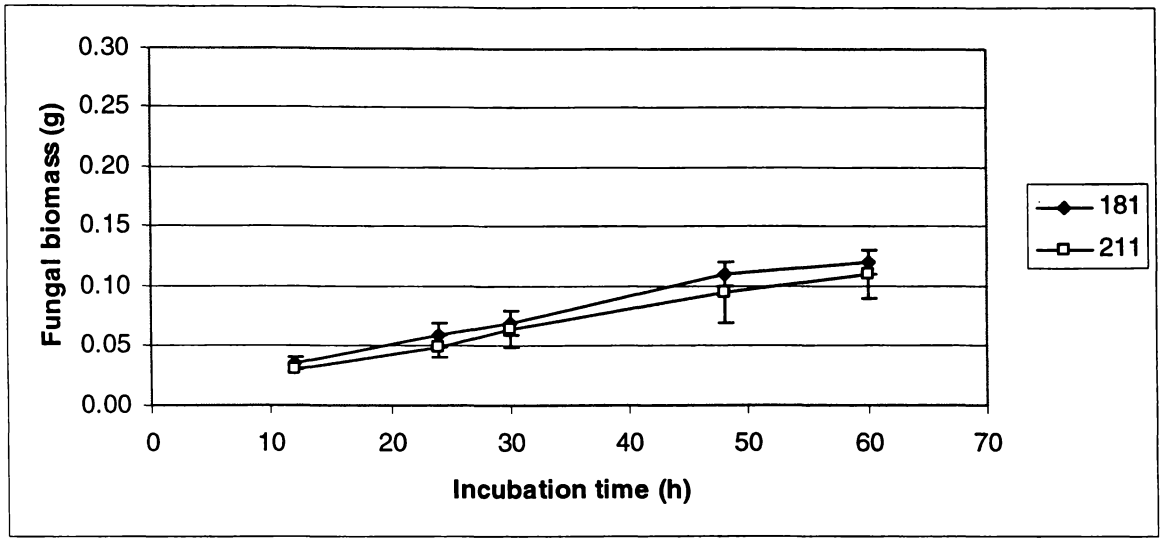


Figure A2.11: Biomass accumulation of *O. ips* #181 and *P. cupulatum* #211 in liquid media at 35°C.

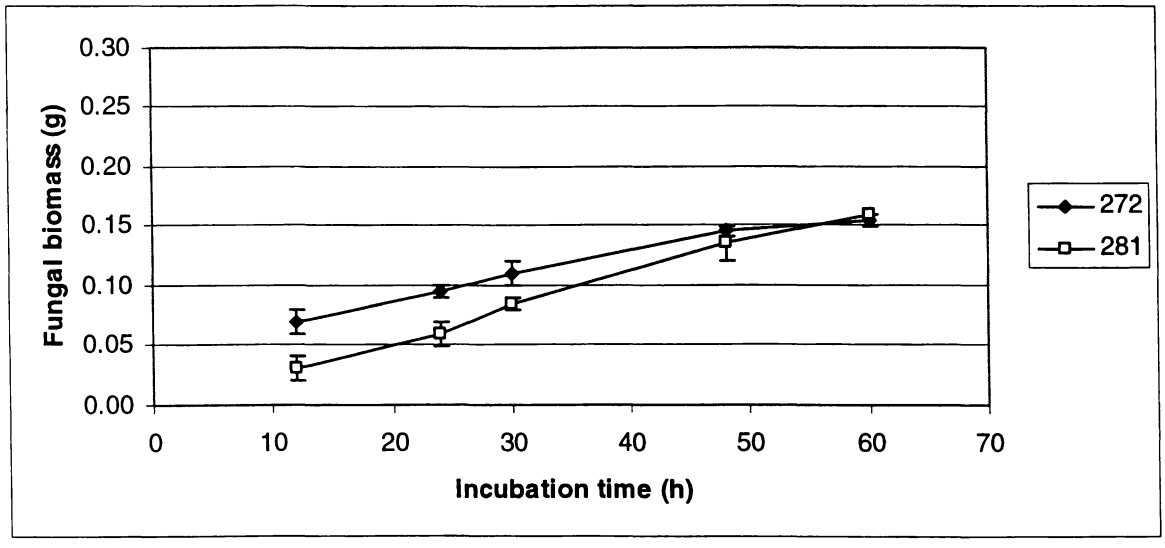


Figure A2.12: Biomass accumulation of *O. piceae* #272 and *L. procerum* #281 in liquid media at 35°C.

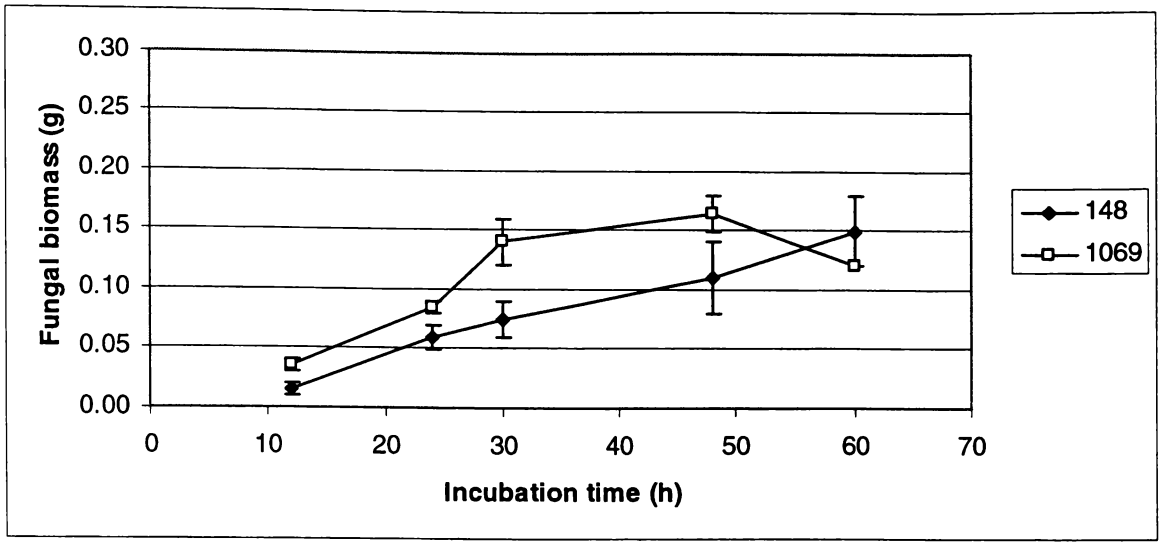


Figure A2.13: Biomass accumulation of *O. floccosum* #148 and *O. querci* #1069 in liquid media at 35°C.

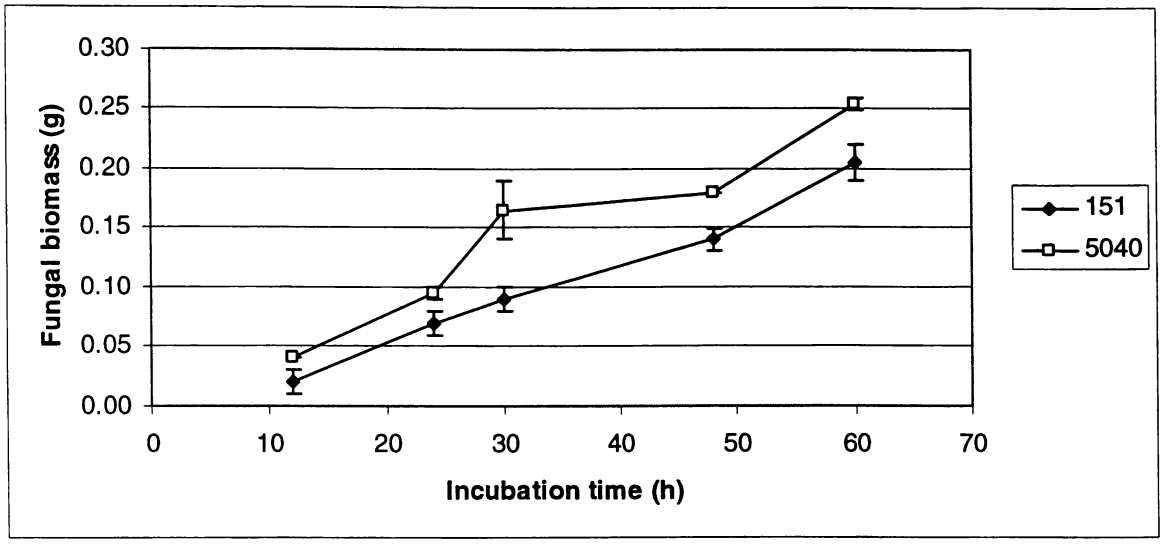


Figure A2.14: Biomass accumulation of *O. pluriannulatum* #151 and #5040 in liquid media at 35°C.

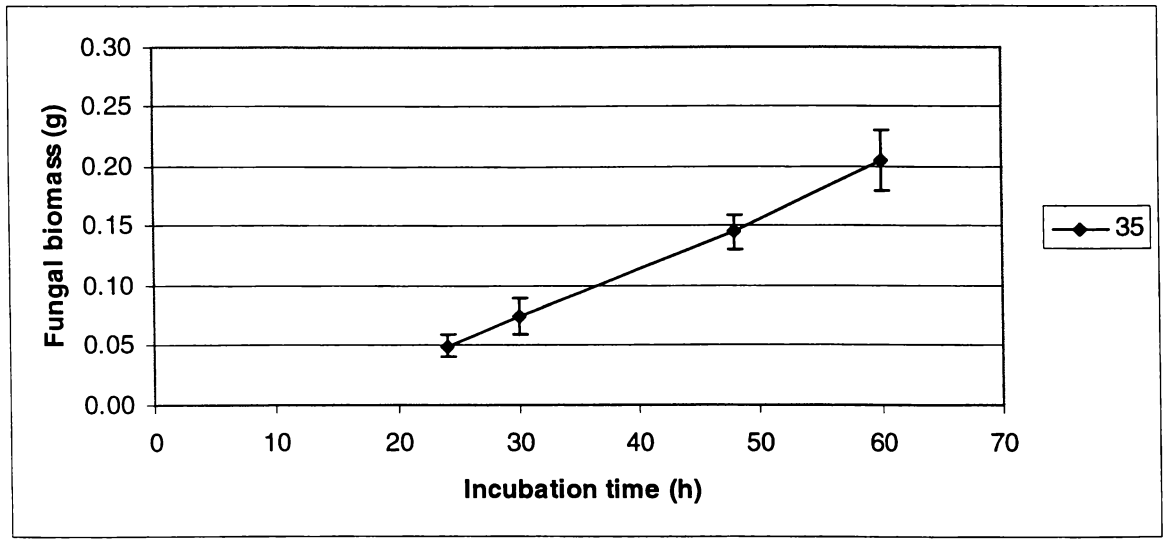


Figure A2.15: Biomass accumulation of *S. sapinea* #35 in liquid media at 35°C.

APPENDIX 3 – ORIGINAL DATA FROM TOUGHNESS AND WEIGHT LOSS DETERMINATIONS.

Contents of Appendix 3:

File name	Exp. no.	Samples / Controls tested	Incubation time
10-11-98	I	*O. floccosum* #138; *S. sapinea* #4	8 weeks
11-11-98	I	*O. pluriannulatum* #151 and #5040	8 weeks
07-12-98	I	*O. floccosum* #138; *S. sapinea* #4	16 weeks
09-12-98	I	*O. pluriannulatum* #151 and #5040	16 weeks
10-12-98	I	*O. pluriannulatum* #151 and #5040 (continued)	16 weeks
29-02-00	II	*L. procerum* #1852, *O. piceae* #272, *O. ips* #308, *S. commune*, *G. trabeum*	16 weeks
07-09-00	III	*O. ips* #294, *O. floccosum* #F40, *O. ips* #308, *O. floccosum* #F13	16 weeks
26-09-00	III	*G. trabeum*, *P. gigantea*, *O. ips* #1191, *O. ips* #P36, *O. pluriannulatum* F3410, *S. sapinea* #35	16 weeks
23-07-01	IV	*G. trabeum*, *O. ips* #308, *S. sapinea* #4	16 weeks
24-07-01	IV	*G. trabeum*, *O. ips* #308, *O. ips* #294, *S. sapinea* #4	16 weeks

							Enter File Name ->					Inch-lbf	Joules	
	Toughness Test						Enter Weight Position ->		5	Machine Constant =		716	80.90	
138, D4 / 8 wks							Enter Initial Angle ->		60					
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
1	DG 33-40a	Control 138	14.24	12.47	114.4	15.1	14.7	52	30	14.2	561	491	18.48	163.6
2	DG 33-40a	Control 138	15.75	13.82	114.5	14.8	14.8	46	30	14.0	628	551	32.79	290.2
3	DG 33-40a	Control 138	15.08	13.25	114.5	14.6	14.4	48	40	13.8	626	550	29.31	259.5
4	DG 33-40a	Control 138	15.49	13.61	114.5	14.6	14.8	47	45	13.8	626	550	30.64	271.2
5	DG 33-40a	Control 138	15.66	13.74	114.5	14.9	14.7	46	10	14.0	624	548	33.40	295.6
6	DG 33-40a	Control 138	14.77	12.96	114.3	14.9	14.3	50	20	14.0	606	532	24.66	218.3
7	DG 33-40a	Control 138	15.56	13.6	114.4	14.8	15.1	44	35	14.4	609	532	36.21	320.5
8	DG 33-40a	Control 138	13.74	12.06	114.4	14.9	14.2	51	0	13.9	568	498	23.22	205.5
9	DG 57-64a	Control 138	12.74	11.15	114.4	14.8	14.9	54	0	14.3	505	442	15.18	134.3
10	DG 57-64a	Control 138	15	13.14	114.3	14.9	14.8	47	50	14.2	595	521	29.51	261.2
11	DG 57-64a	Control 138	13.7	12.03	114.3	14.5	14.4	50	10	13.9	574	504	25.96	229.7
12	DG 57-64a	Control 138	14.46	12.69	114.5	14.6	14.5	48	7	13.9	597	524	30.41	269.2
13	DG 57-64a	Control 138	13.89	12.16	114.4	14.9	14.6	49	45	14.2	558	489	25.52	225.9
14	DG 57-64a	Control 138	13.51	11.82	114.3	14.9	14.4	53	32	14.3	551	482	16.71	147.9
15	DG 57-64a	Control 138	13.6	11.92	114.5	14.7	14.8	48	4	14.1	546	479	29.60	262.0
16	DG 57-64a	Control 138	12.51	10.94	114.4	14.6	14.5	51	56	14.4	517	452	21.15	187.2
17	DG 17-24a	Control 138	14.03	12.27	114.3	14.8	14.8	51	50	14.3	560	490	20.53	181.7
18	DG 17-24a	Control 138	13.83	12.14	114.3	14.4	14.7	48	36	13.9	572	502	29.49	261.0
19	DG 17-24a	Control 138	12.71	11.13	114.2	15.1	14.9	53	45	14.2	495	433	15.31	135.5
20	DG 17-24a	Control 138	12.21	10.7	114.4	14.4	14.6	54	0	14.1	508	445	16.16	143.0
21	DG 17-24a	Control 138	14.47	12.66	114.4	14.7	15.1	51	45	14.3	570	499	20.53	181.7
22	DG 17-24a	Control 138	14.59	12.76	114.5	15	15	51	30	14.3	566	495	20.61	182.4
23	DG 17-24a	Control 138	13.46	11.78	114.3	15	14.4	54	25	14.3	545	477	14.35	127.0
24	DG 17-24a	Control 138	13.11	11.48	114.1	14.4	14.8	54	5	14.2	539	472	15.73	139.2
25	LG 57-64a	Control D4	11.63	10.18	114.2	14.1	14.7	55	10	14.2	491	430	13.45	119.0
26	LG 57-64a	Control D4	11.83	10.39	114.3	14.4	14.9	55	25	13.9	482	424	12.19	107.9
27	LG 57-64a	Control D4	11.84	10.37	114.3	14.4	14.9	54	22	14.2	483	423	14.90	131.9
28	LG 57-64a	Control D4	11.97	10.48	114.4	14.9	14.8	54	35	14.2	474	415	13.70	121.2

29	LG 57-64a	Control D4	11.28	9.86	114.4	15.1	14.6	54	55	14.4	447	391	12.79	113.2
30	LG 57-64a	Control D4	11.02	9.62	114.1	14.8	15.1	54	37	14.6	432	377	13.49	119.4
31	LG 57-64a	Control D4	10.68	9.35	114.2	15	14.8	54	22	14.2	421	369	14.08	124.6
32	LG 57-64a	Control D4	10.87	9.51	114.1	15.1	15	55	46	14.3	421	368	10.42	92.2
33	LG 73-80a	Control D4	11.33	9.9	114.4	14.7	15.2	55	38	14.4	443	387	11.05	97.8
34	LG 73-80a	Control D4												
35	LG 73-80a	Control D4	11.76	10.26	114.2	15.1	14.4	53	53	14.6	474	413	15.51	137.3
36	LG 73-80a	Control D4	11.57	10.14	114.3	14.9	14.6	55	22	14.1	465	408	11.93	105.6
37	LG 73-80a	Control D4	10.87	9.48	114.1	15.2	15.5	56	0	14.7	404	353	9.44	83.6
38	LG 73-80a	Control D4												
39	LG 73-80a	Control D4	11.87	10.41	114.4	14.9	14.6	53	42	14.0	477	418	16.07	142.2
40	LG 73-80a	Control D4	11.68	10.24	114.4	14.6	14.5	54	40	14.1	482	423	14.21	125.8
41	LG 57-64b	D4	12.45	10.91	114.3	15	15.1	54	10	14.1	481	421	14.28	126.4
42	LG 57-64b	D4	12.56	11.01	114.3	15.2	14.4	54	44	14.1	502	440	13.29	117.6
43	LG 57-64b	D4	11.93	10.44	114.3	14.6	14.9	53	47	14.3	480	420	16.04	142.0
44	LG 57-64b	D4	12.01	10.5	114.3	15	14.7	53	20	14.4	477	417	16.68	147.6
45	LG 57-64b	D4	10.95	9.57	114.3	15	14.9	54	48	14.4	429	375	12.94	114.6
46	LG 57-64b	D4	11.14	9.74	114.3	14.8	14.9	53	55	14.4	442	386	15.38	136.1
47	LG 57-64b	D4	10.44	9.11	114.1	15	14.8	55	30	14.6	412	360	11.32	100.2
48	LG 57-64b	D4	9.59	8.39	114.1	14.7	14.9	55	14	14.3	384	336	12.27	108.6
49	LG 73-80b	D4	10.78	9.42	114	15	14.7	55	6	14.4	429	375	12.38	109.6
50	LG 73-80b	D4	11.92	10.4	114	15.4	14.9	54	17	14.6	456	398	13.62	120.6
51	LG 73-80b	D4	11.31	9.9	114.3	15.1	14.9	52	32	14.2	440	385	18.15	160.7
52	LG 73-80b	D4	11.33	9.91	114.3	15.1	14.7	53	55	14.3	447	391	15.12	133.8
53	LG 73-80b	D4	10.97	9.59	114.1	14.7	14.7	54	50	14.4	445	389	13.45	119.1
54	LG 73-80b	D4	11.22	9.84	114.3	14.8	14.8	53	22	14.0	448	393	16.83	149.0
55	LG 73-80b	D4	9.94	8.63	114.1	15.2	14.7	55	12	15.2	390	339	11.89	105.3
56	LG 73-80b	D4	11.45	10.05	114.5	14.3	14.8	52	25	13.9	473	415	20.18	178.6
57	DG 33-40b	138	13.9	12.13	114.5	15.1	14.9	50	20	14.6	540	471	23.19	205.2
58	DG 33-40b	138	15.6	13.62	114.2	14.5	14.5							
59	DG 33-40b	138	14.6	12.73	114.8	14.5	14.7	47	30	14.7	597	520	31.76	281.1
60	DG 33-40b	138	14.63	12.76	114.7	14.9	14.7	51	43	14.7	582	508	20.74	183.5
61	DG 33-40b	138	13.79	12.04	114.4	14.9	14.4	51	45	14.5	562	491	21.09	186.6
62	DG 33-40b	138	15.88	13.85	114.5	14.7	14.8	46	54	14.7	637	556	32.24	285.3
63	DG 33-40b	138	15.77	13.77	114.5	14.9	14.8	47	22	14.5	625	545	30.54	270.3
64	DG 33-40b	138	14.7	12.83	114.7	14.8	14.8	49	30	14.6	585	511	26.02	230.3

65	DG 57-64b	138	15.05	13.11	114.5	15.1	14.6	47	40	14.8	596	519	29.67	262.6
66	DG 57-64b	138	15.01	13.09	114.3	14.9	14.7							
67	DG 57-64b	138	14.14	12.34	114.3	15	14.6	49	39	14.6	565	493	25.49	225.6
68	DG 57-64b	138	14.53	12.65	114.4	14.8	14.7	47	25	14.9	584	508	30.96	274.0
69	DG 57-64b	138	13.73	11.97	114.4	15.1	14.8	52	7	14.7	537	468	19.25	170.4
70	DG 57-64b	138	12.81	11.17	114.5	15	15	52	30	14.7	497	434	18.30	161.9
71	DG 57-64b	138	12.96	11.32	114.4	14.8	14.8	54	3	14.5	517	452	15.16	134.2
72	DG 57-64b	138	13.24	11.56	114.4	14.8	14.6	53	18	14.5	536	468	17.23	152.5
73	DG 17-24b	138	12.22	10.66	114.4	14.7	14.7	54	12	14.6	494	431	15.05	133.2
74	DG 17-24b	138	12.73	11.1	114.4	15.2	15	52	4	14.7	488	426	18.91	167.4
75	DG 17-24b	138	14.45	12.61	114.4	14.4	14.5	48	15	14.6	605	528	30.75	272.1
76	DG 17-24b	138	14.48	12.67	114.6	14.8	14.6	48	55	14.3	585	512	27.73	245.4
77	DG 17-24b	138	14.25	12.43	114.6	14.5	14.9	49	3	14.6	576	502	27.73	245.5
78	DG 17-24b	138	14.14	12.33	114.4	15	15	52	50	14.7	549	479	17.52	155.1
79	DG 17-24b	138	13.94	12.19	114.3	15.1	14.9	52	25	14.4	542	474	18.42	163.1
80	DG 17-24b	138	13.63	11.94	113.9	14.7	15.2	53	50	14.2	536	469	15.44	136.6

							Enter File Name ->				Inch-lbf	Joules	
	Toughness Test						Enter Weight Position ->	5	Machine Constant =		716	80.90	
	151, 5040 / 8 wks						Enter Initial Angle ->	60					
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
1	LG/P 17-24b	Control 5040	11.73	10.17	113.9	15.4	14.9	51	30	15.3	449	389	19.92	176.3
2	LG/P 17-24b	Control 5040	11.36	9.86	114.3	14.9	14.1	52	50	15.2	473	411	18.83	166.7
3	LG/P 17-24b	Control 5040	12	10.41	114.2	15.2	14.8			15.3	467	405		
4	LG/P 17-24b	Control 5040	12.46	10.8	114	15.2	14.6	55	30	15.4	493	427	11.25	99.5
5	LG/P 17-24b	Control 5040	11.33	9.82	114.3	15	15	52	30	15.4	441	382	18.30	161.9
6	LG/P 17-24b	Control 5040	11.27	9.8	114.3	15.2	15.1	50	38	15.0	430	374	21.99	194.6
7	LG/P 17-24b	Control 5040	10.8	9.39	114.3	15.5	14.6	54	25	15.0	418	363	13.45	119.1
8	LG/P 17-24b	Control 5040	11.47	9.97	114.2	15.3	15.1	54	35	15.0	435	378	12.89	114.1
9	LG 129-136a	Control 5040	10.33	8.97	114.2	14.9	13.4	54	48	15.2	453	393	14.54	128.7
10	LG 129-136a	Control 5040	10.81	9.39	114.4	14.8	13.8	54	48	15.1	463	402	14.27	126.3
11	LG 129-136a	Control 5040	10.6	9.22	114.2	14.9	14			15.0	445	387		
12	LG 129-136a	Control 5040	10.83	9.43	114.2	14.9	13.9	52	35	14.8	458	399	19.74	174.7
13	LG 129-136a	Control 5040	10.74	9.33	114.2	15	14.4	53	55	15.1	435	378	15.59	138.0
14	LG 129-136a	Control 5040	10.65	9.26	114.3	15.1	14.2	53	6	15.0	435	378	17.66	156.3
15	LG 129-136a	Control 5040	10.44	9.09	114.3	15.1	14.2	53	35	14.9	426	371	16.47	145.8
16	LG 129-136a	Control 5040	9.93	8.65	114	14.8	14	55	40	14.8	420	366	11.78	104.3
17	LG 153-160a	Control 5040	12.07	10.46	114.1	15.4	14.9	51	10	15.4	461	400	20.66	182.9
18	LG 153-160a	Control 5040	12.01	10.42	114	15.3	14.9	52	4	15.3	462	401	18.85	166.8
19	LG 153-160a	Control 5040	12.36	10.72	113.9	15.4	15.2	52	32	15.3	464	402	17.26	152.8
20	LG 153-160a	Control 5040	11.53	10.02	113.9	15.1	15.2	51	12	15.1	441	383	20.80	184.1
21	LG 153-160a	Control 5040	10.35	9.01	114.1	14.8	14.8	55	6	14.9	414	361	12.56	111.2
22	LG 153-160a	Control 5040	10.99	9.58	114.2	14.9	14.5	55	8	14.7	445	388	12.60	111.5
23	LG 153-160a	Control 5040	11.37	9.92	114.2	14.9	14.7	52	56	14.6	455	397	17.82	157.7
24	LG 153-160a	Control 5040	11.81	10.3	114.3	15.1	15	53	19	14.7	456	398	16.22	143.5
25	LG 97-104a	Control 5040	8.65	7.52	113.9	14.5	15	56	7	15.0	349	304	10.19	90.2
26	LG 97-104a	Control 5040	8.84	7.67	113.8	14.7	14.8	56	48	15.3	357	310	8.37	74.1
27	LG 97-104a	Control 5040	9.21	8.02	114	15.1	14.9	56	7	14.8	359	313	9.64	85.3
28	LG 97-104a	Control 5040	9.16	7.95	114	15.1	14.8	56	32	15.2	360	312	8.68	76.9

29	LG 97-104a	Control 5040	9.85	8.56	113.9	15.3	15.1	56	31	15.1	374	325	8.38	74.2
30	LG 97-104a	Control 5040	9.69	8.4	113.9	15.4	15.1	56	12	15.4	366	317	9.03	79.9
31	LG 97-104a	Control 5040	9.98	8.66	114.2	15.3	15.2	55	17	15.2	376	326	11.19	99.1
32	LG 97-104a	Control 5040	9.91	8.6	114.2	15.2	14.8	56	14	15.2	386	335	9.32	82.5
33	LG 121-128a	Control 151	11.04	9.58	114.2	15	14.2	54	0	15.2	454	394	15.60	138.1
34	LG 121-128a	Control 151	10.64	9.24	114.3	15.1	13.9	53	54	15.2	444	385	16.03	141.9
35	LG 121-128a	Control 151	10.5	9.11	114.3	15	13.8	53	53	15.3	444	385	16.35	144.7
36	LG 121-128a	Control 151	10.56	9.17	114.2	14.9	13.8	52	55	15.2	450	391	19.03	168.4
37	LG 121-128a	Control 151	11.33	9.84	114.3	14.6	13.8	51	11	15.1	492	427	24.18	214.0
38	LG 121-128a	Control 151	11.59	10.07	114.4	14.4	13.8	53	0	15.1	510	443	19.83	175.5
39	LG 121-128a	Control 151	12.21	10.62	114.3	15.2	13.9	53	7	15.0	506	440	17.82	157.7
40	LG 121-128a	Control 151	12.1	10.53	114.3	15.1	14.1	53	16	14.9	497	433	17.38	153.8
41	LG 41-48a	Control 151	10.23	8.89	114.3	15.2	15.4	56	6	15.1	382	332	9.27	82.1
42	LG 41-48a	Control 151	10.12	8.79	114.3	15.2	15	54	5	15.1	388	337	14.27	126.3
43	LG 41-48a	Control 151	10.35	9	114.1	15.2	15.2	52	18	15.0	393	341	18.14	160.6
44	LG 41-48a	Control 151	10.37	9.01	114.3	15.2	15.4	52	21	15.1	388	337	17.79	157.5
45	LG 41-48a	Control 151	10.51	9.17	114.4	15.1	15.4	54	17	14.6	395	345	13.59	120.3
46	LG 41-48a	Control 151	10.66	9.3	114.3	15.1	15.2	55	50	14.6	406	354	10.12	89.6
47	LG 41-48a	Control 151	10.48	9.17	114.4	15.1	15.4	53	56	14.3	394	345	14.39	127.4
48	LG 41-48a	Control 151	10.35	9.02	114.2	14.7	15.5	53	39	14.7	398	347	15.57	137.8
49	LG 113-120a	Control 151	10.45	9.05	114	15.3	15.4	53	57	15.5	389	337	14.06	124.5
50	LG 113-120a	Control 151	10.62	9.21	114.1	15	15.4	55	21	15.3	403	349	11.23	99.4
51	LG 113-120a	Control 151	10.99	9.54	114.2	15.1	15.1	54	22	15.2	422	366	13.66	120.9
52	LG 113-120a	Control 151	10.43	9.05	114.1	15	15.3	55	40	15.2	398	346	10.56	93.4
53	LG 113-120a	Control 151	10.93	9.46	113.9	14.4	14.5	54	48	15.5	460	398	14.17	125.4
54	LG 113-120a	Control 151	10.11	8.77	114.2	15	13.5	55	37	15.3	437	379	12.10	107.1
55	LG 113-120a	Control 151	11.14	9.69	114.2	14.8	14.2	55	12	15.0	464	404	12.83	113.6
56	LG 113-120a	Control 151	11.28	9.83	114.4	15	14.3	54	21	14.8	460	401	14.62	129.4
57	LG 113-120b	151	10.5	9.06	114.2	15.3	15.5	55	10	15.9	388	335	11.24	99.5
58	LG 113-120b	151	11.54	10	114.1	15	15.1	53	42	15.4	447	387	15.38	136.1
59	LG 113-120b	151	10.69	9.23	114.1	14.4	14.2	55	32	15.8	458	396	12.48	110.4
60	LG 113-120b	151	11.35	9.84	114.2	15.2	15.7	53	55	15.3	416	361	14.01	124.0
61	LG 113-120b	151	11.51	9.93	114.1	14.9	15	52	39	15.9	451	389	18.13	160.5
62	LG 113-120b	151	10.83	9.38	114.4	15.6	14.9	54	40	15.5	407	353	12.49	110.5
63	LG 113-120b	151	11.56	10.03	114.4	14.8	14.7	53	38	15.3	464	403	16.29	144.2
64	LG 113-120b	151	10.75	9.3	113.8	15.1	15.3	55	22	15.6	409	354	11.15	98.7

65	LG 41-48b	151	10.01	8.68	114.4	14.9	15.2	54	7	15.3	386	335	14.45	127.9
66	LG 41-48b	151	9.97	8.64	114.4	15.2	15	55	12	15.4	382	331	11.66	103.2
67	LG 41-48b	151	9.92	8.6	114.2	14.7	15.3	55	3	15.3	386	335	12.40	109.7
68	LG 41-48b	151	10.28	8.92	114.3	15.2	15	55	20	15.2	394	342	11.34	100.4
69	LG 41-48b	151	10.24	8.9	114	15.3	14.8	55	14	15.1	397	345	11.61	102.8
70	LG 41-48b	151	9.94	8.63	114.3	15.1	14.8	55	12	15.2	389	338	11.93	105.6
71	LG 41-48b	151	9.97	8.66	114.5	15.3	15	54	40	15.1	379	330	12.78	113.1
72	LG 41-48b	151	10.21	8.87	114.4	15.1	15.2	54	51	15.1	389	338	12.44	110.1
73	LG 121-128b	151	11.76	10.18	114.4	14.7	13.7	53	54	15.5	510	442	16.95	150.0
74	LG 121-128b	151	10.69	9.29	114.5	15.1	14	54	12	15.1	442	384	15.16	134.1
75	LG 121-128b	151	11.42	9.9	114.2	14.8	13.3	53	0	15.4	508	440	19.72	174.6
76	LG 121-128b	151	10.22	8.88	114.3	14.9	13.5	55	0	15.1	445	386	13.90	123.0
77	LG 121-128b	151	11.65	10.11	114	15.2	13.4	54	9	15.2	502	435	15.81	139.9
78	LG 121-128b	151	11.17	9.69	114.2	15	13.7	53	47	15.3	476	413	16.73	148.1
79	LG 121-128b	151	10.77	9.35	114.2	15.1	13.9	51	0	15.2	449	390	23.24	205.7
80	LG 121-128b	151	10.47	9.09	114.4	14.9	13.8	54	33	15.2	445	386	14.78	130.8
81	LG 137-144b	151	11.77	10.19	114.2	15.4	15.1	52	30	15.5	443	384	17.45	154.5
82	LG 137-144b	151	11.46	9.95	114.3	15.3	14.2	54	7	15.2	461	401	14.85	131.4
83	LG 137-144b	151	11.07	9.57	113.8	14.7	14.8	55	8	15.7	447	387	12.61	111.6
84	LG 137-144b	151	11.92	10.33	114.1	15.2	14.9	54	22	15.4	461	400	13.71	121.3
85	LG 137-144b	151	10.6	9.2	114.2	14.8	15.1	54	30	15.2	415	360	13.77	121.9
86	LG 137-144b	151	10.37	9	114.2	15	14.9	53	45	15.2	406	353	15.46	136.9
87	LG 137-144b	151	11.02	9.53	113.8	15.2	14.9	55	5	15.6	428	370	12.01	106.3
88	LG 137-144b	151	12.3	10.68	114.3	15.1	14.9	55	0	15.2	478	415	12.33	109.2
89	LG 97-104b	5040	9.38	8.18	113.9	14.5	15.8	55	56	14.7	359	313	10.12	89.6
90	LG 97-104b	5040	9.35	8.13	113.9	14.9	15.5	55	22	15.0	355	309	11.24	99.5
91	LG 97-104b	5040	9.13	7.93	114.2	15.3	15.2	55	53	15.1	344	299	9.80	86.8
92	LG 97-104b	5040	9.74	8.43	114.2	15.2	15.7	55	34	15.5	357	309	10.31	91.2
93	LG 97-104b	5040	10.41	9.03	114.1	15.3	15.8	55	23	15.3	377	327	10.55	93.3
94	LG 97-104b	5040	10.23	8.86	114.5	15.4	15.6	54	55	15.5	372	322	11.61	102.8
95	LG 97-104b	5040	10.11	8.77	114.3	15.2	15.3	55	5	15.3	380	330	11.70	103.5
96	LG 97-104b	5040	10.72	9.28	114.4	15.3	15.7	54	49	15.5	390	338	11.88	105.1
97	LG 129-136b	5040	9.99	8.65	114.4	15.1	14.1	54	41	15.5	410	355	13.83	122.4
98	LG 129-136b	5040	10.24	8.88	114.4	14.9	14	54	45	15.3	429	372	14.05	124.3
99	LG 129-136b	5040	10.79	9.35	114.5	15	14.2	54	34	15.4	442	383	14.17	125.4
100	LG 129-136b	5040	10.41	9.02	114.5	14.8	14.4	54	53	15.4	427	370	13.46	119.1

101	LG 129-136b	5040	10.25	8.89	114.3	15	14.1	53	49	15.3	424	368	16.17	143.1
102	LG 129-136b	5040	10.58	9.16	114.5	15.1	13.6	53	34	15.5	450	390	17.24	152.6
103	LG 129-136b	5040	10.48	9.09	114.5	14.9	13.6	53	56	15.3	452	392	16.63	147.2
104	LG 129-136b	5040	10.49	9.11	114.4	14.7	13.8	54	55	15.1	452	393	14.11	124.8
105	LG/P 17-24a	5040	12.09	10.49	114.3	15.2	14.7	51	7	15.3	473	411	21.48	190.1
106	LG/P 17-24a	5040	11.9	10.32	114.4	14.8	14.8	51	40	15.3	475	412	20.93	185.2
107	LG/P 17-24a	5040	12.07	10.47	114.4	15.1	14.8	50	47	15.3	472	410	22.32	197.5
108	LG/P 17-24a	5040	12.26	10.65	114.4	15.1	14.9	49	56	15.1	476	414	24.08	213.2
109	LG/P 17-24a	5040	10.24	8.92	114.4	14.9	15.1	54	23	14.8	398	347	13.91	123.1
110	LG/P 17-24a	5040	10.81	9.41	114.4	15.1	14.5	53	47	14.9	432	376	15.65	138.5
111	LG/P 17-24a	5040												
112	LG/P 17-24a	5040	11.02	9.57	114.3	15.3	14.6	54	12	15.2	432	375	14.24	126.0
113	LG 153-160b	5040	12.15	10.54	114.3	15.3	15	54	3	15.3	463	402	14.21	125.8
114	LG 153-160b	5040	11.9	10.31	114.3	15.3	14.6	54	1	15.4	466	404	14.68	129.9
115	LG 153-160b	5040	12.34	10.71	114.4	15.3	15	52	15	15.2	470	408	18.31	162.1
116	LG 153-160b	5040	11.94	10.38	114.4	15.1	15	53	0	15.0	461	401	16.95	150.0
117	LG 153-160b	5040	10.53	9.15	114	14.7	14.9	53	30	15.1	422	366	16.57	146.6
118	LG 153-160b	5040	10.56	9.19	114.3	15.1	14.7	55	19	14.9	416	362	11.73	103.8
119	LG 153-160b	5040	10.69	9.27	114.3	15	14.9	55	13	15.3	418	363	11.94	105.6
120	LG 153-160b	5040	11.23	9.77	114.4	15	15.1	51	0	14.9	433	377	21.61	191.3
121	LG 137-144a	Control 151	12.07	10.47	114.2	15	15	52	47	15.3	470	407	17.64	156.1
122	LG 137-144a	Control 151	12.06	10.47	114.3	15.2	14.6	55	9	15.2	475	413	12.10	107.1
123	LG 137-144a	Control 151	12.07	10.46	114.3	15.2	14.7	55	30	15.4	473	410	11.17	98.9
124	LG 137-144a	Control 151	12.34	10.72	114.2	15.1	14.7	53	32	15.1	487	423	16.03	141.9
125	LG 137-144a	Control 151	11.1	9.61	113.9	14.9	15.3	55	23	15.5	427	370	11.35	100.4
126	LG 137-144a	Control 151	10.7	9.28	114.1	15.2	14.8	56	6	15.3	417	362	9.65	85.4
127	LG 137-144a	Control 151	11.1	9.65	114.2	14.9	15.1	54	37	15.0	432	376	13.35	118.1
128	LG 137-144a	Control 151	10.72	9.32	114.2	15.2	15.1	54	6	15.0	409	356	14.14	125.2

							Enter File Name ->				Inch-lbf	Joules	
	Toughness Test						Enter Weight Position ->	5	Machine Constant =		716	80.90	
D4, 138 / 16 wks							Enter Initial Angle ->	60					
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
129	LG 25-32a	Control D4	12.25	10.67	114.2	15.1	14.9	53	57	14.8	477	415	14.83	131.3
130	LG 25-32a	Control D4	12.41	10.78	114.3	15.4	14.2	51	38	15.1	496	431	20.59	182.3
131	LG 25-32a	Control D4	12.64	11	114.4	15.4	14.8	53	21	14.9	485	422	15.87	140.4
132	LG 25-32a	Control D4	12.45	10.83	114.5	15.3	15	51	17	15.0	474	412	20.47	181.2
133	LG 25-32a	Control D4	11.9	10.35	114.5	14.8	14.9	53	49	15.0	471	410	15.63	138.3
134	LG 25-32a	Control D4	11.99	10.44	114.5	14.9	14.6	52	15	14.8	481	419	19.60	173.4
135	LG 25-32a	Control D4	11.2	9.74	114.6	14.9	14.6	52	32	15.0	449	391	18.91	167.4
136	LG 25-32a	Control D4	10.93	9.51	114.3	15	15	51	55	14.9	425	370	19.65	173.9
137	LG 17-24a	Control D4	10.99	9.57	114.5	14.9	15	53	58	14.8	429	374	15.00	132.8
138	LG 17-24a	Control D4	11.24	9.77	114.5	14.7	14.8	51	3	15.0	451	392	22.63	200.3
139	LG 17-24a	Control D4	10.89	9.47	114.4	15.2	14.9	52	12	15.0	420	366	18.73	165.8
140	LG 17-24a	Control D4	10.78	9.39	114.4	15.1	15	53	52	14.8	416	362	14.93	132.1
141	LG 17-24a	Control D4	9.81	8.53	114.4	14.7	14.7	51	44	15.0	397	345	21.13	187.0
142	LG 17-24a	Control D4	10.6	9.22	114.3	15.2	15.2	53	50	15.0	401	349	14.66	129.8
143	LG 17-24a	Control D4	9.95	8.68	114.3	14.5	14.9	52	22	14.6	403	351	19.74	174.7
144	LG 17-24a	Control D4	10.32	9	114.2	14.8	14.9	54	33	14.7	410	357	13.83	122.4
145	DG 1-8a	Control 138	13.2	11.55	114.3	15.2	14.8	54	0	14.3	513	449	14.66	129.8
146	DG 1-8a	Control 138	13.32	11.65	114.4	14.9	14.7	51	31	14.3	532	465	21.21	187.7
147	DG 1-8a	Control 138	16.79	14.55	114.3	15	14.9	51	0	15.4	657	570	21.90	193.8
148	DG 1-8a	Control 138	16.24	14.08	114.3	14.9	14.7	51	13	15.3	649	562	21.92	194.0
149	DG 1-8a	Control 138	16.6	14.37	114.3	15.1	14.8	49	52	15.5	650	563	24.40	215.9
150	DG 1-8a	Control 138	15.94	13.85	114.4	15.1	14.6	50	52	15.1	632	549	22.43	198.5
151	DG 1-8a	Control 138	12.39	10.77	114.2	14.7	15.2	54	30	15.0	486	422	13.82	122.3
152	DG 1-8a	Control 138	13.2	11.47	114.4	15.1	15	52	32	15.1	509	443	18.03	159.6
153	DG 49-56a	Control 138	16.03	13.92	114.3	14.7	14.9	46	47	15.2	640	556	32.28	285.7
154	DG 49-56a	Control 138	14.84	12.87	114.3	13.9	14.7	50	10	15.3	635	551	27.14	240.2
155	DG 49-56a	Control 138	15.6	13.54	114	14.6	14.8	46	50	15.2	633	550	32.73	289.7
156	DG 49-56a	Control 138	16.51	14.32	114.4	14.9	14.6	46	26	15.3	663	575	33.04	292.4

157	DG 49-56a	Control 138	15.78	13.67	114.3	14.9	14.8	46	3	15.4	626	542	33.43	295.8
158	DG 49-56a	Control 138	15.54	13.47	114.2	15	14.7	49	14	15.4	617	535	26.26	232.4
159	DG 49-56a	Control 138	15.61	13.52	114.3	14.8	15.3	46	31	15.5	603	522	31.68	280.4
160	DG 49-56a	Control 138	15.73	13.64	114.5	15.3	14.7	46	37	15.3	611	530	31.11	275.4
161	DG 65-72a	Control 138	12.92	11.27	114.3	15.1	14.9	54	0	14.6	502	438	14.72	130.2
162	DG 65-72a	Control 138	13.04	11.37	114.4	15	14.5	53	53	14.7	524	457	15.56	137.8
163	DG 65-72a	Control 138	13.17	11.5	114.4	14.8	15	52	54	14.5	519	453	17.73	156.9
164	DG 65-72a	Control 138	13.11	11.45	114.5	14.7	14.7	51	44	14.5	530	463	21.13	187.0
165	DG 65-72a	Control 138	16.65	14.54	114.4	14.8	14.9	45	32	14.5	660	576	34.67	306.8
166	DG 65-72a	Control 138	17.38	15.18	114.6	14.9	14.8	45	14	14.5	688	601	35.18	311.4
167	DG 65-72a	Control 138	17.23	15.04	114.4	14.9	14.6	44	26	14.6	692	604	37.38	330.9
168	DG 65-72a	Control 138	16.48	14.32	114.3	15	14.6	46	17	15.1	658	572	33.03	292.3
169	LG 49-56a	Control D4	12.28	10.75	114.3	15.2	15	51	36	14.2	471	413	19.97	176.7
170	LG 49-56a	Control D4	11.74	10.25	114.3	15.1	15	51	40	14.5	453	396	20.02	177.2
171	LG 49-56a	Control D4	11.62	10.13	114.4	14.7	15	52	47	14.7	461	402	18.19	161.0
172	LG 49-56a	Control D4	12.02	10.5	114.3	14.8	15.3	52	14	14.5	464	406	18.93	167.6
173	LG 49-56a	Control D4	11.94	10.41	114.3	15.1	14.9	52	23	14.7	464	405	18.50	163.8
174	LG 49-56a	Control D4	11.84	10.32	114.3	15	15	52	45	14.7	460	401	17.71	156.8
175	LG 49-56a	Control D4	11.6	10.11	114.2	15.2	14.8	54	2	14.7	452	394	14.59	129.1
176	LG 49-56a	Control D4	12.1	10.57	114.2	15.1	14.6	54	24	14.5	481	420	14.05	124.3
177	DG 73-80a	Control D4	15.35	13.39	114.2	14.7	14.2	46	16	14.6	644	562	35.07	310.4
178	DG 73-80a	Control D4	15.04	13.12	114.5	14.9	13.9	47	26	14.6	634	553	32.37	286.5
179	DG 73-80a	Control D4	14.53	12.67	114.4	14.5	14.3	46	38	14.7	613	534	34.71	307.2
180	DG 73-80a	Control D4	14.8	12.91	114.4	14.7	14.8	50	2	14.6	595	519	25.04	221.6
181	DG 73-80a	Control D4	15.18	13.24	114.1	14.9	14.5	48	14	14.7	616	537	29.21	258.5
182	DG 73-80a	Control D4	15.36	13.4	114.4	14.9	14.7	45	30	14.6	613	535	34.85	308.4
183	DG 73-80a	Control D4	14.63	12.75	114.5	14.6	14.2	46	35	14.7	616	537	34.70	307.2
184	DG 73-80a	Control D4	13.99	12.2	114.4	14.7	14.1	48	41	14.7	590	515	29.58	261.8
185	LG 33-40a	Control D4	10.64	9.32	114.2	15.2	15.2	54	8	14.2	403	353	13.97	123.7
186	LG 33-40a	Control D4	11.39	9.99	114.4	15.2	14.9	53	47	14.0	440	386	15.07	133.4
187	LG 33-40a	Control D4	11.43	10.02	114.4	15.4	14.9	53	14	14.1	435	382	16.03	141.8
188	LG 33-40a	Control D4	11.51	10.06	114.4	14.9	14.8	53	11	14.4	456	399	17.10	151.3
189	LG 33-40a	Control D4	10.88	9.5	113.1	14.9	15.1	52	22	14.5	428	373	18.68	165.3
190	LG 33-40a	Control D4	11.28	9.85	112.3	15	15	54	0	14.5	446	390	14.77	130.7
191	LG 33-40a	Control D4	10.51	9.17	111.6	15.3	15.4	52	32	14.6	400	349	17.21	152.3
192	LG 33-40a	Control D4	11.26	9.84	112.2	14.9	15.4	52	32	14.4	437	382	17.93	158.7

193	LG 1-8a	Control D4	12.58	10.97	114.4	15.1	14.4	53	48	14.7	506	441	15.72	139.1
194	LG 1-8a	Control D4	12.23	10.67	114.2	15.2	14.9	54	44	14.6	473	413	12.84	113.6
195	LG 1-8a	Control D4	12.09	10.56	114.5	14.6	14.9	51	25	14.5	485	424	21.83	193.3
196	LG 1-8a	Control D4	12.18	10.63	114.4	15.1	14.3	52	8	14.6	493	430	19.88	176.0
197	LG 1-8a	Control D4	12.17	10.62	114.3	14.8	14.7	52	33	14.6	489	427	18.94	167.6
198	LG 1-8a	Control D4	12.59	10.99	114.4	15	14.9	50	38	14.6	492	430	22.74	201.3
199	LG 1-8a	Control D4	9.76	8.5	113.8	15.1	14.6	55	42	14.8	389	339	10.87	96.2
200	LG 1-8a	Control D4	9.51	8.29	113.8	15.3	14.8	53	47	14.7	369	322	15.02	133.0
201	DG 1-8b	138	15.07	13.12	114.4	14.8	14.6	52	31	14.9	610	531	19.15	169.5
202	DG 1-8b	138	16.17	14.07	113.8	14.8	14.8	50	51	14.9	649	564	22.87	202.4
203	DG 1-8b	138	15.27	13.31	114.2	14.7	14.5	48	23	14.7	627	547	29.47	260.8
204	DG 1-8b	138	11.88	10.38	114.5	14.5	14.8	54	0	14.5	483	422	15.77	139.6
205	DG 1-8b	138	12.37	10.81	114.5	15.1	14.8	54	7	14.4	483	422	14.54	128.7
206	DG 25-32b	138	15.89	13.86	114.6	14.5	14.8	45	44	14.6	646	564	35.57	314.9
207	DG 25-32b	138	16.37	14.29	114.4	14.9	14.8	46	33	14.6	649	566	32.34	286.2
208	DG 25-32b	138	15.58	13.59	114.3	14.8	14.2	48	48	14.6	649	566	28.79	254.8
209	DG 25-32b	138	16.39	14.28	114.4	15	14.6	44	37	14.8	654	570	36.61	324.0
210	DG 25-32b	138	16.58	14.46	114.4	14.9	14.8	48	32	14.7	657	573	27.94	247.3
211	DG 25-32b	138	16.22	14.14	114.5	14.9	14.7	49	20	14.7	647	564	26.30	232.8
212	DG 25-32b	138	16.16	14.09	114.3	14.9	14.7	48	25	14.7	645	563	28.39	251.3
213	DG 25-32b	138	16.24	14.17	114.6	15.3	14.5	45	52	14.6	639	557	33.14	293.3
214	DG 9-16b	138	15.94	13.87	114.5	15	14.8	47	35	14.9	627	546	29.76	263.4
215	DG 9-16b	138	15.32	13.34	114.5	15.2	13.2	54	33	14.8	667	581	14.98	132.6
216	DG 9-16b	138	15.36	13.36	114.5	14.8	14.3	51	25	15.0	634	551	22.28	197.2
217	DG 9-16b	138	16.11	14.04	114.5	14.9	14.9	47	33	14.7	634	552	29.94	265.0
218	DG 9-16b	138	14.83	12.91	114.2	14.6	14.9	46	27	14.9	597	520	33.37	295.4
219	DG 9-16b	138	15.01	13.08	114.5	15.1	14.8	47	50	14.8	587	511	28.91	255.8
220	DG 9-16b	138	15.49	13.48	114.3	15	14.8	49	30	14.9	610	531	25.48	225.5
221	DG 9-16b	138	15.72	13.68	114.4	15.1	14.8	49	8	14.9	615	535	26.04	230.5
222	DG 65-72b	138	13.3	11.61	114.4	15.1	15.4	51	44	14.6	500	436	19.35	171.3
223	DG 65-72b	138	12.89	11.23	114.5	15.1	15	53	4	14.8	497	433	16.80	148.7
224	DG 65-72b	138	12.94	11.28	114.2	14.9	14.9	53	12	14.7	510	445	16.94	150.0
225	DG 65-72b	138	12.95	11.29	114.5	14.9	14.3	50	48	14.7	531	463	23.54	208.4
226	DG 65-72b	138	16.62	14.5	114.5	15	14.7	48	53	14.6	658	574	27.05	239.4
227	DG 65-72b	138	17.16	14.97	114.6	14.9	14.8	46	1	14.6	679	592	33.50	296.5
228	DG 65-72b	138	16.91	14.74	114.7	14.9	14.4	45	45	14.7	687	599	35.02	310.0

229	DG 65-72b	138	16.15	14.05	114.6	15	14.4	49	15	14.9	652	568	26.77	236.9
230	DG 49-56b	138	15.81	13.73	114.3	15	14.8	49	48	15.1	623	541	24.80	219.5
231	DG 49-56b	138	15.81	13.71	114.4	14.8	14.9	48	45	15.3	627	543	27.55	243.9
232	DG 49-56b	138	16.35	14.18	114.4	15	14.8	49	55	15.3	644	558	24.54	217.2
233	DG 49-56b	138	16.55	14.37	114.5	14.9	14.8	46	17	15.2	655	569	32.92	291.4
234	DG 49-56b	138	14.94	12.94	114.4	14.3	15.1	50	12	15.5	605	524	25.21	223.1
235	DG 49-56b	138	15.54	13.46	114.2	15.4	14.8	48	33	15.5	597	517	26.52	234.7
236	DG 49-56b	138	15.75	13.64	114.3	15.2	14.9	45	47	15.5	608	527	32.75	289.9
237	DG 49-56b	138	15.69	13.6	114.3	15	14.6	49	4	15.4	627	543	26.82	237.4
238	LG 25-32b	D4												
239	LG 25-32b	D4												
240	LG 25-32b	D4												
241	LG 25-32b	D4												
242	LG 25-32b	D4												
243	LG 25-32b	D4												
244	LG 25-32b	D4												
245	LG 25-32b	D4												
246	LG 49-56b	D4												
247	LG 49-56b	D4												
248	LG 49-56b	D4												
249	LG 49-56b	D4												
250	LG 49-56b	D4												
251	LG 49-56b	D4												
252	LG 49-56b	D4												
253	LG 49-56b	D4												
254	LG 33-40b	D4	9.77	8.5	110.7	15.1	14.6	53	9	14.9	400	348	17.06	151.0
255	LG 33-40b	D4	10.39	9.04	109.6	15.3	14.9	54	1	14.9	416	362	14.38	127.3
256	LG 33-40b	D4	10.49	9.13	111.7	15.2	15	52	37	14.9	412	358	17.66	156.3
257	LG 33-40b	D4	10.35	9.01	111.3	14.9	14.7	53	55	14.9	425	370	15.43	136.6
258	LG 33-40b	D4	11.11	9.69	114.4	15.3	14.7	54	5	14.7	432	377	14.42	127.6
259	LG 33-40b	D4	11.62	10.14	114.3	15	15	53	26	14.6	452	394	16.11	142.6
260	LG 33-40b	D4	11.3	9.86	112	15.1	14.7	54	57	14.6	455	397	12.62	111.7
261	LG 33-40b	D4	10.91	9.51	114.3	15.2	15.3	52	37	14.7	410	358	17.31	153.2
262	LG 17-24b	D4	10.79	9.39	114.3	15.2	15.2	55	20	14.9	409	356	11.19	99.0
263	LG 17-24b	D4	9.89	8.62	111.7	15	15.1	52	16	14.7	391	341	18.71	165.6
264	LG 17-24b	D4	9.91	8.62	111.9	15.2	15.1	55	7	15.0	386	336	11.77	104.2

265	LG 17-24b	D4	10.14	8.83	114.5	15.3	14.8	52	0	14.8	391	341	19.13	169.3
266	LG 17-24b	D4	10.11	8.82	114.4	14.5	14.9	54	4	14.6	409	357	15.50	137.2
267	LG 17-24b	D4	10.7	9.33	114.3	15.3	15	52	31	14.7	408	356	17.71	156.7
268	LG 17-24b	D4	10.41	9.08	112.3	14.9	14.7	54	0	14.6	423	369	15.23	134.8
269	LG 17-24b	D4	10.69	9.33	111.9	15	14.6	53	32	14.6	436	381	16.31	144.3
270	DG 73-80b	D4	15.29	13.31	114.4	14.9	14.2	49	0	14.9	632	550	28.02	248.0
271	DG 73-80b	D4	15.1	13.16	114.6	14.9	14.7	46	7	14.7	602	524	33.51	296.6
272	DG 73-80b	D4	14.93	12.99	113.1	14.7	14.7	49	22	14.9	611	532	26.78	237.0
273	DG 73-80b	D4	15.3	13.32	113.1	15	14.3	46	50	14.9	631	549	32.49	287.6
274	DG 73-80b	D4	14.73	12.83	114.4	14.6	14.8	46	43	14.8	596	519	33.00	292.0
275	DG 73-80b	D4	14.94	13	114.5	15	14.5	46	53	14.9	600	522	31.93	282.6
276	DG 73-80b	D4	15.51	13.52	114.3	14.8	14.9	45	39	14.7	615	536	34.42	304.6
277	DG 73-80b	D4	15.41	13.42	112.9	14.9	14.5	44	53	14.8	632	550	36.67	324.6
278	LG 1-8b	D4	9.03	7.84	110.7	14.7	14.8	55	21	15.2	375	326	12.06	106.7
279	LG 1-8b	D4	9.11	7.9	110.3	14.9	15.1	54	48	15.3	367	318	12.91	114.2
280	LG 1-8b	D4	12.56	10.95	114.5	15.2	15.1	53	31	14.7	478	417	15.49	137.1
281	LG 1-8b	D4	13.01	11.34	114.4	15.3	15	52	0	14.7	496	432	18.87	167.0
282	LG 1-8b	D4	12.45	10.85	114.6	15.6	14.4	50	15	14.7	484	421	23.00	203.6
283	LG 1-8b	D4	12.28	10.69	114.6	15	14.8	52	32	14.9	483	420	18.47	163.4
284	LG 1-8b	D4	12.79	11.11	114.2	15.2	15.2	53	40	15.1	485	421	15.04	133.1
285	LG 1-8b	D4	12.72	11.08	114.4	14.4	14.9	51	14	14.8	518	451	22.76	201.4

							Enter File Name ->				Inch-lbf	Joules	
	Toughness Test						Enter Weight Position ->	5	Machine Constant =		716	80.90	
151, 5040 / 16 wks							Enter Initial Angle ->	60					
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
283	LG 9-16b	Control 151	11.94	10.33	114.4	15.2	15	52	30	15.6	458	396	17.93	158.7
284	LG 9-16b	Control 151	11.72	10.15	114.3	15	15	54	7	15.5	456	395	14.49	128.3
285	LG 9-16b	Control 151	11.12	9.61	114.4	15.2	14.5	53	46	15.7	441	381	15.53	137.4
286	LG 9-16b	Control 151	11.37	9.82	114.4	14.9	14.7	53	30	15.8	454	392	16.45	145.6
287	LG 9-16b	Control 151	10.94	9.46	114.2	15	14.6	54	24	15.6	437	378	14.19	125.6
288	LG 9-16b	Control 151	10.72	9.28	114.4	15	14.9	55	48	15.5	419	363	10.51	93.1
289	LG 9-16b	Control 151	10.8	9.35	114.3	15.1	14.8	54	23	15.5	423	366	13.90	123.0
290	LG 9-16b	Control 151	10.56	9.13	114.2	14.9	15	54	46	15.7	414	358	13.07	115.7
291	LG/P 9-16b	Control 151	10.23	8.86	114.3	15.2	14.9	55	19	15.5	395	342	11.46	101.4
292	LG/P 9-16b	Control 151	10.04	8.68	114	14.7	14.4	55	45	15.7	416	360	11.35	100.5
293	LG/P 9-16b	Control 151	10.54	9.12	114.4	14.8	14.3	55	13	15.6	435	377	12.70	112.4
294	LG/P 9-16b	Control 151	10.31	8.92	114.2	15.1	14.2	55	0	15.6	421	364	12.94	114.5
295	LG/P 9-16b	Control 151	10.09	8.73	114.4	15	14.3							
296	LG/P 9-16b	Control 151	8.99	7.79	114.3	15.2	13.4	55	30	15.4	386	335	12.25	108.4
297	LG/P 9-16b	Control 151	9.23	7.99	114.3	15	13.7	55	30	15.5	393	340	12.23	108.3
298	LG/P 9-16b	Control 151	9.91	8.61	114.4	14.9	12.5	55	18	15.1	465	404	14.13	125.1
299	LG/P 25-32b	Control 151	12.19	10.54	114.3	15.1	14.7	54	37	15.7	480	415	13.43	118.9
300	LG/P 25-32b	Control 151	11.99	10.36	114.3	15.2	14.7	51	15	15.7	469	406	21.18	187.4
301	LG/P 25-32b	Control 151	12.38	10.72	114.4	15	14.9	50	33	15.5	484	419	22.93	203.0
302	LG/P 25-32b	Control 151	12.73	11.02	114.4	15.1	15	43	39	15.5	491	425	37.23	329.5
303	LG/P 25-32b	Control 151	11.97	10.36	114.4	15	14.9	49	48	15.5	468	405	24.63	218.0
304	LG/P 25-32b	Control 151	12.01	10.4	114.3	15.1	14.9	46	50	15.5	467	404	30.86	273.2
305	LG/P 25-32b	Control 151	11.91	10.32	114.3	15.1	14.5	48	50	15.4	476	412	27.26	241.3
306	LG/P 25-32b	Control 151	11.68	10.12	114.3	15	14.3	49	22	15.4	476	413	26.68	236.2
307	LG 65-72b	Control 151	11.27	9.74	114	15.2	15.2	53	45	15.7	428	370	14.85	131.4
308	LG 65-72b	Control 151	12.01	10.37	114.2	15.2	14.8	50	41	15.8	467	404	22.32	197.5
309	LG 65-72b	Control 151	11.57	10.02	114.6	14.8	15.1	47	55	15.5	452	391	29.04	257.0
310	LG 65-72b	Control 151	10	8.65	114	14.7	15.2	52	10	15.6	393	340	19.42	171.9

311	LG 65-72b	Control 151	10.38	8.98	114.4	15.2	14.8	52	40	15.6	403	349	17.78	157.4
312	LG 65-72b	Control 151	11.41	9.87	114.5	15.3	15.3	45	55	15.6	426	368	31.31	277.1
313	LG 65-72b	Control 151	9.65	8.37	113.9	15.1	15.1	55	33	15.3	372	322	10.87	96.2
314	LG 65-72b	Control 151	10.02	8.69	114.3	14.9	15.4	46	45	15.3	382	331	30.66	271.4
315	LG/P 33-40a	Control 5040	11.07	9.61	114.3	14.8	13.6	52	0	15.2	481	418	21.91	193.9
316	LG/P 33-40a	Control 5040	11.41	9.91	114.2	15.1	14.7	53	15	15.1	450	391	16.71	147.9
317	LG/P 33-40a	Control 5040	11.5	9.98	114.4	15.3	14.8							
318	LG/P 33-40a	Control 5040	11.45	9.94	114.4	14.9	15.2	53	0	15.2	442	384	17.08	151.2
319	LG/P 33-40a	Control 5040	11.8	10.22	114.2	15.2	14.6							
320	LG/P 33-40a	Control 5040	11.84	10.27	114.3	15	14.3							
321	LG/P 33-40a	Control 5040	12.03	10.43	114.4	15.2	14.7	49	38	15.3	471	408	24.84	219.8
322	LG/P 33-40a	Control 5040	12.31	10.67	114.2	15.1	15.1	47	53	15.4	473	410	28.23	249.8
323	DG/P 1-8a	Control 5040	13.59	11.77	114.3	14.5	14.4	51	35	15.5	569	493	22.41	198.4
324	DG/P 1-8a	Control 5040	13.84	12	114.4	14.1	14.6	52	8	15.3	588	510	21.65	191.6
325	DG/P 1-8a	Control 5040	14.14	12.26	114.5	14.4	15	45	19	15.3	572	496	36.41	322.2
326	DG/P 1-8a	Control 5040	14.15	12.27	114.4	14.7	14.7	44	40	15.3	572	496	37.41	331.1
327	DG/P 1-8a	Control 5040	13.68	11.85	114.3	14.7	14.7	49	30	15.4	554	480	26.47	234.3
328	DG/P 1-8a	Control 5040	13.8	11.95	114.4	14.9	14.5	49	32	15.5	558	483	26.20	231.9
329	DG/P 1-8a	Control 5040	14.05	12.17	114.5	15.1	14.8	46	13	15.4	549	476	32.39	286.7
330	DG/P 1-8a	Control 5040	13.84	12	114.4	14.9	14.7	47	52	15.3	552	479	29.63	262.3
331	LG/P 1-8a	Control 5040	10.31	8.94	114.3	15	14	54	36	15.3	430	372	14.29	126.5
332	LG/P 1-8a	Control 5040	10.77	9.33	114.1	14.8	14.6			15.4	437	378	88.23	780.9
333	LG/P 1-8a	Control 5040	11.46	9.93	114.1	14.8	15	44	30	15.4	452	392	36.63	324.2
334	LG/P 1-8a	Control 5040	11.62	10.07	114.1	15.1	15	50	31	15.4	450	390	22.62	200.2
335	LG/P 1-8a	Control 5040	10.96	9.49	114.2	14.6	15.9	40	10	15.5	413	358	43.71	386.9
336	LG/P 1-8a	Control 5040	10.64	9.19	113.9	14.7	16							
337	LG/P 1-8a	Control 5040	11.77	10.18	114	15	16.3							
338	LG/P 1-8a	Control 5040	11.83	10.24	113.9	15.1	16.5							
339	DG/P 9-15a	Control 5040	14.25	12.34	114.4	15.1	15.1							
340	DG/P 9-15a	Control 5040	16.02	13.86	114.3	14.9	15	54	23	15.6	627	543	14.00	123.9
341	DG/P 9-15a	Control 5040	16.26	14.06	113.5	14.8	15							
342	DG/P 9-15a	Control 5040	16.35	14.13	114.6	14.9	14.7							
343	DG/P 9-15a	Control 5040	16.62	14.39	114.3	14.8	14.8	47	38	15.5	664	575	30.27	267.9
344	DG/P 9-15a	Control 5040	14.91	12.93	114.5	14.6	14.6	51	27	15.3	611	530	22.20	196.5
345	DG/P 9-15a	Control 5040	15.33	13.3	114.5	14.9	14.7	46	13	15.3	611	530	33.29	294.6
346	DG/P 9-15a	Control 5040	15.4	13.37	114.4	14.7	14.9	47	7	15.2	615	534	31.54	279.2

						Enter File Name ->						Inch-lbf	Joules		
	Toughness Test					Enter Weight Position ->		5	Machine Constant =			716	80.90		
151, 5040 / 16 wks						Enter Initial Angle ->		60							
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness	
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)	
1	LG/P 25-32b	151	12.02	10.52	113.9	15.2	15.1	52	12	14.3	460	402	18.49	163.6	
2	LG/P 25-32b	151	11.16	9.76	113.7	15.3	14.4	54	44	14.3	446	390	13.15	116.4	
3	LG/P 25-32b	151	12.02	10.5	113.7	15.3	14.8	52	55	14.5	467	408	17.03	150.7	
4	LG/P 25-32b	151	12.28	10.74	113.6	15.2	14.6	53	23	14.3	487	426	16.33	144.6	
5	LG/P 25-32b	151	12.19	10.66	114.3	15.2	14.9	52	32	14.4	471	412	17.97	159.0	
6	LG/P 25-32b	151	12.02	10.53	113.9	15.2	14.6	52	52	14.2	476	417	17.56	155.4	
7	LG/P 25-32b	151	11.75	10.27	113.9	15.2	14.3	55	40	14.4	475	415	11.07	97.9	
8	LG/P 25-32b	151	12	10.51	113.4	15.2	14.8	53	15	14.2	470	412	16.43	145.4	
9	LG/P 9-16a	151	9.01	7.89	113	14.9	14	55	55	14.2	382	335	11.00	97.4	
10	LG/P 9-16a	151	9.16	8.02	113	15.2	14.3	56	0	14.2	373	327	10.23	90.6	
11	LG/P 9-16a	151	10.62	9.31	113.8	14.9	14.5	55	16	14.1	432	379	12.27	108.6	
12	LG/P 9-16a	151	10.32	9.06	113.5	15.1	14.6	55	30	13.9	412	362	11.36	100.5	
13	LG/P 9-16a	151	10.37	9.12	113.5	15.1	15.2	55	30	13.7	398	350	10.91	96.6	
14	LG/P 9-16a	151	9.57	8.47	113.7	15	13.7	55	39	13.0	410	363	11.83	104.7	
15	LG/P 9-16a	151	9.49	8.4	113.8	15	13.9	55	41	13.0	400	354	11.58	102.5	
16	LG/P 9-16a	151	10.49	9.25	113.9	15	13.3	55	20	13.4	462	407	13.05	115.5	
17	LG 65-72a	151	10.79	9.44	113.2	15.2	14.7	54	7	14.3	427	373	14.49	128.2	
18	LG 65-72a	151	10.67	9.36	113.9	15.1	14.9	54	31	14.0	416	365	13.49	119.4	
19	LG 65-72a	151	9.67	8.48	113.4	14.9	14.9	55	15	14.0	384	337	11.98	106.0	
20	LG 65-72a	151	10.22	8.96	113.8	15.4	15	54	42	14.1	389	341	12.58	111.3	
21	LG 65-72a	151	10.44	9.13	113.6	15.2	15.3	52	57	14.3	395	346	16.56	146.6	
22	LG 65-72a	151	9.36	8.19	112.9	14.8	14.6	56	7	14.3	384	336	10.15	89.8	
23	LG 65-72a	151	9.19	8.06	112.9	14.7	14.3	55	42	14.0	387	340	11.57	102.4	
24	LG 65-72a	151	10.96	9.61	113.7	15.3	15.1	54	12	14.0	417	366	13.77	121.9	
25	LG 9-16a	151	11.62	10.21	113.3	15.3	15	52	33	13.8	447	393	17.63	156.1	
26	LG 9-16a	151	11.36	9.99	113.8	15.2	14.8	54	0	13.7	444	390	14.66	129.8	
27	LG 9-16a	151	11.52	10.13	114	15.2	15	49	40	13.7	443	390	24.27	214.8	
28	LG 9-16a	151	11.15	9.81	114.1	15	15	51	7	13.7	434	382	21.49	190.2	
29	LG 9-16a	151	10.9	9.57	114.2	15.1	14.8	53	54	13.9	427	375	15.05	133.2	

30	LG 9-16a	151	10.47	9.2	114.4	15.1	15	49	10	13.8	404	355	25.62	226.8	
31	LG 9-16a	151	10.21	8.97	114.4	15.1	14.7	49	33	13.8	402	353	25.28	223.7	
32	LG 9-16a	151	10.03	8.78	114.3	14.7	14.8	53	30	14.2	403	353	16.68	147.6	
33	LG/P 33-40b	5040	9.87	8.63	114.6	15	12.9	54	6	14.4	445	389	16.90	149.5	
34	LG/P 33-40b	5040	11.16	9.76	114.4	15.1	14.9	38	23	14.3	434	379	47.59	421.2	
35	LG/P 33-40b	5040	11.23	9.83	114.5	15.3	14.8	50	50	14.2	433	379	21.76	192.6	
36	LG/P 33-40b	5040	11.02	9.66	114.6	15.2	14.8	46	37	14.1	427	375	31.22	276.3	
37	LG/P 33-40b	5040	12.13	10.62	114.3	15.2	15.1	42	57	14.2	462	405	37.98	336.1	
38	LG/P 33-40b	5040	11.55	10.12	114.4	15.1	14.3	39	19	14.1	468	410	47.80	423.0	
39	LG/P 33-40b	5040	11.94	10.46	114.5	15.2	14.9	52	6	14.1	460	403	18.96	167.8	
40	LG/P 33-40b	5040	11.8	10.34	114.4	15.1	14.9	52	26	14.1	458	402	18.39	162.7	
41	DG/P 1-8b	5040	13.53	11.86	113.8	15	14.6	50	0	14.1	543	476	24.68	218.4	
42	DG/P 1-8b	5040	13.62	11.91	114.4	14.9	14.9	50	8	14.4	536	469	24.13	213.6	
43	DG/P 1-8b	5040	13.45	11.78	114.2	15.1	14.8	52	33	14.2	527	462	18.24	161.4	
44	DG/P 1-8b	5040	13.77	12.05	114.5	15.2	14.7	51	7	14.3	538	471	21.48	190.1	
45	DG/P 1-8b	5040	13.43	11.75	114.1	14.7	14.9	50	38	14.3	537	470	23.46	207.7	
46	DG/P 1-8b	5040	13.19	11.54	114.6	15	14.3	52	5	14.3	537	469	20.21	178.9	
47	DG/P 1-8b	5040	13.4	11.71	114.5	14.7	14.8	48	30	14.4	538	470	28.61	253.2	
48	DG/P 1-8b	5040	13.76	12.01	114	15.1	14.7	50	32	14.6	544	475	23.04	204.0	
49	LG/P 1-8b	5040	10.91	9.49	114	14.9	14.8	52	33	15.0	434	377	18.62	164.8	
50	LG/P 1-8b	5040	11.28	9.87	114.4	14.9	14.9	50	27	14.3	444	389	23.40	207.1	
51	LG/P 1-8b	5040	10.86	9.51	114.2	15.1	14.9	53	50	14.2	423	370	15.11	133.7	
52	LG/P 1-8b	5040	10.67	9.35	114.3	15.2	14.3	45	25	14.1	429	376	34.91	309.0	
53	LG/P 1-8b	5040	10.47	9.19	114.2	14.8	15.4	50	18	13.9	402	353	23.22	205.5	
54	LG/P 1-8b	5040	9.94	8.7	113.8	15.2	15								
55	LG/P 1-8b	5040	10.35	9.08	114.2	15.2	15.3	53	55	14.0	390	342	14.38	127.2	
56	LG/P 1-8b	5040	10.51	9.21	114.1	15.2	15.5	49	19	14.1	391	343	24.23	214.4	

							Enter File Name ->				Inch-lbf	Joules	
	Toughness Test						Enter Weight Position ->	5	Machine Constant =		716	80.90	
	16 wks						Enter Initial Angle ->	60					
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
1	73-80b	Control #1852	8.95	7.8	114.2	15	14.5	54	30	14.7	360	314	14.04	124.3
2	73-80b	Control #1852	9.29	8.12	114.3	15.2	14.7	54	52	14.4	364	318	12.69	112.4
3	73-80b	Control #1852	8.99	7.82	114.1	15	14.9	55	21	15.0	353	307	11.61	102.8
4	73-80b	Control #1852	9.87	8.64	114.3	15	15	55	47	14.2	384	336	10.48	92.8
5	73-80b	Control #1852	9.56	8.31	114	15.2	14.9	53	30	15.0	370	322	15.73	139.3
6	73-80b	Control #1852	9.65	8.35	114.2	15.5	15	54	15	15.6	363	314	13.47	119.2
7	73-80b	Control #1852	10.1	8.77	114.5	15.3	14.5	54	56	14.7	396	345	12.58	111.3
8	73-80b	Control #1852	9.65	8.41	114.4	15	14.8	54	25	14.7	380	331	13.96	123.6
9	57-64b	Control #1852	10	8.71	114.4	15.1	15.1	54	0	15.3	385	334	14.52	128.5
10	57-64b	Control #1852	10.1	8.82	114.4	14.9	14.9	54	22	14.5	398	347	14.13	125.1
11	57-64b	Control #1852	9.89	8.61	114.4	15.2	14.7	53	18	14.9	387	337	16.42	145.3
12	57-64b	Control #1852	9.54	8.29	114.4	14.7	15.2	54	40	15.1	373	324	13.42	118.7
13	57-64b	Control #1852	9.61	8.38	114.5	15.2	14.3	54	0	14.7	386	337	15.18	134.3
14	57-64b	Control #1852	9.52	8.28	114.3	14.9	14.3	55	0	15.0	391	340	13.12	116.1
15	57-64b	Control #1852	10.1	8.79	114.3	15.1	14.3	53	21	14.6	408	356	16.93	149.8
16	57-64b	Control #1852	9.96	8.7	114.3	15.2	14.4	53	43	14.5	398	348	15.76	139.5
17	25-32b	Control #1852	9.39	8.19	114.4	15.2	14.6	54	48	14.7	370	323	12.94	114.6
18	25-32b	Control #1852	9.52	8.3	114.4	15.3	14.7	51	30	14.7	370	323	20.40	180.5
19	25-32b	Control #1852	9.38	8.19	114.3	15	14.6	53	37	14.5	375	327	16.11	142.5
20	25-32b	Control #1852	9.18	7.98	114.4	14.3	14.7	53	24	15.0	382	332	17.78	157.4
21	25-32b	Control #1852	9.47	8.24	114.3	14.5	14.8	52	30	14.9	386	336	19.54	173.0
22	25-32b	Control #1852	9.45	8.27	114.3	14.8	14.6	53	26	14.3	383	335	16.90	149.6
23	25-32b	Control #1852	9.17	8.02	114.4	14.6	14.5	54	7	14.3	379	331	15.63	138.3
24	25-32b	Control #1852	9.49	8.26	114.2	15.2	14.7	54	7	14.9	372	324	14.49	128.2
25	DG 121-128b	Control #1852	14.1	12.3	114.5	15.1	14.8	48	45	14.6	552	481	26.89	238.0
26	DG 121-128b	Control #1852	14.1	12.3	114.4	14.8	14.6	49	0	14.3	570	498	27.54	243.7
27	DG 121-128b	Control #1852	14.3	12.5	114.5	14.9	14.6	50	7	14.6	575	501	24.66	218.3
28	DG 121-128b	Control #1852	16.1	14	114.5	14.8	14.6	46	37	14.4	649	567	32.98	291.9
29	DG 121-128b	Control #1852	15.7	13.7	114.6	14.6	14.6	45	20	14.4	644	562	36.58	323.8

30	DG 121-128b	Control #1852	15.5	13.5	114.4	14.9	14.6	45	15	14.3	622	544	35.63	315.3
31	DG 121-128b	Control #1852	15.3	13.4	114.5	14.7	14.8	46	52	14.3	616	539	32.31	286.0
32	DG 121-128b	Control #1852	14.1	12.3	114.5	14.8	14.9	50	45	14.5	558	487	22.95	203.1
33	DG 105-112b	Control #308	16.2	14.2	114.5	14.8	14.5	44	5	14.3	658	576	38.79	343.3
34	DG 105-112b	Control #308	15	13.1	114.5	15	14.2	46	17	14.4	614	537	33.96	300.6
35	DG 105-112b	Control #308	16	14	114.6	15	14.9	45	38	14.6	624	545	33.74	298.7
36	DG 105-112b	Control #308	15.6	13.7	114.6	14.8	14.8	47	10	14.4	623	544	31.31	277.1
37	DG 105-112b	Control #308	16.5	14.5	114.3	14.3	14.7	45	35	14.1	686	601	36.94	326.9
38	DG 105-112b	Control #308	16.9	14.8	114.5	14.4	14.9	43	22	14.5	688	601	40.94	362.4
39	DG 105-112b	Control #308	15.3	13.3	114.6	14.6	14.8	43	55	14.7	616	537	39.17	346.7
40	DG 105-112b	Control #308	15.9	13.9	114.6	14.7	14.4	44	37	14.2	656	574	38.30	339.0
41	17-24b	Control #308	9.17	7.98	114.3	14.8	15.1	54	0	14.9	359	312	14.98	132.6
42	17-24b	Control #308	11.5	10	114	15.5	15.3	52	30	14.8	425	370	17.05	150.9
43	17-24b	Control #308	11.2	9.73	113.9	14.9	15	54	20	15.0	440	382	14.12	125.0
44	17-24b	Control #308	11.3	9.82	114.1	14.7	14.9	54	35	15.1	452	393	13.89	123.0
45	17-24b	Control #308	11.4	9.93	113.9	14.7	15.1	53	35	15.0	452	393	16.15	142.9
46	17-24b	Control #308	12	10.4	113.8	15.2	15.2	51	45	15.1	455	395	19.37	171.4
47	17-24b	Control #308	10.4	9.12	113.9	15.1	15.2	53	7	14.5	399	349	16.46	145.7
48	17-24b	Control #308	11.6	10.1	113.8	15.2	15	55	23	15.1	447	388	11.22	99.3
49	65-72b	Control #308	9.72	8.49	114.3	15.5	15	53	36	14.5	366	319	14.94	132.2
50	65-72b	Control #308	9.83	8.57	114.3	14.9	15.2	53	45	14.7	380	331	15.32	135.6
51	65-72b	Control #308	10.5	9.16	114.5	15.3	15.1	50	55	14.4	396	346	21.14	187.1
52	65-72b	Control #308	10.3	8.95	114.6	15.2	14.9	53	30	15.0	396	345	15.73	139.3
53	65-72b	Control #308	9.24	8.11	114.4	15.1	14.4	53	35	13.9	371	326	16.24	143.8
54	65-72b	Control #308	8.88	7.78	114.3	14.9	14.6	53	35	14.1	357	313	16.36	144.8
55	65-72b	Control #308	9.19	8.02	114.5	15.2	14.9	52	40	14.6	354	309	17.66	156.3
56	65-72b	Control #308	9.78	8.55	114.3	15.4	14.8	51	18	14.4	375	328	20.50	181.5
57	1-8b	Control #308	10.2	8.88	114.1	15.3	14.9	51	55	14.9	392	341	19.19	169.8
58	1-8b	Control #308	9.43	8.2	114	15.1	14.5	54	21	15.0	378	329	14.27	126.3
59	1-8b	Control #308	10.2	8.83	114	15.3	14.9	52	9	15.1	391	340	18.66	165.1
60	1-8b	Control #308	10.5	9.12	114.1	15.3	15	53	52	15.4	402	348	14.63	129.5
61	1-8b	Control #308	10	8.69	114	15.2	14.9	53	8	15.3	388	337	16.59	146.8
62	1-8b	Control #308	9.93	8.61	114	15.3	14.6	53	50	15.3	390	338	15.11	133.7
63	1-8b	Control #308	10.5	9.12	113.9	15.3	14.8	51	5	14.9	406	354	21.20	187.6
64	1-8b	Control #308	10.4	9	113.9	15.3	14.8	53	35	15.0	401	349	15.49	137.1
65	DG 137-144b	Control #272	15.9	13.9	114.5	14.6	14.7	42	50	14.4	646	565	41.76	369.6

66	DG 137-144b	Control #272	15.7	13.7	114.5	14.6	14.5	44	20	14.5	649	566	39.06	345.7
67	DG 137-144b	Control #272	16	14	114.4	14.8	14.7	43	30	14.6	643	561	39.50	349.6
68	DG 137-144b	Control #272	16.8	14.7	114.5	14.8	14.8	40	53	14.4	669	585	44.57	394.5
69	DG 137-144b	Control #272	17.2	15	114.5	15	15	42	34	14.5	666	582	39.78	352.1
70	DG 137-144b	Control #272	17	14.9	114.5	15	15.1	42	10	14.5	656	573	40.31	356.8
71	DG 137-144b	Control #272	16.7	14.6	114.5	14.8	14.6	42	11	14.2	675	591	42.53	376.4
72	DG 137-144b	Control #272	15.6	13.6	114.4	14.9	14.6	46	0	14.4	626	547	33.99	300.9
73	129-136b	Control #272	9.53	8.31	113.9	15.2	14.8	50	45	14.7	372	324	22.17	196.2
74	129-136b	Control #272	9.26	8.09	114.6	15	14.5	50	45	14.5	372	325	23.09	204.4
75	129-136b	Control #272	9.63	8.42	114.6	15.3	14.7	51	22	14.4	374	327	20.70	183.2
76	129-136b	Control #272	9.65	8.42	114.6	15.2	14.7	51	30	14.6	377	329	20.61	182.4
77	129-136b	Control #272	9.85	8.58	114	15	15	52	51	14.8	384	335	17.48	154.7
78	129-136b	Control #272	10.3	8.9	114	14.8	14.7	51	20	15.2	413	359	21.87	193.6
79	129-136b	Control #272	9.97	8.66	113.9	15.2	15	51	40	15.1	384	333	19.82	175.4
80	129-136b	Control #272	11.1	9.61	113.8	14.8	15.2	50	42	15.4	433	375	22.61	200.1
81	33-40b	Control #272	9.7	8.47	114.4	14.9	14.5	50	30	14.5	392	343	23.93	211.8
82	33-40b	Control #272	9.88	8.63	114.5	14.9	14.8	51	32	14.5	391	342	21.03	186.1
83	33-40b	Control #272	9.78	8.55	114.5	15.1	14.6	52	18	14.4	387	339	19.08	168.9
84	33-40b	Control #272	9.82	8.58	114.6	15.2	14.5	51	10	14.5	389	340	21.66	191.7
85	33-40b	Control #272	9.94	8.68	114.6	14.8	15.1	50	20	14.5	388	339	23.60	208.9
86	33-40b	Control #272	9.29	8.11	114.5	14.9	14.2	51	48	14.5	383	335	21.26	188.2
87	33-40b	Control #272	9.67	8.45	114.6	15.1	14.3	51	12	14.4	391	341	22.11	195.7
88	33-40b	Control #272	9.78	8.56	114.6	14.9	14.7	50	0	14.3	390	341	24.77	219.2
89	49-56b	Control #272	9.19	8.02	114.4	14.8	14.6	51	36	14.6	372	324	21.38	189.2
90	49-56b	Control #272	8.54	7.46	114.5	14.2	14.7	51	35	14.5	357	312	22.68	200.7
91	49-56b	Control #272	8.85	7.7	114.4	14.8	14.3	51	0	14.9	366	318	23.30	206.2
92	49-56b	Control #272	9.74	8.52	114.4	15.2	14.8	51	20	14.3	378	331	20.85	184.5
93	49-56b	Control #272	9.27	8.12	114.4	14.8	14.5	51	10	14.2	378	331	22.58	199.8
94	49-56b	Control #272	9.33	8.18	114.3	15.3	14.8	51	25	14.1	360	316	20.45	181.0
95	49-56b	Control #272	9.73	8.53	114.4	14.7	14.6	50	8	14.1	396	347	25.14	222.5
96	49-56b	Control #272	9.99	8.75	114.5	14.9	14.9	51	30	14.2	393	344	20.96	185.6
97	153-160b	Control Schizo	9.21	8.09	114.7	14.9	14.7	50	24	13.8	367	322	23.84	211.0
98	153-160b	Control Schizo	8.9	7.8	114.5	14.9	14.2	50	37	14.1	367	322	24.15	213.8
99	153-160b	Control Schizo	8.99	7.87	114.7	14.5	14.8	51	30	14.2	365	320	22.01	194.8
100	153-160b	Control Schizo	8.68	7.62	114.5	14.6	14.8	51	5	13.9	351	308	22.79	201.7
101	153-160b	Control Schizo	9.34	8.15	114.5	15	14.6	50	22	14.6	372	325	23.83	210.9

102	153-160b	Control Schizo	9.63	8.42	114.3	15	14.7	51	7	14.4	382	334	21.93	194.1
103	153-160b	Control Schizo	9.72	8.51	114.5	14.8	14.8	51	45	14.2	388	339	20.73	183.5
104	153-160b	Control Schizo	9.58	8.4	114.5	14.7	14.9	51	18	14.0	382	335	21.88	193.7
105	DG 153-160b	Control Schizo	13.6	11.9	114.5	14.8	14.8	43	36	14.2	540	473	39.02	345.4
106	DG 153-160b	Control Schizo	14.2	12.4	114.3	14.9	14.9	42	45	14.3	560	490	40.10	354.9
107	DG 153-160b	Control Schizo	15.8	13.8	114.4	14.6	14.8	46	0	14.1	638	559	34.61	306.3
108	DG 153-160b	Control Schizo	14.5	12.7	114.5	14.8	14.5	49	40	14.0	588	516	26.16	231.5
109	DG 153-160b	Control Schizo	13.7	12	114.3	15.2	14.4	43	7	14.2	548	480	39.48	349.4
110	DG 153-160b	Control Schizo	16	14	114.3	15.1	14.7	44	35	14.0	631	553	36.06	319.2
111	DG 153-160b	Control Schizo	16	14	114.4	14.9	14.5	43	48	13.9	647	568	38.99	345.1
112	DG 129-136b	Control Schizo	15.7	13.8	114.5	14.9	14.7	43	32	14.0	625	548	39.02	345.4
113	DG 129-136b	Control Schizo	17	14.9	114.6	14.8	15.1	39	5	14.0	663	582	47.13	417.1
114	DG 129-136b	Control Schizo	16.4	14.4	114.4	14.9	14.5	42	26	14.3	664	581	41.86	370.5
115	DG 129-136b	Control Schizo	16.6	14.6	114.6	14.8	14.6	40	47	14.2	672	588	45.38	401.7
116	DG 129-136b	Control Schizo	14.8	12.9	114.5	14.9	14.8	42	47	14.4	586	512	40.30	356.7
117	DG 129-136b	Control Schizo	15.3	13.4	114.5	14.8	14.7	44	6	14.3	615	538	38.23	338.4
118	DG 129-136b	Control Schizo	14.7	12.9	114.6	14.7	14.8	40	52	14.2	591	518	45.07	398.9
119	DG 129-136b	Control Schizo	16	14	114.5	14.8	14.6	42	17	14.3	646	565	42.32	374.6
120	DG 89-96b	Control Schizo	14.5	12.6	114.4	14.5	15.1	42	27	14.6	578	505	41.89	370.7
121	DG 89-96b	Control Schizo	14.4	12.6	114.5	14.7	14.5	43	48	14.6	591	516	39.82	352.4
122	DG 89-96b	Control Schizo	15	13.1	114.5	14.8	15.1	41	40	14.4	585	512	42.15	373.0
123	DG 89-96b	Control Schizo	14.6	12.8	114.4	14.4	14.6	41	35	14.6	608	531	45.65	404.1
124	DG 89-96b	Control Schizo	15.5	13.5	114.4	14.4	14.8	42	5	14.6	635	554	43.98	389.2
125	DG 89-96b	Control Schizo	15.9	13.8	114.5	15.1	14.7	47	47	14.8	624	544	29.21	258.6
126	DG 89-96b	Control Schizo	16.2	14.1	114.1	15	15.1	46	12	14.8	627	546	32.11	284.2
127	DG 89-96b	Control Schizo	16.3	14.2	114.5	14.8	14.8	43	6	14.7	650	566	40.07	354.6
128	9-16b	Control Gloeo	9.51	8.28	114	14.3	15	49	50	14.9	389	339	26.26	232.5
129	9-16b	Control Gloeo	10.6	9.21	114	15.4	14.9	49	44	15.0	405	352	23.80	210.6
130	9-16b	Control Gloeo	10	8.74	114	15.4	14.8	51	10	14.6	386	336	20.80	184.1
131	9-16b	Control Gloeo	10.2	8.88	114.1	15.1	14.8	50	16	14.4	398	348	23.49	207.9
132	9-16b	Control Gloeo	10.3	8.9	114.1	15.3	14.9	50	45	15.2	394	342	21.80	192.9
133	9-16b	Control Gloeo	10.2	8.88	113.9	15	15	51	17	14.9	398	347	21.11	186.8
134	9-16b	Control Gloeo	10.3	8.98	114	14.9	15	50	36	14.8	405	352	22.90	202.7
135	9-16b	Control Gloeo	10.2	8.9	113.9	15.2	15	50	0	14.9	394	343	23.53	208.3
136	DG 145-152b	Control Gloeo	15.3	13.4	114.3	15.1	15	43	0	14.6	592	516	38.52	341.0
137	DG 145-152b	Control Gloeo	14.7	12.8	114.3	14.3	14.2	48	36	14.7	632	551	30.86	273.2

138	DG 145-152b	Control Gloeo	14.8	12.9	114.3	14.4	14.7	41	30	14.7	611	533	45.52	402.9
139	DG 145-152b	Control Gloeo	16.2	14.2	114.5	15	14.7	39	27	14.3	642	562	46.72	413.5
140	DG 145-152b	Control Gloeo	16.6	14.5	114.5	14.9	14.7	39	8	14.2	662	580	47.82	423.2
141	DG 145-152b	Control Gloeo	16.2	14.2	114.5	15.1	14.9	41	30	14.5	629	549	41.73	369.4
142	DG 145-152b	Control Gloeo	14.7	12.8	113.9	13.9	14.7	45	30	14.7	630	550	38.80	343.4
143	LG 105-112b	Control Gloeo	10.4	9.06	114.2	15.3	15.8	49	30	14.3	375	328	23.15	204.9
144	LG 105-112b	Control Gloeo	10.1	8.82	114.3	14.8	15.6	50	8	14.3	382	334	23.29	206.1
145	LG 105-112b	Control Gloeo	10.6	9.31	114.4	14.8	15.7	51	45	14.1	400	350	19.54	173.0
146	LG 105-112b	Control Gloeo	9.73	8.53	114.5	15.2	15.8	49	48	14.1	354	310	22.76	201.4
147	LG 105-112b	Control Gloeo	10.3	9.01	114.5	15.2	15.5	50	30	14.0	381	334	21.71	192.1
148	LG 105-112b	Control Gloeo	10.1	8.85	114.5	15.2	15.2	50	30	14.1	382	335	22.13	195.9
149	LG 105-112b	Control Gloeo	10.7	9.31	114	15.2	15.5	50	55	14.4	397	347	20.81	184.2
150	LG 105-112b	Control Gloeo	10.1	8.87	114.1	15	15.2	50	12	14.3	390	341	23.26	205.9
151	41-48b	Control Gloeo	9.97	8.73	114.5	15.3	14.8	50	34	14.2	385	337	22.35	197.9
152	41-48b	Control Gloeo	10.6	9.24	114.5	15	14.8	49	10	14.3	415	364	26.23	232.2
153	41-48b	Control Gloeo	8.9	7.81	114.3	14.8	15.1	51	40	14.0	348	306	20.51	181.6
154	41-48b	Control Gloeo	10.2	8.98	114.5	14.9	14.8	50	30	14.0	406	356	23.44	207.5
155	41-48b	Control Gloeo	9.43	8.25	114.5	15.4	14.8	51	7	14.3	361	316	20.91	185.1
156	41-48b	Control Gloeo	9.85	8.61	114.3	15.4	14.9	51	12	14.4	376	328	20.59	182.2
157	41-48b	Control Gloeo	9.17	8.05	114.5	15.2	14.7	50	20	13.9	358	315	23.26	205.9
158	41-48b	Control Gloeo	10.1	8.88	114.2	15.2	14.8	50	48	14.1	394	346	22.06	195.2
159	49-56a	# 272	9.31	8.11	114.3	14.7	14.7	50	30	14.8	377	328	24.10	213.3
160	49-56a	# 272	9.09	7.95	114.7	14.7	14.7	50	52	14.3	367	321	23.22	205.5
161	49-56a	# 272	9.59	8.37	114.4	14.8	14.4	50	8	14.6	393	343	25.23	223.3
162	49-56a	# 272	9.49	8.27	114.4	15.1	14.5	50	51	14.8	379	330	22.63	200.3
163	49-56a	# 272	9.88	8.62	114.5	14.9	14.6	50	22	14.6	397	346	24.08	213.1
164	49-56a	# 272	9.63	8.41	114.5	15.3	14.7	50	3	14.5	374	327	23.66	209.4
165	49-56a	# 272	10.1	8.75	114.4	14.8	14.6	50	7	15.0	407	354	24.92	220.6
166	49-56a	# 272	9.1	7.94	114.5	15	14.7	50	55	14.6	360	314	22.39	198.2
167	33-40a	# 272	10.1	8.77	114.5	15.2	15	48	47	15.1	387	336	26.19	231.8
168	33-40a	# 272	9.35	8.14	114.6	14.6	15	50	51	14.9	373	324	23.04	203.9
169	33-40a	# 272	10.3	8.94	114.5	15.2	14.9	49	32	15.1	397	345	24.72	218.8
170	33-40a	# 272	9.65	8.4	114.5	15	14.5	50	0	14.9	387	337	24.85	219.9
171	33-40a	# 272	9.46	8.25	114.6	15.1	14.5	50	30	14.7	377	329	23.44	207.5
172	33-40a	# 272	9.53	8.32	114.4	15.4	14.3	50	17	14.5	378	330	23.55	208.4
173	33-40a	# 272	9.81	8.56	114.5	15.1	14.7	49	43	14.6	386	337	24.90	220.4

174	33-40a	# 272	9.54	8.35	114.5	15.3	14.8	50	20	14.3	368	322	22.87	202.4
175	DG 137-144a	# 272	14.6	12.8	114.5	14.9	14.8	44	35	14.6	579	505	36.56	323.6
176	DG 137-144a	# 272	15	13	114.6	14.8	15	43	5	14.6	588	513	39.57	350.2
177	DG 137-144a	# 272	15.4	13.4	114.5	14.8	14.9	40	37	14.6	610	532	44.80	396.5
178	DG 137-144a	# 272	15.2	13.2	114.6	14.9	15	41	23	14.7	591	516	42.55	376.6
179	DG 137-144a	# 272	15.5	13.5	114.5	14.2	14.5	43	42	14.7	657	573	42.23	373.8
180	DG 137-144a	# 272	16	13.9	114.7	14.6	14.5	42	54	14.7	658	574	42.20	373.5
181	DG 137-144a	# 272	16.7	14.5	114.5	14.8	14.8	40	36	14.7	664	579	45.13	399.5
182	DG 137-144a	# 272	15.8	13.8	114.3	14.6	14.6	42	38	14.7	648	565	42.48	375.9
183	129-136a	# 272	9.44	8.26	114.5	15.3	14.8	50	24	14.3	364	319	22.73	201.1
184	129-136a	# 272	9.5	8.29	114.5	15.1	14.2	49	5	14.6	387	338	27.26	241.2
185	129-136a	# 272	9.63	8.42	114.5	15.4	14.6	49	30	14.4	374	327	24.80	219.5
186	129-136a	# 272	9.69	8.45	114.3	15.4	14.8	48	44	14.7	372	324	26.12	231.2
187	129-136a	# 272	9.99	8.67	114.1	14.9	14.8	49	11	15.2	397	345	26.47	234.3
188	129-136a	# 272	9.76	8.46	114.2	14.9	15	49	30	15.4	382	331	25.40	224.8
189	129-136a	# 272	9.38	8.16	114.2	15.2	14.9	48	47	15.0	363	315	26.37	233.4
190	129-136a	# 272	8.95	7.81	114.2	15.2	14.9	50	12	14.6	346	302	23.25	205.8
191	DG 121-128a	# 1852	13.6	11.9	114.6	14.8	14.9	45	30	14.3	538	470	34.74	307.5
192	DG 121-128a	# 1852	15.8	13.8	114.5	14.8	14.9	40	23	14.2	625	547	45.25	400.5
193	DG 121-128a	# 1852	15.7	13.8	114.5	14.9	14.4	40	0	14.1	641	561	47.11	416.9
194	DG 121-128a	# 1852	15.8	13.9	114.9	14.6	14.6	40	54	14.1	646	566	46.11	408.1
195	DG 121-128a	# 1852	15.9	13.9	114.5	14.8	14.6	41	44	14.1	643	563	43.45	384.6
196	DG 121-128a	# 1852	13.7	12	114.5	15	14.9	47	22	14.1	534	468	30.03	265.8
197	DG 121-128a	# 1852	13.4	11.8	114.6	14.3	15	46	40	14.2	546	478	33.73	298.6
198	DG 121-128a	# 1852	13.9	12.1	114.5	14.8	15	46	39	14.3	545	477	32.02	283.4
199	25-32a	# 1852	9.51	8.32	114.6	15.2	14.6	50	8	14.3	374	327	23.88	211.3
200	25-32a	# 1852	9.57	8.38	114.6	15	14.7	49	16	14.2	379	332	26.19	231.8
201	25-32a	# 1852	8.99	7.89	114.5	15.1	14.6	49	52	13.9	356	313	24.73	218.9
202	25-32a	# 1852	9.23	8.1	114.6	15.1	14.5	50	5	14.0	368	323	24.40	216.0
203	25-32a	# 1852	9.33	8.19	114.5	15.2	14.5	48	26	13.9	370	325	27.88	246.7
204	25-32a	# 1852	9.19	8.06	114.6	15.2	14.4	54	37	14.0	366	321	13.57	120.1
205	25-32a	# 1852	9.14	8.01	114.6	15.1	14.5	54	47	14.1	364	319	13.21	116.9
206	25-32a	# 1852	9.39	8.24	114.5	15.2	14.4	54	50	14.0	375	329	13.04	115.4
207	73-80a	# 1852	9.43	8.26	114.5	15.2	14.9	55	31	14.2	364	319	10.98	97.2
208	73-80a	# 1852	9.42	8.25	114.5	15.4	15	54	18	14.2	356	312	13.49	119.4
209	73-80a	# 1852	9.2	8.05	114.5	14.8	14.8	55	47	14.3	367	321	10.85	96.0

210	73-80a	# 1852	9.59	8.38	114.5	15.1	14.5	55	22	14.4	383	334	11.77	104.2
211	73-80a	# 1852	9.74	8.5	114.3	15.3	15.2	55	51	14.6	366	320	9.88	87.4
212	73-80a	# 1852	9.1	7.94	114.2	15.5	15	55	48	14.6	343	299	9.93	87.9
213	73-80a	# 1852	9.82	8.63	114.6	15.3	14.8	55	0	13.8	378	333	12.17	107.7
214	73-80a	# 1852	9.43	8.26	114.5	15	14.7	55	4	14.2	374	327	12.47	110.3
215	57-64a	# 1852	9.66	8.47	114.5	14.9	15.1	54	23	14.0	375	329	13.91	123.1
216	57-64a	# 1852	9.63	8.42	114.5	15.1	15.1	54	50	14.4	369	323	12.56	111.2
217	57-64a	# 1852	10.1	8.81	114.4	15.2	15.3	54	12	14.2	378	331	13.73	121.5
218	57-64a	# 1852	9.25	8.12	114.7	14.9	14.5	55	25	13.9	373	328	11.89	105.2
219	57-64a	# 1852	9.43	8.3	114.6	15.3	14.1	55	0	13.6	381	336	12.77	113.0
220	57-64a	# 1852	9.54	8.38	114.7	15.2	14.8	55	23	13.8	370	325	11.37	100.7
221	57-64a	# 1852	9.71	8.53	114.6	15.2	15	55	44	13.8	372	326	10.39	92.0
222	57-64a	# 1852	9.54	8.39	114.5	15.3	14.3	56	3	13.7	381	335	10.01	88.6
223	DG 105-112a	# 308	16.2	14.2	114.7	14.9	14.5	46	45	13.9	652	572	32.56	288.2
224	DG 105-112a	# 308	16.4	14.4	114.7	14.6	14.8	44	53	14.0	661	580	37.07	328.1
225	DG 105-112a	# 308	14.3	12.5	114.5	15	14.7	49	7	14.5	567	495	26.53	234.8
226	DG 105-112a	# 308	16.4	14.3	114.5	14.7	14.6			14.4	666	582		0.0
227	DG 105-112a	# 308	16.5	14.4	114.6	14.9	14.7	48	0	14.6	659	575	29.34	259.6
228	DG 105-112a	# 308	16.6	14.5	115	14.7	14.9	45	23	14.7	659	575	35.36	312.9
229	DG 105-112a	# 308	16.5	14.4	114.5	14.9	15.1	48	48	14.6	641	559	26.79	237.2
230	DG 105-112a	# 308	16.4	14.3	114.8	15	14.3	44	54	14.5	665	581	36.76	325.4
231	65-72a	# 308	9.74	8.49	114.5	15.1	14.7	56	0	14.7	383	334	10.06	89.0
232	65-72a	# 308	9.9	8.63	114.5	15.3	14.8	55	30	14.7	382	333	10.98	97.2
233	65-72a	# 308	10.2	8.91	114.6	15.4	15.3	55	48	14.6	378	330	9.83	87.0
234	65-72a	# 308	10.3	8.94	114.6	15.4	15.3	54	15	14.7	380	331	13.34	118.1
235	65-72a	# 308	9.7	8.46	114.4	15	14.7	55	15	14.7	385	335	12.02	106.3
236	65-72a	# 308	8.96	7.81	114.6	15	14.8	55	5	14.7	352	307	12.34	109.2
237	65-72a	# 308	8.92	7.8	114.4	15.2	14.6	54	45	14.4	351	307	13.06	115.6
238	65-72a	# 308	9.34	8.13	114.5	15.2	14.7	55	34	14.9	365	318	11.01	97.4
239	17-24a	# 308	11.2	9.67	113.9	14.6	15.1	54	30	15.3	444	385	14.06	124.5
240	17-24a	# 308	10.8	9.36	113.8	15.6	15.2	54	7	15.0	399	347	13.46	119.1
241	17-24a	# 308	11.8	10.2	113.9	15.2	15	52	13	15.3	453	393	18.57	164.4
242	17-24a	# 308	9.55	8.32	113.9	15.2	15.3	53	48	14.8	361	314	14.64	129.6
243	17-24a	# 308	11.9	10.3	113.8	15.3	15.1	53	45	15.5	454	393	14.80	131.0
244	17-24a	# 308	12	10.4	113.8	15	15.3	54	43	15.1	459	399	12.80	113.3
245	17-24a	# 308	11.6	10.1	114	14.7	15	54	25	15.4	463	401	14.21	125.8

246	17-24a	# 308	11.3	9.83	113.5	15.4	15.4	54	0	15.0	420	365	13.81	122.2
247	1-8a	# 308	9.36	8.15	114.2	15.2	15.1	55	0	14.8	357	311	12.05	106.6
248	1-8a	# 308	9.96	8.65	114.3	15.4	14.7	55	6	15.1	385	334	11.89	105.2
249	1-8a	# 308	9.7	8.46	114.4	15.1	14.9	54	10	14.7	377	329	14.32	126.7
250	1-8a	# 308	9.71	8.45	114.3	15.1	15	53	38	14.9	375	326	15.48	137.0
251	1-8a	# 308	9.29	8.09	114.3	15	15	54	37	14.8	361	315	13.30	117.7
252	1-8a	# 308	10.4	9.02	114.1	15.3	15	55	30	14.9	396	344	10.83	95.9
253	1-8a	# 308	9.83	8.57	114.2	15.3	15	54	45	14.7	375	327	12.59	111.4
254	1-8a	# 308	10.1	8.81	114.3	15.3	14.8	55	45	14.8	391	340	10.39	91.9
255	DG 89-96a	Schizo	16	14	114.4	15.1	15.1	53	26	14.5	615	537	15.84	140.2
256	DG 89-96a	Schizo	15.2	13.3	114.4	15.1	14.4	53	10	14.5	611	533	17.26	152.7
257	DG 89-96a	Schizo	15.1	13.2	114	14.9	14.8	47	10	14.5	601	525	30.99	274.3
258	DG 89-96a	Schizo	13.5	11.9	114.6	14.6	14.7	50	54	14.2	550	482	23.39	207.0
259	DG 89-96a	Schizo	15.2	13.3	114.4	14.5	15	47	30	14.3	610	534	31.13	275.5
260	DG 89-96a	Schizo	13.8	12.1	114.6	14.7	15	47	53	14.3	547	478	29.62	262.1
261	DG 89-96a	Schizo	13.7	12	114.3	15	14.7	54	13	14.4	544	475	14.54	128.7
262	DG 89-96a	Schizo	14.1	12.4	114.3	14.9	14.8	46	10	14.2	560	490	33.17	293.6
263	DG 129-136a	Schizo	15.7	13.7	114.5	14.6	14.8	45	5	14.2	633	555	36.64	324.2
264	DG 129-136a	Schizo	14.8	13	114.8	14.5	14.4	45	47	14.3	619	542	36.45	322.6
265	DG 129-136a	Schizo	16.4	14.3	114.7	15	14.6	46	30	14.1	651	571	32.55	288.1
266	DG 129-136a	Schizo	15.5	13.6	114.9	14.4	14.7	44	15	14.3	639	559	39.55	350.0
267	DG 129-136a	Schizo	15.5	13.5	114.7	14.9	14.2	47	18	14.2	637	558	31.99	283.1
268	DG 129-136a	Schizo	15.7	13.8	114.6	14.8	15.4	46	40	14.2	601	526	31.16	275.8
269	DG 129-136a	Schizo	15.7	13.7	114.7	14.8	14.4	47	9	14.2	640	561	32.22	285.2
270	DG 129-136a	Schizo	16.4	14.4	114.7	14.9	14.6	46	32	14.0	658	577	32.82	290.5
271	DG 153-160a	Schizo	16.2	14.2	114.8	15	14.6	46	26	14.0	645	566	32.70	289.4
272	DG 153-160a	Schizo	16.1	14.1	114.6	15.1	14.8	49	25	14.1	629	552	25.41	224.9
273	DG 153-160a	Schizo	16.6	14.6	114.3	14.9	14.9	48	32	14.1	656	575	27.75	245.6
274	DG 153-160a	Schizo	13.5	11.8	114.5	15	15	52	47	14.2	525	460	17.64	156.1
275	DG 153-160a	Schizo	14.1	12.4	114.8	15.1	14.8	50	45	14.2	550	482	22.40	198.2
276	DG 153-160a	Schizo	13.6	11.9	114	14.6	14.5	51	5	14.1	564	494	23.26	205.9
277	DG 153-160a	Schizo	14.1	12.3	114.5	15.1	14.6	51	48	14.1	557	488	20.26	179.3
278	153-160a	Schizo	9.42	8.27	114.7	14.7	14.8	54	5	13.9	377	331	15.24	134.8
279	153-160a	Schizo	9.36	8.23	114.7	14.9	14.3	54	14	13.7	383	337	15.06	133.3
280	153-160a	Schizo	9.81	8.62	114.7	14.9	14.7	55	20	13.8	390	343	11.93	105.6
281	153-160a	Schizo	9.64	8.44	114.8	14.9	14.4	54	45	14.2	391	343	13.66	120.9

282	153-160a	Schizo	9.47	8.31	114.6	15.1	14.9	54	48	14.0	367	322	12.81	113.4
283	153-160a	Schizo	10	8.76	114.5	15.1	15	54	33	14.4	386	338	13.32	117.9
284	153-160a	Schizo	9.61	8.4	114.5	15.1	13.8	55	17	14.4	403	352	12.58	111.4
285	153-160a	Schizo	9.68	8.46	114.4	15	15	55	15	14.4	376	329	11.78	104.2
286	LG 105-112a	Gloeo	8.3	7.24	112.7	14.2	14.7	57	48	14.6	353	308	6.14	54.4
287	LG 105-112a	Gloeo	9.77	8.48	113.5	14.6	15	57	37	15.2	393	341	6.24	55.2
288	LG 105-112a	Gloeo	9.48	8.25	112.7	14.7	14.7	58	8	14.9	389	339	4.95	43.8
289	LG 105-112a	Gloeo	8.99	7.85	114.2	14.8	14.8	57	10	14.5	359	314	7.35	65.0
290	LG 105-112a	Gloeo	8.97	7.8	113.2	14.4	14.7	57	33	15.0	374	326	6.69	59.2
291	LG 105-112a	Gloeo	8.05	6.96	112.3	14.3	14.4	57	52	15.7	348	301	6.02	53.3
292	LG 105-112a	Gloeo	9.5	8.28	112.7	14.5	14.9	57	31	14.7	390	340	6.61	58.5
293	LG 105-112a	Gloeo	9.2	7.98	112	14.6	14.6	57	15	15.3	385	334	7.38	65.4
294	DG 145-152a	Gloeo	16.1	14	114.5	15.4	14.9	47	56	15.0	614	534	27.64	244.7
295	DG 145-152a	Gloeo	17.2	15	114.6	15	15.8	43	52	14.7	633	552	35.29	312.3
296	DG 145-152a	Gloeo	17.5	15.2	114.4	15.3	15.3	50	23	14.8	653	568	22.02	194.9
297	DG 145-152a	Gloeo	16.9	14.8	114.5	15.1	15.5	52	8	14.6	631	551	18.34	162.3
298	DG 145-152a	Gloeo	15.3	13.4	114.3	15.1	14.5	50	55	14.7	612	534	22.47	198.9
299	DG 145-152a	Gloeo	14.8	12.9	114.5	14.6	14.7	54	23	14.5	600	524	14.74	130.5
300	DG 145-152a	Gloeo	14.1	12.3	114.5	15	14.2	54	30	14.8	577	503	14.34	126.9
301	41-80a	Gloeo	8.34	7.25	113.8	14.6	14.8	57	48	15.0	339	295	5.84	51.7
302	41-80a	Gloeo	6.49	5.66	113.3	13.6	14.1	58	0	14.7	299	261	6.23	55.1
303	41-80a	Gloeo	8.24	7.17	112	15	14.4	57	37	14.9	341	296	6.24	55.2
304	41-80a	Gloeo	8.35	7.16	112.5	14.8	14.9	57	15	16.6	337	289	7.08	62.7
305	41-80a	Gloeo	6.44	5.2	113.5	14.8	14	58	5	23.8	274	221	5.28	46.7
306	41-80a	Gloeo	6.78	5.99	113.6	14.2	14.3			13.2	294	260		0.0
307	41-80a	Gloeo	7.75	6.81	112.8	14.5	14.4			13.8	329	289		0.0
308	41-80a	Gloeo	8.38	7.35	112.3	14.5	14.7			14.0	350	307		0.0
309	9-16a	Gloeo	9.33	8.14	113.2	15.2	14.7	57	45	14.6	369	322	5.65	50.0
310	9-16a	Gloeo	9.63	8.43	114.2	15.1	14.9	56	55	14.2	375	328	7.69	68.0
311	9-16a	Gloeo	9.75	8.53	114.2	15.1	15	57	0	14.3	377	330	7.43	65.8
312	9-16a	Gloeo	9.6	8.41	113.5	14.9	15.3	57	20	14.1	371	325	6.62	58.6
313	9-16a	Gloeo	9.37	8.19	113.9	15.1	14.7	56	54	14.4	371	324	7.83	69.3
314	9-16a	Gloeo	9.63	8.42	114.1	14.8	14.9	56	18	14.4	383	335	9.48	83.9
315	9-16a	Gloeo	9.36	8.17	113	15.1	14.6	57	23	14.6	376	328	6.67	59.1
316	9-16a	Gloeo	8.65	7.55	112.8	14.9	14.7	55	30	14.6	350	306	11.52	101.9

						Enter File Name ->						Inch-lbf	Joules	
	Toughness Test					Enter Weight Position ->		5	Machine Constant =			716	80.90	
						Enter Initial Angle ->		60						
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
1	36a	294 Control	10.37	8.99	115.6	13.6	14.9	54	13	15.4	443	384	16.69	147.7
2	36a	294 Control	10.54	9.14	115.4	14.7	14.6	54	55	15.3	426	369	13.33	118.0
3	36a	294 Control	11.1	9.62	115.4	14.5	14.9	54	25	15.4	445	386	14.61	129.3
4	36a	294 Control	11.01	9.56	114.8	14.3	15.1	53	40	15.2	444	386	16.64	147.3
5	36a	294 Control	10.72	9.32	115	13.8	15	55	17	15.0	450	392	13.30	117.7
6	36a	294 Control	11.01	9.56	115.1	14.1	14.6	55	52	15.2	465	403	11.62	102.9
7	36a	294 Control	10.18	8.83	113.7	14.3	14.3	54	31	15.3	438	380	15.29	135.3
8	36a	294 Control	10.3	8.93	115	14.5	14.6	52	37	15.3	423	367	19.51	172.7
9	88a	294 Control	12.76	11.08	115.1	14.5	14.2	51	24	15.2	538	468	23.20	205.3
10	88a	294 Control	13.15	11.43	114.8	14.1	14.9	48	30	15.0	545	474	30.30	268.2
11	88a	294 Control	13.08	11.36	115	14.2	14.1	50	16	15.1	568	493	27.12	240.0
12	88a	294 Control	13.15	11.41	114.8	14.4	14.5	49	57	15.2	549	476	26.59	235.4
13	88a	294 Control	13.2	11.48	114.9	14.3	14.7	51	4	15.0	547	475	23.73	210.1
14	88a	294 Control	13.14	11.43	114.3	14.5	14.4	49	5	15.0	551	479	28.62	253.3
15	88a	294 Control	12.99	11.3	115.4	14.3	14.5	49	44	15.0	543	472	27.42	242.7
16	88a	294 Control	13.12	11.41	115.2	14.4	14.2	48	18	15.0	557	484	31.27	276.8
17	93a	294 Control	11.67	10.15	114.8	14.4	14.6	52	0	15.0	484	421	21.29	188.5
18	93a	294 Control	11.89	10.35	115.2	14.2	14.9	52	3	14.9	488	425	21.19	187.6
19	93a	294 Control	13.89	12.04	114.7	14.5	14.8	48	26	15.4	564	489	29.37	260.0
20	93a	294 Control	14.06	12.22	115.1	14.3	14.6	46	3	15.1	585	509	36.11	319.6
21	93a	294 Control	14.95	12.99	115.1	14.3	14.7	45	44	15.1	618	537	36.59	323.9
22	93a	294 Control	13.49	11.72	114.5	14	14.4	48	30	15.1	584	508	31.70	280.6
23	93a	294 Control	14.32	12.42	115.1	14.2	14.7	49	32	15.3	596	517	27.84	246.4
24	93a	294 Control	13.57	11.79	115	14.2	14.7	49	7	15.1	565	491	28.87	255.5
25	86a	294 Control	13.36	0	114.8	14.4	14.4	49	48	#DIV/0!	561	0	27.15	240.3
26	86a	294 Control	12.18	0	114.7	13.8	14.9	54	30	#DIV/0!	516	0	15.55	137.6
27	86a	294 Control	13.01	0	115.4	13.8	14.9	51	32	#DIV/0!	548	0	23.51	208.1
28	86a	294 Control	12	0	115.1	14	14.7	53	50	#DIV/0!	507	0	17.21	152.4
29	86a	294 Control	12.79	0	115.1	13.9	14.3	52	0	#DIV/0!	559	0	22.96	203.2

30	86a	294 Control	12.12	0	114.7	14.3	14.5	53	30	#DIV/0!	510	0	17.77	157.2
31	86a	294 Control	13.36	0	115.7	14.2	14.8	49	2	#DIV/0!	549	0	28.88	255.6
32	86a	294 Control	12.78	0	115.4	14.2	14.7	53	31	#DIV/0!	531	0	17.67	156.4
33	71a	294 Control	10.74	9.33	115.3	14	14.7	56	0	15.1	453	393	11.30	100.1
34	71a	294 Control	11.03	9.57	115.4	14.3	14.6	54	22	15.3	458	397	15.37	136.0
35	71a	294 Control	11.72	10.17	115.5	14.4	14.7	54	57	15.2	479	416	13.58	120.2
36	71a	294 Control	10.59	9.18	115	14.3	15	55	50	15.4	429	372	11.16	98.8
37	71a	294 Control	11.84	10.31	114.5	14	14.3	55	4	14.8	517	450	14.26	126.2
38	71a	294 Control	11.28	9.77	114.5	14.3	15.2	55	32	15.5	453	393	11.78	104.3
39	71a	294 Control	10.45	9.1	115.4	13.9	14.4	56	3	14.8	452	394	11.53	102.0
40	71a	294 Control	10.41	9.05	115.5	14.5	14.8	55	16	15.0	420	365	12.53	110.9
41	24a	F40 Control	14.93	12.95	115.2	14.3	14.8	52	10	15.3	612	531	20.81	184.2
42	24a	F40 Control	14	12.18	115.3	14.4	14.6	47	27	14.9	578	502	32.44	287.1
43	24a	F40 Control	12.81	11.13	115.1	14.2	14.3	54	52	15.1	548	476	14.50	128.3
44	24a	F40 Control	13.35	11.61	115.2	14.4	14.5	53	25	15.0	555	483	17.79	157.5
45	24a	F40 Control	12.84	11.16	115.5	14.7	14.7	50	48	15.1	514	447	23.38	207.0
46	24a	F40 Control	13.33	11.61	114.5	14.3	14.6	53	0	14.8	558	486	18.95	167.7
47	24a	F40 Control	12.72	11.05	115.2	14.1	14.9	52	22	15.1	526	457	20.61	182.4
48	24a	F40 Control	13.4	11.66	115.4	14.2	14.8	53	30	14.9	553	481	17.60	155.7
49	84a	F40 Control	12.76	11.13	115.7	14	14.9	52	13	14.6	529	461	21.23	187.9
50	84a	F40 Control	12.69	11.07	115.3	14.4	14.7	52	16	14.6	520	454	20.48	181.2
51	84a	F40 Control	11.76	10.24	115.1	14.1	15	54	5	14.8	483	421	16.03	141.9
52	84a	F40 Control	11.57	10.08	114.7	14	15	54	5	14.8	480	418	16.21	143.5
53	84a	F40 Control	11.72	10.2	115.3	14.4	14.5	53	49	14.9	487	424	16.75	148.3
54	84a	F40 Control	11.75	10.24	114.9	14	14.7	53	55	14.7	497	433	16.99	150.4
55	84a	F40 Control	12.01	10.46	115.5	14.1	14.8	52	45	14.8	498	434	19.75	174.8
56	84a	F40 Control	11.58	10.08	115.3	13.9	14.9	53	53	14.9	485	422	17.04	150.8
57	83a	F40 Control	13.51	11.78	114.8	14.1	14.8	49	19	14.7	564	492	28.49	252.2
58	83a	F40 Control	12.06	10.52	115.5	14.4	14.8	54	30	14.6	490	427	14.66	129.7
59	83a	F40 Control	11.52	10.03	115	13.9	14.9	55	0	14.9	484	421	14.02	124.1
60	83a	F40 Control	12.81	11.16	115.2	14.2	14.7	50	22	14.8	533	464	25.76	228.0
61	83a	F40 Control	12.56	10.95	114.9	14.2	14.9	49	50	14.7	517	450	26.73	236.6
62	83a	F40 Control	12.77	11.13	115.3	14.1	14.6	51	44	14.7	538	469	22.69	200.8
63	83a	F40 Control	13.46	11.73	115	14.3	14.7	49	53	14.7	557	485	26.68	236.1
64	83a	F40 Control	11.75	10.24	115.2	13.6	14.4	51	41	14.7	521	454	24.47	216.6
65	87a	F40 Control	13.44	0	115.1	14.4	14.7	48	37	#DIV/0!	552	0	29.45	260.7

66	87a	F40 Control	12.89	0	115.2	14.1	14.4	49	39	#DIV/0!	551	0	28.43	251.6
67	87a	F40 Control	13.42	0	115	14.3	14.8	48	35	#DIV/0!	551	0	29.65	262.4
68	87a	F40 Control	12.27	0	115.3	13.9	14.7	52	33	#DIV/0!	521	0	20.87	184.7
69	87a	F40 Control	13.12	0	114.8	14.2	13.8	50	20	#DIV/0!	583	0	27.53	243.6
70	87a	F40 Control	12.93	0	114.6	14.2	14.7	49	48	#DIV/0!	541	0	27.18	240.5
71	87a	F40 Control	13.36	0	114.8	14.4	14.7	48	45	#DIV/0!	550	0	29.13	257.9
72	87a	F40 Control	13.24	0	114.5	14.3	14.9	51	51	#DIV/0!	543	0	21.47	190.0
73	37a	F40 Control	10.56	9.23	115	14.1	14.8	50	2	14.4	440	385	26.70	236.4
74	37a	F40 Control	11.28	9.81	115.3	14.4	14.7	53	45	15.0	462	402	16.69	147.8
75	37a	F40 Control	13.31	11.55	115.2	14.2	14.8	48	38	15.2	550	477	29.85	264.2
76	37a	F40 Control	12.65	11	115.5	14.3	14.9	53	6	15.0	514	447	18.31	162.1
77	37a	F40 Control	11.88	10.31	115.6	14.9	14.6	52	5	15.2	472	410	20.00	177.0
78	37a	F40 Control	12.15	10.57	115.1	13.8	14.7	54	7	14.9	520	453	16.82	148.9
79	37a	F40 Control	10.33	9	114.8	13.8	14.1	54	43	14.8	462	403	15.80	139.9
80	37a	F40 Control	10.53	9.19	114.7	13.8	14.5	55	34	14.6	459	400	12.96	114.7
81	72a	308 Control	9.97	8.65	115	14.1	14.5	54	55	15.3	424	368	14.32	126.7
82	72a	308 Control	10.92	9.47	115.6	13.7	15	54	38	15.3	460	399	15.25	135.0
83	72a	308 Control	10.35	8.97	114.9	14.1	15.1	55	47	15.4	423	367	11.46	101.4
84	72a	308 Control	11.34	9.86	115.2	14.1	14.7	54	45	15.0	475	413	14.57	129.0
85	72a	308 Control	10.71	9.28	114.5	14.5	14.5	55	12	15.4	445	385	12.97	114.8
86	72a	308 Control	11.3	9.81	115.3	14.3	15.1	55	15	15.2	454	394	12.59	111.5
87	72a	308 Control	11.31	9.81	115.3	14.6	14.4	55	4	15.3	467	405	13.27	117.4
88	72a	308 Control	10.59	9.19	115.2	14.3	14.9	55	0	15.2	431	374	13.42	118.7
89	32a	308 Control	10.63	9.27	115.4	14.4	14.9	54	56	14.7	429	374	13.44	119.0
90	32a	308 Control	13.64	11.88	115.6	14	14.4	52	15	14.8	585	510	21.88	193.6
91	32a	308 Control	13.44	11.7	115.4	13.8	14	52	30	14.9	603	525	22.30	197.4
92	32a	308 Control	13	11.31	115.5	14.2	14.4	51	32	14.9	550	479	23.28	206.0
93	32a	308 Control	14.04	12.2	115.4	14.2	14.4	47	56	15.1	595	517	32.42	287.0
94	32a	308 Control	13.54	11.77	115.7	14	14.6	53	16	15.0	573	498	18.86	166.9
95	32a	308 Control	13.48	11.72	115.6	13.8	14.2	51	20	15.0	595	517	25.22	223.3
96	32a	308 Control	11.08	9.63	115	14.5	14.9	54	18	15.1	446	388	14.91	132.0
97	66a	308 Control	13.1	11.41	115.6	14.2	14.7	51	10	14.8	543	473	23.74	210.1
98	66a	308 Control	11.51	10.01	115.3	14.2	14.6	52	33	15.0	482	419	20.33	179.9
99	66a	308 Control	13.36	11.65	114.6	14.2	14.6	50	51	14.7	562	490	24.71	218.7
100	66a	308 Control	12.06	10.5	116	14.2	14.9	53	36	14.9	491	428	17.22	152.4
101	66a	308 Control	11.76	10.23	115.2	13.9	14.6	53	45	15.0	503	438	17.75	157.1

102	66a	308 Control	11.77	10.25	115.4	14.2	14.5	52	22	14.8	495	431	20.95	185.4
103	66a	308 Control	12.07	10.51	115.2	14.1	14.7	53	18	14.8	505	440	18.44	163.2
104	66a	308 Control	13.17	11.47	115.3	14.4	14.7	49	23	14.8	540	470	27.61	244.3
105	44a	308 Control	13.38	0	115.7	14.7	14.6	49	24	#DIV/0!	539	0	26.89	238.0
106	44a	308 Control	14.34	0	114.8	13.7	14.2	50	56	#DIV/0!	642	0	26.62	235.6
107	44a	308 Control	12.89	0	115.5	14.4	14.7	54	23	#DIV/0!	527	0	15.06	133.3
108	44a	308 Control	12.74	0	115.5	14.3	14.7	52	50	#DIV/0!	525	0	19.25	170.3
109	44a	308 Control	13.57	0	115.1	14.4	14.5	51	36	#DIV/0!	565	0	22.46	198.8
110	44a	308 Control	13.6	0	115.1	14.4	14.6	49	48	#DIV/0!	562	0	26.78	237.0
111	44a	308 Control	12.76	0	115.2	14	14.7	50	2	#DIV/0!	538	0	27.18	240.6
112	44a	308 Control	15.19	0	114.5	13.9	14.7	47	52	#DIV/0!	649	0	32.99	292.0
113	81a	308 Control	11.83	10.31	115	14.1	14.6	54	40	14.7	500	436	14.90	131.8
114	81a	308 Control	13.15	11.48	115.2	14	14.9	53	0	14.5	547	478	19.18	169.8
115	81a	308 Control	13.42	11.73	115	14	14.7	49	7	14.4	567	496	29.51	261.2
116	81a	308 Control	12.84	11.22	114.9	14.1	14.5	51	33	14.4	547	478	23.33	206.5
117	81a	308 Control	13.3	11.61	115.2	14.3	14.5	50	37	14.6	557	486	25.20	223.1
118	81a	308 Control	12.67	11.06	115	13.7	14.4	52	35	14.6	558	487	21.69	192.0
119	81a	308 Control	12.36	10.8	114.5	14.6	14.8	52	50	14.4	500	437	18.51	163.9
120	81a	308 Control	10.75	9.38	115.5	14.4	14.8	55	21	14.6	437	381	12.45	110.2
121	74a	F13 Control	11.65	10.19	115.3	13.9	14.3	54	7	14.3	508	445	17.10	151.3
122	74a	F13 Control	10.79	9.41	115.3	14.5	14.7	53	37	14.7	439	383	16.86	149.2
123	74a	F13 Control	11.07	9.68	115.3	14.2	14.2	54	15	14.4	476	416	16.29	144.2
124	74a	F13 Control	10.29	8.98	115.5	14.5	14.2	53	38	14.6	433	378	17.41	154.1
125	74a	F13 Control	10.97	9.53	115.4	13.9	14.9	55	21	15.1	459	399	13.06	115.6
126	74a	F13 Control	11.06	9.65	115	14.3	14.6	55	7	14.6	461	402	13.38	118.4
127	74a	F13 Control	10.76	9.38	114.6	14.1	14.7	54	33	14.7	453	395	15.11	133.7
128	74a	F13 Control	10.3	9	115.2	14.2	14.6	55	0	14.4	431	377	13.84	122.5
129	73a	F13 Control	10.63	9.27	115	14.4	14.9	55	30	14.7	431	376	11.98	106.0
130	73a	F13 Control	11.31	9.87	114.8	14.4	14.9	54	50	14.6	459	401	13.70	121.3
131	73a	F13 Control	10.2	8.91	115.3	14.2	14	54	30	14.5	445	389	15.83	140.1
132	73a	F13 Control	11.93	10.36	114.9	14.7	15.1	55	10	15.2	468	406	12.27	108.6
133	73a	F13 Control	11.33	9.89	115	14.4	14.9	54	15	14.6	459	401	15.20	134.5
134	73a	F13 Control	10.76	9.37	114.6	14.2	14.8	54	28	14.8	447	389	15.06	133.3
135	73a	F13 Control	10.65	9.28	114.3	14.6	14.7	54	38	14.8	434	378	14.11	124.8
136	73a	F13 Control	10.6	9.28	115.3	14.3	14.9	55	10	14.2	431	378	12.98	114.9
137	89a	F13 Control	13.15	11.47	115	14.6	14.8	48	49	14.6	529	462	28.17	249.3

138	89a	F13 Control	12.83	11.19	114.5	14.6	14.5	48	33	14.7	529	462	29.39	260.1
139	89a	F13 Control	12.75	11.12	114.7	14.4	14.5	49	16	14.7	532	464	28.27	250.3
140	89a	F13 Control	14.38	12.55	114.9	14.1	14.6	46	33	14.6	608	531	35.70	316.0
141	89a	F13 Control	13.74	12	114.6	14	14.5	49	58	14.5	591	516	27.73	245.4
142	89a	F13 Control	12.48	10.91	114.6	14.6	14.1	49	31	14.4	529	462	27.85	246.5
143	89a	F13 Control	13.21	11.55	114.7	14.5	14.8	49	11	14.4	537	469	27.60	244.3
144	89a	F13 Control	12.8	11.19	114.3	14.4	14.3	49	47	14.4	544	475	27.38	242.3
145	91a	F13 Control	12.38	0	115.4	14.2	14.5	47	33	#DIV/0!	521	0	33.14	293.3
146	91a	F13 Control	12.26	0	115.7	14	14.5	51	5	#DIV/0!	522	0	24.82	219.7
147	91a	F13 Control	12.96	0	115.4	14.2	14.4	50	6	#DIV/0!	549	0	26.98	238.8
148	91a	F13 Control	12.2	0	115.3	14.2	14.5	50	37	#DIV/0!	514	0	25.48	225.5
149	91a	F13 Control	13.78	0	115.3	14.4	14.8	50	18	#DIV/0!	561	0	25.20	223.0
150	91a	F13 Control	12.75	0	115.8	14.2	14.7	49	9	#DIV/0!	527	0	28.79	254.8
151	91a	F13 Control	12.76	0	115.5	14.6	14.3	52	50	#DIV/0!	529	0	19.16	169.6
152	91a	F13 Control	13.46	0	115.2	14.1	14.2	56	3	#DIV/0!	584	0	11.43	101.2
153	18a	F13 Control	11.91	10.36	115.6	14.2	14.6	52	12	15.0	497	432	21.24	188.0
154	18a	F13 Control	12.35	10.74	115.2	13.8	14.7	50	37	15.0	528	460	26.26	232.5
155	18a	F13 Control	11.89	10.34	114.3	14	14.6	54	17	15.0	509	443	16.11	142.6
156	18a	F13 Control	11.88	10.35	114.9	14.2	14.5	50	18	14.8	502	437	26.28	232.6
157	18a	F13 Control	11.72	10.2	115.5	13.7	14.6	53	7	14.9	507	442	19.92	176.3
158	18a	F13 Control	11.65	10.16	115.3	14	14.8	54	0	14.7	488	425	16.65	147.4
159	18a	F13 Control	11.63	10.14	114.8	14	14.7	51	43	14.7	492	429	22.83	202.1
160	18a	F13 Control	11.78	10.28	115.4	14.2	14.2	53	21	14.6	506	442	18.75	165.9
161	73b	F13 Samples	11.39	9.87	114.7	14.4	15	55	24	15.4	460	398	12.16	107.6
162	73b	F13 Samples	10.97	9.47	114.9	14.6	14.8	54	20	15.8	442	381	14.77	130.7
163	73b	F13 Samples	10.92	9.47	114.6	14.6	14.8	54	14	15.3	441	382	15.02	132.9
164	73b	F13 Samples	10.63	9.19	115.3	14.1	14.4	55	48	15.7	454	393	11.97	106.0
165	73b	F13 Samples	10.35	8.96	115.6	14.2	14.8	55	10	15.5	426	369	13.21	116.9
166	73b	F13 Samples	10.94	9.48	115.2	14.2	14.7	54	10	15.4	455	394	15.96	141.3
167	73b	F13 Samples	10.5	9.1	115.3	14.2	14.9	54	5	15.4	430	373	15.96	141.3
168	73b	F13 Samples	10.95	9.45	113.7	14.6	15.1	54	35	15.9	437	377	13.86	122.6
169	74b	F13 Samples	10.45	9.05	115	14.4	13.9	54	8	15.5	454	393	16.61	147.0
170	74b	F13 Samples	10.07	8.72	115.5	14.3	14.5	55	10	15.5	420	364	13.34	118.1
171	74b	F13 Samples	10.32	8.93	115.4	13.9	15.1	55	10	15.6	426	369	13.38	118.4
172	74b	F13 Samples	13.3	11.55	115.1	13.9	14.7	52	43	15.2	566	491	20.42	180.7
173	74b	F13 Samples	11.72	10.18	114.8	14.3	15.2	56	10	15.1	470	408	10.15	89.8

174	74b	F13 Samples	11.1	9.64	115.2	14.4	14.4	51	20	15.1	465	404	23.29	206.1
175	74b	F13 Samples	10.82	9.38	114.5	14.2	15.1	54	56	15.4	441	382	13.56	120.0
176	74b	F13 Samples	11.47	9.96	115.2	14.5	15	54	30	15.2	458	398	14.31	126.6
177	91b	F13 Samples	14.15	0	115.7	14.5	14.9	50	13	#DIV/0!	566	0	24.96	221.0
178	91b	F13 Samples	14.33	0	115.4	14.2	14.7	48	22	#DIV/0!	595	0	30.71	271.8
179	91b	F13 Samples	13.31	0	115.4	14.5	14.5	53	20	#DIV/0!	549	0	17.82	157.7
180	91b	F13 Samples	14.16	0	115.4	14.1	14.8	53	43	#DIV/0!	588	0	17.22	152.4
181	91b	F13 Samples	14.34	0	115.3	14.2	14.4	48	45	#DIV/0!	608	0	30.39	269.0
182	91b	F13 Samples	13.91	0	115.4	13.9	14.4	49	17	#DIV/0!	602	0	30.03	265.7
183	91b	F13 Samples	14.34	0	115.5	14.3	14.7	48	44	#DIV/0!	591	0	29.49	261.0
184	91b	F13 Samples	14.31	0	115.3	14	14.8	50	47	#DIV/0!	599	0	25.09	222.0
185	18b	F13 Samples	12.33	10.7	115.2	13.7	14.8	50	16	15.2	528	458	27.31	241.7
186	18b	F13 Samples	11.71	10.18	114.8	14	14.6	53	13	15.0	499	434	19.00	168.1
187	18b	F13 Samples	12.29	10.68	114.9	14.1	14.7	52	16	15.1	516	448	21.15	187.2
188	18b	F13 Samples	12.1	10.5	114.3	13.9	14.7	52	26	15.2	518	450	21.18	187.5
189	18b	F13 Samples	11.96	10.39	115.1	14.1	14.8	53	23	15.1	498	433	18.10	160.2
190	18b	F13 Samples	12.7	11.03	115.5	14.2	14.8	50	27	15.1	523	454	25.38	224.6
191	18b	F13 Samples	11.67	10.14	115.5	13.5	14.9	54	20	15.1	502	436	16.56	146.5
192	18b	F13 Samples	12.3	10.69	114.9	14.2	14.6	51	3	15.1	516	449	24.20	214.2
193	89b	F13 Samples	14.01	12.18	114.5	14.3	14.7	49	40	15.0	582	506	27.21	240.8
194	89b	F13 Samples	14.03	12.2	114.9	14.4	14.6	47	52	15.0	581	505	31.45	278.4
195	89b	F13 Samples	14.05	12.21	114.6	14.4	14.8	48	6	15.1	575	500	30.48	269.8
196	89b	F13 Samples	13.56	11.77	115	14.4	14.4	49	30	15.2	569	494	27.89	246.9
197	89b	F13 Samples	13.9	12.09	115.3	14.3	14.7	50	24	15.0	573	499	25.40	224.8
198	89b	F13 Samples	13.61	11.83	115.1	14.6	14.1	48	42	15.0	574	499	29.86	264.2
199	89b	F13 Samples	13.86	12.03	114.7	14.1	14.5	46	30	15.2	591	513	36.07	319.2
200	89b	F13 Samples	14.04	12.21	114.9	14.1	14.6	47	39	15.0	594	516	33.03	292.3
201	37b	F40 Samples	10.99	9.56	115.5	14.4	14.6	54	8	15.0	453	394	15.81	140.0
202	37b	F40 Samples	11.15	9.67	114.4	13.9	15	52	45	15.3	467	405	19.93	176.4
203	37b	F40 Samples	10.77	9.35	114.8	13.8	14.6	54	16	15.2	466	404	16.52	146.2
204	37b	F40 Samples	12.68	11	115.4	14.1	15.1	53	0	15.3	516	448	18.72	165.7
205	37b	F40 Samples	13.1	11.35	115.3	14.5	15.1	48	35	15.4	519	450	28.45	251.8
206	37b	F40 Samples	12.42	10.77	115.3	14.7	14.6	50	51	15.3	502	435	23.42	207.3
207	37b	F40 Samples	10.91	9.47	115.7	14	14.9	53	48	15.2	452	392	17.07	151.1
208	37b	F40 Samples	11.7	10.16	115.7	14.3	14.4	52	5	15.2	491	426	21.61	191.2
209	24b	F40 Samples	13.71	11.88	115.3	14.5	15.2	48	45	15.4	540	467	27.88	246.7

210	24b	F40 Samples	15.53	13.48	115.1	14.3	14.9	51	36	15.2	633	550	22.09	195.5
211	24b	F40 Samples	12.3	10.63	115.4	14	14.1	53	0	15.7	540	467	20.27	179.4
212	24b	F40 Samples	13.08	11.35	115.3	14.3	14.9	52	18	15.2	532	462	20.34	180.0
213	24b	F40 Samples	13.03	11.31	114.8	14.4	14.5	52	56	15.2	544	472	19.04	168.6
214	24b	F40 Samples	13.33	11.56	115.5	14.6	14.9	50	38	15.3	531	460	23.71	209.9
215	24b	F40 Samples	13.56	11.77	114.9	14.4	15.1	49	46	15.2	543	471	25.97	229.9
216	24b	F40 Samples	13.58	11.8	115.5	13.9	15.1	52	27	15.1	560	487	20.58	182.1
217	83b	F40 Samples	11.01	9.57	115.5	14.6	14.3	55	0	15.0	457	397	13.54	119.8
218	83b	F40 Samples	10.08	8.75	115.3	13.8	14.6	56	0	15.2	434	377	11.64	103.0
219	83b	F40 Samples	11.17	9.71	115.4	14.3	14.5	52	45	15.0	467	406	19.73	174.6
220	83b	F40 Samples	10.39	9.04	114.8	14.4	14.7	53	36	14.9	428	372	17.08	151.2
221	83b	F40 Samples	12.16	10.6	115.4	14.5	14.1	55	0	14.7	515	449	13.88	122.8
222	83b	F40 Samples	12.18	10.56	115	14.2	14.4	51	47	15.3	518	449	22.63	200.3
223	83b	F40 Samples	11.94	10.36	115.3	13.8	14.3	52	43	15.3	525	455	21.23	187.9
224	83b	F40 Samples	12	10.41	114.9	14.1	14.2	51	30	15.3	522	453	23.96	212.0
225	84b	F40 Samples	11.8	10.21	115.7	14	14.6	53	30	15.6	499	432	18.23	161.4
226	84b	F40 Samples	12	10.39	115.3	14.4	14.8	53	30	15.5	488	423	17.22	152.4
227	84b	F40 Samples	10.96	9.48	114.6	14.1	14.4	55	15	15.6	471	407	13.50	119.5
228	84b	F40 Samples	10.51	9.07	114.8	13.8	14.6	55	34	15.9	454	392	12.87	113.9
229	84b	F40 Samples	10.86	9.38	115.3	14.3	14.8	54	45	15.8	445	384	14.16	125.3
230	84b	F40 Samples	11.22	9.69	114.7	14.1	14.8	55	0	15.8	469	405	13.80	122.2
231	84b	F40 Samples	10.91	9.44	115.7	14	14.7	55	47	15.6	458	396	11.90	105.3
232	84b	F40 Samples	10.52	9.09	115.2	14.2	14.5	55	50	15.7	444	383	11.67	103.3
233	87b	F40 Samples	12.73	0	114.6	14.4	13.9	49	2	#DIV/0!	555	0	30.09	266.3
234	87b	F40 Samples	13.41	0	115.4	14.1	14.6	49	6	#DIV/0!	564	0	29.43	260.5
235	87b	F40 Samples	13.09	0	115.1	14.3	14.4	49	11	#DIV/0!	552	0	28.99	256.6
236	87b	F40 Samples	11.51	0	115.4	14	14.7	53	46	#DIV/0!	485	0	17.39	153.9
237	87b	F40 Samples	14.12	0	115	14.4	14.4	48	16	#DIV/0!	592	0	30.92	273.7
238	87b	F40 Samples	11.76	0	114.5	14.3	13.8	51	38	#DIV/0!	520	0	23.76	210.3
239	87b	F40 Samples	14	0	115	14.5	14	46	48	#DIV/0!	600	0	35.05	310.2
240	87b	F40 Samples	12.04	0	114.5	14.4	14.1	54	0	#DIV/0!	518	0	16.73	148.1
241	66b	308 Samples	11.4	9.9	445	14.2	14.4	53	13	15.2	125	109	18.84	166.8
242	66b	308 Samples	13.35	11.59	115.2	14.2	14.9	46	42	15.2	548	476	34.25	303.1
243	66b	308 Samples	11.6	10.05	115.3	14.4	14.5	50	55	15.4	482	417	24.18	214.0
244	66b	308 Samples	12.81	11.11	115.1	13.8	14.8	49	30	15.3	545	473	28.98	256.5
245	66b	308 Samples	13.38	11.61	115.7	14.2	14.6	49	0	15.2	558	484	29.36	259.8

246	66b	308 Samples	12.06	10.44	114.7	14.4	14.9	50	18	15.5	490	424	25.03	221.5
247	66b	308 Samples	12.84	11.15	115.1	14	14.7	51	38	15.2	542	471	23.05	204.0
248	66b	308 Samples	12.71	11.03	115.3	13.7	14.7	53	35	15.2	547	475	18.49	163.7
249	81b	308 Samples	12.48	10.84	115.5	14.4	14.4	50	8	15.1	521	453	26.32	232.9
250	81b	308 Samples	13.19	11.45	114.9	13.9	14.5	53	25	15.2	570	494	18.79	166.3
251	81b	308 Samples	12.75	11.04	114.9	14.3	15.1	49	40	15.5	514	445	26.49	234.5
252	81b	308 Samples	11.4	9.86	115.3	14.5	14.3	55	9	15.6	477	412	13.28	117.6
253	81b	308 Samples	11.49	9.98	115	14.1	14.5	52	11	15.1	489	424	21.66	191.7
254	81b	308 Samples	13.31	11.55	114.8	14.2	14.4	51	2	15.2	567	492	24.58	217.5
255	81b	308 Samples	10.97	9.5	115.1	14.2	14.6	55	52	15.5	460	398	11.50	101.8
256	81b	308 Samples	11.9	10.32	115	14	14.2	51	45	15.3	521	451	23.54	208.4
257	72b	308 Samples	10.73	9.24	115	14.6	15.2	54	27	16.1	420	362	14.09	124.7
258	72b	308 Samples	10.84	9.37	114	14.6	15.1	55	7	15.7	431	373	12.53	110.9
259	72b	308 Samples	10.76	9.31	115.2	14.3	14.7	55	0	15.6	444	384	13.60	120.4
260	72b	308 Samples	11.21	9.68	114.9	14.5	14.8	56	6	15.8	455	393	10.38	91.8
261	72b	308 Samples	10.23	8.86	115.3	13.5	14.9	55	26	15.5	441	382	13.43	118.8
262	72b	308 Samples	10.5	9.07	114.9	14.2	14.5	55	9	15.8	444	383	13.53	119.7
263	72b	308 Samples	10.51	9.08	115.2	14.3	14.9	54	36	15.7	428	370	14.46	127.9
264	72b	308 Samples	10.61	9.19	115.2	14.2	15.1	55	7	15.5	430	372	13.08	115.8
265	44b	308 Samples	11.67	0	115.5	14.5	14.7	53	43	#DIV/0!	474	0	16.60	146.9
266	44b	308 Samples	11.97	0	115.5	14.5	14	54	12	#DIV/0!	511	0	16.14	142.8
267	44b	308 Samples	13.4	0	114.4	14	14.6	51	10	#DIV/0!	573	0	24.43	216.2
268	44b	308 Samples	13.41	0	114.9	13.7	14.6	50	35	#DIV/0!	583	0	26.83	237.5
269	44b	308 Samples	12.36	0	114.7	14.2	15	52	5	#DIV/0!	506	0	20.97	185.6
270	44b	308 Samples	12.36	0	115.3	14.6	14.6	53	6	#DIV/0!	503	0	18.10	160.2
271	44b	308 Samples	13.13	0	114.8	14.4	15	50	5	#DIV/0!	530	0	25.39	224.7
272	44b	308 Samples	12.39	0	115.2	14.4	14.7	52	10	#DIV/0!	508	0	20.73	183.5
273	32b	308 Samples	10.55	9.12	115	14.4	14.7	53	23	15.7	433	375	17.64	156.1
274	32b	308 Samples	13.07	11.34	115.2	13.9	14.9	50	42	15.3	548	475	25.41	224.9
275	32b	308 Samples	13.29	11.53	115.2	14.2	14.5	50	30	15.3	560	486	25.77	228.1
276	32b	308 Samples	13.89	12.02	115.3	14.3	14.8	52	43	15.6	569	493	19.41	171.8
277	32b	308 Samples	13.34	11.56	115.1	14	14.6	50	8	15.4	567	491	27.11	240.0
278	32b	308 Samples	14.02	12.16	115.3	14.2	14.4	51	11	15.3	595	516	24.19	214.1
279	32b	308 Samples	10.31	8.92	115.1	14.5	15	54	56	15.6	412	356	13.21	116.9
280	32b	308 Samples	12.88	11.17	115.3	14.3	14.3	49	15	15.3	546	474	29.02	256.9
281	93b	294 Samples	12.55	10.9	115.1	14.3	14.8	50	22	15.1	515	447	25.31	224.0

282	93b	294 Samples	12.47	10.83	115	14.3	14.4	51	11	15.1	527	457	23.93	211.8
283	93b	294 Samples	13.78	11.97	115.3	14.2	14.8	47	43	15.1	569	494	32.07	283.8
284	93b	294 Samples	14.31	12.39	114.9	14.5	15	48	11	15.5	573	496	29.56	261.6
285	93b	294 Samples	14.23	12.33	114.8	14.7	15	49	33	15.4	562	487	25.82	228.6
286	93b	294 Samples	13.91	12.07	114.8	14.1	14.5	48	26	15.2	593	514	31.31	277.1
287	93b	294 Samples	13.39	11.63	115.1	14.2	14.4	47	0	15.1	569	494	34.71	307.2
288	93b	294 Samples	13.59	11.79	115.4	14.2	14.4	53	23	15.3	576	500	18.40	162.8
289	88b	294 Samples	12.86	11.16	115.3	14.5	14.3	50	57	15.2	538	467	24.17	214.0
290	88b	294 Samples	12.52	10.87	114.6	14.5	13.9	51	35	15.2	542	471	23.22	205.5
291	88b	294 Samples	13	11.29	115.6	14.1	14.3	49	17	15.1	558	484	29.58	261.8
292	88b	294 Samples	13.01	11.31	115.2	14.3	14.4	50	53	15.0	548	477	24.70	218.6
293	88b	294 Samples	12.93	11.23	115.4	14.3	14.2	48	52	15.1	552	479	30.19	267.2
294	88b	294 Samples	12.65	11.01	115.2	13.9	14.2	50	51	14.9	556	484	26.26	232.4
295	88b	294 Samples	12.45	10.83	114.9	14.3	13.7	52	15	15.0	553	481	22.25	197.0
296	88b	294 Samples	12.96	11.25	115.5	14.1	14.5	51	6	15.2	549	476	24.50	216.9
297	86b	294 Samples	12.32	0	115.5	14.5	14.9	53	44	#DIV/0!	494	0	16.34	144.6
298	86b	294 Samples	13.04	0	115.8	14.4	14.1	51	15	#DIV/0!	555	0	24.00	212.4
299	86b	294 Samples	11.91	0	114.7	14.4	14.8	54	21	#DIV/0!	487	0	15.04	133.1
300	86b	294 Samples	12.75	0	115.3	14.2	14.2	51	32	#DIV/0!	548	0	23.61	208.9
301	86b	294 Samples	11.23	0	115	14	14.6	54	30	#DIV/0!	478	0	15.52	137.3
302	86b	294 Samples	12.54	0	115.5	13.8	13.4	49	53	#DIV/0!	587	0	30.92	273.7
303	86b	294 Samples	11.33	0	114.3	14.1	14.1	54	50	#DIV/0!	499	0	14.96	132.4
304	86b	294 Samples	13.39	0	114.9	14.3	14	47	35	#DIV/0!	582	0	33.87	299.7
305	71b	294 Samples	10.65	9.24	115.4	14.6	14.6	54	15	15.3	433	376	15.18	134.4
306	71b	294 Samples	10.36	9	115.2	14.1	14.9	55	53	15.1	428	372	11.34	100.4
307	71b	294 Samples	10.86	9.4	114.8	14.3	14.9	55	36	15.5	444	384	11.85	104.8
308	71b	294 Samples	11.17	9.69	115.3	14.3	15.1	52	20	15.3	449	389	19.98	176.9
309	71b	294 Samples	10.48	9.03	114.3	14.4	14.7	55	10	16.1	433	373	13.02	115.2
310	71b	294 Samples	10.86	9.37	114.5	14.3	14.8	55	48	15.9	448	387	11.40	100.9
311	71b	294 Samples	10.82	9.37	115.3	14.4	15	53	46	15.5	434	376	16.32	144.4
312	71b	294 Samples	10.99	9.51	115	14.2	15.1	52	56	15.6	446	386	18.69	165.4
313	36b	294 Samples	10.77	9.33	115.5	13.6	15	52	50	15.4	457	396	20.38	180.4
314	36b	294 Samples	11.02	9.56	115	14	14.6	56	7	15.3	469	407	11.06	97.9
315	36b	294 Samples	10.82	9.36	115	14.3	14.8	52	44	15.6	445	385	19.37	171.4
316	36b	294 Samples	10.69	9.24	114.2	14.4	14.6	53	0	15.7	445	385	18.74	165.9
317	36b	294 Samples	10.97	9.53	115.5	14.4	14.8	53	43	15.1	446	387	16.67	147.5

318	36b	294 Samples	11.2	9.68	115.4	14.6	15.1	52	15	15.7	440	380	19.55	173.1
319	36b	294 Samples	10.92	9.47	115.5	14.4	15	53	50	15.3	438	380	16.15	142.9
320	36b	294 Samples	11.49	9.97	115	14.2	15.2	54	6	15.2	463	402	15.61	138.1

							Enter File Name ->					Inch-lbf	Joules	
	Toughness Test						Enter Weight Position ->	5	Machine Constant =			716	80.90	
							Enter Initial Angle ->	60						
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
321	59a	G. tr. Control	13.52	11.69	115.5	14	14.3	54	35	15.7	585	506	15.61	138.2
322	59a	G. tr. Control	12.82	11.09	115.2	14	14.8	53	18	15.6	537	465	18.52	163.9
323	59a	G. tr. Control	13.25	11.48	115.7	13	14.5	51	2	15.4	608	526	27.98	247.6
324	59a	G. tr. Control	14.32	12.42	115.8	14.2	14.7	48	7	15.3	592	514	31.32	277.2
325	59a	G. tr. Control	14.07	12.2	115.5	13.9	14.2	49	20	15.3	617	535	30.32	268.3
326	59a	G. tr. Control	13.21	11.45	114.9	14.3	14	50	3	15.4	574	498	27.58	244.1
327	59a	G. tr. Control	12.83	11.12	115.5	14.4	14.7	50	31	15.4	525	455	24.84	219.8
328	59a	G. tr. Control	13.62	11.79	115.4	13.6	14.5	49	21	15.5	599	518	30.66	271.4
329	1a	G. tr. Control	11.16	0	115.7	14.3	14	50	46	#DIV/0!	482	0	25.71	227.5
330	1a	G. tr. Control	12.34	0	115.2	14.4	14.2	55	43	#DIV/0!	524	0	11.98	106.0
331	1a	G. tr. Control	11.13	0	115.7	14.2	13.7	53	23	#DIV/0!	494	0	19.34	171.1
332	1a	G. tr. Control	11.28	0	115.2	14.4	14.2	53	3	#DIV/0!	479	0	19.14	169.4
333	1a	G. tr. Control	10.7	0	115.7	14.3	13.6	52	57	#DIV/0!	476	0	20.48	181.2
334	1a	G. tr. Control	11.76	0	115.6	14.8	14.4	53	20	#DIV/0!	477	0	17.38	153.9
335	1a	G. tr. Control	11.84	0	115.5	14.5	14.4	54	10	#DIV/0!	491	0	15.78	139.6
336	1a	G. tr. Control	11.36	0	115.3	14.3	14.8	52	35	#DIV/0!	466	0	19.75	174.8
337	15a	G. tr. Control	11.65	10.1	115.4	14.3	14.4	54	57	15.3	490	425	14.02	124.1
338	15a	G. tr. Control	11.51	9.98	115.2	14.6	14.6	55	22	15.3	469	406	12.31	109.0
339	15a	G. tr. Control	12.01	10.42	114.5	14.7	14.7	54	57	15.3	485	421	13.16	116.5
340	15a	G. tr. Control	11.29	9.8	115.1	13.8	14	55	30	15.2	508	441	13.62	120.5
341	15a	G. tr. Control	11.32	9.83	115.3	14.6	14.2	54	52	15.2	474	411	13.99	123.8
342	15a	G. tr. Control	12.6	10.93	115.1	14.6	15	53	48	15.3	500	434	15.89	140.7
343	15a	G. tr. Control	12.65	10.97	114.7	14.3	14.7	54	36	15.3	525	455	14.65	129.7
344	15a	G. tr. Control	11.21	9.72	114.3	13.8	14.4	56	8	15.3	494	428	11.41	101.0
345	13a	G. tr. Control	11.7	10.14	115.1	14.3	14.4	56	0	15.4	494	428	11.17	98.8
346	13a	G. tr. Control	12.28	10.62	115	14.3	14.8	54	20	15.6	505	436	15.25	135.0
347	13a	G. tr. Control	13	11.28	115.2	14.6	14.7	55	4	15.2	526	456	13.00	115.0
348	13a	G. tr. Control	11.61	10.07	115.1	14.2	14.2	55	36	15.3	500	434	12.57	111.2

349	13a	G. tr. Control	12.44	10.77	115.3	14.7	14.8	55	22	15.5	496	429	12.02	106.4
350	13a	G. tr. Control	12.38	10.74	115.2	14.3	14.7	54	23	15.3	511	444	15.22	134.7
351	13a	G. tr. Control	12.71	11	114.8	14.4	14.9	54	34	15.5	516	447	14.39	127.3
352	13a	G. tr. Control	12.72	11.02	115.1	14.4	14.9	55	17	15.4	515	446	12.54	111.0
353	94a	G. tr. Control	12.51	10.84	115.4	14.1	14.3	53	8	15.4	538	466	19.41	171.8
354	94a	G. tr. Control	12.43	10.78	115	14.2	14.8	51	12	15.3	514	446	23.49	207.9
355	94a	G. tr. Control	11.68	10.14	115.2	13.6	14.9	54	45	15.2	500	434	15.20	134.5
356	94a	G. tr. Control	12.8	11.11	115.8	14.4	14.7	50	30	15.2	522	453	24.88	220.2
357	94a	G. tr. Control	11.91	10.36	115	14.4	14.7	52	3	15.0	489	426	21.02	186.1
358	94a	G. tr. Control	11.42	9.91	115.3	14.2	14.5	53	30	15.2	481	417	17.96	159.0
359	94a	G. tr. Control	12.74	11.07	115.4	14.1	13.7	48	10	15.1	572	497	33.84	299.5
360	94a	G. tr. Control	12.07	10.49	115.2	14.1	13.7	52	6	15.1	542	471	23.16	205.0
361	11a	P.gi. Control	12.03	10.42	114.9	13.9	14.6	55	34	15.5	516	447	12.72	112.6
362	11a	P.gi. Control	12.04	10.44	115.4	14.3	14.5	55	8	15.3	503	436	13.43	118.9
363	11a	P.gi. Control	11.73	10.19	115.4	14.5	14.7	55	30	15.1	477	414	12.01	106.3
364	11a	P.gi. Control	12.39	10.74	115.1	14.3	14.9	55	3	15.4	505	438	13.29	117.6
365	11a	P.gi. Control	11.96	10.37	115.3	14.5	14.2	55	6	15.3	504	437	13.51	119.6
366	11a	P.gi. Control	12.26	10.63	114.9	14.7	15	54	37	15.3	484	420	13.72	121.4
367	11a	P.gi. Control	11.9	10.32	114.7	14.4	14.5	55	37	15.3	497	431	12.00	106.2
368	11a	P.gi. Control	12.09	10.49	114.9	14.7	14.7	54	37	15.3	487	422	14.00	123.9
369	7a	P.gi. Control	11.95	10.39	115.5	14.5	14.1	55	5	15.0	506	440	13.65	120.8
370	7a	P.gi. Control	11.94	10.38	114.9	14.4	14.8	55	25	15.0	488	424	12.28	108.7
371	7a	P.gi. Control	11.87	10.31	115.1	14.4	14.9	55	20	15.1	481	417	12.41	109.8
372	7a	P.gi. Control	12.03	10.46	115.6	14.5	15	55	18	15.0	478	416	12.28	108.7
373	7a	P.gi. Control	12.06	10.48	115.3	14.5	14.2	54	14	15.1	508	441	15.82	140.0
374	7a	P.gi. Control	11.28	9.81	114.9	14.3	14.2	55	0	15.0	483	420	14.08	124.6
375	7a	P.gi. Control	11.74	10.2	114.8	14.2	14	55	22	15.1	514	447	13.40	118.6
376	7a	P.gi. Control	11.19	9.7	115.2	14.1	14.6	55	30	15.4	472	409	12.63	111.8
377	12a	P.gi. Control	11.95	10.38	115.3	14.4	14.9	54	50	15.1	483	420	13.70	121.3
378	12a	P.gi. Control	12.03	10.45	115.2	14.3	15	55	23	15.1	487	423	12.33	109.1
379	12a	P.gi. Control	11.92	10.34	115.1	14.3	14.6	55	51	15.3	496	430	11.42	101.1
380	12a	P.gi. Control	12.55	10.89	115.5	14.7	14.9	54	52	15.2	496	430	13.19	116.7
381	12a	P.gi. Control	12.55	10.9	114.7	14.6	14.9	54	10	15.1	503	437	15.09	133.5
382	12a	P.gi. Control	12.94	11.25	115.1	14.4	14.8	54	38	15.0	528	459	14.31	126.7
383	12a	P.gi. Control	12.82	11.13	115.3	14.5	14.7	54	2	15.2	522	453	15.79	139.8
384	12a	P.gi. Control	12.41	10.78	115.3	14.4	14.9	54	23	15.1	502	436	14.86	131.5

385	14a	P.gi. Control	11.87	0	115	14.4	14.5	54	12	#DIV/0!	494	0	15.75	139.4
386	14a	P.gi. Control	12.57	0	115.1	14.4	14.7	54	55	#DIV/0!	516	0	13.67	121.0
387	14a	P.gi. Control	11.89	0	115.5	14.7	14.5	54	38	#DIV/0!	483	0	14.15	125.2
388	14a	P.gi. Control	12.75	0	115.2	14.6	14.1	54	3	#DIV/0!	538	0	16.25	143.8
389	14a	P.gi. Control	11.27	0	115.1	14.6	14.4	54	43	#DIV/0!	466	0	14.18	125.5
390	14a	P.gi. Control	12.39	0	115	14.3	14.7	55	31	#DIV/0!	513	0	12.23	108.2
391	14a	P.gi. Control	13.27	0	114.4	14.3	14.4	54	45	#DIV/0!	563	0	14.55	128.8
392	14a	P.gi. Control	12.5	0	115.2	14.3	14.7	55	3	#DIV/0!	516	0	13.47	119.2
393	6a	P.gi. Control	12.09	10.52	115.1	14.3	14.8	55	10	14.9	496	432	13.07	115.7
394	6a	P.gi. Control	12.59	10.95	114.9	14.6	14.9	55	39	15.0	504	438	11.34	100.4
395	6a	P.gi. Control	12.37	10.76	115	14.4	15	55	53	15.0	498	433	10.91	96.5
396	6a	P.gi. Control	12.2	10.63	115.5	14	15	50	20	14.8	503	438	25.89	229.1
397	6a	P.gi. Control	11.67	10.16	115.5	14.2	14.2	54	57	14.9	501	436	14.37	127.2
398	6a	P.gi. Control	12	10.45	115	14.6	14.3	54	53	14.8	500	435	13.84	122.5
399	6a	P.gi. Control	11.02	9.59	115	14.2	13.9	55	30	14.9	485	422	13.12	116.1
400	6a	P.gi. Control	12.12	10.55	115	14.5	14.6	53	17	14.9	498	433	17.82	157.7
401	57a	1191 Control	12.59	10.92	115.4	14.2	14.6	53	52	15.3	526	456	16.87	149.3
402	57a	1191 Control	13.05	11.35	115.2	14	14.6	50	45	15.0	554	482	25.52	225.8
403	57a	1191 Control	13.6	11.82	115.6	14.3	14.6	52	2	15.1	563	490	21.44	189.7
404	57a	1191 Control	12.55	10.91	115.7	13.8	14.7	53	9	15.0	535	465	19.47	172.3
405	57a	1191 Control	12.35	10.75	115.2	14	14.1	51	57	14.9	543	473	23.16	205.0
406	57a	1191 Control	12.78	11.08	115	14.5	14.9	52	37	15.3	514	446	19.12	169.2
407	57a	1191 Control	13.23	11.5	115.4	13.9	14.8	52	32	15.0	557	484	20.77	183.8
408	57a	1191 Control	12.44	10.82	115.7	14.1	14.5	53	6	15.0	526	457	19.23	170.2
409	58a	1191 Control	12.66	10.97	114.7	14.4	14.7	50	50	15.4	521	452	24.06	212.9
410	58a	1191 Control	12.71	11.01	114.9	14.2	14.4	53	48	15.4	541	469	17.28	152.9
411	58a	1191 Control	13.27	11.48	115.1	13.7	14.9	51	15	15.6	565	489	24.53	217.1
412	58a	1191 Control	14.17	12.28	115.3	14.3	14.8	47	50	15.4	581	503	31.44	278.3
413	58a	1191 Control	13.72	11.9	114.5	14.1	14.7	50	2	15.3	578	501	26.89	238.0
414	58a	1191 Control	14.22	12.35	115.4	13.6	14.5	49	35	15.1	625	543	30.04	265.8
415	58a	1191 Control	12.83	11.12	114.6	14.2	14.7	51	30	15.4	536	465	22.89	202.6
416	58a	1191 Control	12.15	10.52	115.2	14	14.7	53	8	15.5	512	444	19.09	168.9
417	34a	1191 Control	10.69	9.23	114.7	13.6	15.2	56	14	15.8	451	389	10.78	95.4
418	34a	1191 Control	10.71	9.26	115.1	14.5	14.8	54	57	15.7	434	375	13.35	118.1
419	34a	1191 Control	10.43	9.05	115.1	14	14.8	54	12	15.2	437	379	16.11	142.6
420	34a	1191 Control	10.99	9.49	115.3	14.4	15	55	22	15.8	441	381	12.24	108.4

421	34a	1191 Control	10.88	9.44	114.6	14.4	14.9	54	34	15.3	442	384	14.39	127.3
422	34a	1191 Control	10.76	9.33	114.4	14.1	15	53	45	15.3	445	386	16.90	149.6
423	34a	1191 Control	10.83	9.4	115.2	14	14.6	54	40	15.2	460	399	15.06	133.3
424	34a	1191 Control	11	9.55	115.5	13.7	14.8	56	32	15.2	470	408	10.09	89.3
425	90a	1191 Control	14.59	0	115.2	14.2	14.7	45	30	#DIV/0!	607	0	37.54	332.2
426	90a	1191 Control	14.01	0	115	14	14.7	48	49	#DIV/0!	592	0	30.26	267.9
427	90a	1191 Control	13.86	0	115.2	14.2	14.6	48	24	#DIV/0!	580	0	30.84	272.9
428	90a	1191 Control	11.74	0	115.2	13.8	15	53	47	#DIV/0!	492	0	17.38	153.9
429	90a	1191 Control	13.83	0	115.2	13.9	14.4	48	31	#DIV/0!	600	0	32.01	283.3
430	90a	1191 Control	13.55	0	115.7	14.2	14.6	48	18	#DIV/0!	565	0	31.08	275.1
431	90a	1191 Control	12.97	0	114.7	14	14.4	47	40	#DIV/0!	561	0	33.81	299.3
432	90a	1191 Control	12.01	0	114.9	14	14.3	50	9	#DIV/0!	522	0	27.64	244.6
433	55a	1191 Control	12.19	10.61	115.7	14.1	14.2	51	6	14.9	526	458	25.02	221.5
434	55a	1191 Control	12.77	11.04	115	14	14.8	54	35	15.7	536	463	15.08	133.5
435	55a	1191 Control	11.72	10.19	115.5	13.9	13.7	53	34	15.0	533	463	19.45	172.2
436	55a	1191 Control	12.32	10.67	114.8	14.7	14.5	54	36	15.5	503	436	14.23	126.0
437	55a	1191 Control	13.25	11.51	115.3	13.7	14.7	49	22	15.1	571	496	29.86	264.3
438	55a	1191 Control	13.45	11.68	115.8	14.3	13.9	48	53	15.2	584	507	30.80	272.6
439	55a	1191 Control	12.88	11.2	115.3	14.4	14.5	50	26	15.0	535	465	25.39	224.7
440	55a	1191 Control	13.42	11.65	115.7	13.6	14.2	50	45	15.2	601	521	27.44	242.8
441	25a	F3410 Control	13.29	11.55	115	14.1	14.9	51	30	15.1	550	478	22.83	202.1
442	25a	F3410 Control	12.56	10.89	115.4	14.3	14.9	54	35	15.3	511	443	14.50	128.3
443	25a	F3410 Control	13.66	11.86	115.3	14.3	14.7	52	12	15.2	564	489	20.87	184.7
444	25a	F3410 Control	12.94	11.25	114.6	14.3	14.5	52	16	15.0	545	473	20.98	185.7
445	25a	F3410 Control	13.21	11.47	114.8	13.8	14.6	52	17	15.2	571	496	21.97	194.5
446	25a	F3410 Control	13.04	11.32	114.7	14.2	14.6	54	12	15.2	548	476	15.98	141.4
447	25a	F3410 Control	13.12	11.37	115.2	14.3	14.8	52	33	15.4	538	466	19.84	175.6
448	25a	F3410 Control	12.76	11.11	114.7	14	14.6	52	21	14.9	544	474	21.31	188.6
449	63a	F3410 Control	13.87	12.08	115.2	14.2	14.7	51	58	14.8	577	502	21.69	192.0
450	63a	F3410 Control	13.1	11.4	115.4	14.1	14.6	50	53	14.9	551	480	24.89	220.3
451	63a	F3410 Control	13.63	11.86	115.8	14	14.8	49	47	14.9	568	494	27.63	244.6
452	63a	F3410 Control	14.47	12.56	115.4	14.3	14.9	49	55	15.2	588	511	26.24	232.2
453	63a	F3410 Control	13.64	11.87	114.7	14.3	14.4	50	9	14.9	578	503	26.56	235.1
454	63a	F3410 Control	14.18	12.34	115.1	14.2	14.8	47	37	14.9	586	510	32.31	285.9
455	63a	F3410 Control	14.1	12.28	115.5	12.9	14.8	51	6	14.8	639	557	27.54	243.8
456	63a	F3410 Control	13.51	11.72	115.5	14	14.7	50	3	15.3	568	493	27.14	240.2

457	27a	F3410 Control	13.27	0	115.3	13.8	14.9	52	37	#DIV/0!	560	0	20.64	182.7
458	27a	F3410 Control	13.57	0	115.3	14.4	14.5	51	9	#DIV/0!	564	0	23.59	208.8
459	27a	F3410 Control	12.51	0	115.6	14	14.6	51	52	#DIV/0!	529	0	22.59	200.0
460	27a	F3410 Control	13.31	0	115.5	14.6	14.8	52	0	#DIV/0!	533	0	20.56	182.0
461	27a	F3410 Control	12.8	0	115.2	14.6	14.9	50	56	#DIV/0!	511	0	23.00	203.5
462	27a	F3410 Control	12.28	0	115.4	13.5	14.5	53	55	#DIV/0!	544	0	18.22	161.3
463	27a	F3410 Control	13.41	0	115.3	14.7	14.7	51	35	#DIV/0!	538	0	21.50	190.3
464	27a	F3410 Control	13.01	0	115.5	14.2	14.3	53	53	#DIV/0!	555	0	17.18	152.0
465	21a	F3410 Control	12.26	10.67	115.6	14.1	14.8	50	30	14.9	508	442	25.53	226.0
466	21a	F3410 Control	11.41	9.92	115.3	14	13.7	51	43	15.0	516	449	24.50	216.8
467	21a	F3410 Control	11.84	10.23	115.5	13.6	14.4	52	4	15.7	523	452	23.39	207.1
468	21a	F3410 Control	12.04	10.43	115.1	14.1	14.8	49	25	15.4	501	434	28.24	250.0
469	21a	F3410 Control	11.44	9.9	114.7	14.4	14.5	51	4	15.6	478	413	23.80	210.7
470	21a	F3410 Control	12.18	10.61	115.5	14.4	14.4	54	50	14.8	509	443	14.18	125.5
471	21a	F3410 Control	12.21	10.62	115.8	14.2	14.6	51	35	15.0	509	442	22.83	202.1
472	21a	F3410 Control	11.68	10.17	115.2	14.4	13.6	51	40	14.8	518	451	23.76	210.3
473	3a	35 Control	11.27	9.83	115.6	14	14.2	54	47	14.6	490	428	15.16	134.2
474	3a	35 Control	12.5	10.9	114.9	14.1	14.7	54	45	14.7	525	458	14.57	129.0
475	3a	35 Control	11.81	10.3	115.1	14.5	14.3	54	55	14.7	495	432	13.90	123.1
476	3a	35 Control	11.51	10.03	115.1	14.3	14.6	54	4	14.8	479	417	16.16	143.0
477	3a	35 Control	11.77	10.26	115.2	14.2	14.9	52	47	14.7	483	421	19.32	171.0
478	3a	35 Control	11.8	10.28	115.2	14.2	14.8	52	18	14.8	487	425	20.70	183.2
479	3a	35 Control	11.1	9.67	114.8	14.4	13.6	54	17	14.8	494	430	16.56	146.5
480	3a	35 Control	12.18	10.63	114.8	14.1	14.4	excl.		14.6	523	456	#VALUE!	#VALUE!
481	56a	35 Control	13.19	11.5	115.1	14.6	13.7	52	19	14.7	573	500	21.37	189.2
482	56a	35 Control	13.28	11.57	115.4	14.2	13.9	52	50	14.8	583	508	20.58	182.1
483	56a	35 Control	13.06	11.38	115.2	13.2	14.5	54	10	14.8	592	516	18.11	160.3
484	56a	35 Control	13.98	12.17	115.7	13.8	14.5	52	44	14.9	604	526	20.89	184.9
485	56a	35 Control	13.07	11.35	115.8	14	14.9	52	52	15.2	541	470	19.53	172.9
486	56a	35 Control	13.56	11.81	115.2	14.3	14.5	50	5	14.8	568	494	26.54	234.9
487	56a	35 Control	11.78	10.25	115.1	13.7	13.9	55	7	14.9	537	468	15.02	132.9
488	56a	35 Control	12.54	10.93	115.2	14.4	14.6	54	37	14.7	518	451	14.55	128.8
489	48a	35 Control	12.56	10.93	115	14.3	14.4	52	33	14.9	530	462	20.39	180.5
490	48a	35 Control	12.38	10.77	115.2	14	14.2	53	22	14.9	541	470	19.12	169.2
491	48a	35 Control	12.83	11.18	115.2	14.4	14.7	53	57	14.8	526	458	16.18	143.2
492	48a	35 Control	12.99	11.29	115.4	14.6	15.1	53	17	15.1	511	444	17.05	150.9

493	48a	35 Control	13.31	11.61	114.9	14.3	14.5	52	28	14.6	559	487	20.46	181.1
494	48a	35 Control	12.94	11.28	115.4	14.4	14.5	53	13	14.7	537	468	18.31	162.1
495	48a	35 Control	12.49	10.84	114.8	13.9	14.5	54	12	15.2	540	468	16.63	147.2
496	48a	35 Control	12.81	11.1	115.1	13.7	14.5	52	3	15.4	560	485	23.02	203.7
497	49a	35 Control	13.57	0	115.2	14.4	14.4	50	26	#DIV/0!	568	0	25.57	226.3
498	49a	35 Control	13.17	0	114.7	13.9	14.6	51	20	#DIV/0!	566	0	24.26	214.7
499	49a	35 Control	13.57	0	115.5	14.3	14.5	48	39	#DIV/0!	567	0	30.10	266.4
500	49a	35 Control	14.35	0	115.5	13.9	14.6	49	40	#DIV/0!	612	0	28.63	253.4
501	49a	35 Control	13.15	0	115.4	14.3	14.6	54	3	#DIV/0!	546	0	16.20	143.4
502	49a	35 Control	13.15	0	114.7	14.3	14.6	51	52	#DIV/0!	549	0	21.86	193.5
503	49a	35 Control	13.88	0	115.1	14.3	14.8	50	30	#DIV/0!	570	0	24.98	221.1
504	49a	35 Control	13.85	0	115.3	14.3	14.8	50	35	#DIV/0!	568	0	24.77	219.3
505	30a	35 Control	13.47	11.72	115.4	14.5	14.5	51	30	14.9	555	483	22.47	198.9
506	30a	35 Control	12.75	11.06	115.2	14.6	15	52	27	15.3	505	438	19.20	169.9
507	30a	35 Control	12.81	11.07	115.6	13.9	14.5	53	40	15.7	550	475	18.10	160.2
508	30a	35 Control	12.38	10.72	115.3	13.9	14.8	54	19	15.5	522	452	15.98	141.4
509	30a	35 Control	13	11.31	115.3	14.5	14.6	52	27	14.9	533	463	19.94	176.4
510	30a	35 Control	12.56	10.92	115.7	14.4	14.8	52	47	15.0	509	443	19.04	168.5
511	30a	35 Control	12.69	11.03	115.2	14.1	14.5	53	36	15.0	539	468	17.89	158.3
512	30a	35 Control	13.19	11.47	115.1	14.3	14.6	51	44	15.0	549	477	22.20	196.5
513	85a	P36 Control	13.58	11.81	115.1	14.1	14.8	53	14	15.0	565	492	18.49	163.7
514	85a	P36 Control	12.55	10.92	114.9	14.1	14.5	51	39	14.9	534	465	23.07	204.2
515	85a	P36 Control	13.49	11.74	115.4	14.2	14.6	49	24	14.9	564	491	28.36	251.0
516	85a	P36 Control	12.25	10.69	115	13.4	14.8	54	14	14.6	537	469	17.15	151.8
517	85a	P36 Control	12.37	10.75	114.6	14.3	14.7	53	37	15.1	513	446	17.22	152.4
518	85a	P36 Control	12.58	10.97	114.5	14.2	14.8	49	44	14.7	523	456	27.16	240.4
519	85a	P36 Control	12.4	10.79	115.1	14	14.8	54	50	14.9	520	452	14.41	127.5
520	85a	P36 Control	12.55	10.93	114.8	14.1	14.9	55	0	14.8	520	453	13.71	121.4
521	28a	P36 Control	12.75	11.09	115	14	14.7	50	26	15.0	539	469	26.16	231.5
522	28a	P36 Control	12.94	11.25	115	14.3	14.5	50	46	15.0	543	472	24.82	219.7
523	28a	P36 Control	12.94	11.24	115.4	14	14.5	51	45	15.1	552	480	23.06	204.1
524	28a	P36 Control	12.99	11.3	114.7	14.1	14.6	51	30	15.0	550	479	23.30	206.2
525	28a	P36 Control	12.9	11.22	115.3	14.3	14.4	52	15	15.0	543	473	21.17	187.4
526	28a	P36 Control	12.84	11.18	115.8	14	14.4	51	35	14.8	550	479	23.66	209.4
527	28a	P36 Control	12.46	10.84	115.3	13.9	14.6	53	3	14.9	533	463	19.66	174.0
528	28a	P36 Control	13.06	11.36	115.5	13.9	14.7	51	53	15.0	553	481	22.64	200.4

529	26a	P36 Control	13.72	11.93	115.4	14.8	14.9	51	36	15.0	539	469	20.95	185.4
530	26a	P36 Control	13.27	11.56	115.3	14.3	15	51	46	14.8	537	467	21.53	190.5
531	26a	P36 Control	12.19	10.62	114.6	13.4	14.2	52	15	14.8	559	487	23.74	210.1
532	26a	P36 Control	12.47	10.86	115.2	13.9	14.9	51	53	14.8	523	455	22.34	197.7
533	26a	P36 Control	12.7	11.06	114.5	14.4	14.9	52	21	14.8	517	450	19.99	177.0
534	26a	P36 Control	13.13	11.43	115	14.5	14.7	52	0	14.9	536	466	20.92	185.2
535	26a	P36 Control	12.05	10.5	115.1	13.4	14.6	54	21	14.8	535	466	17.04	150.8
536	26a	P36 Control	12.87	11.18	114.7	14.5	14.8	53	23	15.1	523	454	17.33	153.4
537	52a	P36 Control	13.53	0	114.6	14.3	14.6	56	12	#DIV/0!	565	0	10.48	92.7
538	52a	P36 Control	12.15	0	114.3	14.3	14.1	56	40	#DIV/0!	527	0	9.54	84.4
539	52a	P36 Control	12.19	0	115.2	14.3	14.3	51	8	#DIV/0!	517	0	24.23	214.4
540	52a	P36 Control	10.68	0	114.7	14.6	14	53	30	#DIV/0!	456	0	17.82	157.7
541	52a	P36 Control	12.11	0	115.6	14	15	53	37	#DIV/0!	499	0	17.44	154.4
542	52a	P36 Control	12.48	0	115	14.6	14.4	52	2	#DIV/0!	516	0	21.05	186.3
543	52a	P36 Control	12.2	0	115.2	14.2	14.6	55	21	#DIV/0!	511	0	12.90	114.2
544	52a	P36 Control	12.1	0	114.3	14.5	14.8	53	53	#DIV/0!	493	0	16.07	142.2
545	53a	P36 Control	11.7	10.18	114.8	13.6	14.8	54	8	14.9	506	441	17.04	150.8
546	53a	P36 Control	12.32	10.71	115.2	14.3	14.8	55	4	15.0	505	439	13.33	118.0
547	53a	P36 Control	11.88	10.3	114.7	13.7	14.8	55	0	15.3	511	443	14.43	127.7
548	53a	P36 Control	10.85	9.4	114.6	13.5	14.7	56	12	15.4	477	413	11.37	100.7
549	53a	P36 Control	11.95	10.34	115.2	14.4	14.4	54	53	15.6	500	433	14.04	124.3
550	53a	P36 Control	11.05	9.58	115.1	14.6	14.3	54	10	15.3	460	399	15.72	139.1
551	53a	P36 Control	12.68	11.02	115	14.3	15	54	40	15.1	514	447	14.19	125.6
552	53a	P36 Control	12.29	10.69	114.8	14.4	15	55	34	15.0	496	431	11.73	103.8
553	49b	35 Sample	14.72	0	114.6	14.2	14.7	47	22	#DIV/0!	615	0	33.13	293.2
554	49b	35 Sample	14.06	0	114.7	13.6	14.4	51	30	#DIV/0!	626	0	24.98	221.1
555	49b	35 Sample	14.26	0	115.1	14.1	14.9	48	45	#DIV/0!	590	0	29.69	262.8
556	49b	35 Sample	14.85	0	115.3	14.6	15	46	16	#DIV/0!	588	0	33.56	297.0
557	49b	35 Sample	12.73	0	115.3	14.3	14.4	50	51	#DIV/0!	536	0	24.78	219.3
558	49b	35 Sample	14.56	0	115.5	14.1	14.6	47	30	#DIV/0!	612	0	33.39	295.6
559	49b	35 Sample	14.35	0	115.4	14.2	14.6	48	12	#DIV/0!	600	0	31.33	277.3
560	49b	35 Sample	12.75	0	115.6	13.9	14.7	51	37	#DIV/0!	540	0	23.35	206.7
561	30b	35 Sample	12.88	11.19	115.3	14.1	14	54	5	15.1	566	492	17.18	152.0
562	30b	35 Sample	13.46	11.68	115.3	13.9	14.7	52	30	15.2	571	496	21.00	185.9
563	30b	35 Sample	12.9	11.22	115.3	13.7	15	50	15	15.0	544	474	26.98	238.8
564	30b	35 Sample	14.39	12.51	115.7	13.7	14.7	49	36	15.0	618	537	29.25	258.9

565	30b	35 Sample	13.94	12.12	115.3	14.2	14.7	53	13	15.0	579	504	18.46	163.4
566	30b	35 Sample	14.43	12.53	115.5	14.3	14.6	47	37	15.2	598	520	32.39	286.7
567	30b	35 Sample	13.67	11.87	115	14.1	14.6	49	33	15.2	577	501	28.30	250.4
568	30b	35 Sample	12.9	11.19	115.6	14.1	14.4	54	0	15.3	550	477	16.93	149.8
569	56b	35 Sample	13.67	11.87	115.3	13.9	14.2	50	48	15.2	601	522	26.39	233.6
570	56b	35 Sample	14.18	12.31	115.6	14.1	14.7	49	55	15.2	592	514	27.18	240.6
571	56b	35 Sample	13.36	11.6	115.4	14.2	14.5	51	51	15.2	562	488	22.30	197.4
572	56b	35 Sample	13.77	11.95	115.6	14.3	14.9	50	22	15.2	559	485	25.14	222.5
573	56b	35 Sample	14.75	12.81	115	14.2	14.4	52	55	15.1	627	545	19.64	173.8
574	56b	35 Sample	12.32	10.7	115	14.5	14.8	55	50	15.1	499	434	11.07	98.0
575	56b	35 Sample	13.22	11.46	114.8	14	14.8	50	33	15.4	556	482	25.68	227.3
576	56b	35 Sample	12.21	10.61	115.5	13.3	14.9	53	13	15.1	533	464	20.15	178.3
577	3b	35 Sample	12.27	10.68	115.3	14.1	14.9	53	3	14.9	507	441	18.84	166.8
578	3b	35 Sample	11.99	10.44	115.2	14.4	14.5	53	2	14.8	498	434	18.79	166.3
579	3b	35 Sample	13.25	11.53	114.7	14.2	14.7	50	53	14.9	553	482	24.46	216.5
580	3b	35 Sample	11.72	10.21	115.1	14.4	14.6	51	7	14.8	484	422	23.52	208.1
581	3b	35 Sample	11.66	10.08	115	14.4	14.8	51	36	15.7	476	411	22.00	194.7
582	3b	35 Sample	12.1	10.48	115.2	14.2	14.7	50	22	15.5	503	436	25.76	228.0
583	3b	35 Sample	12.28	10.62	114.7	14.2	13.9	56	53	15.6	542	469	9.16	81.0
584	3b	35 Sample	11.51	9.96	115.5	14.2	14	53	30	15.6	501	434	18.60	164.6
585	48b	35 Sample	13.41	11.62	115	13.6	14.9	52	4	15.4	575	499	22.61	200.1
586	48b	35 Sample	13.01	11.26	115.5	14.1	14.7	50	30	15.5	543	470	25.70	227.5
587	48b	35 Sample	12.86	11.1	115.1	14.6	14.7	52	32	15.9	521	449	19.38	171.6
588	48b	35 Sample	14.03	12.15	115.4	14.2	14.7	48	18	15.5	582	504	30.87	273.2
589	48b	35 Sample	13.95	12.09	114.9	14.3	14.6	48	30	15.4	582	504	30.26	267.8
590	48b	35 Sample	12.66	10.97	114.9	14.5	14.4	51	15	15.4	528	457	23.25	205.8
591	48b	35 Sample	13.09	11.35	114.9	14.5	14.9	49	53	15.3	527	457	25.76	228.0
592	48b	35 Sample	13.22	11.45	114.8	13.8	14.6	52	47	15.5	572	495	20.61	182.4
593	52b	P 36 Sample	12.76	0	115.1	14.7	14.7	55	51	#DIV/0!	513	0	10.87	96.2
594	52b	P 36 Sample	12.34	0	115.5	14.2	14.5	55	46	#DIV/0!	519	0	11.85	104.9
595	52b	P 36 Sample	13.01	0	114.1	14.5	15.2	54	24	#DIV/0!	517	0	14.37	127.2
596	52b	P 36 Sample	13.05	0	114.6	14.3	14.9	54	52	#DIV/0!	534	0	13.76	121.8
597	52b	P 36 Sample	12.96	0	115	14.4	14.4	52	57	#DIV/0!	543	0	19.13	169.3
598	52b	P 36 Sample	12.73	0	115.2	14.6	14.9	52	23	#DIV/0!	508	0	19.49	172.5
599	52b	P 36 Sample	12.44	0	114.4	14.6	14.7	50	13	#DIV/0!	507	0	25.04	221.6
600	52b	P 36 Sample	11.57	0	113.6	14.2	14.6	52	3	#DIV/0!	491	0	21.63	191.4

601	28b	P 36 Sample	13.75	11.9	115.3	13.8	15	50	13	15.5	576	499	26.77	236.9
602	28b	P 36 Sample	14.19	12.3	114.9	13.8	14.2	51	7	15.4	630	546	25.82	228.5
603	28b	P 36 Sample	13.97	12.11	115.1	13.8	14.6	53	30	15.4	602	522	18.64	165.0
604	28b	P 36 Sample	13.98	12.13	114.6	13.8	14.7	49	45	15.3	601	522	28.53	252.5
605	28b	P 36 Sample	13.12	11.39	114.7	14	13.7	54	8	15.2	596	518	17.60	155.8
606	28b	P 36 Sample	13.42	11.65	115.1	14.2	14.7	49	15	15.2	559	485	28.54	252.6
607	28b	P 36 Sample	13.88	12.05	115.5	13.9	14.9	49	28	15.2	580	504	28.56	252.7
608	28b	P 36 Sample	14.31	12.43	115.4	13.9	14.7	50	50	15.1	607	527	25.41	224.9
609	85b	P 36 Sample	13.29	11.55	115.6	14.3	14.5	51	15	15.1	554	482	23.59	208.8
610	85b	P 36 Sample	11.43	9.93	114.5	14.3	14.4	54	21	15.1	485	421	15.63	138.3
611	85b	P 36 Sample	11.46	9.96	114.8	13.9	14.3	55	21	15.1	502	436	13.61	120.5
612	85b	P 36 Sample	11.61	10.09	115.3	14.3	14.2	54	4	15.1	496	431	16.62	147.1
613	85b	P 36 Sample	11.21	9.73	115.2	14	14.5	54	36	15.2	479	416	15.35	135.9
614	85b	P 36 Sample	11.37	9.86	115	14.3	14.4	54	33	15.3	480	416	15.09	133.6
615	85b	P 36 Sample	10.9	9.46	115.3	13.8	14.6	55	26	15.2	469	407	13.24	117.2
616	85b	P 36 Sample	10.82	9.4	115	14.3	14.3	53	0	15.1	460	400	19.34	171.2
617	26b	P 36 Sample	12.35	10.72	114.8	13.3	14.9	53	40	15.2	543	471	18.86	166.9
618	26b	P 36 Sample	13.9	12.03	114.8	14.5	15.2	50	57	15.5	549	475	22.74	201.3
619	26b	P 36 Sample	13.22	11.47	114.5	14.4	14.6	51	35	15.3	549	476	22.34	197.8
620	26b	P 36 Sample	13.61	11.79	114.6	14.5	14.9	50	30	15.4	550	476	24.29	214.9
621	26b	P 36 Sample	13.15	11.41	114.8	13.4	14.7	51	24	15.2	582	505	25.31	224.0
622	26b	P 36 Sample	12.9	11.18	115	13.7	14.5	53	25	15.4	565	489	19.22	170.1
623	26b	P 36 Sample	12.42	10.77	115.5	14.2	14.7	52	15	15.3	515	447	20.97	185.6
624	26b	P 36 Sample	14.2	12.33	115.3	14.5	14.7	49	0	15.2	578	502	28.23	249.9
625	53b	P 36 Sample	11.07	9.6	114.2	13.6	14.6	52	49	15.3	488	423	20.99	185.7
626	53b	P 36 Sample	12.31	10.71	115.1	14.1	14.9	51	34	14.9	509	443	22.66	200.6
627	53b	P 36 Sample	11.82	10.26	114.7	13.9	15	51	47	15.2	494	429	22.45	198.7
628	53b	P 36 Sample	11.61	10.09	114.7	14.1	14.8	52	43	15.1	485	422	19.84	175.6
629	53b	P 36 Sample	12.17	10.58	114.8	14.5	14.6	53	56	15.0	501	435	16.16	143.0
630	53b	P 36 Sample	12.06	10.48	115	14	15.1	54	14	15.1	496	431	15.71	139.0
631	53b	P 36 Sample	12.44	10.85	114.9	14.2	14.5	52	57	14.7	526	459	19.42	171.9
632	53b	P 36 Sample	11.85	10.33	114.8	13.8	14.4	53	20	14.7	519	453	19.37	171.4
633	58b	1191 Sample	14.63	12.71	115.5	14.1	14.5	46	0	15.1	620	538	37.27	329.9
634	58b	1191 Sample	13.15	11.38	114.5	14.2	14.8	49	30	15.6	546	473	27.73	245.5
635	58b	1191 Sample	13.86	11.93	114.9	13.7	14.5	52	0	16.2	607	523	23.15	204.9
636	58b	1191 Sample	13.41	11.63	115.1	13.5	14.1	51	48	15.3	612	531	24.94	220.7

637	58b	1191 Sample	13.14	11.41	114.9	13.9	14.8	51	7	15.2	556	483	24.50	216.8
638	58b	1191 Sample	13.46	11.67	115	13.8	14.6	51	0	15.3	581	504	25.42	225.0
639	58b	1191 Sample	13.28	11.5	115.1	14.2	14.7	52	33	15.5	553	479	20.19	178.7
640	58b	1191 Sample	13.62	11.82	114.8	14.1	14.6	51	27	15.2	576	500	23.43	207.4
641	90b	1191 Sample	14.27	0	114.8	14	14.5	45	35	#DIV/0!	612	0	38.70	342.5
642	90b	1191 Sample	13	0	115.2	13.6	15.1	50	20	#DIV/0!	550	0	26.89	238.0
643	90b	1191 Sample	12.43	0	115	14.2	14.8	52	17	#DIV/0!	514	0	20.74	183.6
644	90b	1191 Sample	13.85	0	115.1	14.1	14.6	47	36	#DIV/0!	585	0	33.15	293.4
645	90b	1191 Sample	13.95	0	115.1	13.9	14.7	50	7	#DIV/0!	593	0	27.27	241.4
646	90b	1191 Sample	14.37	0	115.3	14.2	14.6	47	8	#DIV/0!	601	0	33.92	300.2
647	90b	1191 Sample	14.59	0	114.6	14.1	14.7	46	50	#DIV/0!	614	0	34.78	307.8
648	90b	1191 Sample	13.27	0	115.1	14.2	14.6	49	36	#DIV/0!	556	0	27.86	246.6
649	34b	1191 Sample	11.32	9.83	114.7	14.2	15	53	44	15.2	463	402	16.76	148.4
650	34b	1191 Sample	10.64	9.25	115	13.9	14.8	54	25	15.0	450	391	15.71	139.0
651	34b	1191 Sample	10.61	9.23	115.3	14	14.3	55	45	15.0	460	400	12.33	109.1
652	34b	1191 Sample	10.74	9.28	115.7	14.3	14.8	54	5	15.7	439	379	15.90	140.7
653	34b	1191 Sample	11.03	9.57	115.3	13.8	14.7	56	24	15.3	472	409	10.43	92.3
654	34b	1191 Sample	11.07	9.61	114.4	13.9	14.8	53	8	15.2	470	408	19.17	169.7
655	34b	1191 Sample	10.39	8.98	115.1	14.6	15.2	54	42	15.7	407	352	13.48	119.3
656	34b	1191 Sample	10.78	9.3	114.8	13.7	14.8	56	58	15.9	463	400	8.85	78.3
657	55b	1191 Sample	11.95	10.32	114.9	14	15	52	12	15.8	495	428	21.13	187.0
658	55b	1191 Sample	12.38	10.72	115.6	13.7	14.6	52	33	15.5	535	464	21.49	190.2
659	55b	1191 Sample	11.99	10.4	115.4	13.8	14	52	0	15.3	538	466	23.71	209.9
660	55b	1191 Sample	13.69	11.87	115.2	13.9	14.6	51	36	15.3	586	508	23.55	208.5
661	55b	1191 Sample	12.35	10.72	115.4	14	15.1	51	24	15.2	506	439	23.03	203.8
662	55b	1191 Sample	13.74	11.91	114.6	14	14.5	48	7	15.4	591	512	32.45	287.2
663	55b	1191 Sample	14.38	12.47	115.2	14.1	14.7	47	57	15.3	602	522	32.07	283.8
664	55b	1191 Sample	13.41	11.59	115.3	14.7	14.7	52	23	15.7	538	465	19.55	173.0
665	57b	1191 Sample	13.69	11.87	115.1	13.8	14.8	54	0	15.3	582	505	17.03	150.7
666	57b	1191 Sample	13.96	12.09	115.4	13.7	14.7	52	21	15.5	601	520	21.89	193.7
667	57b	1191 Sample	12.92	11.18	115.3	14.2	15.1	52	15	15.6	523	452	20.41	180.6
668	57b	1191 Sample	12.99	11.26	115.5	13.9	14.5	51	11	15.4	558	484	24.83	219.8
669	57b	1191 Sample	12.89	11.15	114.9	14.4	14.5	52	0	15.6	537	465	21.44	189.8
670	57b	1191 Sample	13.13	11.39	115.2	14	14.8	52	10	15.3	550	477	21.50	190.3
671	57b	1191 Sample	13.37	11.6	115.7	14.4	15	52	22	15.3	535	464	19.82	175.4
672	57b	1191 Sample	13.68	11.87	115.5	14.1	14.8	52	0	15.2	568	492	21.70	192.1

673	21b	F3410 Sample	11.94	10.39	115.6	14	14.7	53	32	14.9	502	437	18.02	159.5
674	21b	F3410 Sample	12.35	10.75	115	14.2	14.8	51	43	14.9	511	445	22.19	196.4
675	21b	F3410 Sample	12.07	10.5	115.6	13.8	14.7	53	50	15.0	515	448	17.60	155.8
676	21b	F3410 Sample	11.66	10.14	114.6	14.1	14.6	54	0	15.0	494	430	16.69	147.8
677	21b	F3410 Sample	12.17	10.58	115.5	14.1	14.7	53	26	15.0	508	442	18.09	160.1
678	21b	F3410 Sample	12.36	10.76	115.5	14.1	14.7	53	56	14.9	516	449	16.76	148.3
679	21b	F3410 Sample	12.62	10.98	115.5	14.3	15.1	54	47	14.9	506	440	13.79	122.1
680	21b	F3410 Sample	12.62	10.97	115.5	14.3	14.8	50	47	15.0	516	449	24.28	214.9
681	27b	F3410 Sample	13.86	0	115.6	14	14.8	50	36	#DIV/0!	579	0	25.56	226.2
682	27b	F3410 Sample	13.51	0	115.3	14.6	15	52	42	#DIV/0!	535	0	18.59	164.5
683	27b	F3410 Sample	13.29	0	115.3	13.7	14.6	53	7	#DIV/0!	576	0	19.92	176.3
684	27b	F3410 Sample	13.99	0	115.5	14.2	14.8	51	23	#DIV/0!	576	0	23.03	203.8
685	27b	F3410 Sample	14.21	0	115.3	14.3	15.1	50	51	#DIV/0!	571	0	23.63	209.2
686	27b	F3410 Sample	12.77	0	115.4	13.2	14.9	54	22	#DIV/0!	563	0	17.04	150.8
687	27b	F3410 Sample	14	0	115.4	14.1	14.6	51	36	#DIV/0!	589	0	23.04	203.9
688	27b	F3410 Sample	13.15	0	115.3	14.3	14.3	52	33	#DIV/0!	558	0	20.53	181.7
689	63b	F3410 Sample	13.73	11.95	115.5	13.9	14.7	52	2	14.9	582	506	22.25	196.9
690	63b	F3410 Sample	13.62	11.86	115.3	13.4	14.9	50	25	14.8	592	515	27.66	244.8
691	63b	F3410 Sample	14.13	12.27	115.6	14.1	15	48	55	15.2	578	502	29.09	257.5
692	63b	F3410 Sample	13.96	12.15	115	14.2	14.7	47	8	14.9	582	506	33.68	298.1
693	63b	F3410 Sample	14.05	12.24	115.3	14	14.8	49	38	14.8	588	512	28.01	247.9
694	63b	F3410 Sample	14.04	12.19	115.2	14.2	14.6	52	37	15.2	588	510	20.16	178.4
695	63b	F3410 Sample	14.29	12.43	115.2	14	14.8	48	32	15.0	599	521	30.76	272.3
696	63b	F3410 Sample	13.77	11.99	115	14.1	14.7	51	49	14.8	578	503	22.32	197.6
697	25b	F3410 Sample	12.94	11.24	115	14.3	15.1	51	58	15.1	521	453	20.89	184.9
698	25b	F3410 Sample	13.81	12	115.4	14.4	14.3	53	35	15.1	581	505	17.60	155.8
699	25b	F3410 Sample	13.28	11.55	114.8	13.8	14.6	53	27	15.0	574	499	18.78	166.2
700	25b	F3410 Sample	13.42	11.67	114.5	13.9	14.9	50	6	15.0	566	492	26.95	238.5
701	25b	F3410 Sample	12.96	11.26	115.2	14.3	14.9	52	50	15.1	528	459	18.99	168.1
702	25b	F3410 Sample	13.35	11.61	114.5	14.1	14.6	51	46	15.0	566	493	22.61	200.1
703	25b	F3410 Sample	12.6	10.95	114.5	14.3	14.9	53	6	15.1	516	449	18.31	162.1
704	25b	F3410 Sample	13.2	11.47	115.1	14	14.6	53	45	15.1	561	488	17.56	155.4
705	59b	G.tr. Sample	12.27	10.7	115.4	13.3	14.6	57	5	14.7	548	478	9.04	80.0
706	59b	G.tr. Sample	14.16	12.34	115.2	14	14.8	50	20	14.7	593	517	26.24	232.2
707	59b	G.tr. Sample	13.58	11.83	115.1	13.9	13.9	55	22	14.8	611	532	13.95	123.5
708	59b	G.tr. Sample	13.16	11.45	115.3	14.1	14.7	55	10	14.9	551	479	13.45	119.0

709	59b	G.tr. Sample	13.57	11.83	115.7	13.6	14.4	50	51	14.7	599	522	26.78	237.0
710	59b	G.tr. Sample	12.82	11.19	115.3	13.7	13.9	55	55	14.6	584	510	12.61	111.6
711	59b	G.tr. Sample	13.47	11.73	115.1	13.9	14.4	53	44	14.8	585	509	18.05	159.7
712	59b	G.tr. Sample	13.35	11.63	115.5	14	13.8	55	24	14.8	598	521	13.80	122.2
713	15b	G.tr. Sample	11.24	9.8	115.4	14.4	14.4	56	17	14.7	470	410	10.28	91.0
714	15b	G.tr. Sample	12.09	10.53	114.6	14.4	14.7	56	0	14.8	498	434	10.82	95.8
715	15b	G.tr. Sample	11.65	10.15	115.2	13.8	14.9	56	2	14.8	492	428	11.31	100.1
716	15b	G.tr. Sample	11.7	10.2	114.7	14.7	14.8	56	0	14.7	469	409	10.41	92.2
717	15b	G.tr. Sample	11.81	10.3	114.7	14.5	14	55	55	14.7	507	442	11.47	101.5
718	15b	G.tr. Sample	12.29	10.68	115.2	14.3	14.8	56	15	15.1	504	438	10.20	90.3
719	15b	G.tr. Sample	11.61	10.14	115.3	13.7	14.6	56	23	14.5	503	440	10.66	94.4
720	15b	G.tr. Sample	11.88	10.37	114.9	14.4	14.6	56	0	14.6	492	429	10.90	96.4
721	13b	G.tr. Sample	11.23	9.76	115.3	14.2	14.2	56	30	15.1	483	420	10.05	88.9
722	13b	G.tr. Sample	12.03	10.52	114.8	14.6	14.9	55	39	14.4	482	421	11.34	100.4
723	13b	G.tr. Sample	12.37	10.78	115.2	14.3	14.6	55	30	14.7	514	448	12.36	109.4
724	13b	G.tr. Sample	12.57	10.94	114.1	14.6	14.8	55	37	14.9	510	444	11.51	101.8
725	13b	G.tr. Sample	11.54	10.04	115.4	14.5	14.7	56	0	14.9	469	408	10.71	94.8
726	13b	G.tr. Sample	12.1	10.52	115.3	14.3	14.9	55	50	15.0	493	428	11.23	99.4
727	13b	G.tr. Sample	11.83	10.31	115.3	14.1	14.8	56	19	14.7	492	428	10.24	90.7
728	13b	G.tr. Sample	11.59	10.09	114.9	14	15.1	56	12	14.9	477	415	10.47	92.6
729	1b	G.tr. Sample	11.77	0	115.6	14.1	14.4	54	58	#DIV/0!	501	0	14.28	126.4
730	1b	G.tr. Sample	11.29	0	115.2	13.6	14.6	55	32	#DIV/0!	494	0	13.26	117.3
731	1b	G.tr. Sample	11.97	0	115.5	13.8	14.9	55	21	#DIV/0!	504	0	13.21	116.9
732	1b	G.tr. Sample	11.83	0	115.1	14.1	14.2	55	35	#DIV/0!	513	0	12.75	112.8
733	1b	G.tr. Sample	12.72	0	115.3	13.9	14.5	55	0	#DIV/0!	547	0	14.40	127.5
734	1b	G.tr. Sample	11.45	0	115.5	14.2	14.4	55	20	#DIV/0!	485	0	13.12	116.1
735	1b	G.tr. Sample	11.45	0	115.6	13.7	14.4	56	32	#DIV/0!	502	0	10.37	91.8
736	1b	G.tr. Sample	11.67	0	115.4	14	14.6	57	21	#DIV/0!	495	0	7.60	67.2
737	94b	G.tr. Sample	12.45	10.87	115	13.5	14.6	53	56	14.5	549	480	18.05	159.7
738	94b	G.tr. Sample	12.74	11.12	115.4	14	14.4	53	32	14.6	548	478	18.39	162.8
739	94b	G.tr. Sample	13.36	11.66	115.5	14.6	14.5	51	52	14.6	546	477	21.32	188.7
740	94b	G.tr. Sample	12.06	10.52	115.3	14.2	14.4	53	45	14.6	512	446	17.42	154.1
741	94b	G.tr. Sample	12.67	11.05	115.2	14.1	14.7	53	38	14.7	531	463	17.56	155.4
742	94b	G.tr. Sample	11.45	9.98	115.2	14.2	14.5	55	4	14.7	483	421	13.76	121.7
743	94b	G.tr. Sample	9.85	8.58	115.3	14.2	14.1	56	32	14.8	427	372	10.02	88.7
744	94b	G.tr. Sample	11.69	10.15	114.9	14.5	14.2	56	41	15.2	494	429	9.23	81.7

745	14b	P.gi. Sample	11.57	0	115.2	14.3	14.2	55	37	#DIV/0!	495	0	12.38	109.6
746	14b	P.gi. Sample	12.14	0	115.2	14.1	14.7	55	48	#DIV/0!	508	0	11.73	103.8
747	14b	P.gi. Sample	13.04	0	114.4	14.1	14.6	56	35	#DIV/0!	554	0	9.65	85.4
748	14b	P.gi. Sample	11.54	0	114.9	13.9	14.5	55	53	#DIV/0!	498	0	11.92	105.5
749	14b	P.gi. Sample	12.08	0	114.4	14.1	14.6	55	45	#DIV/0!	513	0	11.94	105.7
750	14b	P.gi. Sample	11.61	0	114.6	14.1	13.9	55	0	#DIV/0!	517	0	14.70	130.1
751	14b	P.gi. Sample	12.48	0	115.4	14.4	14.4	56	0	#DIV/0!	522	0	11.05	97.8
752	14b	P.gi. Sample	11.62	0	115.3	14.4	14.7	56	12	#DIV/0!	476	0	10.29	91.1
753	6b	P.gi. Sample	11.53	10.03	114.6	14.1	14.9	56	48	15.0	479	417	8.86	78.4
754	6b	P.gi. Sample	11.56	10.05	115	14.3	15	56	46	15.0	469	407	8.70	77.0
755	6b	P.gi. Sample	11.96	10.4	115.2	14.2	14.6	56	15	15.0	501	435	10.45	92.5
756	6b	P.gi. Sample	11.21	9.76	114.8	14	14.3	56	18	14.9	488	425	10.77	95.3
757	6b	P.gi. Sample	12.43	10.83	114.9	14.1	14.7	54	46	14.8	522	455	14.53	128.6
758	6b	P.gi. Sample	12.35	10.72	114.4	14.3	14.6	54	45	15.2	517	449	14.36	127.1
759	6b	P.gi. Sample	10.28	8.97	115.1	13.9	14.6	55	24	14.6	440	384	13.19	116.7
760	6b	P.gi. Sample	12.48	10.87	114.8	14.4	14.9	55	30	14.8	507	441	11.98	106.0
761	11b	P.gi. Sample	11.2	9.75	114.6	14	14.6	56	5	14.9	478	416	11.15	98.7
762	11b	P.gi. Sample	10.88	9.47	114.5	14.3	14.7	55	48	14.9	452	393	11.47	101.6
763	11b	P.gi. Sample	12.46	10.85	115	14.5	14.6	55	0	14.8	512	446	13.40	118.6
764	11b	P.gi. Sample	10.86	9.44	114.2	13.8	14.9	56	10	15.0	462	402	10.94	96.8
765	11b	P.gi. Sample	12.02	10.45	114.8	14.2	14.2	55	30	15.0	519	451	12.84	113.7
766	11b	P.gi. Sample	12.62	10.97	115.1	14	14.7	54	47	15.0	533	463	14.64	129.6
767	11b	P.gi. Sample	12.21	10.61	114.3	14.1	14.6	55	32	15.1	519	451	12.54	111.0
768	11b	P.gi. Sample	10.73	9.29	114.6	14.3	14.2	56	0	15.5	461	399	11.33	100.2
769	7b	P.gi. Sample	12.38	10.76	115.3	14.3	14.6	55	12	15.1	514	447	13.16	116.5
770	7b	P.gi. Sample	11.8	10.25	114.7	14.3	14.8	56	3	15.1	486	422	10.73	95.0
771	7b	P.gi. Sample	11.54	10.01	115.3	14.3	14.5	56	10	15.3	483	419	10.64	94.2
772	7b	P.gi. Sample	12.06	10.48	115.1	14.3	15	56	15	15.1	488	424	10.06	89.1
773	7b	P.gi. Sample	11.71	10.17	114.9	14.1	14.8	56	9	15.1	488	424	10.70	94.7
774	7b	P.gi. Sample	12.3	10.68	114.8	14.3	14.7	54	28	15.2	510	443	15.00	132.8
775	7b	P.gi. Sample	11.34	9.84	114.9	14.2	14.5	56	2	15.2	479	416	11.12	98.4
776	7b	P.gi. Sample	11.75	10.21	114.9	14.4	14.4	56	20	15.1	493	429	10.15	89.8
777	12b	P.gi. Sample	11.93	10.38	115.1	14.3	14.8	55	56	14.9	490	426	11.04	97.7
778	12b	P.gi. Sample	11.04	9.61	114.8	14.1	14.5	55	48	14.9	470	409	11.89	105.2
779	12b	P.gi. Sample	11.81	10.27	114.9	14.4	14.6	55	40	15.0	489	425	11.78	104.3
780	12b	P.gi. Sample	10.99	9.59	114.8	14.2	14.6	55	57	14.6	462	403	11.27	99.8

781	12b	P.gi. Sample	11.72	10.21	115.1	14.3	14.6	55	43	14.8	488	425	11.78	104.2
782	12b	P.gi. Sample	11.55	10.07	115.2	14.2	14.4	55	42	14.7	490	427	12.12	107.2
783	12b	P.gi. Sample	10.86	9.46	115.3	14.1	14.3	56	28	14.8	467	407	10.18	90.1
784	12b	P.gi. Sample	12.05	10.52	115.4	14.3	14.8	55	48	14.5	493	431	11.40	100.9

23-7-01finished

							Enter File Name ->				Inch-lbf	Joules	
	Toughness Test						Enter Weight Position ->	5	Machine Constant =		716	80.90	
							Enter Initial Angle ->	60					
Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
1	70b	*G. trabeum*	11.52	9.91	115.3	14.9	14	55	48	16.2	479	412	11.31	100.1
2	70b	*G. trabeum*	7.87	6.7	112.3	14.8	14.1	57	20	17.5	336	286	7.26	64.3
3	70b	*G. trabeum*	10.69	9.24	113.1	14.6	14	56	12	15.7	462	400	10.58	93.6
4	70b	*G. trabeum*	9.17	7.9	114.1	14.7	13.8	57	23	16.1	396	341	7.36	65.1
5	70b	*G. trabeum*	10.02	8.22	114.5	14.9	14.1	57	2	21.9	417	342	7.98	70.7
6	70b	*G. trabeum*	9.76	8.41	114.8	14.1	13.4	57	53	16.1	450	388	6.56	58.0
7	70b	*G. trabeum*	9.43	8.13	113.6	15	14.1	58	20	16.0	392	338	4.47	39.6
8	70b	*G. trabeum*	9.94	8.34	114.7	14.1	13.4	56	5	19.2	459	385	12.02	106.3
9	17b	*G. trabeum*	8.01	6.98	115.4	13.7	12.5	58	50	14.8	405	353	4.07	36.0
10	17b	*G. trabeum*	9.69	8.36	114.7	14.2	13.6	56	0	15.9	437	377	11.95	105.8
11	17b	*G. trabeum*	10.32	8.94	115.2	14.4	13.7	54	6	15.4	454	393	16.94	150.0
12	17b	*G. trabeum*	10.37	8.98	114.2	14	14	54	38	15.5	463	401	15.80	139.9
13	17b	*G. trabeum*	10.29	8.92	115.3	14.7	13.4	53	27	15.4	453	393	18.56	164.3
14	17b	*G. trabeum*	8.87	7.71	115	13.5	13.2	57	51	15.0	433	376	7.23	64.0
15	17b	*G. trabeum*	11.02	9.55	114.7	14.8	13.8	54	30	15.4	470	408	15.07	133.3
16	17b	*G. trabeum*	9.93	8.59	115.2	14.4	14.1	57	45	15.6	425	367	6.41	56.7
17	54b	*G. trabeum*	9.76	8.15	114.2	14.5	13.6	56	48	19.8	433	362	9.30	82.3
18	54b	*G. trabeum*	10.36	8.92	115.3	14.4	14.6	54	0	16.1	427	368	16.16	143.0
19	54b	*G. trabeum*	10.38	8.96	115	13.8	14.2	56	0	15.8	461	398	11.97	105.9
20	54b	*G. trabeum*	10.98	9.49	114.7	14.7	14.3	56	45	15.7	455	394	8.79	77.8
21	8b	*G. trabeum*	10.04	8.72	114.8	14.4	13.8	58	30	15.1	440	382	4.38	38.8
22	8b	*G. trabeum*	12.56	10.83	114.9	15	14.5	56	42	16.0	503	433	8.53	75.5
23	8b	*G. trabeum*	11.2	9.71	114.9	14.9	13.9	56	32	15.3	471	408	9.44	83.5
24	8b	*G. trabeum*	11.45	9.93	114.7	14.9	14.3	56	45	15.3	469	406	8.61	76.2
25	8b	*G. trabeum*	12.17	10.52	114.4	14.6	13.9	57	30	15.7	524	453	7.06	62.5
26	8b	*G. trabeum*	12.79	11.06	115.2	14.6	14.4	54	50	15.6	528	457	13.88	122.8
27	8b	*G. trabeum*	9.09	7.87	114.8	15	13	57	32	15.5	406	352	7.15	63.2
28	8b	*G. trabeum*	10.06	8.74	114.4	14.7	13.3	57	33	15.1	450	391	7.16	63.3

29	79b	*G. trabeum*	9.16	8.01	114.8	14.3	13.6	58	47	14.4	410	359	3.65	32.3
30	79b	*G. trabeum*	11.71	10.18	115.4	13.9	13.7	54	55	15.0	533	463	15.49	137.1
31	79b	*G. trabeum*	10.62	9.21	114.2	14.3	14.2	56	18	15.3	458	397	10.49	92.9
32	79b	*G. trabeum*	12.21	10.56	115.1	14.7	13.6	53	49	15.6	531	459	17.30	153.1
33	79b	*G. trabeum*	11.75	10.16	115.3	14.7	13.8	54	8	15.6	502	434	16.21	143.4
34	79b	*G. trabeum*	12.95	11.22	115.8	14.2	13.9	54	20	15.4	567	491	16.41	145.3
35	79b	*G. trabeum*	11.7	10.16	115.4	14.4	14.1	56	40	15.2	499	434	9.44	83.5
36	79b	*G. trabeum*	9.34	7.93	114.3	14.2	14.4	57	0	17.8	400	339	8.51	75.4
37	61b	*G. trabeum*	13.27	11.5	115.5	15	13.9	54	52	15.4	551	478	13.70	121.3
38	61b	*G. trabeum*	12.3	10.62	115.1	14.5	14.2	53	42	15.8	519	448	17.23	152.5
39	61b	*G. trabeum*	12.85	11.16	115.2	14.9	14.3	54	37	15.1	524	455	14.09	124.7
40	61b	*G. trabeum*	12.26	10.62	114.3	14.9	14.3	55	17	15.4	503	436	12.40	109.7
41	61b	*G. trabeum*	12.17	10.56	115.1	15	13.9	53	56	15.2	507	440	16.11	142.6
42	61b	*G. trabeum*	11.3	9.83	115.2	13.8	14.1	58	0	15.0	504	439	6.09	53.9
43	61b	*G. trabeum*	10.69	9.08	114.5	14.3	13.4	57	13	17.7	487	414	8.41	74.4
44	61b	*G. trabeum*	13.16	11.4	114.4	14.7	14.5	55	45	15.4	540	468	11.28	99.8
45	54b	*G. trabeum*	10.67	9.27	114.8	15	14	54	35	15.1	443	385	14.33	126.9
46	54b	*G. trabeum*	9.13	7.67	114.2	14.6	14.1	57	0	19.0	388	326	8.33	73.7
47	54b	*G. trabeum*	8.2	7.14	114.2	14.1	13.8	58	25	14.8	369	321	4.78	42.3
48	54b	*G. trabeum*	12.36	10.71	114.7	14.9	14.4	54	50	15.4	502	435	13.45	119.0
49	69b	*O. ips* 308	13.65	11.71	114.9	15.2	14.2	52	35	16.6	550	472	18.74	165.8
50	69b	*O. ips* 308	12	10.36	115.3	14.9	14.2	51	32	15.8	492	425	21.92	194.0
51	69b	*O. ips* 308	11.81	10.2	115.5	14.6	14	52	4	15.8	500	432	21.56	190.9
52	69b	*O. ips* 308	13.47	11.65	115.3	14.9	13.9	51	32	15.6	564	488	22.39	198.2
53	69b	*O. ips* 308	12.14	10.5	114.8	14.9	14.1	55	10	15.6	503	435	12.87	113.9
54	69b	*O. ips* 308	13.32	11.51	114.7	15	14.5	47	5	15.7	534	461	31.49	278.7
55	42b	*O. ips* 308	14.2	12.26	115.2	14.9	14.5	53	0	15.8	571	493	17.90	158.5
56	42b	*O. ips* 308	13.2	11.42	114.6	14.7	14.2	53	17	15.6	552	477	17.94	158.8
57	42b	*O. ips* 308	12.72	11	115.5	14.6	14	53	16	15.6	539	466	18.43	163.2
58	42b	*O. ips* 308	13.39	11.46	114.6	14.9	14.5	52	55	16.8	541	463	18.11	160.3
59	42b	*O. ips* 308	13.34	11.51	115.2	14.9	14.4	52	21	15.9	540	466	19.62	173.7
60	42b	*O. ips* 308	13.38	11.54	115.5	14.8	14.4	52	38	15.9	544	469	19.13	169.3
61	43b	*O. ips* 308	12.6	10.85	115.5	15	14.7	50	43	16.1	495	426	22.86	202.3
62	43b	*O. ips* 308	12.14	10.47	115.4	14.4	14.4	49	56	16.0	507	438	26.82	237.4
63	41b	*O. ips* 308	13.04	11.23	115.4	14.8	14.4	53	17	16.1	530	457	17.51	155.0
64	41b	*O. ips* 308	13.1	11.28	115.5	14.9	14.5	52	18	16.1	525	452	19.61	173.6

65	41b	*O. ips* 308	12.48	10.76	115.3	15.2	14.6	51	17	16.0	488	421	21.25	188.0
66	41b	*O. ips* 308	12.97	11.19	115.6	15.1	14.6	50	12	15.9	509	439	23.97	212.1
67	41b	*O. ips* 308	12.46	10.74	115.2	15	13.8	53	46	16.0	523	450	16.65	147.4
68	41b	*O. ips* 308	12.59	10.91	115.6	14.9	14.3	51	0	15.4	511	443	23.06	204.1
69	41b	*O. ips* 308	12.97	11.18	115.1	14.9	14.5	50	30	16.0	522	450	23.93	211.8
70	41b	*O. ips* 308	12.98	11.22	113.8	15.1	14.6	52	49	15.7	517	447	17.85	158.0
71	67b	*O. ips* 308	13.43	11.61	115.4	15	14.5	49	50	15.7	535	463	25.24	223.4
72	67b	*O. ips* 308	12	10.39	115.7	14.8	14.1	54	25	15.5	497	430	14.96	132.4
73	67b	*O. ips* 308	11.63	10.05	115.5	14.6	14.7	52	50	15.7	469	405	18.64	165.0
74	67b	*O. ips* 308	13.05	11.31	115.2	15	13.9	49	20	15.4	543	471	27.53	243.7
75	67b	*O. ips* 308	12.1	10.48	115.1	15	14	56	32	15.5	501	434	9.27	82.1
76	67b	*O. ips* 308	12.22	10.6	114.9	14.4	14	52	57	15.3	528	458	19.68	174.2
77	75b	*O. ips* 308	11.32	9.81	115.5	14.8	14.5	52	38	15.4	457	396	19.00	168.1
78	75b	*O. ips* 308	11.16	9.66	114.8	14.7	14.2	53	25	15.5	466	403	17.60	155.8
79	75b	*O. ips* 308	10.39	8.99	114.7	14.2	14.6	54	23	15.6	437	378	15.49	137.1
80	75b	*O. ips* 308	12.74	11.04	114.4	14.9	13.5	53	32	15.4	554	480	17.82	157.7
81	75b	*O. ips* 308	13.32	11.54	115.7	14.4	14.7	52	35	15.4	544	471	19.67	174.1
82	75b	*O. ips* 308	12.56	10.89	115.3	14.7	14.4	53	22	15.3	515	446	17.48	154.7
83	92b	*S. sapinea* 4	12.27	10.53	115.4	14.7	14	50	45	16.5	517	443	24.68	218.4
84	92b	*S. sapinea* 4	14.43	12.35	115.6	14.8	14.3	48	37	16.8	590	505	29.02	256.9
85	92b	*S. sapinea* 4	14.71	12.63	115.5	14.7	14.3	47	14	16.5	606	520	32.59	288.5
86	92b	*S. sapinea* 4	12.73	10.93	114.9	14.6	14.3	51	10	16.5	531	456	23.38	206.9
87	92b	*S. sapinea* 4	14.4	12.36	114.8	14.7	14	48	23	16.5	610	523	30.52	270.2
88	92b	*S. sapinea* 4	14.68	12.62	115.7	14.7	14.1	47	18	16.3	612	526	32.90	291.2
89	92b	*S. sapinea* 4	14.05	12.07	115.5	14.9	14.2	51	17	16.4	575	494	22.53	199.4
90	92b	*S. sapinea* 4	14.93	12.85	114.7	14.8	14.3	47	20	16.2	615	529	32.02	283.4
91	16b	*S. sapinea* 4	12.27	10.57	115.4	14.9	13.7	53	25	16.1	521	449	17.86	158.1
92	16b	*S. sapinea* 4	11.85	10.19	115.6	14.8	13.6	51	26	16.3	509	438	23.38	206.9
93	16b	*S. sapinea* 4	11.41	9.8	115.4	14.3	13.9	52	2	16.4	497	427	22.52	199.3
94	16b	*S. sapinea* 4	12.23	10.5	114.5	14.8	14.1	54	25	16.5	512	439	14.96	132.4
95	16b	*S. sapinea* 4	12.12	10.42	115.2	14.5	14.5	54	46	16.3	500	430	14.10	124.8
96	16b	*S. sapinea* 4	12.64	10.83	115.6	15.1	14.5	54	32	16.7	499	428	13.82	122.3
97	16b	*S. sapinea* 4	12.76	10.94	115.3	14.9	14.2	55	48	16.6	523	448	11.15	98.7
98	16b	*S. sapinea* 4	12.53	10.76	115.2	14.7	14.5	55	21	16.4	510	438	12.31	109.0
99	78b	*S. sapinea* 4	11.51	9.89	115.1	14.4	14.5	50	7	16.4	479	412	26.18	231.7
100	78b	*S. sapinea* 4	13.92	11.98	114.9	14.8	14.3	48	43	16.2	572	493	28.79	254.8

101	78b	*S. sapinea* 4	12.85	11.04	115.4	14.9	14.1	51	16	16.4	530	455	22.73	201.2
102	78b	*S. sapinea* 4	12.09	10.39	115.5	14.2	14.5	51	47	16.4	508	437	22.47	198.9
103	78b	*S. sapinea* 4	12.21	10.47	115.4	14.5	14.6	52	3	16.6	500	429	20.94	185.3
104	78b	*S. sapinea* 4	13.97	12	115.1	14.6	14.4	49	26	16.4	577	496	27.47	243.1
105	78b	*S. sapinea* 4	13.32	11.45	115	14.2	14.2	51	6	16.3	574	494	24.75	219.1
106	78b	*S. sapinea* 4	12.26	10.54	114.9	14.7	14.3	52	0	16.3	508	436	21.06	186.4
107	38b	*S. sapinea* 4	13.43	11.47	115.6	14.5	14	53	18	17.1	572	489	18.54	164.1
108	38b	*S. sapinea* 4	13.79	11.83	114.9	14.9	14.6	50	5	16.6	552	473	24.74	219.0
109	38b	*S. sapinea* 4	13.63	11.67	115.2	15	14.6	52	56	16.8	540	463	17.76	157.2
110	38b	*S. sapinea* 4	13.99	12.02	114.6	14.8	14.6	52	32	16.4	565	485	19.11	169.1
111	38b	*S. sapinea* 4	12.93	11.1	114.9	15.1	14.6	51	39	16.5	510	438	20.61	182.4
112	38b	*S. sapinea* 4	12.75	10.96	115.1	14.8	13.7	52	0	16.3	546	470	21.75	192.5
113	38b	*S. sapinea* 4	12.71	10.92	115.3	14.8	13.8	52	21	16.4	540	464	20.69	183.1
114	38b	*S. sapinea* 4	13.93	11.98	114.4	15	14.6	50	18	16.3	556	478	23.98	212.3
115	103b	*S. sapinea* 4	11.53	9.92	115.1	14	14	53	10	16.2	511	440	19.95	176.6
116	103b	*S. sapinea* 4	11.29	9.71	115.2	14.4	14	55	36	16.3	486	418	12.47	110.4
117	103b	*S. sapinea* 4	13.51	11.54	114.3	15	14.4	51	50	17.1	547	467	20.67	182.9
118	103b	*S. sapinea* 4	12.74	10.9	115.3	14.9	13.9	51	26	16.9	534	456	22.64	200.4
119	103b	*S. sapinea* 4	12.44	10.65	115.6	14.8	13.7	53	50	16.8	531	454	16.95	150.0
120	103b	*S. sapinea* 4	11.11	9.51	115.1	14.7	14.1	54	13	16.8	466	399	15.64	138.5
121	103b	*S. sapinea* 4	12.73	10.89	115.1	14.8	14	51	51	16.9	534	457	21.66	191.7
122	103b	*S. sapinea* 4	11.53	9.84	114.8	15.1	14.2	54	7	17.2	468	400	15.15	134.1
123	104b	*S. sapinea* 4	12.32	10.55	115.5	14.7	13.8	52	15	16.8	526	450	21.17	187.4
124	104b	*S. sapinea* 4	13.19	11.31	115.3	14.6	14.6	52	23	16.6	537	460	19.89	176.0
125	104b	*S. sapinea* 4	12.08	10.36	115.7	14.6	13.7	52	49	16.6	522	448	20.04	177.4
126	104b	*S. sapinea* 4	11.89	10.22	115.3	14.8	13.4	53	50	16.3	520	447	17.33	153.4
127	104b	*S. sapinea* 4	11.87	10.16	115.2	14.8	13.2	52	33	16.8	527	451	21.09	186.7
128	104b	*S. sapinea* 4	11.39	9.77	114.9	14.4	13.8	53	42	16.6	499	428	17.92	158.6
129	104b	*S. sapinea* 4	11.97	10.28	115.2	14.6	13.8	53	35	16.4	516	443	17.86	158.0
130	104b	*S. sapinea* 4	12.35	10.6	115.7	14.1	14.7	53	6	16.5	515	442	18.97	167.9
131	41a	Controls *O. ips*	13.34	11.6	115.2	15.1	14.4	50	51	15.0	533	463	22.78	201.6
132	41a	Controls *O. ips*	11.82	10.26	115.1	14.6	13.7	53	10	15.2	513	446	19.11	169.1
133	41a	Controls *O. ips*	12.28	10.67	115	14.8	14.4	50	38	15.1	501	435	24.02	212.6
134	41a	Controls *O. ips*	13.38	11.6	115.4	15	14.3	51	41	15.3	541	469	21.18	187.4
135	41a	Controls *O. ips*	13.7	11.89	115.4	14.6	14.5	53	2	15.2	561	487	18.39	162.8
136	41a	Controls *O. ips*	13.63	11.83	113.9	14.7	14.5	51	52	15.2	561	487	21.10	186.7

137	41a	Controls *O. ips*	13.78	11.96	115.8	14.8	14.4	51	37	15.2	558	485	21.63	191.5
138	41a	Controls *O. ips*	13.87	12.05	115.6	14.8	14.4	53	23	15.1	563	489	17.26	152.7
139	42a	Controls *O. ips*	13.18	11.45	115.3	14.7	14.6	49	23	15.1	533	463	26.93	238.3
140	42a	Controls *O. ips*	13.4	11.64	115.5	14.9	14.2	53	56	15.1	548	476	15.93	141.0
141	42a	Controls *O. ips*	13.07	11.31	115.6	15.1	14.4	54	0	15.6	520	450	15.23	134.8
142	42a	Controls *O. ips*	14	12.18	115.5	14.8	14.1	52	15	14.9	581	505	20.50	181.5
143	42a	Controls *O. ips*	12.68	10.99	115.3	14.8	14.5	55	0	15.4	512	444	13.07	115.7
144	42a	Controls *O. ips*	13.61	11.82	115.2	15.2	14.7	53	14	15.1	529	459	16.58	146.7
145	42a	Controls *O. ips*	13.13	11.41	114.8	15.1	14.2	53	58	15.1	533	464	15.52	137.4
146	42a	Controls *O. ips*	13.75	11.92	114.9	14.9	14.5	52	55	15.4	554	480	18.11	160.3
147	43a	Controls *O. ips*	12.94	11.26	115.4	14.9	14.2	53	20	14.9	530	461	17.45	154.4
148	43a	Controls *O. ips*	13.65	11.85	115.1	14.5	14.5	53	54	15.2	564	490	16.36	144.8
149	43a	Controls *O. ips*	13.58	11.81	115.6	14.8	13.9	54	20	15.0	571	497	15.40	136.3
150	43a	Controls *O. ips*	13.09	11.35	114.9	14.5	14	50	3	15.3	561	487	26.99	238.9
151	43a	Controls *O. ips*	12.56	10.9	115.1	13.8	14.7	53	42	15.2	538	467	17.97	159.0
152	43a	Controls *O. ips*	14.5	12.61	115.4	14.4	14.5	54	36	15.0	602	523	14.70	130.1
153	43a	Controls *O. ips*	13.35	11.6	115.6	14.5	14.8	53	0	15.1	538	468	18.29	161.9
154	43a	Controls *O. ips*	13.46	11.71	115.4	14.3	14.3	53	32	14.9	570	496	17.93	158.7
155	67a	Controls *O. ips*	11.42	9.94	115.3	14.7	14.3	54	8	14.9	471	410	15.64	138.4
156	67a	Controls *O. ips*	11.83	10.29	115.2	14.9	14.6	52	0	15.0	472	411	20.20	178.8
157	67a	Controls *O. ips*	11.38	9.91	114.8	14.5	13.7	53	32	14.8	499	435	18.31	162.1
158	67a	Controls *O. ips*	13.38	11.64	114.9	14.6	14.1	52	0	14.9	566	492	21.58	191.0
159	67a	Controls *O. ips*	12.34	10.71	114.8	14.9	13.9	54	33	15.2	519	450	14.67	129.9
160	67a	Controls *O. ips*	13.22	11.51	115.6	14.5	14.2	50	48	14.9	555	484	24.72	218.8
161	67a	Controls *O. ips*	13.45	11.71	115.5	15.2	14.6	51	11	14.9	525	457	21.48	190.1
162	67a	Controls *O. ips*	11.01	9.58	115	14.2	14	56	38	14.9	482	419	9.81	86.8
163	75a	Controls *O. ips*	11.9	10.36	115.3	14.6	14.6	54	43	14.9	484	422	13.99	123.8
164	75a	Controls *O. ips*	12.3	10.71	115.1	14.7	14.1	54	55	14.8	516	449	13.81	122.2
165	75a	Controls *O. ips*	11.23	9.78	115	14.5	14.3	53	47	14.8	471	410	16.89	149.5
166	75a	Controls *O. ips*	12.79	11.13	115.2	14.5	14.5	54	6	14.9	528	460	15.84	140.2
167	75a	Controls *O. ips*	12.17	10.6	114.7	14.5	13.6	54	8	14.8	538	469	16.80	148.7
168	75a	Controls *O. ips*	10.82	9.38	115.5	14.3	14.6	53	30	15.4	449	389	17.64	156.2
169	75a	Controls *O. ips*	11.41	9.9	115.1	14.9	14.4	54	37	15.3	462	401	14.00	123.9
170	75a	Controls *O. ips*	10.79	9.38	114.8	14.6	14.5	54	27	15.0	444	386	14.77	130.7
171	69a	Controls *O. ips*	12.82	11.14	114.9	14.7	14.2	51	40	15.1	535	464	22.04	195.1
172	69a	Controls *O. ips*	12.15	10.53	115	15	14.4	54	22	15.4	489	424	14.47	128.1

173	69a	Controls *O. ips*	12.43	10.81	115.3	15	14.2	53	7	15.0	506	440	17.80	157.6
174	69a	Controls *O. ips*	12.36	10.79	114.6	14.9	14.3	52	37	14.6	506	442	19.10	169.1
175	69a	Controls *O. ips*	11.12	9.66	115.4	15.1	14.5	55	31	15.1	440	382	11.40	100.9
176	69a	Controls *O. ips*	11.97	10.41	114.8	14.7	14.2	53	21	15.0	500	434	17.77	157.3
177	69a	Controls *O. ips*	12.88	11.14	115.4	14.5	14.1	56	15	15.6	546	472	10.48	92.8
178	69a	Controls *O. ips*	12.43	10.84	115.4	14.7	14	54	32	14.7	523	456	14.92	132.0
179	61a	Contr. *G. trab.*	13.61	11.82	115.5	14.7	14.2	47	44	15.1	565	490	31.64	280.1
180	61a	Contr. *G. trab.*	13.93	12.08	115.2	14.8	13.7	49	30	15.3	596	517	28.10	248.7
181	61a	Contr. *G. trab.*	13.55	11.76	115.4	14.5	14.2	46	54	15.2	570	495	34.32	303.8
182	61a	Contr. *G. trab.*	14.15	12.25	115.5	14.9	14.1	49	30	15.5	583	505	27.02	239.2
183	61a	Contr. *G. trab.*	13.43	11.65	115.5	14.8	13.9	51	17	15.3	565	490	23.26	205.8
184	61a	Contr. *G. trab.*	13.87	12.03	114.9	14.6	14.4	52	4	15.3	574	498	20.97	185.6
185	61a	Contr. *G. trab.*	15.04	13.08	115.7	14.9	14.4	46	32	15.0	606	527	33.28	294.5
186	61a	Contr. *G. trab.*	14.71	12.78	115.3	14.9	14.5	53	30	15.1	591	513	16.67	147.6
187	8a	Contr. *G. trab.*	12.47	10.83	115.3	15.1	14.5	54	48	15.1	494	429	13.17	116.5
188	8a	Contr. *G. trab.*	11.43	9.92	115.1	13.9	14.3	54	47	15.2	500	434	15.22	134.7
189	8a	Contr. *G. trab.*	10.63	9.21	115.4	13.6	13.8	55	32	15.4	491	425	14.03	124.1
190	8a	Contr. *G. trab.*	11.93	10.38	115	14.2	14.5	55	20	14.9	504	438	13.03	115.3
191	8a	Contr. *G. trab.*	11	9.56	115.1	14.3	13.9	56	18	15.1	481	418	10.72	94.9
192	8a	Contr. *G. trab.*	12.51	10.88	115.4	15	14.6	54	47	15.0	495	431	13.25	117.3
193	8a	Contr. *G. trab.*	11.67	10.16	115.2	14.2	14.3	54	33	14.9	499	434	15.36	136.0
194	8a	Contr. *G. trab.*	12.99	11.29	115.3	15	14.6	53	39	15.1	514	447	16.02	141.8
195	79a	Contr. *G. trab.*	13.43	11.68	114.9	14.9	14.6	50	0	15.0	537	467	24.94	220.7
196	79a	Contr. *G. trab.*	11.28	9.8	115.4	14.6	13.8	53	31	15.1	485	421	18.03	159.6
197	79a	Contr. *G. trab.*	11.97	10.42	115	14.5	14.3	49	43	14.9	502	437	27.25	241.2
198	79a	Contr. *G. trab.*	12.73	11.06	115.8	14.5	14	53	4	15.1	542	470	19.16	169.6
199	79a	Contr. *G. trab.*	11.93	10.39	115.5	14.2	14	54	20	14.8	520	452	16.30	144.2
200	79a	Contr. *G. trab.*	13.27	11.56	114.9	14.5	14.4	50	57	14.8	553	482	24.01	212.5
201	79a	Contr. *G. trab.*	13.23	11.48	115.4	15	14.2	53	56	15.2	538	467	15.77	139.6
202	79a	Contr. *G. trab.*	12.7	10.97	115.5	14.5	14.1	52	0	15.8	538	465	21.81	193.1
203	70a	Contr. *G. trab.*	11.75	10.17	114.8	14.5	14.5	54	23	15.5	487	421	15.10	133.7
204	70a	Contr. *G. trab.*	10.47	9.1	115.5	14.7	14.4	55	55	15.1	428	372	10.92	96.6
205	70a	Contr. *G. trab.*	11.31	9.84	115.2	14.3	13.5	53	3	14.9	509	442	20.35	180.1
206	70a	Contr. *G. trab.*	12.07	10.5	115.4	14.8	14.1	54	19	15.0	501	436	15.22	134.7
207	70a	Contr. *G. trab.*	10.69	9.26	115.6	15	14.2	56	6	15.4	434	376	10.26	90.8
208	70a	Contr. *G. trab.*	12.13	10.5	115	14.8	13.4	55	52	15.5	532	460	11.75	104.0

209	70a	Contr. *G. trab.*	11.07	9.61	115.3	14.8	14.2	54	44	15.2	457	397	14.04	124.3
210	70a	Contr. *G. trab.*	11.83	10.28	114.6	14.8	14.3	50	8	15.1	488	424	25.40	224.8
211	17a	Contr. *G. trab.*	11.73	10.22	115.5	14.3	13.9	52	48	14.8	511	445	20.44	180.9
212	17a	Contr. *G. trab.*	11.66	10.16	115.8	14.3	13.6	54	6	14.8	518	451	17.25	152.7
213	17a	Contr. *G. trab.*	11.68	10.18	115.2	14.7	13.9	52	5	14.7	496	432	21.45	189.8
214	17a	Contr. *G. trab.*	12.31	10.75	115.5	14.5	14	51	30	14.5	525	458	23.27	206.0
215	17a	Contr. *G. trab.*	11.5	10.01	115.7	14.3	14	50	42	14.9	496	432	25.88	229.1
216	17a	Contr. *G. trab.*	12.16	10.58	115.3	15	13.6	52	7	14.9	517	450	21.16	187.3
217	17a	Contr. *G. trab.*	11.97	10.42	115.4	14.6	14.4	50	15	14.9	493	429	25.48	225.5
218	17a	Contr. *G. trab.*	12.46	10.84	115.4	14.5	14.2	53	11	14.9	524	456	18.59	164.5
219	54a	Contr. *G. trab.*	12.79	11.12	115.4	14.6	14.7	51	30	15.0	516	449	21.93	194.1
220	54a	Contr. *G. trab.*	12.8	11.13	115.7	14.4	14.3	52	38	15.0	537	467	20.09	177.9
221	54a	Contr. *G. trab.*	12.3	10.72	114.8	15	14.4	50	51	14.7	496	432	23.02	203.7
222	54a	Contr. *G. trab.*	12.42	10.82	114.6	15	14.4	52	27	14.8	502	437	19.18	169.8
223	54a	Contr. *G. trab.*	12.91	11.24	115.2	14.6	14.3	53	31	14.9	537	467	17.40	154.0
224	54a	Contr. *G. trab.*	12.09	10.55	115.4	14.1	14.2	54	14	14.6	523	457	16.52	146.2
225	54a	Contr. *G. trab.*	12.04	10.5	116	14.1	14.3	51	48	14.7	515	449	22.99	203.5
226	54a	Contr. *G. trab.*	12.21	10.64	114.9	14.3	14.2	51	8	14.8	523	456	24.40	215.9
227	104a	Contr. *S. sap.*	12.02	10.47	114.5	13.9	13.9	51	14	14.8	543	473	25.76	228.0
228	104a	Contr. *S. sap.*	12.37	10.76	115.1	13.9	13.8	51	15	15.0	560	487	25.90	229.2
229	104a	Contr. *S. sap.*	12.81	11.14	115.2	14.7	14.6	52	17	15.0	518	451	19.93	176.4
230	104a	Contr. *S. sap.*	12.38	10.77	115.6	13.9	13.9	49	16	14.9	554	482	31.15	275.7
231	104a	Contr. *S. sap.*	12.31	10.7	114.7	14.8	13.9	53	25	15.0	522	453	17.79	157.5
232	104a	Contr. *S. sap.*	11.88	10.33	115.2	14.2	13.4	50	58	15.0	542	471	26.60	235.4
233	104a	Contr. *S. sap.*	12.4	10.79	115.3	14.2	13.8	52	37	14.9	549	478	21.32	188.7
234	104a	Contr. *S. sap.*	12.52	10.9	115.7	14.1	13.7	49	30	14.9	560	488	30.29	268.1
235	78a	Contr. *S. sap.*	10.85	9.44	115.4	14.4	14.2	52	37	14.9	460	400	20.28	179.5
236	78a	Contr. *S. sap.*	12.51	10.9	114.8	14.4	14.2	51	0	14.8	533	464	24.48	216.6
237	78a	Contr. *S. sap.*	10.72	9.31	115	14.8	14.5	55	22	15.1	434	377	12.14	107.4
238	78a	Contr. *S. sap.*	12.3	10.71	115	14.2	14.1	53	20	14.8	534	465	18.92	167.5
239	78a	Contr. *S. sap.*	12.94	11.27	115.2	14.7	14.3	50	56	14.8	534	465	23.71	209.8
240	78a	Contr. *S. sap.*	13.32	11.61	115.4	14.4	14.5	51	49	14.7	553	482	21.91	193.9
241	78a	Contr. *S. sap.*	13.34	11.61	115	14.8	14.6	52	17	14.9	537	467	19.72	174.5
242	78a	Contr. *S. sap.*	12.79	11.09	114.7	14.3	14.5	49	28	15.3	538	466	28.08	248.6
243	38a	Contr. *S. sap.*	12.61	10.92	115.5	15	13.9	52	47	15.5	524	453	19.03	168.4
244	38a	Contr. *S. sap.*	12.8	11.09	115.3	14.9	14.7	52	23	15.4	507	439	19.14	169.4

245	38a	Contr. *S. sap.*	11.92	10.34	114.8	14.6	14	53	18	15.3	508	441	18.35	162.4
246	38a	Contr. *S. sap.*	11.75	10.2	115.1	14.1	14.7	50	10	15.2	493	428	26.55	235.0
247	38a	Contr. *S. sap.*	12.28	10.64	115.5	15	14	51	34	15.4	506	439	21.92	194.0
248	38a	Contr. *S. sap.*	13.21	11.43	115	14.9	15	54	3	15.6	514	445	14.80	131.0
249	38a	Contr. *S. sap.*	13.04	11.32	114.9	15.1	14.7	51	53	15.2	511	444	19.92	176.3
250	38a	Contr. *S. sap.*	13.38	11.62	114.6	15	14.7	52	13	15.1	529	460	19.34	171.2
251	103a	Contr. *S. sap.*	11.51	9.99	114.3	14.1	14.6	54	4	15.2	489	425	16.52	146.2
252	103a	Contr. *S. sap.*	11.74	10.2	115.1	14.4	14	54	3	15.1	506	440	16.72	148.0
253	103a	Contr. *S. sap.*	11.79	10.26	115.6	14.2	14	52	6	14.9	513	446	22.42	198.4
254	103a	Contr. *S. sap.*	12.55	10.91	115.8	14.7	13.8	54	42	15.0	534	464	14.69	130.0
255	103a	Contr. *S. sap.*	12.75	11.08	115	14.7	14.3	52	30	15.1	527	458	19.80	175.3
256	103a	Contr. *S. sap.*	12.73	11.05	115.4	14.4	14.1	53	45	15.2	543	472	17.41	154.1
257	103a	Contr. *S. sap.*	12.01	10.41	115.3	14.6	14.3	53	2	15.4	499	432	18.65	165.0
258	103a	Contr. *S. sap.*	11.36	9.87	115.5	14.3	13.8	51	20	15.1	498	433	24.57	217.4
259	92a	Contr. *S. sap.*	12.35	10.73	115.5	14.3	14.1	50	5	15.1	530	461	27.30	241.6
260	92a	Contr. *S. sap.*	12.26	10.64	115.7	14.5	14.1	50	38	15.2	518	450	25.32	224.1
261	92a	Contr. *S. sap.*	14.3	12.44	115.4	14.5	14	46	54	15.0	610	531	34.81	308.1
262	92a	Contr. *S. sap.*	14.39	12.5	115.2	14.8	14.3	49	6	15.1	590	513	27.88	246.7
263	92a	Contr. *S. sap.*	13.76	11.94	115.4	14.4	14.1	51	56	15.2	587	510	22.22	196.7
264	92a	Contr. *S. sap.*	13.04	11.31	115.8	14.3	14.3	50	36	15.3	551	478	25.60	226.6
265	92a	Contr. *S. sap.*	12.26	10.63	114.5	14.6	14.1	51	52	15.3	520	451	21.92	194.0
266	92a	Contr. *S. sap.*	11.78	10.22	115.1	14.4	13.8	50	30	15.3	515	447	26.50	234.6
267	16a	Contr. *S. sap.*	11.73	10.2	114.8	14.9	14.1	54	38	15.0	486	423	14.25	126.1
268	16a	Contr. *S. sap.*	12.17	10.56	115.6	14.9	14.2	54	30	15.2	498	432	14.49	128.3
269	16a	Contr. *S. sap.*	11.6	10.08	115.4	14.6	13.7	49	10	15.1	503	437	29.55	261.5
270	16a	Contr. *S. sap.*	12.29	10.68	115.5	14.6	14.6	53	52	15.1	499	434	16.16	143.0
271	16a	Contr. *S. sap.*	12.4	10.75	115.6	14.9	14.4	54	14	15.3	500	433	14.96	132.4
272	16a	Contr. *S. sap.*	11.73	10.21	115.3	14	13.8	51	36	14.9	527	458	24.64	218.1
273	16a	Contr. *S. sap.*	12.11	10.57	115.5	14.4	14.6	54	49	14.6	499	435	14.03	124.1
274	16a	Contr. *S. sap.*	11.79	10.27	115.6	14.6	14.3	49	18	14.8	489	426	27.98	247.7

Toughness Test

Enter File Name -> 24-7-01

Enter Weight Position -> 5

Enter Initial Angle -> 60

Machine Constant = Inch-lbf 716 Joules 80.90

Lab No	Specimen ID	Identification	w1	wo	Length	Width (Breadth)	Height (Depth)	Final Reading		Moisture Content	Db	Dn	Toughness	Toughness
			(g)	(g)	(mm)	(mm)	(mm)	Degrees	Minutes	(%)	(kg/m3)	(kg/m3)	(Joules)	(in/lbf)
1	68a	Controls 308	12.69	10.82	114.5	14.6	13.8	54	45	17.3	550	469	14.71	130.2
2	68a	Controls 308	11.81	10.11	114.5	14.6	14.1	53	3	16.8	501	429	18.87	167.0
3	68a	Controls 308	11.92	10.17	115.5	14.7	14.5	53	2	17.2	484	413	18.20	161.1
4	68a	Controls 308	11.64	9.94	115.1	14.9	14.5	54	23	17.1	468	400	14.48	128.2
5	68a	Controls 308	13	11.13	115.2	14.7	14.6	49	45	16.8	526	450	26.06	230.6
6	68a	Controls 308	13.03	11.11	115.1	15.1	14.5	54	8	17.3	517	441	14.80	131.0
7	68a	Controls 308	11.12	9.52	114.8	14.5	12.8	53	33	16.8	522	447	19.55	173.1
8	68a	Controls 308	11.49	9.83	114.9	14.7	14	51	43	16.9	486	416	22.23	196.8
9	50a	Controls 308	13.19	11.28	115.5	14.4	14.6	51	31	16.9	543	465	22.51	199.2
10	50a	Controls 308	12.9	11.05	115	14.6	14.2	53	16	16.7	541	463	18.17	160.9
11	50a	Controls 308	13.46	11.53	115.6	14.7	14.3	48	2	16.7	554	474	30.71	271.8
12	50a	Controls 308	12.8	10.95	115	14.5	14.4	52	37	16.9	533	456	19.79	175.1
13	50a	Controls 308	13.6	11.63	114.4	15	14.6	53	54	16.9	543	464	15.42	136.4
14	50a	Controls 308	13.03	11.15	115.4	14.3	14.4	50	23	16.9	548	469	25.97	229.9
15	50a	Controls 308	13.21	11.32	115.3	14.6	14.1	52	38	16.7	557	477	19.95	176.6
16	50a	Controls 308	13.64	11.66	114.8	14.7	14.5	52	56	17.0	557	477	18.45	163.3
17	2a	Controls 308	12.41	10.64	115.3	15	14.4	52	52	16.6	498	427	18.17	160.8
18	2a	Controls 308	12.44	10.67	115.2	14.8	14.4	49	54	16.6	507	435	25.79	228.2
19	2a	Controls 308	12.37	10.61	115.1	14.5	14.4	54	4	16.6	515	441	16.04	141.9
20	2a	Controls 308	11.97	10.27	115.6	14.3	14.6	54	30	16.6	496	426	15.02	132.9
21	2a	Controls 308	12.32	10.58	115.4	14.8	14.3	53	38	16.4	504	433	16.75	148.2
22	2a	Controls 308	12.09	10.38	115.4	14.8	14.2	52	18	16.5	499	428	20.23	179.1
23	2a	Controls 308	12.23	10.48	115.5	14.6	14.2	53	53	16.7	511	438	16.57	146.7
24	2a	Controls 308	12.2	10.46	114.5	14.9	14.4	49	37	16.6	497	426	26.19	231.8
25	97a	Controls 308	13.33	11.46	115.2	14.7	14.2	50	48	16.3	554	477	24.21	214.2
26	97a	Controls 308	13.71	11.76	115	14.8	14.6	49	50	16.6	552	473	25.59	226.5
27	97a	Controls 308	11.21	9.6	114.3	14.6	14.6	53	48	16.8	460	394	16.33	144.5
28	97a	Controls 308	12.74	10.92	114.9	14.8	14.5	52	38	16.7	517	443	19.00	168.1

29	97a	Controls 308	12.19	10.44	115.6	15.1	13.9	53	56	16.8	502	430	15.94	141.1
30	97a	Controls 308	11.94	10.25	115.5	14.5	14.5	53	6	16.5	492	422	18.42	163.0
31	97a	Controls 308	12.34	10.58	115.2	14.7	14.2	49	38	16.6	513	440	27.08	239.6
32	97a	Controls 308	12.15	10.4	115.2	15.1	14.1	53	11	16.8	495	424	17.58	155.6
33	22a	Controls 308	13.83	11.84	115.5	14.4	14.2	54	7	16.8	586	501	16.30	144.3
34	22a	Controls 308	13.56	11.63	115.4	14.5	14.4	52	37	16.6	563	483	19.79	175.1
35	22a	Controls 308	13.55	11.61	115.2	14.8	14.2	52	30	16.7	560	480	19.73	174.6
36	22a	Controls 308	13.21	11.35	115.2	14.8	14.2	50	2	16.4	546	469	25.83	228.6
37	22a	Controls 308	14.66	12.59	115	14.8	14.4	46	23	16.4	598	514	33.96	300.6
38	22a	Controls 308	14.23	12.18	114.9	14.8	14.2	49	2	16.8	589	504	28.23	249.9
39	22a	Controls 308	12.22	10.51	115.8	14.8	13.9	50	36	16.3	513	441	24.97	221.0
40	22a	Controls 308	11.28	9.69	114.8	14.5	13.2	53	37	16.4	513	441	18.77	166.1
41	76a	Controls 294	11.85	10.19	115.6	14.5	14.4	53	13	16.3	491	422	18.24	161.5
42	76a	Controls 294	11.63	9.93	115.3	14.8	13.1	55	38	17.1	520	444	12.68	112.2
43	76a	Controls 294	11.05	9.47	115.3	14.2	14	55	48	16.7	482	413	12.18	107.8
44	76a	Controls 294	10.9	9.3	115.3	15.1	14.4	54	21	17.2	435	371	14.37	127.2
45	76a	Controls 294	11.68	10.01	115.2	14.8	13.6	52	17	16.7	504	432	21.17	187.4
46	76a	Controls 294	9.72	8.33	115.1	15	13.7	55	12	16.7	411	352	13.02	115.3
47	76a	Controls 294	13.16	11.29	115.7	15.1	14.6	51	25	16.6	516	443	21.15	187.2
48	76a	Controls 294	11.68	9.99	115.4	14.5	14.8	53	46	16.9	472	403	16.36	144.8
49	31a	Controls 294	13.8	11.81	115.5	14.3	14.3	49	36	16.9	584	500	28.14	249.1
50	31a	Controls 294	13.25	11.35	115.3	14.7	14.4	53	8	16.7	543	465	18.07	159.9
51	31a	Controls 294	13.4	11.52	115.4	14.5	14.5	52	34	16.3	552	475	19.78	175.0
52	31a	Controls 294	13.47	11.56	115.5	14.6	14.3	55	21	16.5	559	479	12.61	111.6
53	31a	Controls 294	13.18	11.3	115.8	14.5	14.4	50	15	16.6	545	467	25.75	227.9
54	31a	Controls 294	13.2	11.3	115.1	14.9	14.3	50	31	16.8	538	461	24.22	214.4
55	31a	Controls 294	13.38	11.46	115.3	14.8	14.2	53	0	16.8	552	473	18.47	163.5
56	31a	Controls 294	13.26	11.36	115.4	14.8	14.3	53	49	16.7	543	465	16.28	144.1
57	4a	Controls 294	11.61	9.97	115.6	14.9	13.9	54	43	16.4	485	416	14.24	126.0
58	4a	Controls 294	11.93	10.26	115.5	14.9	14.4	53	13	16.3	481	414	17.49	154.8
59	4a	Controls 294	11.44	9.87	114.9	14.9	14	54	42	15.9	477	412	14.18	125.5
60	4a	Controls 294	11.06	9.52	115.2	14.5	14.2	53	13	16.2	466	401	18.50	163.7
61	4a	Controls 294	11.47	9.87	115.1	14.6	14.2	52	23	16.2	481	414	20.45	181.0
62	4a	Controls 294	11.17	9.62	115.5	14.3	14.2	53	6	16.1	476	410	19.21	170.0
63	4a	Controls 294	10.91	9.42	115.8	14.4	14.3	54	50	15.8	458	395	14.28	126.4
64	4a	Controls 294	10.96	9.45	115.5	14	14	53	14	16.0	484	417	19.76	174.9

65	98a	Controls 294	11.61	9.97	114.9	14.5	14.4	54	43	16.4	484	416	14.33	126.9
66	98a	Controls 294	10.38	8.93	115.3	14.6	14.4	55	33	16.2	428	368	12.00	106.2
67	98a	Controls 294	11.99	10.34	115.1	14.6	14.1	51	10	16.0	506	436	23.71	209.8
68	98a	Controls 294	11.31	9.74	115.5	14.8	13.9	53	56	16.1	476	410	16.45	145.6
69	98a	Controls 294	11.76	10.14	115.3	14.8	14.6	53	6	16.0	472	407	17.72	156.8
70	98a	Controls 294	11.06	9.54	115.7	14.4	14.4	52	37	15.9	461	398	20.00	177.0
71	98a	Controls 294	12.08	10.37	114.7	14.8	14.6	53	48	16.5	487	418	15.99	141.5
72	98a	Controls 294	12.55	10.83	115	14.7	14	52	22	15.9	530	458	20.57	182.0
73	40a	Controls 294	13.07	11.32	115.2	15.1	14.5	53	8	15.5	518	449	17.22	152.4
74	40a	Controls 294	11.91	10.54	115.8	14.8	14	52	45	13.0	496	439	19.38	171.5
75	40a	Controls 294	12.83	11.08	115.2	14.9	14.6	53	25	15.8	512	442	16.76	148.4
76	40a	Controls 294	12.2	10.53	115.6	15	14.6	52	5	15.9	482	416	19.79	175.2
77	40a	Controls 294	12.79	10.99	115.6	15	14.4	53	6	16.4	512	440	17.60	155.8
78	40a	Controls 294	12.81	11.04	115.4	15.1	14	52	32	16.0	525	453	19.32	171.0
79	40a	Controls 294	12.36	10.63	115.3	14.6	14.7	50	45	16.3	499	430	23.75	210.2
80	40a	Controls 294	11.42	9.84	115.2	14.8	14.2	53	9	16.1	472	406	18.09	160.1
81	101a	Controls S. sap.	13.27	11.45	115.3	14.9	14	54	2	15.9	552	476	15.90	140.7
82	101a	Controls S. sap.	13.87	11.97	115	14.8	13.8	51	46	15.9	591	510	22.19	196.4
83	101a	Controls S. sap.	12.71	10.97	114.2	14.4	13.9	51	36	15.9	556	480	23.43	207.3
84	101a	Controls S. sap.	12.5	10.79	115	15	13.9	53	47	15.8	521	450	16.49	146.0
85	101a	Controls S. sap.	12.53	10.83	115.3	15	14	52	37	15.7	517	447	19.31	170.9
86	101a	Controls S. sap.	13.46	11.62	115	14.9	14.1	53	45	15.8	557	481	16.51	146.1
87	101a	Controls S. sap.	12.7	10.9	114.8	14.7	13.9	53	8	16.5	541	465	18.72	165.7
88	101a	Controls S. sap.	11.99	10.33	114.5	14.2	14.5	54	0	16.1	509	438	16.63	147.2
89	64a	Controls S. sap.	14.26	12.26	114.8	14.7	14.3	52	13	16.3	591	508	20.51	181.6
90	64a	Controls S. sap.	13.84	11.91	115.8	14.8	14.4	48	22	16.2	561	483	29.41	260.3
91	64a	Controls S. sap.	13.84	11.9	114.7	14.7	14.4	49	12	16.3	570	490	27.74	245.5
92	64a	Controls S. sap.	13.51	11.61	115.4	14.2	13.8	52	34	16.4	597	513	21.46	190.0
93	64a	Controls S. sap.	13.25	11.37	115.5	14.3	14.2	51	48	16.5	565	485	22.65	200.5
94	64a	Controls S. sap.	12.19	10.48	115.2	14.7	14.1	53	0	16.3	511	439	18.80	166.4
95	64a	Controls S. sap.	12.53	10.78	114.7	14.6	14.3	49	52	16.2	523	450	26.60	235.4
96	64a	Controls S. sap.	14.05	12.08	115.1	14.8	14.5	52	7	16.3	569	489	20.27	179.4
97	51a	Controls S. sap.	13.5	11.58	115	14.9	13.6	54	47	16.6	579	497	14.37	127.2
98	51a	Controls S. sap.	13.06	11.15	115.5	15	14.2	55	8	17.1	531	453	12.74	112.7
99	51a	Controls S. sap.	14.03	11.98	115.3	14.8	14.4	57	0	17.1	571	488	7.99	70.7
100	51a	Controls S. sap.	12.64	10.84	115.7	14.4	14.3	52	56	16.6	531	455	19.31	170.9

101	51a	Controls S. sap.	12.81	10.96	115.5	14.7	14.4	53	0	16.9	524	448	18.41	162.9
102	51a	Controls S. sap.	12.71	10.86	114.5	15.1	14.2	55	33	17.0	518	442	11.55	102.3
103	51a	Controls S. sap.	13.36	11.44	115.5	14.7	14.4	53	48	16.8	546	468	16.38	145.0
104	51a	Controls S. sap.	13.22	11.3	115.5	14.9	14.8	53	15	17.0	519	444	16.94	149.9
105	33a	Controls S. sap.	10.29	8.81	115.3	14.3	13.7	53	32	16.8	456	390	18.71	165.6
106	33a	Controls S. sap.	10.63	9.04	115.6	14.9	13.9	54	21	17.6	444	378	15.19	134.5
107	33a	Controls S. sap.	10.76	9.17	115.7	14.6	14.3	53	33	17.3	445	380	17.32	153.3
108	33a	Controls S. sap.	10.62	9.08	115.5	14.9	14.1	53	30	17.0	438	374	17.15	151.8
109	33a	Controls S. sap.	10.92	9.36	115.5	14.7	14.3	53	35	16.7	450	386	17.05	150.9
110	33a	Controls S. sap.	10.68	9.15	115.2	14.5	14.3	53	30	16.7	447	383	17.63	156.1
111	33a	Controls S. sap.	10.71	9.18	115.8	14.6	14.1	53	36	16.7	449	385	17.43	154.3
112	33a	Controls S. sap.	13.31	11.41	115.6	13.8	13.9	52	38	16.7	600	515	22.08	195.4
113	80a	Controls S. sap.	13.71	11.78	115.2	14.9	14.5	53	6	16.4	551	473	17.66	156.3
114	80a	Controls S. sap.	11.76	10.1	115.2	14.4	14.5	53	8	16.4	489	420	18.53	164.0
115	80a	Controls S. sap.	13.12	11.29	115.4	14.3	13.8	56	27	16.2	576	496	10.37	91.8
116	80a	Controls S. sap.	10.23	8.79	115.2	14.4	14.3	52	25	16.4	431	371	20.66	182.8
117	80a	Controls S. sap.	13.41	11.52	115.6	14.2	14	52	8	16.4	584	501	22.33	197.6
118	80a	Controls S. sap.	11.93	10.24	114.8	14.3	14.5	50	46	16.5	501	430	24.82	219.7
119	80a	Controls S. sap.	11.21	9.63	115.7	14.2	14.5	53	32	16.4	471	404	17.87	158.2
120	80a	Controls S. sap.	12.67	10.89	114.4	14.4	14.4	51	48	16.3	534	459	22.10	195.6
121	39a	Contr. *G.trab.*	13.27	11.39	115.5	15.1	14.5	50	57	16.5	525	450	22.39	198.2
122	39a	Contr. *G.trab.*	12.67	10.88	115.1	14.7	14.7	51	13	16.5	509	437	22.38	198.1
123	39a	Contr. *G.trab.*	13.11	11.25	115.4	14.8	14.6	52	2	16.5	526	451	20.33	179.9
124	39a	Contr. *G.trab.*	12.59	10.81	114.5	14.9	14.6	50	2	16.5	505	434	24.86	220.0
125	39a	Contr. *G.trab.*	12.75	10.96	115.5	14.9	14.3	53	41	16.3	518	445	16.45	145.6
126	39a	Contr. *G.trab.*	12.98	11.13	115.6	15	14.6	54	57	16.6	513	440	12.84	113.6
127	39a	Contr. *G.trab.*	13.09	11.25	115.5	15.1	14.1	55	30	16.4	532	457	11.76	104.1
128	39a	Contr. *G.trab.*	12.04	10.34	114.8	14.8	14.2	52	16	16.4	499	429	20.32	179.8
129	45a	Contr. *G.trab.*	13.32	11.47	115.8	14.2	14.3	53	39	16.1	566	488	17.81	157.6
130	45a	Contr. *G.trab.*	14.07	12.11	115.6	14.5	13.8	53	6	16.2	608	524	19.35	171.3
131	45a	Contr. *G.trab.*	14.59	12.57	114.8	14.7	14	49	0	16.1	618	532	29.02	256.9
132	45a	Contr. *G.trab.*	14.38	12.38	114.9	14.8	13.9	49	51	16.2	608	524	26.84	237.5
133	45a	Contr. *G.trab.*	13.65	11.74	115.6	14.3	14.2	52	49	16.3	582	500	19.97	176.7
134	45a	Contr. *G.trab.*	14.53	12.52	115.7	14.6	13.9	49	51	16.1	619	533	27.41	242.6
135	45a	Contr. *G.trab.*	15.11	13.02	115.3	14.5	14	50	54	16.1	646	556	24.82	219.7
136	45a	Contr. *G.trab.*	14.42	12.42	115.3	14.8	13.9	53	12	16.1	608	524	18.35	162.4

137	65a	Contr. *G.trab.*	11.92	10.28	115.2	14.7	14	52	27	16.0	503	434	20.35	180.1
138	65a	Contr. *G.trab.*	11.86	10.22	115.2	14.9	14.4	49	58	16.0	480	413	25.36	224.5
139	65a	Contr. *G.trab.*	13.54	11.67	115.4	14.4	14.1	57	30	16.0	578	498	7.11	62.9
140	65a	Contr. *G.trab.*	10.5	9.03	114.8	14.3	13.5	53	30	16.3	474	407	19.08	168.9
141	65a	Contr. *G.trab.*	11.57	9.97	115.6	14.3	13.9	52	33	16.0	504	434	21.12	187.0
142	65a	Contr. *G.trab.*	11.43	9.85	114.9	14.2	13.3	56	40	16.0	527	454	10.22	90.5
143	65a	Contr. *G.trab.*	13.02	11.22	114.5	14.9	14.2	52	0	16.0	537	463	20.77	183.8
144	65a	Contr. *G.trab.*	11.52	9.94	115.6	14.4	14.3	52	36	15.9	484	418	20.18	178.6
145	77a	Contr. *G.trab.*	13.03	11.24	115.6	14.7	14.6	52	53	15.9	525	453	18.45	163.3
146	77a	Contr. *G.trab.*	11.32	9.78	114.7	14.7	13.9	51	6	15.7	483	417	23.97	212.1
147	77a	Contr. *G.trab.*	11.86	10.21	115.1	14.5	14.4	54	14	16.2	493	425	15.60	138.1
148	77a	Contr. *G.trab.*	12.62	10.88	115.7	14.4	14.1	50	36	16.0	537	463	25.68	227.3
149	77a	Contr. *G.trab.*	12.23	10.55	115.6	14.5	14.6	53	47	15.9	500	431	16.55	146.4
150	77a	Contr. *G.trab.*	12.28	10.52	115.4	14.7	13.7	53	50	16.7	528	453	17.13	151.6
151	77a	Contr. *G.trab.*	11.86	10.13	114.7	14.4	14.3	55	32	17.1	502	429	12.39	109.7
152	77a	Contr. *G.trab.*	12.7	10.91	115	14.7	13.8	50	17	16.4	544	468	26.22	232.1
153	82a	Contr. *G.trab.*	12.39	10.65	115.7	14.7	14.2	51	12	16.3	513	441	23.21	205.4
154	82a	Contr. *G.trab.*	12.86	11.05	115.1	14.7	14.3	48	51	16.4	532	457	28.77	254.6
155	82a	Contr. *G.trab.*	13.76	11.85	115.5	14.8	14.6	48	36	16.1	551	475	28.47	251.9
156	82a	Contr. *G.trab.*	13.46	11.58	115.2	14.7	14	49	24	16.2	568	488	28.04	248.2
157	82a	Contr. *G.trab.*	12.01	10.31	115.3	14.6	14.5	51	18	16.5	492	422	22.73	201.1
158	82a	Contr. *G.trab.*	12.75	10.96	115.7	14.5	14.5	54	50	16.3	524	451	13.93	123.3
159	82a	Contr. *G.trab.*	13.13	11.31	115.2	14.9	14.3	51	22	16.1	535	461	22.17	196.2
160	82a	Contr. *G.trab.*	12.55	10.8	114.8	14.6	13.5	53	7	16.2	555	477	19.53	172.8
161	97b	*O. ips* 308	13.72	11.83	115.3	14.8	14.4	48	41	16.0	558	481	28.67	253.7
162	97b	*O. ips* 308	13.44	11.6	115.2	14.8	14.2	50	35	15.9	555	479	24.49	216.7
163	97b	*O. ips* 308	12.84	11.07	115.4	14.8	13.5	51	46	16.0	557	480	22.68	200.8
164	97b	*O. ips* 308	13.16	11.34	115.3	14.6	13.9	51	0	16.0	562	485	24.48	216.7
165	97b	*O. ips* 308	12.7	10.94	114.7	14.4	14.4	52	5	16.1	534	460	21.37	189.2
166	97b	*O. ips* 308	11.91	10.27	115.3	15	14	52	21	16.0	492	424	19.98	176.8
167	97b	*O. ips* 308	12.16	10.49	115	14.8	14.8	50	2	15.9	483	416	24.78	219.3
168	97b	*O. ips* 308	13.2	11.39	115	14.6	14.2	49	52	15.9	554	478	26.79	237.1
169	50b	*O. ips* 308	13.55	11.65	114.9	15	14.5	53	11	16.3	542	466	17.27	152.9
170	50b	*O. ips* 308	12.91	11.12	115.3	15	14.6	52	27	16.1	511	440	18.92	167.4
171	50b	*O. ips* 308	13.15	11.31	115.2	15.2	14.6	49	12	16.3	514	442	25.98	229.9
172	50b	*O. ips* 308	13.3	11.45	114.3	15	14.7	53	30	16.2	528	454	16.28	144.1

173	50b	*O. ips* 308	12.66	10.88	114.9	14.4	14.3	51	54	16.4	535	460	22.00	194.7
174	50b	*O. ips* 308	14.58	12.55	115.5	14.7	14.4	50	42	16.2	596	513	24.11	213.4
175	50b	*O. ips* 308	13.7	11.82	115.1	14.4	13.9	49	30	15.9	595	513	28.90	255.8
176	50b	*O. ips* 308	13.75	11.84	115.5	14.8	14.6	50	38	16.1	551	474	23.70	209.7
177	22b	*O. ips* 308	14.63	12.59	115	15	14.5	52	31	16.2	585	503	18.89	167.2
178	22b	*O. ips* 308	11.48	9.89	114.3	14.8	13.3	53	40	16.1	510	440	17.91	158.6
179	22b	*O. ips* 308	13.54	11.65	115.2	15	14.2	50	30	16.2	552	475	24.18	214.0
180	22b	*O. ips* 308	13.73	11.82	115.5	15.2	14.5	51	46	16.2	539	464	20.27	179.4
181	22b	*O. ips* 308	13.88	11.96	114.8	14.6	14	49	32	16.1	592	510	28.00	247.8
182	22b	*O. ips* 308	13.43	11.57	115.2	15	14.3	49	49	16.1	543	468	25.63	226.8
183	22b	*O. ips* 308	12.29	10.62	115.7	15	13.9	53	0	15.7	509	440	18.48	163.6
184	22b	*O. ips* 308	13.59	11.72	115.5	15.2	14.3	49	47	16.0	541	467	25.19	222.9
185	2b	*O. ips* 308	12.22	10.56	115.1	15.1	14.5	51	8	15.7	485	419	21.96	194.4
186	2b	*O. ips* 308	12.14	10.51	114.8	14.9	14.5	50	45	15.5	489	424	23.33	206.5
187	2b	*O. ips* 308	12.11	10.47	115.3	14.9	14.3	53	30	15.7	493	426	16.91	149.6
188	2b	*O. ips* 308	10.72	9.27	115.5	13.5	14.5	53	19	15.6	474	410	19.95	176.5
189	2b	*O. ips* 308	11.42	9.86	115.7	14.7	14.6	51	0	15.8	460	397	23.06	204.1
190	2b	*O. ips* 308	12.2	10.54	115.3	14.9	14.4	54	6	15.7	493	426	15.29	135.4
191	2b	*O. ips* 308	12.37	10.68	115.5	14.3	14.5	51	7	15.8	517	446	23.93	211.8
192	2b	*O. ips* 308	11.32	9.79	115.5	14.3	14.7	51	32	15.6	466	403	22.56	199.7
193	68b	*O. ips* 308	12.48	10.78	114.9	15.1	14.2	52	30	15.8	507	438	19.13	169.3
194	68b	*O. ips* 308	13.03	11.23	114	14.9	14.1	48	47	16.0	544	469	28.73	254.3
195	68b	*O. ips* 308	11.4	9.81	114.9	14.5	13	54	35	16.2	526	453	16.27	144.0
196	68b	*O. ips* 308	13.77	11.88	115	15.2	14.7	47	48	15.9	536	462	28.88	255.6
197	68b	*O. ips* 308	12.18	10.52	115.4	14.7	14.3	49	39	15.8	502	434	26.85	237.6
198	68b	*O. ips* 308	11.95	10.32	115.1	14.6	14.2	53	33	15.8	501	432	17.44	154.4
199	68b	*O. ips* 308	12.71	10.99	115.3	14.8	13.9	51	23	15.7	536	463	23.00	203.6
200	68b	*O. ips* 308	11.77	10.16	114.7	14.6	14.1	53	50	15.8	498	430	16.82	148.9
201	4b	*O. ips* 294	11.96	10.36	115.5	14.9	14.2	55	6	15.4	489	424	12.95	114.7
202	4b	*O. ips* 294	11.7	10.14	115.1	14.9	14.2	54	45	15.4	480	416	13.85	122.6
203	4b	*O. ips* 294	11.76	10.18	115.2	14.3	14.1	55	36	15.5	506	438	12.52	110.8
204	4b	*O. ips* 294	11.99	10.38	114.6	14.7	14	53	47	15.5	508	440	16.89	149.5
205	4b	*O. ips* 294	11.88	10.28	115.4	14.7	14.2	54	12	15.6	493	427	15.58	137.9
206	4b	*O. ips* 294	11.42	9.89	115.7	14.5	13.8	54	50	15.5	493	427	14.64	129.5
207	4b	*O. ips* 294	11.51	9.95	115.5	13.8	14.2	54	47	15.7	509	440	15.50	137.2
208	4b	*O. ips* 294	12.16	10.51	115.2	14.8	14.4	51	39	15.7	495	428	21.55	190.8

209	31b	*O. ips* 294	13.84	11.94	115.1	14.6	14	50	22	15.9	588	508	25.91	229.3
210	31b	*O. ips* 294	13.76	11.88	115.5	14.8	13.8	50	2	15.8	583	504	26.57	235.2
211	31b	*O. ips* 294	13.99	12.08	115.7	14.9	14.1	50	50	15.8	576	497	23.79	210.6
212	31b	*O. ips* 294	14.01	12.1	115.2	14.9	14	50	6	15.8	583	504	25.76	228.0
213	31b	*O. ips* 294	14.36	12.41	115.8	14.6	14.2	51	0	15.7	598	517	23.96	212.1
214	31b	*O. ips* 294	13.94	12	115.5	14.4	14.2	49	40	16.2	590	508	27.87	246.7
215	31b	*O. ips* 294	14.06	12.1	115.5	14.4	14.4	52	37	16.2	587	505	20.00	177.0
216	31b	*O. ips* 294	13.59	11.68	114.7	14.9	14.1	51	55	16.4	564	485	21.12	186.9
217	76b	*O. ips* 294	11.05	9.49	115.3	14.6	14.3	54	25	16.4	459	394	15.07	133.3
218	76b	*O. ips* 294	12.16	10.46	115.3	14.7	14	53	57	16.3	512	441	16.46	145.6
219	76b	*O. ips* 294	9.75	8.33	114.8	14.6	13.8	55	23	17.0	422	360	12.98	114.9
220	76b	*O. ips* 294	12.21	10.48	115.5	13.5	14.2	53	24	16.5	551	473	20.12	178.1
221	76b	*O. ips* 294	10.98	9.41	115	14.4	13.7	54	35	16.7	484	415	15.60	138.1
222	76b	*O. ips* 294	13.21	11.38	115.2	14.8	14.6	50	21	16.1	531	457	24.37	215.7
223	76b	*O. ips* 294	14.09	12.15	115.4	14.3	14.5	49	48	16.0	589	508	27.25	241.2
224	76b	*O. ips* 294	10.75	9.26	115.5	14.4	13.3	54	21	16.1	486	419	16.74	148.2
225	40b	*O. ips* 294	13.4	11.58	115	15	13.9	52	18	15.7	559	483	20.25	179.2
226	40b	*O. ips* 294	12.57	10.84	115.7	14.9	13.9	53	25	16.0	525	452	17.61	155.8
227	40b	*O. ips* 294	13.02	11.21	115.3	14.8	14.5	50	54	16.1	526	453	23.22	205.5
228	40b	*O. ips* 294	12.54	10.81	115.4	14.7	14.6	52	46	16.0	506	436	18.73	165.8
229	40b	*O. ips* 294	12.64	10.89	115.1	15.2	14.2	51	20	16.1	509	438	21.73	192.3
230	40b	*O. ips* 294	13.31	11.49	115.1	14.8	14.4	52	56	15.8	543	468	18.38	162.7
231	40b	*O. ips* 294	13.45	11.61	115.2	15.1	14.6	48	36	15.8	530	457	27.60	244.2
232	40b	*O. ips* 294	13.33	11.5	115.7	15.1	14.3	50	6	15.9	534	460	24.71	218.7
233	98b	*O. ips* 294	13.58	11.72	115.3	14.7	14.5	49	47	15.9	553	477	26.16	231.5
234	98b	*O. ips* 294	12.72	10.97	115.4	14.5	14.2	48	40	16.0	535	462	30.04	265.9
235	98b	*O. ips* 294	13.28	11.45	115	14.8	14.2	48	2	16.0	549	474	30.61	270.9
236	98b	*O. ips* 294	12.97	11.19	114.6	14.6	14.2	51	46	15.9	546	471	22.02	194.9
237	98b	*O. ips* 294	12.24	10.57	115	14.4	14.1	52	35	15.8	524	453	20.51	181.5
238	98b	*O. ips* 294	13.1	11.3	115.3	14.7	14	51	7	15.9	552	476	23.75	210.2
239	98b	*O. ips* 294	12.69	10.95	115.5	14.5	13.9	48	45	15.9	545	470	30.48	269.8
240	98b	*O. ips* 294	12.74	10.98	115.7	14.9	14.3	50	34	16.0	517	445	24.10	213.3
241	64b	*S. sapinea*	13.93	12.02	115.3	14.7	14.3	49	43	15.9	575	496	26.68	236.2
242	64b	*S. sapinea*	13.62	11.78	114.9	14.5	14.3	49	52	15.6	572	494	26.88	237.9
243	64b	*S. sapinea*	14.57	12.59	115.4	14.8	14.3	48	31	15.7	597	515	29.26	259.0
244	64b	*S. sapinea*	13.87	12	114.8	14.7	14.3	46	37	15.6	575	497	34.02	301.1

245	64b	*S. sapinea*	13.05	11.29	115.4	14.8	13.9	52	56	15.6	550	476	19.04	168.5
246	64b	*S. sapinea*	13.98	12.07	115.3	14.5	14.6	49	21	15.8	573	494	27.58	244.1
247	64b	*S. sapinea*	12.83	11.11	115.2	14.7	13.8	51	52	15.5	549	475	22.17	196.2
248	64b	*S. sapinea*	13.66	11.82	114.8	14.4	14.4	47	44	15.6	574	497	32.21	285.1
249	101b	*S. sapinea*	11.07	9.59	114.6	14.4	13.1	54	18	15.4	512	444	17.14	151.7
250	101b	*S. sapinea*	10.88	9.42	114.3	14.7	12.2	54	0	15.5	531	460	18.73	165.8
251	101b	*S. sapinea*	13.57	11.72	115.2	14.9	13.9	50	56	15.8	569	491	23.89	211.4
252	101b	*S. sapinea*	11.36	9.82	115.2	14.5	13.9	54	16	15.7	489	423	16.07	142.3
253	101b	*S. sapinea*	12.49	10.79	114.8	14.7	13.8	53	55	15.8	536	463	16.78	148.5
254	101b	*S. sapinea*	11.7	10.11	115	14.5	13.9	54	0	15.7	505	436	16.79	148.6
255	101b	*S. sapinea*	11.35	9.83	114.8	14.8	14.1	53	23	15.5	474	410	17.62	156.0
256	101b	*S. sapinea*	11.85	10.26	114.8	14.7	13.9	52	54	15.5	505	437	19.33	171.1
257	33b	*S. sapinea*	10.6	9.17	115.3	14.6	14.3	53	33	15.6	440	381	17.32	153.3
258	33b	*S. sapinea*	11.09	9.59	115.1	14.9	13.8	53	52	15.6	469	405	16.57	146.6
259	33b	*S. sapinea*	13.03	11.26	115.5	15	14.1	50	32	15.7	533	461	24.27	214.8
260	33b	*S. sapinea*	10.68	9.23	115.6	14.3	14	52	50	15.7	461	399	20.21	178.9
261	33b	*S. sapinea*	11.04	9.54	115.6	15	13.9	54	7	15.7	458	396	15.64	138.4
262	33b	*S. sapinea*	10.22	8.82	115	14.3	14.4	53	30	15.9	432	372	17.89	158.3
263	33b	*S. sapinea*	10.94	9.46	115.3	15	14.2	54	43	15.6	445	385	13.79	122.1
264	33b	*S. sapinea*	10.06	8.69	115.2	14.4	13.7	53	35	15.8	443	382	18.37	162.6
265	51b	*S. sapinea*	13.2	11.39	115.4	14.8	14.4	50	43	15.9	537	463	23.82	210.9
266	51b	*S. sapinea*	12.93	11.18	115.1	14.6	14.6	50	6	15.7	527	456	25.49	225.6
267	51b	*S. sapinea*	13.88	12	115.7	14.8	14.1	48	48	15.7	575	497	29.00	256.6
268	51b	*S. sapinea*	12.21	10.56	114.7	14.5	14.3	51	46	15.6	513	444	22.10	195.6
269	51b	*S. sapinea*	12.24	10.56	115	14.4	13.9	52	27	15.9	532	459	21.16	187.3
270	51b	*S. sapinea*	12.65	10.93	115.1	14.7	14.3	52	57	15.7	523	452	18.66	165.2
271	51b	*S. sapinea*	13.29	11.49	115.7	14.8	14.3	51	2	15.7	543	469	23.22	205.5
272	51b	*S. sapinea*	13.15	11.32	115.4	15.1	14.4	53	10	16.2	524	451	17.26	152.7
273	80b	*S. sapinea*	12.72	10.97	115.4	14.9	14	50	26	16.0	528	456	24.95	220.8
274	80b	*S. sapinea*	11.29	9.72	114.6	14.4	14.5	51	7	16.2	472	406	23.68	209.6
275	80b	*S. sapinea*	13.87	11.97	115	14.8	14.5	49	28	15.9	562	485	26.63	235.7
276	80b	*S. sapinea*	13.07	11.26	115.5	14.7	14.4	54	37	16.1	535	461	14.29	126.5
277	80b	*S. sapinea*	13.38	11.57	115.7	14.7	13.8	55	31	15.6	570	493	12.48	110.5
278	80b	*S. sapinea*	12.76	11.01	115.6	14.8	14.4	47	20	15.9	518	447	31.80	281.4
279	80b	*S. sapinea*	13.86	11.98	115.2	14.7	14.3	50	3	15.7	572	495	25.87	229.0
280	80b	*S. sapinea*	12.17	10.52	115.3	14.6	14.1	51	20	15.7	513	443	23.29	206.1

281	77b	*G. trabeum*	11.28	9.63	114.3	14.5	14	53	7	17.1	486	415	19.03	168.4
282	77b	*G. trabeum*	11.39	9.75	114	14.6	14.3	55	50	16.8	479	410	11.33	100.3
283	77b	*G. trabeum*	11.08	9.44	115.4	14.4	14	53	10	17.4	476	406	19.10	169.0
284	77b	*G. trabeum*	11.72	10.04	114.6	14.8	14.4	52	27	16.7	480	411	19.58	173.3
285	77b	*G. trabeum*	11.78	10.06	114.7	14.8	14.6	52	37	17.1	475	406	18.91	167.3
286	77b	*G. trabeum*	11.15	9.54	113.3	14.7	14.3	53	7	16.9	468	401	18.24	161.4
287	77b	*G. trabeum*	11.69	9.96	114.1	14.6	14.6	49	44	17.4	481	410	26.37	233.4
288	77b	*G. trabeum*	12.36	10.59	114.7	14.8	13.6	52	45	16.7	535	459	19.95	176.5
289	65b	*G. trabeum*	11.85	10.15	114.4	15	13.8	54	17	16.7	500	429	15.32	135.6
290	65b	*G. trabeum*	11.91	10.27	115.5	14.2	13.7	53	42	16.0	530	457	18.45	163.3
291	65b	*G. trabeum*	12.51	10.78	115.6	14.7	14.2	53	25	16.0	518	447	17.60	155.8
292	65b	*G. trabeum*	12.34	10.63	114.8	14.9	13.8	53	6	16.1	523	450	18.55	164.2
293	65b	*G. trabeum*	11.42	9.82	114.3	15	13.6	52	38	16.3	490	421	19.84	175.6
294	65b	*G. trabeum*	12.95	11.16	115.3	14.8	14	54	49	16.0	542	467	14.02	124.1
295	65b	*G. trabeum*	11.58	9.99	113.9	15	13.3	56	7	15.9	510	440	10.91	96.6
296	65b	*G. trabeum*	11.59	9.98	113.1	14.9	14.5	50	58	16.1	474	408	22.82	202.0
297	45b	*G. trabeum*	12.2	10.41	114.3	14.8	14.1	52	52	17.2	511	436	18.94	167.7
298	45b	*G. trabeum*	12.95	11.09	115.4	14.7	14.5	50	47	16.8	526	451	23.75	210.2
299	45b	*G. trabeum*	13.01	11.11	114.3	14.8	14.1	53	30	17.1	545	466	17.33	153.3
300	45b	*G. trabeum*	11.77	10.06	115.1	14.6	14.6	55	43	17.0	480	410	11.40	100.9
301	45b	*G. trabeum*	12.25	10.48	115.8	14.8	13.8	53	10	16.9	518	443	18.57	164.4
302	45b	*G. trabeum*	12.34	10.6	114.7	14.8	13.8	53	50	16.4	527	452	16.83	148.9
303	45b	*G. trabeum*	12.26	10.5	114.7	14.6	13.8	54	17	16.8	531	454	15.97	141.4
304	45b	*G. trabeum*	11.33	9.73	115.3	14.4	14.3	54	57	16.4	477	410	13.96	123.6
305	39b	*G. trabeum*	12.65	10.86	114.2	15.1	14.3	55	18	16.5	513	440	12.10	107.1
306	39b	*G. trabeum*	12.75	10.92	115.5	14.6	14.6	54	57	16.8	518	444	13.39	118.5
307	39b	*G. trabeum*	13.17	11.32	115	14.8	14.2	54	26	16.3	545	468	14.81	131.1
308	39b	*G. trabeum*	12.61	10.83	114.9	15.2	14.6	55	45	16.4	495	425	10.64	94.1
309	39b	*G. trabeum*	12.13	10.45	114.6	14.9	14.2	55	52	16.1	500	431	10.97	97.1
310	39b	*G. trabeum*	12.71	10.95	114.5	14.9	14.4	53	45	16.1	517	446	16.17	143.1
311	39b	*G. trabeum*	12.36	10.59	114.7	15.1	13.9	54	32	16.7	513	440	14.42	127.6
312	39b	*G. trabeum*	13.27	11.37	115.8	14.5	14.6	53	7	16.7	541	464	18.25	161.5
313	82b	*G. trabeum*	11.91	10.2	114.7	14.7	14.5	53	53	16.8	487	417	16.06	142.1
314	82b	*G. trabeum*	10.54	9.03	114.5	14.8	14.6	55	17	16.7	426	365	12.27	108.6
315	82b	*G. trabeum*	12.08	10.39	114.5	14	14.4	54	50	16.3	523	450	14.81	131.1
316	82b	*G. trabeum*	10.54	9.06	114.4	14	14.1	54	48	16.3	467	401	15.22	134.7

317	82b	*G. trabeum*	13.97	12.04	115.1	14.9	14.6	49	4	16.0	558	481	27.10	239.9
318	82b	*G. trabeum*	10.19	8.8	113.8	14.2	14.5	56	1	15.8	435	376	11.17	98.8
319	82b	*G. trabeum*	14.64	12.63	115.3	14.8	14.3	46	56	15.9	600	518	32.94	291.6
320	82b	*G. trabeum*	11.36	9.76	115.4	14.9	13.5	54	32	16.4	489	420	15.15	134.1