A Comparative Study of *Pyrenophora teres* Drechs. f. *teres* and *Pyrenophora teres* Drechs. f. *maculata* Smedeg. cause of "net" and "spot" type net blotch respectively on New Zealand Barley

Daphne Geraldine Carvalho

Submitted in fulfilment for the degree of M.Sc.
School of Biological Sciences
Victoria University of Wellington

1990

ABSTRACT

Net blotch is caused by *Pyrenophora teres* Drechs. (stat. conid. *Drechslera teres* (Sacc.) Shoem., syn. *Helminthosporium teres* Sacc.). *P. teres* produces symptoms which appear initially as small necrotic spots and streaks on the leaf. These increase to produce the characteristic net-like symptoms, which have given rise to the name net blotch.

Sometimes, lesions develop from small necrotic spots, to form elliptical lesions. This is the "spot" type of *P. teres* and was first noticed in 1967 in isolates from North America, Mexico, Israel and Holland. It was thought that these isolates were mutants of *P. teres*. Since 1969 however, other workers have reported similar observations widely occurring in Norway, Denmark and Finland. Based on minor morphological differences, Ito and Kuribayashi proposed a new species, called *P. japonica*.

Smedegård-Petersen disagreed, and showed that the spot-producing isolate represents a deviating type of *P. teres*, only differing from the usual "net" type in the symptoms induced on barley plants. He based his reasoning on morphological, cultural and genetical investigations. Consequently, Smedegård-Petersen described two new forms of the fungus, *Pyrenophora teres* Drechs. f. *teres* Smedeg., which produces the usual net lesions, and *Pyrenophora teres* Drechs. f. *maculata* Smedeg., which produces well defined dark brown circular or elliptical lesions without netting.

The aim of the research undertaken in the present study was to conduct a comparative study on the morphology and fitness of a range of New Zealand "net" and "spot" type isolates. An attempt was also made at crossing a "net" type with a "spot" type. Although Smedegård-Petersen had stated that there was no morphological difference between the "net" and "spot" types, this project was undertaken because no research had been done on New Zealand isolates. Furthermore, different features were studied using different methods not used by other workers in studying *P. teres*.

The only morphological difference that was distinctive was that the "spot" types of *P. teres* formed coremial strands, which were fan-like in morphology, which produced conidia in culture, and the "net" types did not. There was no way to tell the "net" isolates apart from the "spot" isolates, based on conidia colour, length, width, volume or the number of cells per conidium. One fact that did emerge, was that the longest conidia had the greatest number of cells per conidium and the reverse was also true.

The germination of monoconidial isolates showed that there were no major differences in branching between the two types of *P. teres*. However, it was revealed that two germ tubes were capable of emerging from one cell in the "spot" isolates. All cells in a conidium in both the "net" and "spot" types were able to germinate, cells that germinated tended to be at opposite ends, and the first cell to germinate in a conidium was usually the cell at the hilum.

Examination of the growth rates showed that there were no significant differences in the growth rates of the "net" and "spot" types when grown on MEA+B. The "spot" types were able to penetrate cellulose faster than the "net" types and hence may produce cellulase

faster as well. ANT148, which had previously been an unknown type, was proved to be a "spot" type in the pathogenicity tests. It may have been the source of the New Zealand "spot" type inoculum because the seed it came from was imported into New Zealand in 1984, two years prior to the discovery of the "spot" type of *P. teres* in the South Island.

Both forms of *P. teres* penetrated the leaf through the epidermal cell wall, and occasionally entered through the stomata. Even though the "spot" type may be present inside the leaf, the symptoms are not usually manifested until later, compared with the "net" type where the symptoms tend to be an indication of the amount of hyphae present in the leaf.

In the screening of the progeny from the crossing, the "spot" type of *P. teres* had lost up to 78.9% of its resistance to triadimenol and flutriafol, when compared to the sensitivity tests carried out in 1986 and 1987. It is hypothesised that 13Y, the "net" type is dominant, and the "spot" type, KF₂, recessive, as none of the progeny had any resistance to triadimenol or flutriafol, after undergoing somatic recombination.

It was concluded that the "spot" and "net" types are two types of the same species, and there was not enough evidence to suggest otherwise. Further studies should be done, using more current isolates of the "net" and "spot" types of *P. teres*, and the old *D. japonica* isolates from New Zealand, to establish if the cultures identified as *D. japonica*, are different in any way.

Contents

INTRODUCTION	1
AIMS	3
Examination of colony morphology	3
Examination and measurement of conidia	3
Examination of germinated monoconidial isolates	3
Comparison of the growth rates of the inoculum types	3
Cellulase (cell-wall degrading enzyme) production in <i>D. teres</i>	4
Examination of fungal penetration and development of <i>D. teres</i> in the leaf	4
Pathogenicity tests	4
Crossing of a "net" type with a "spot" type	4
LITERATURE REVIEW	6
METHODS	12
Materials Used	12
Collecting and Naming of the Isolate Types	13
Comparison of Isolates of <i>D. teres</i>	14
a) Isolating <i>D. teres</i>	14
b) Colony Morphology and Colony Growth Rate	15
c) Examination and measurement of conidia	15
d) Preparation of fungal specimens for SEM (Scanning Electron Microscopy)	17
e) Method for Promoting the Formation of Conidia	18
f) Making Monoconidial Isolates for Examination Under a Scanning Electron Microscope	19
g) Making Monoconidial Isolates for Examination Under the Compound Microscope	19
h) Modified Method for Making Monoconidial Isolates for Examination Under the Compound Microscope	20
i) Growth Rates of Fungal Colonies	21
j) Cellulase Production (Cell-Wall Degrading Enzymes) in <i>D.</i> <i>teres</i>	21
Studies of Barley Leaf Infection	22
a) Growing Barley	22
b) Inoculating Barley Plants With <i>Drechslera teres</i>	23
c) Examination of Fungal Penetration and Development of Hyphae	25
Somatic Hybridisation Studies	26
The Crossing of a "Net" Type and a "Spot" Type	26
a) Co-infection of barley plants	26
b) Crossing on WA+B barley leaf decoction media	27

RESULTS	29
Comparison of isolates of <i>D. teres</i>	29
Examination of colony morphology	29
Examination and measurement of conidia	31
Examination of germinated monoconidial isolates	39
Growth rates of the different inoculum types	41
Cellulase Production	43
Studies of Barley Leaf Infection	44
Pathogenicity Tests	44
Examination of fungal penetration and development of <i>D. teres</i> in the leaf	46
Somatic Hybridisation Studies	51
The crossing of a triadimenol and flutriafol sensitive "net" type with a non-sensitive "spot" type of <i>D. teres</i> and testing the resultant progeny on fungicides	51
Crossing on barley plants	53
Crossing on MEA+B barley decoction	53
DISCUSSION	59
Examination of colony morphology	59
Examination and measurement of conidia	60
Examination of germinated monoconidial isolates	61
Growth rates of the different inoculum types	62
Cellulase production	62
Pathogenicity tests	62
Examination of fungal penetration and development of <i>D. teres</i> in the leaf	64
Crossing of a "net" type with a "spot" type	66
CONCLUSIONS	73
Examination of colony morphology	73
Examination and measurement of conidia	73
Examination of germinated monoconidial isolates	74
Growth rates of the different inoculum types	74
Cellulase production	74
Pathogenicity tests	75
Examination of fungal penetration and development of <i>D. teres</i> in the leaf	75
Crossing of a "net" type with a "spot" type	75
GLOSSARY	79
ACKNOWLEDGEMENTS	80
APPENDICES	81
Appendix 1: Preparation of Malt Extract Agar + Benomyl (MEA + B)	81
Appendix 2: Preparation of Water Agar + Benomyl (WA + B)	81

Appendix 3: Preparation of Water Agar + Benomyl (WA + B) barley leaf decoction	82
Appendix 4: Preparation of MEA + B with fungicide	82
Appendix 5: Source of EBI fungicides	83
Appendix 6: Preparation of Clear Lactophenol	83
Appendix 7: Preparation of Lactophenol-Cotton Blue	84
Appendix 8: NUV (Near Ultra Violet) Light	84
Appendix 9: Peat-Sand Pot Mix	84
Appendix 10: Details on the morphological description of <i>D. teres</i> isolates	85
Appendix 11: Complete data on the length, width, number of cells per conidiospore and colour of all inoculum types examined	86
Appendix 12: Complete data on the measurement of the diameters of all the inoculum types, following inoculation	104

Tables

Table 1: Isolate origin	13
Table 2: Seed used to provide hosts in all experiments - their source, condition and name used to denote the seed	13
Table 3: The etching on a haemocytometer	16
Table 4: Test grid showing the pattern of application of inoculum to cultivars	24
Table 5: Information on the inoculum types 13Y and KF ₂ used in the crossing experiment	26
Table 6: Morphological description of colonies based on comparison of the overall appearance of a number of physical characteristics	30
Table 7: Mean and standard deviation (for a population based on a sample) for the lengths, widths, volumes, number of cells per conidium and predominant colour for each inoculum type	32
Table 8: The ranges of the length, width, volume and number of cells per conidium	33
Table 9: A summary of the decisions of whether the means were equal, based on the ANOVA tests for the conidia characteristics examined	38
Table 10: Single conidia, germinated on Cellophane placed on MEA+B, examined after 24 and 48 hours, using a compound microscope	39
Table 11: Mean diameters of colonies (mm) following inoculation (days)	42
Table 12: Demonstration of cellulase production	43
Table 13: Degree of infection and type of symptoms on barley plants one week after inoculation	45
Table 14: Degree of infection and type of symptoms on barley plants two weeks after inoculation	45
Table 15: Examination of barley leaf segments harvested three days after inoculation, cleared and stained	48
Table 16: Examination of barley leaf segments harvested three weeks after inoculation, cleared and stained	50
Table 17: Mean colony diameters (mm)* of the "net" type and "spot" type of <i>D. teres</i> and the 15 isolates of their resultant crosses on 50 and 100 ppm triadimenol	54
Table 18: Sensitivity of 1973-1986 isolates of <i>D. teres</i> to triadimenol carried out in November 1986 (Sheridan and Nendick, 1987). Measurements show mean colony diameter (mm)*	55
Table 19: Mean colony diameters (mm)* of the "net" type and "spot" type of <i>D. teres</i> and the 15 isolates of their resultant crosses on 10 and 50 ppm flutriafol	56
Table 20: Sensitivity of isolates from farmers crops to flutriafol, 1986-1987 survey (Sheridan and Nendick, 1987)	57
Table 21: The increase in sensitivity (%), or decrease in resistance for triadimenol and flutriafol, in the period between 1986-87 and 1990	67
Table 22: The quantities of fungicide required to make up the different concentrations of triadimenol and flutriafol used	83
Table 23: Details on triadimenol and flutriafol	83

Table 24: Morphological description of colonies based on comparison of the overall appearance of a number of physical characteristics	85
Table 25: H88 leaf - dimensions, number of cells and colour of conidiospores	86
Table 26: T83 seed - dimensions, number of cells and colour of conidiospores	89
Table 27: T88 leaf - Dimensions, number of cells and colour of conidiospores	92
Table 28: Z87 seed - Dimensions, number of cells and colour of conidiospores	95
Table 29: K87 seed - Dimensions, number of cells and colour of conidiospores	98
Table 30: K88 seed - dimensions, number of cells and colour of conidiospores	101
Table 31: ANT148 - growth rates	104
Table 32: H88 - growth rates	105
Table 33: T83 - growth rates	105
Table 34: T88 - growth rates	106
Table 35: Z87 - growth rates	106
Table 36: K87 - growth rates	107
Table 37: K88 - growth rates	107

Graphs

Graph 1: Mean lengths of 100 conidiospores	33
Graph 2: Mean widths of 100 conidiospores	34
Graph 3: Estimated mean volumes of 100 conidiospores	35
Graph 4: Mean number of cells per 100 conidia	36
Graph 5: Growth rates of the different inoculum types over the 9 day period	42
Graph 6: Cellulase production by germinating conidiospores	44
Graph 7: Comparing 1986 and 1990 triadimenol sensitivity tests	67
Graph 8: Comparing 1987 and 1990 flutriafol sensitivity tests	68

Photographs

Photograph 1: How the inoculated barley plants were grown in a glasshouse, with plastic bags on for the duration of the experiment	24
Photograph 2: The method of crossing 13Y and KF ₂ on WA+B barley leaf decoction media	28
Photograph 3: The difference in morphology of the inoculum types studied, T83, T88, H88, Z87, K87, K88 and ANT148	31
Photograph 4: SEM photo of seven day old fungal growth of H88 comprising of thick hyphae which make up the conidiophores, thin hyphae and conidia . . .	32
Photograph 5: T83 - 24 hours growth, shows the good branching pattern, and germ tubes emerging from opposite ends of the conidium	40
Photograph 6: K87 - 24 hours growth, showing two germ tubes emerging from one cell	41
Photograph 7: An example of "net" type symptoms on barley	46
Photograph 8: An example of "spot" type symptoms on barley	47
Photograph 9: K88 barley plants showing both "net" symptoms from the inoculation, and "spot" type symptoms which carried through from the infected seed.	47
Photograph 10: Barley plants inoculated with ANT148, which is a "spot" type of <i>D. teres</i>	48
Photograph 11: Appresorium at the end of an invading thin-walled hypha, growing on a barley leaf surface	49
Photograph 12: Connections that form between conidia, and between conidia and germ tubes	52
Photograph 13: Connections that form between conidia	52
Photograph 14: Growth of 13Y and KF ₂ on triadimenol (50 and 100 ppm) and flutriafol (10 and 50 ppm), four weeks after inoculation Note: The cross progeny had the same morphology and growth rate as 13Y, the sensitive "net" type	58
Photograph 15: A comparison of plates freshly subcultured from monoconidial isolates, with plates repeatedly subcultured by cutting a plug of hyphae and conidia	60
Photograph 16: K87 - 24 hours growth, showing appresoria (club-shaped) penetrating Cellophane (regenerated cellulose) to grow underneath, on the MEA+B	63
Photograph 17: Growth of the cross types under NUV light, on 50 ppm triadimenol and 10 ppm flutriafol, six weeks after inoculation	70
Photograph 18: Growth of the cross types, in an incubator (dark), after eight weeks of growth on 50 and 100 ppm triadimenol, and 10 and 50 ppm flutriafol . . .	71

INTRODUCTION

The fungal plant pathogen *Pyrenophora teres* Drechs. (stat. conid. *Drechslera teres* (Sacc.) Shoem., syn. *Helminthosporium teres* Sacc.) is the cause of net blotch of barley. The term net blotch refers to the dark brown necrotic streaks which form a net-like pattern on leaves of infected plants. Infected parts of barley leaves sometimes develop a water-soaked appearance and are also often yellowed in the later stages of infection. The primary source of infection of net blotch is carried as mycelium within the seed coat, or as conidia on the seed surface.

In 1931, a new species *Pyrenophora japonica* was described (Ito and Kuribayashi, 1931). It was very closely related to *P. teres*, but the main difference between *P. japonica* and *P. teres* were the symptoms produced on barley, with *P. japonica* producing distinct elliptical spots instead of netting. Morphologically there were only few and inconspicuous differences. *P. japonica* has been described as causing eye-spot lesions on the leaves of *Hordeum*, and has also been recorded on *Secale* and other grasses (Ellis, 1976; Ellis and Ellis, 1985).

Working with isolates of *Pyrenophora teres* from North America, Mexico, Israel and Holland, McDonald (1967) obtained two isolates which instead of the usual net lesions produced well defined spot lesions similar to *P. japonica* symptoms. He considered the two isolates as mutants of *P. teres*. However, similar examples were later found to be widely occurring in Norway (Hansen and Magnus, 1969), Denmark (Smedegård-Petersen, 1971) and Finland (Makela, 1972). In fact this type of *P. teres* was more common in occurrence in Norway than the ordinary type of *P. teres*.

Based on morphological, cultural and genetic investigations, Smedegård-Petersen (1971) showed that the spot-producing isolates represent a deviating type of *P. teres*. It only differed from the usual net type in the symptoms induced on barley. Consequently Smedegård-Petersen described two new forms of the species. *Pyrenophora teres* Drechs. f. *teres* which, produces the usual net lesions, and *Pyrenophora teres* Drechs. f. *maculata* Smedeg., which, produces well defined dark brown circular or elliptical lesions without netting. Common to both forms is that the affected tissue becomes chlorotic, often with areas of water-soaked tissue, although this varies among isolates.

Isolates of *P. teres* causing the characteristic net blotch symptoms were designated "net types" by Smedegård-Petersen (1971), and isolates causing spot symptoms were termed "spot types". The symptoms were referred to as net and spot symptoms. In this thesis the terms "net type" and "spot type" are used to differentiate between the two forms of *P. teres*.

Leaf stripe, caused by *Drechslera graminea* was identified in New Zealand, for what is believed to be the first time, in 1975 (Arnst, 1976; Arnst, et al, 1978). It was found on plants grown from imported seed of new barley cultivars, sown in the South Island. Earlier, Kirk (1906) recorded a disease which, he called leaf-blotch, and identified the pathogen as *Helminthosporium gramineum* (= *D. graminea*). However, it is thought that this was probably net blotch, as the only noted symptoms were on the lower leaves, which

is characteristic of net blotch. Leaf stripe and net blotch have probably been confused by many early workers in New Zealand, and reports of the incidence of and prevalence of net blotch and leaf stripe prior to 1975 may be unreliable. A survey in the lower North Island by Sheridan and Spencer (1975) failed to find leaf stripe, and they also reported that net blotch was present in over 80% of barley crops.

The "spot" type of *D. teres* was identified for the first time in New Zealand in crops on the cultivars Triumph and Kym, grown in North Otago, in the South Island in January 1986 (Sheridan and Sheridan, 1986).

Fungicide resistance studies have shown that the sensitivity of *D. teres* f. *teres*, the "net" type of net blotch, to EBI (Ergosterol Biosynthesis Inhibitors) fungicides has not changed since the early 1980's. This means the population is still highly sensitive to imazalil, propiconazole and prochloraz. Many isolates of *D. teres* f. *maculata*, the "spot" type of net blotch, however, showed reduced sensitivity to all three fungicides as well as high resistance to triadimenol, flutriafol and nuarimol. Eradication of the "spot" type from commercial crops should be attempted according to Sheridan and Nendick because of the possibility of hybrids combining the aggressiveness of the "net" types with the reduced EBI sensitivity of the "spot" types (Sheridan and Nendick, 1987).

Based on the results of genetic studies, Smedegård-Petersen (1976) had suggested that the capacity of producing "net" and "spot" lesions in *D.teres* was determined by two allelic pairs, Ss (spot) and Nn (net) which, control the production of the toxins A and B which are important factors in the development of the disease symptoms of the "net" type and the "spot" type on barley.

Although Smedegård-Petersen had concluded that there was no morphological difference between the "net" type and the "spot" type (Smedegård-Petersen, 1971), his research was conducted almost two decades ago. In the elapsed time, the "spot" type has been identified in other countries like Australia in 1977 (Khan, 1982), and New Zealand in 1986 (Sheridan and Sheridan, 1986). In the elapsed time there have been problems with the development of resistance of the "spot" types to EBI fungicides and the changes in pathogenicity of *D. teres* relating to changes in barley cultivars grown (Khan, 1982). Research work examining many aspects of the morphology and fitness of the "net" type and the "spot" type of *D. teres* was undertaken. Fitness, is considered here to include all factors favourable to the production of progeny (Schepers, 1985). No previous research had been done in this field in New Zealand, using New Zealand isolates. Many new characteristics were also examined, using different methods not used by Smedegård-Petersen. In the present study the possibility of hybridisation, in New Zealand, between the fungicide sensitive "net" type with the non-sensitive "spot" type, also required investigation.

AIMS

The aim of the series of experiments that follows, was to critically examine and compare the morphology and fitness of the "net" types and the "spot" types of *D. teres*. All factors favourable to the production of progeny are considered to represent the fitness of an isolate (Schepers, 1985). The experiments describing how this was done are explained in detail in the Methods section.

Examination of colony morphology

There are obvious differences in the morphologies of the different inoculum types, especially between the "net" types and "spot" types. The aim here was to examine critically the morphology of the "net" and "spot" types in culture by comparing many characteristics. This included the overall appearance of the fungus in culture, whether the aerial growth was coremial or fluffy, the general colour of the fungus in culture (white or grey), the ability or inability of the fungus to sporulate readily on MEA+B (refer to Appendix 1 for recipe) and the production of spermatogonia and pseudopycnidia in culture.

Examination and measurement of conidia

Two methods were used to examine the morphology of the conidia. The first involved using a Scanning Electron Microscope (SEM), and the second was by using a light microscope. Features which were noted under the SEM were the length and width of conidia. Under the light microscope, 100 randomly picked conidia from each inoculum type were measured for the length and width, and noted for the number of cells per conidium and the colour of each conidium. The volume of the conidia was estimated using the length and width measurements with the assumption that a conidium was cylindrical in shape.

Examination of germinated monoconidial isolates

Again two methods were used in examining the germinating monoconidial isolates. One involved using the SEM and the other used the light microscope. Here, the aim of the experiment, using both methods, was to note the number and position of cells which had germinated after a 24 and 48 hour period, and to note any pattern in the germinating hyphae, also after a 24 and 48 hour period.

Comparison of the growth rates of the inoculum types

The different inoculum types were all grown at the same time, under the same conditions in an incubator, in petri dishes. Their growth was measured every day by measuring the diameter of a colony. Each inoculum type was replicated five times, and measured for nine consecutive days, or until growth reached the edge of the petri dish.

Cellulase (cell-wall degrading enzyme) production in *D. teres*

The reason for studying the cellulase production of the inoculum types was because the rate at which a *D. teres* isolate is able to produce a cell-wall degrading enzyme once it has started to grow is critical in determining whether or not it becomes established and multiplies in the host tissue. In this experiment the time (in days) it took for a single conidium to germinate and then penetrate a piece of Cellophane was noted for each inoculum type.

Examination of fungal penetration and development of *D. teres* in the leaf

This experiment was aimed at examining the inoculated leaf pieces from the pathogenicity tests under the light microscope to study and compare the growth pattern and germination of the spores and hyphae, and the method of penetration of the different inoculum types.

Pathogenicity tests

Each of the seven *D. teres* isolate types were inoculated onto the six barley cultivars following a test square pattern to determine the ability of each isolate type to infect each different barley cultivar. The reaction of the barley cultivars to the inoculum types, the symptoms produced by the inoculum types on the barley cultivars and the degree of infection by each inoculum type on each cultivar was also carefully noted.

Crossing of a "net" type with a "spot" type

Here, two attempts were made at crossing a "net" type with a "spot" type, whose levels of resistance to the fungicides triadimenol and flutriafol had been previously determined. The "net" type isolate was sensitive to both triadimenol and flutriafol, and grew on 10 ppm triadimenol but did not grow on 10 ppm flutriafol. The "spot" type isolate had reduced sensitivity to both triadimenol and flutriafol, and grew on 100 ppm triadimenol and 50 ppm flutriafol.

The "net" type and the "spot" type crossing was attempted by:

1. spraying the two inoculum types on the same barley plant. Isolates made from single conidia picked off the leaf pieces which had been placed on MEA+B (refer to Appendix 1 for recipe) were tested using a sensitivity bioassay, for their levels of resistance to the fungicides triadimenol and flutriafol. Any levels of fungicide resistance which were different from the parental types would be considered successful hybrids.
2. Another method used in an attempt to cross the two inoculum types was to place four plugs of each inoculum type on WA+B barley leaf decoction (refer to Appendix 3 for recipe) in a petri dish. The sensitivity bioassay for fungicide

resistance was also carried out on isolates which had been made from single conidia picked from the area between the two different plug types growing in the petri dish. Again, any levels of fungicide resistance in isolates which were different from that of the parental types would be deemed to be due to the formation of successful hybrids.

The isolates made from both cross methods were inoculated onto a barley cultivar to examine the symptoms produced, and checked for parental type symptoms or any intermediate symptoms between the "net" type and the "spot" type.

LITERATURE REVIEW

Net blotch is caused by *Pyrenophora teres* Drechs. (stat. conid. *Drechslera teres* (Sacc.) Shoem., syn. *Helminthosporium teres* Sacc.). *D. teres* produces symptoms which appear initially as small necrotic spots and streaks on the leaf which increase quickly to form narrow, dark brown, longitudinal and transverse streaks to produce the characteristic net-like symptoms which have given rise to the name, net blotch. The affected parts of the leaf turn brown and the adjoining parts of the tissue become chlorotic. Occasionally the initial lesions develop as greenish, water-soaked areas in which the dark netting later becomes evident. These symptoms vary in the reaction of different barley (*Hordeum vulgare* L.) cultivars to the variations in the types of *D. teres* (Smedegård-Petersen, 1971).

Net blotch and leaf stripe (caused by *Drechslera graminea* (Rabenh. ex. Schlecht.) Shoemaker), are not always readily distinguishable and have probably been confused and misidentified in New Zealand (Sheridan, 1975; Arnst, 1976). Net blotch may have been in New Zealand as far back as 1906. Leaf stripe caused by *Drechslera graminea* is rather similar in appearance to net blotch and it is highly likely that many early workers in New Zealand were confusing the two symptoms, and that many reports of the incidence and prevalence of net blotch and leaf stripe may be unreliable (Sheridan, 1975; Arnst, 1976).

Earlier, Kirk (1906) recorded a new disease which he called leaf-blotch, and identified the pathogen as *Helminthosporium gramineum* (= *D. graminea*). However it is thought now that this pathogen was probably *D. teres*, as the only noted symptoms were on the lower leaves, which is characteristic of net blotch. Neill (1928) described the disease as widespread, but he referred to the symptoms as being small lesions on the lower leaves, again uncharacteristic of leaf stripe. Of the two specimens of *D. graminea* deposited in the herbarium at DSIR Auckland, one was identified as *D. teres*, while the other was not identifiable (Arnst, 1976). The first reliable record of leaf stripe was identified in New Zealand in 1975 (Arnst, 1976), on plants grown from imported seed of new barley cultivars, sown in the South Island. A survey in the lower North Island by Sheridan and Spencer (1975), failed to find leaf stripe, and they also reported that net blotch was present in over 80% of barley crops.

Organo-mercury seed treatments were withdrawn in New Zealand in 1973, and imported seed were then treated with captan or benomyl-containing seed treatments, neither of which controlled net blotch or leaf stripe (Arnst *et al*, 1978). It is possible that net blotch was present in New Zealand for many years and had become destructive and widespread since the withdrawal of mercury-based seed treatments in 1973. Leaf stripe may have been introduced much later in the 70's on imported lines of barley.

Sometimes, instead of the usual net-like symptoms, lesions develop to form small elliptical necrotic spots. This is the "spot" type of *D. teres*. The "spot" type of *D. teres* was discovered for the first time in New Zealand, in crops on the cultivars Triumph and Kym, grown in North Otago, in the South Island, in January 1986 (Sheridan and Sheridan, 1986).

A "spot" type disease on barley was originally attributed by Ito and Kuribayashi in 1931, as *Pyrenophora japonica*, a new species which was very closely related to *P. teres*. *P. japonica* and *P. teres* were described as being very similar in morphology. The width and colour of conidia and conidiophores and the size of ascospores were the main criteria for separation (Ito and Kuribayashi, 1931). The conidia of *P. japonica* are 15-18 µm in width, while those of *P. teres* are 15-22.5 µm in width. The conidiophores of *P. japonica* 6.5-9 µm in width, while those of *P. teres* 7.5-9.5 µm in width. Ascospores of *P. japonica* measure 40-60 x 17.5-30 µm, compared with 40-62.5 x 17.5-27.5 µm in *P. teres* (Ito and Kuribayashi, 1931). The main difference was the symptoms produced on barley. *P. japonica* produced distinct elliptical spots instead of the usual netting. *P. japonica* has also been described as causing eye-spot lesions on *Hordeum* leaves, and has also been recorded on *Secale* and other grasses (Ellis, 1976; Ellis and Ellis, 1985).

McDonald (1967), who was working with isolates from Mexico, Israel, Holland and North America obtained two isolates which produced symptoms similar to the *P. japonica* type. He however, considered the two isolates to be mutant strains of *P. teres*. Two other workers, Hansen and Magnus (1969) also demonstrated the presence of a type of *P. teres* which instead of netting, produced similar symptoms to *P. japonica*. In fact, this form was more common in Norway than the "net" type of *P. teres*. In New Zealand, Dr J.E. Sheridan identified *P. japonica* on wild barley grass, *Hordeum murinum* L., in the 1977-78 growing season (Sheridan, 1978), and on barley leaf in the 1974-75 and 1975-76 growing seasons (Sheridan, 1977). The assessments were carried out in the Wairarapa region of the North Island. The identifications were also confirmed by M.B. Ellis of the Commonwealth Mycological Institute, Kew, England.

McDonald conducted crossing experiments on *P. teres*, in order to study the variability and inheritance of morphological mutants (1967). He discovered that cultures ranged from black, conidial types to white, perithecial types. Conidia were produced most abundantly by the darker isolates, and all types produced protoperithecia and spermatogonia. Some wild-type isolates retained their exact parental appearance through successive generations, whereas others varied slightly in appearance. Sectors appeared in some cultures derived from ascospores or conidia, and monoconidial transfers from these mixed colonies yielded both the parental type and pure colonies of a variant. Instability was particularly noticeable in the first few generations of subculturing and was more evident in cultures obtained by mycelial transfer than those obtained from single conidia.

McDonald concluded that the differences in cultural appearance, principally in the amount and placement of tufting, in the wild-type isolates of *P. teres* from infected barley were considered to be within the range normally expected in a heterothallic species of fungus. Particular attention was paid to grey, wooly isolates because it was believed earlier that these cultures which produced the "spot" type lesion, might be *P. japonica* (Ito and Kuribayashi, 1931). The symptoms they illustrated for *P. japonica* were similar to isolates from Manitoba and Israel. As the culture from Manitoba crossed with a known isolate of *P. teres*, McDonald decided that isolates of the *P. japonica* type were a mutant strain of *P. teres*.

Smedegård-Petersen (1971) doubted that the small differences in spore size between *P. japonica* and *P. teres* could be regarded as valid criteria for the separation into two species. He described two new forms of the species, *Pyrenophora teres* Drechs. f. *teres* Smedeg., which produces the ordinary net-like lesions, and *Pyrenophora teres* Drechs. f. *maculata* Smedeg., which produces the dark brown circular or elliptical lesions, without the netting (Smedegård-Petersen, 1971). Common to both forms is that affected tissue becomes chlorotic, often with areas of water-soaked tissue. His decision that the spot producing isolates represented a deviating type of *P. teres*, was based on morphological, cultural and genetic investigations.

A second difference between *P. japonica* and *P. teres* emphasized by Ito and Kuribayashi (1931) is that *P. teres* in culture forms claw-like hyphal bands, whereas *P. japonica* forms fan-like hyphal bands. Again, Smedegård-Petersen disagreed with Ito and Kuribayashi for employing the two kinds of hyphal bands as criteria for separating two species, since both kinds occur in Danish isolates of *P. teres* (Smedegård-Petersen, 1971).

Further studies by Smedegård-Petersen (1976, 1977), indicate that *P. teres* produces two toxins which may be involved in the disease syndrome of net blotch of barley. These toxins do not seem to determine pathogenicity of *P. teres*, but contribute to the virulence of individual isolates. Toxins A and B, were isolated from a cell-free, sterile culture filtrate of *P. teres* by extraction, gel filtration and ion-exchange chromatography. Without the presence of the pathogen, each of the purified toxins incites the most important symptoms of the disease when introduced into healthy barley leaves. After hyphal penetration, chlorosis and water-soaking developed rapidly in advance of the hyphae, indicating the operation of diffusible substances. The toxins were not specific to the "net" or "spot" types. Both toxins A and B were isolated from the "net" and "spot" types. Toxin A is the most potent, producing symptoms in concentrations as low as 25 µg/ml. The most virulent isolates produce the highest concentrations of toxin in vitro. Both toxins were isolated from infected barley leaves, but not from healthy barley leaves.

Interspecific cross-fertility resulting in fertile progeny was demonstrated between the two species *Pyrenophora teres* and *Pyrenophora graminea* by Smedegård-Petersen (1976). The inheritance of genetic factors which, determine the expression of lesion symptoms, pathogenicity and sexual compatibility in *Pyrenophora teres* and *Pyrenophora graminea* was subsequently studied by analysis of ascospore progeny from intraspecific crosses and interspecific crosses. The crosses were between: *P. teres* N ("net" type) X *P. teres* S ("spot" type); *P. graminea* X *P. teres* S; *P. graminea* X *P. teres* N. The capacity of *P. teres* and *P. graminea* to produce spot, net and stripe symptoms on the barley cultivar "Wing" was shown to be controlled by three gene pairs Ss, Nn and Gg respectively, in three loci. The loci for Ss and Nn segregate independently and those for Ss and Gg are closely linked. Results from the backcrossing experiments suggested that pathogenicity and apathogenicity of *P. teres* on "Wing" was controlled by a single gene pair.

D. teres produces pseudothecia after harvest, on straw and stubble of infected barley plants. They are at first sub-epidermal, then erumpent, semi-globose when young, but at maturity flask-shaped with one or more short ostiolar beaks. The blackish-brown walls bear dark, rigid setae on top of which are often formed conidiophores and conidia. The asci are

hyaline, clavate or cylindrical, rounded at the apex and 30-60 x 200-300 µm. They are bitunicate and contain 2-8 ascospores, usually 8, pale yellowish, ellipsoidal or oval ascospores with 3-5 transverse septa and one longitudinal septum, which always occurs in one of the middle cells. The ascospores are clearly constricted at the septa (Smith *et al*, 1988).

Conidia develop in the tips of successive lateral proliferations of light brown conidiophores, that occur singly, or in groups of 2-3. The conidia, which measure 10-25 x 40-160 µm, are cylindrical, rounded at the ends, and often with the basal cell slightly inflated. They are sub-hyaline to straw-coloured, smooth and usually with 2-7 transverse septa. On nutrient-rich substrates in culture, the mycelium often produces characteristic vertical erect mycelial tufts (Smith *et al*, 1988).

D. teres also produces pseudopycnidia which occur abundantly on leaves and straw in nature as well as in culture. The pseudopycnidia are globose, with a short ostiole and measure 60-170 µm. Pseudopycnosporos are hyaline, non-septate, ellipsoidal or spherical. At maturity they are exuded in a slimy matrix appearing as a grey or white drop on top of the pseudopycnidium. Apparently the pseudopycnosporos are unable to infect plants directly, but they germinate to produce mycelium in culture. Their role in the disease cycle is not known (Smith *et. al.*, 1988).

The disease is seed-borne and can be introduced to new areas with infected seed, which is the main source of primary inoculum in New Zealand. Infested stubble and straw left on top of the soil from one growing season to the next, is also an important source of inoculum from which primary infections are established. The fungus overwinters as mycelium or pseudothecia. Volunteer plants of infected winter barley or overwintering spring may also be important primary sources of inoculum.

Sporulation and infection require 10-30 hours of high humidity. Light promotes the production of conidia and sporulation is diurnal. Sporulation occurs over a wide temperature range with an optimum of 20°C. Conidia are large and are dispersed with the wind over short distances. Most conidia settle within 3-8 m from the source but some conidia may be transported over longer distances with strong wind and air-currents.

The development of epidemics is dependent on the amount of inoculum, periods with high humidity and host susceptibility. Longer periods with warm and dry weather will stop or delay epidemics, even if all other factors are favourable (Smith *et. al.*, 1988).

Penetration of barley leaves by *D. teres*, occurs directly through the epidermis and rarely through the stomata (Van Caesele and Grumbles, 1979). This is accomplished by both enzymatic hydrolysis of the cuticle and underlying cell wall layers and by pressure exerted by the penetrating and expanding hypha (Keon and Hargreaves, 1983). It was supposed that cuticle formed a more or less continuous, intact impermeable layer over leaves, except for stomata and other natural openings. However it has been shown that channels containing pectic substances traverse the cuticle of apple and permit the passage of materials (Roberts *et al*, 1948). There are also ectodesmata, protoplasmic continuations in the outer walls of the epidermal cells serving for excretion to the outer leaf surface (Hall and Donaldson, 1962). It is possible that minute cracks caused by internal stress and

strains, or soft channels occur in the cuticle and facilitate passage of the narrow infection hypha.

Having penetrated through the cuticle, the infection hypha has to grow through the underlying outer wall of the epidermal cell, which consists basically of a network of cellulose microfibrils, in a matrix consisting principally of pectic materials with small amounts of arabans, galactans, xylans and other substances (Tarr, 1972; Tribe, 1987). In contrast to cuticular penetration, it appears that penetration by the fungus of cellulose and pectin, is largely enzymic, the infection growing quickly through the softened materials. Many plant pathogens produce pectolytic enzymes and cellulases, and it may be that pectic components in the cell wall are quickly degraded, thus allowing rapid progress of the invading hypha, whereas the cellulose breakdown is a much slower process and proceeds for some time after invasion.

Studies have shown that after penetrating the epidermal cell, *D. teres* produces a large infection vesicle which gives rise to one or sometimes two intracellular hyphae (Van Caesele and Grumbles, 1979). However, Keon and Hargreaves (1983) have stated that penetration of the outer epidermal wall was followed by the development of a primary vesicle within the epidermal cell and the subsequent formation of a secondary vesicle. Following the development of the secondary vesicle, infection hyphae were initiated either within the epidermal cell or during penetration through the lower epidermal wall. Hyphae spread down through the leaf tissue intracellularly for one or two cell layers and intercellularly thereafter (Van Caesele and Grumbles, 1979; Keon and Hargreaves, 1983).

The physical evidence from the studies on the ultrastructure and cytology of *D. teres*, in the penetration of the host, supports the studies of Smedegård-Petersen (1976, 1977) on the production of toxins by the fungus. It is the toxins that are believed to be responsible for the symptoms produced on host plants. Studies by Van Caesele and Grumbles (1979), showed that at sites where *D. teres* had penetrated only four or five cell layers deep, the cellular contents of the mesophyll cells were relatively unaffected despite the presence of intercellular hyphae. Keon and Hargreaves (1983) reported that the lack of direct contact between intercellular hyphae and cells in the chlorotic tissue, and also the different types of ultrastructural damage observed in lesion tissue and chlorotic tissue, indicate that different mechanisms of cellular disruption occur. Death of cells in the chlorotic tissue may be caused by diffusible toxic metabolites emanating from the fungal hyphae, or may be due to the presence of the pathogen.

Net blotch was the most important disease of barley in New Zealand until fairly recently. Since the main source of primary inoculum is infected seed, to achieve good control of the disease, effective seed treatments must be used, with good crop husbandry practice and the use of foliar sprays which, is an additional control measure. Organo-mercury seed treatments were highly effective in controlling seed-borne diseases prior to the mid 60's. The late 1960's saw the gradual withdrawal of these highly toxic chemicals, until in 1973 mercury was finally banned from use. For a period there was little or no treatment to replace the outlawed mercury compounds, then captan-containing treatments were introduced. However, these ineffective treatments led to widespread outbreaks of disease in cereal crops and an epidemic of net blotch in barley crops in the 1976-77 season. Yield losses in the North Island were over 20% (Sheridan and Grbavac, 1979).

Systemic seed treatments were registered for use in New Zealand in 1977-78. These systemic fungicides penetrate the seed coat and become part of the internal plant organism. They are used for controlling seed borne fungi as well as internal pathogens. Ergosterol biosynthesis inhibitors (EBI's) are a class of systemic fungicides. They are organic chemicals which inhibit the ergosterol biosynthesis in fungal organisms. As ergosterol is the dominant fungal sterol, which is an important component of fungal cell or mycelial membranes, EBI's owe their fungitoxicity to their ability to inhibit ergosterol formation (Mercer, 1984). The effectiveness of EBI's is mainly due to the high acropetal systemic properties and the vapour phase activity. They have a broad spectrum of activity and exhibit the highest efficacy of commercial fungicides known to date, as seed treatments, foliar fungicides and post harvest fruit treatments.

Since the introduction of EBI's in New Zealand, the level of infection of barley crops by *D. teres* has gradually decreased. A disease survey in the Wairarapa in the 1987-88 growing season showed that net blotch was found in 9 crops (16%) and incidence ranged from a trace to 41%. Only the "net" type of *D. teres* was found in commercial crops (Sheridan and Nendick, 1988). In the 1988-89 (Sheridan and Nendick, 1989) and 1989-90 (Sheridan, 1990) disease survey in the Wairarapa, net blotch was not found in any of the commercial crops.

The lowering incidence of net blotch could be attributed in part to the weather which has seen warmer temperatures with less rainfall in the last two years. However, the winter of 1989 has been cooler and accompanied by rainfall. This has resulted in an outbreak of the "net" type of *D. teres* in the field trials carried out in the Wairarapa by Dr J.E. Sheridan (Sheridan, 1990). Whether this means there will be an increase in the incidence of net blotch in commercial crops remains to be seen, and is probably dependant on the continuation of the climatic conditions which, have been favourable for the growth of *D. teres* and the ability to control the "spot" type of *D. teres* which has reduced sensitivity to EBI's.

METHODS

Materials Used:

diseased barley seed
infected and uninfected barley seed
freeze dryer
Janola (sodium hypochlorite)
MEA+B (refer to Appendix 1 for recipe)
NUV light source (refer to Appendix 8 for emission and specifications)
Whatman No.1 filter paper
haemocytometer
colourless lactophenol
lactophenol cotton-blue
6% glutaraldehyde
1% osmium tetroxide (OsO_4)
0.1% sodium cacodylate buffer (pH 7.1-7.4)
acetone solutions (30, 50, 70, 90 and 100%)
SEM aluminium mounting stubs
conductive silver paint
colloidal graphite
Cellophane (regenerated cellulose and bought from a commercial stationery store)
peat-sand pot-mix (refer to Appendix 9 for recipe)
clear plastic bags (30 x 45 cm)
thermohygrograph
atomiser (Quickfit)
magnetic stirrer
No. 11 nylon mesh
glacial acetic acid
glycerol (glycerine)

Collecting and Naming of the Isolate Types

Table 1: Isolate origin

Cultivar and Plant Part	Year	Source	<i>D. teres</i> type	Inoculum Type Name
Hassan, leaf	1988	Wairarapa	"Net"	H88
Triumph, seed	1983	Wairarapa	"Net"	T83
Triumph, leaf	1988	Wairarapa	"Net"	T88
Zephyr, seed	1987	Wairarapa	"Net"	Z87
Kym, seed	1987	Wairarapa	"Spot"	K87
Kym, leaf	1988	Wairarapa	"Spot"	K88
ANT, seed	1984	Denmark	Unknown	ANT148*

* The inoculum ANT148 was provided by Dr J E Sheridan. It was isolated from ANT seed whose country of origin was Denmark.

The infected barley leaves were frozen in a freezer for a few days, before being freeze dried and stored in a bell-jar with silica gel, in the dark. The infected barley leaves were frozen in a freezer first to prevent any fungal growth from exploding when freeze dried. These freeze dried leaves were used as the inoculum source for the experiments. The infected seed was stored in a cool, dark cupboard and was used as an inoculum source for the experiments, and for growing seedlings in the pathogenicity tests.

Table 2: Seed used to provide hosts in all experiments - their source, condition and name used to denote the seed

Seed	Year	Source	Treatment	Name Used
Hassan	1989	Seed Testing Station, Plm. Nth.	Baytan IM	H89
Triumph	1987	Wairarapa	Untreated	T87
Triumph	1989	Seed Testing Station, Plm. Nth.	Baytan IM	T89
Zephyr	1987	Wairarapa	Untreated	Z87
Kym	1987	Wairarapa	Untreated	K87
Kym	1988	Wairarapa	Untreated	K88
ANT	1986	Wairarapa	Untreated	ANT86

Comparison of Isolates of *D. teres*

a) Isolating *D. teres*

In conducting all the experiments, the inocula were made from single conidia picked from a plate of fungal growth. The reason for this was that *D. teres* appeared to be unstable in its morphological appearance, when a culture was subcultured repeatedly, using a plug of hyphae and conidia. There was also the uncertainty of conducting experiments on a changing inoculum type. Therefore, the diseased leaf and seed used as the inoculum sources were stored for future use in case the inoculum type being studied changed in morphology or structure.

1. Pieces of freeze dried infected barley leaves were cut to 2 cm long sections and placed into a 50 ml conical flask.
2. Leaf sections were surface sterilised by the addition of Janola (active ingredient-sodium hypochlorite) and sterilised distilled water in a 1:3 proportion (Sheridan, 1980). A drop of Tween 80 was added to break down surface tension. Leaves were swirled around in the solution for 1 minute, then rinsed five times in sterile distilled water, or until there was no hint of Janola on the leaf sections. Longer treatment with sodium hypochlorite reduces the levels of *D. teres*.
3. The leaf sections were then dried between two pieces of sterile filter paper, which had been sterilised in an oven at 160°C for 1½-2 hours (Sheridan, 1980), in a pyrex petri dish.
4. The infected seed was surface sterilised and dried in the same way as the infected leaf sections.
5. Leaf sections and seed were then plated out on MEA+B in petri dishes under sterile conditions in a sterile room. 10 seeds were plated per petri dish and 8 leaf sections per petri dish. For good isolation results at least five plates of each inoculum source were made.
6. Plates were incubated for 3-4 days at 18-25°C (room temperature) under NUV irradiation, under a 12 hours on-12 hours off regime.
7. With the aid of a dissecting microscope, a sterile needle was used to pick up a clump of hyphae and conidia, and this was placed on a plate of WA.
8. This enabled a single conidium to be picked up easily off the WA, using a sterile dissecting needle, and this was plated out on MEA+B in a petri dish. Each plate was derived from only one conidium.
9. The plates were then incubated under a NUV light under a 12 hours on-12 hours off regime, at 18-25°C (room temperature) for growth and sporulation to occur.

10. Ten plates were made by picking a conidium off the original plate and plating it on a fresh plate of MEA+B. These plates were then used as the inoculum source in all the experiments. The original plates made were sealed with "Gladwrap" strips and stored in a polythene bag.
11. If new plates were needed, then they were made by subculturing, but using only one conidium, not a plug of hyphae and conidia.

b) Colony Morphology and Colony Growth Rate

1. Plugs (5 mm in diameter) were cut at random from the edge of 7 day old plates of sporulating fungal growth on MEA+B (refer to Appendix 1) and plated upside down, on fresh plates of MEA+B.
2. 20 plates were prepared for each inoculum type.
3. Plates were placed under a NUV light (refer to Appendix 8 for emission and specifications), under a 12 hours on-12 hours off regime, for three days at room temperature which varied between 18-25°C to encourage sporulation, then incubated in an incubator at 20°C.
4. Plates were examined on a daily basis and notes made on sporulation ability, colour of colonies above agar and in agar, production of pseudopycnidia, production of spermagonia, production and colour of tufts or coremia.
5. Plates were also measured on a daily basis to determine the rate of growth of the different inoculum types. Measurements were carried out by measuring two diameters of growth at right angles to each other and taking the mean of the two measurements.

c) Examination and measurement of conidia

1. A tuft of mycelium and conidia were picked off a plate of fungal growth and placed in a drop of clear lactophenol on a haemocytometer and teased with dissecting needles to break up any clumps. A coverslip was placed over the drop.
2. The reason for using the haemocytometer was to ensure randomness in selecting conidia for measuring. The haemocytometer has etchings on the surface which form a 12 x 12 square, as shown, within a 3 x 3 mm area:

Table 3: The etching on a haemocytometer were assigned column and row numbers as shown below

	1	2	3	4	5	6	7	8	9	10	11	12
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												

- Using a random number generator (on a calculator in this experiment) or the random number tables, numbers were written down and then blocked off in a series of four numbers. For example: 4253, 0671, 3675, 1057 and so on. Based on these numbers, the conidia found in the square 4 x 2 were picked and measured. If more than one conidium was found, all conidia in that square were picked and measured. In cases where the number 0671 came up the square 6 x 7 and 6 x 1 were picked. Where the first two digits were 10, 11 or 12 in 1057 for example, the square 10 x 5 and 10 x 7 were picked. When a square which had been previously selected appeared, it was omitted.
- The measurements and observations made were the length, which was measured from one end to the other; the width, which was measured across the widest point which was usually around the middle; the number of cells per conidium and the colour and texture of the conidium. 100 conidia were measured for each inoculum type.

5. The volume of a conidium was estimated using the width and length measured. Assuming a conidium resembled a cylinder, the volume was estimated using the formula for a cylinder:

$$\text{Volume} = \pi \times \text{radius}^2 \times \text{height}$$

Changing the formula to suit the conditions the following was used:

$$\text{Volume} = \pi \times (\text{width}/2)^2 \times \text{length}$$

6. To eliminate any bias a new tuft of conidia and hyphae were picked off after 25 conidium measurements so a range of conidia were sampled.

d) Preparation of fungal specimens for SEM (Scanning Electron Microscopy) Critical Point Drying Method (Cole and Samson, 1979)

Method:

- 1 The plates of sporulating fungal growth were fixed by flooding 7 day old culture plates with 6% glutaraldehyde and 1% osmium tetroxide (OsO_4), each prepared in 0.1% sodium cacodylate buffer (pH 7.1-7.4).
- 2 Small blocks of agar and mycelium (0.5 x 0.5 mm), were excised from the flooded culture and placed in vials of fresh cool fixative. The vials were left in the dark for 24 hours.
3. Samples were washed ten times in 0.1% sodium cacodylate buffer, then dehydrated in a graded acetone series (30, 50, 70, 90 and 100%) for 10 minutes each.
4. The blocks of agar with fungi were removed from 100% acetone and loaded into a Polaron critical point drying apparatus. The dryer was immediately filled with liquid CO_2 which was flushed through for 5 minutes and then filled and sealed for 1 hour, followed by a second 5 minute wash with CO_2 . After this, the chamber was partially emptied and the pressure increased to 1200 p.s.i. by heating with water at 32°C . The CO_2 was then slowly vented, the drying chamber opened, and the samples removed.
5. The dried specimens removed from the Polaron critical point drier were immediately mounted on SEM aluminium stubs with double-sided Sellotape.
6. Conductive silver paint was applied to the uncovered surface of the double-sided Sellotape, to increase conductivity.
7. Samples were then coated with 100 Å of gold in a Dynavac Sputter Coater (model SC 150), prior to viewing under the electron microscope.

Vapour Fixation Method (Tiedt, L R; Jooste, W J; Hamilton-Atwell, V L, 1987)

1. Blocks of sporulating cultures, 1 cm², were excised from 7 day old plates and carefully transferred right side up, to the inner surface of sterile (3.5 cm diameter) disposable petri dish covers.
2. The covers with 4 or 5 adherent agar blocks were inverted over sterile disposable petri dish bottoms containing 3 ml of 2% osmium tetroxide (OsO₄), previously left to warm up to room temperature. This step and the following steps which involved osmium tetroxide were carried out in a fume cupboard.
3. After 2 days the bottoms of the petri dishes, and any remaining osmium tetroxide were removed and discarded. The covers with the samples were left for a further 3 days to air dry.
4. After 3 days air drying, the covers with samples were put into a bell-jar with silica gel for a further 3 days to remove any remaining moisture. It was extremely important for the samples to be absolutely dry, or they exploded when bombarded with electrons under the scanning electron microscope.
5. The samples were gently removed from the covers of the petri dishes and mounted on SEM aluminium stubs using colloidal graphite as an adhesive.
6. Samples were then coated with 100 Å of gold in a Dynavac Sputter Coater (model SC 150) prior to viewing under the scanning electron microscope.

e) Method for Promoting the Formation of Conidia

Conidia did not form easily, especially in some isolates studied which were poor sporulating forms. Therefore, a method was needed which would promote the sporulation of the inoculum types, as many of the experiments involved using single conidia. Another problem was that the inoculum sources were contaminated by *Drechslera sorokiniana* and many methods to increase sporulation involved using diseased leaf material. Since methods to increase sporulation were not selective for *D. teres*, or involved periods of incubation in complex media, a simple method had to be found which increased the sporulation capability of pure cultures of *D. teres* which had been isolated from the original impure inoculum sources.

Various nutrient media were experimented with, and one which was successful in significantly increasing sporulation was WA+B (refer to Appendix 1) with a decoction of fresh, healthy barley leaves (refer to Appendix 3) during incubation under 12 hour on-12 hour off cycles for 3-4 days at 18-25°C (room temperature) for sporulation to occur.

f) Making Monoconidial Isolates for Examination Under a Scanning Electron Microscope

Monoconidial isolates were made in the same way as for examination under the compound microscope. Initial experimentation with preparing samples showed that the germinating hyphae tended to grow underneath the agar level. Therefore any isolates made were placed on a piece of Cellophane, as germinating conidia did not tend to penetrate Cellophane within 48 hours, the duration of the experiment.

1. Cellophane (regenerated cellulose) was cut into 1 cm² pieces and placed in a sterile petri dish. 99% alcohol (ethanol) was added to sterilise the Cellophane pieces.
2. After 24 hours the excess alcohol was drained off and the Cellophane pieces rinsed three times in sterile distilled water and left to dry in the petri dish in a sterile room.
3. The Cellophane pieces were then placed on the surface of MEA+B in a petri dish. About 15 pieces of Cellophane were placed in each petri dish.
4. Single conidia were picked off the fungal growth and a conidium was placed on each piece of Cellophane.
5. After 24 or 48 hours the germinating conidia were carefully excised by cutting out a 1 cm² agar block with Cellophane, which was placed top side up on the cover of a 3.5 cm sterile disposable petri dish. Four or five blocks of agar were placed on each cover.
6. The samples were fixed using the Vapour Fixation Method (Tiedt, L R; Jooste, W J; Hamilton-Atwell, V L, 1987) as described in the Preparation of Fungal Specimens for Scanning Electron Microscopy section.
7. The samples were gently removed from the covers of the petri dishes and mounted on SEM aluminium stubs using colloidal graphite as an adhesive.
8. Samples were then coated with 100 Å of gold in a Dynavac Sputter Coater (model SC 150) prior to viewing under the scanning electron microscope.
9. Five samples were prepared for each inoculum type and five for each sampling time of 24 or 48 hours.

g) Making Monoconidial Isolates for Examination Under the Compound Microscope

In this experiment the conidial isolates were placed on Cellophane pieces which were then stuck on to slides. The slides were then examined under a compound microscope.

1. Pieces of Cellophane were cut into 1 cm² pieces. The pieces were put into a petri dish sterilised as in Making Monoconidial Isolates for Examination Under the Scanning Electron Microscope.
2. Glass slides, glass petri dishes and Whatman No.1 (9 cm in diameter) filter paper were packed into a biscuit tin and sterilised in an oven at 160°C for 1½-2 hours (Sheridan, 1980). The glassware was left to cool before removing from the oven.
3. Under sterile conditions, using aseptic techniques, a small piece of double sided tape (1 cm²) was applied to a sterile slide, and then a piece of Cellophane was applied to the double sided tape. The Cellophane was smoothed out on the slide as much as possible.
4. A sheet of filter paper was placed in each glass petri dish and 2 ml of sterile distilled water was applied to the filter paper, to provide moisture.
5. A conidium was removed (as described in Making Monoconidial Isolates) and placed on the piece of colourless Cellophane (regenerated cellulose) on the slide.
6. The slide was placed inside the petri dish lined with filter paper, and the conidium left to germinate for 24 and 48 hours.
7. The slides were then removed after the desired time, and fixed with clear lactophenol (refer to Appendix 6 for recipe). A coverslip was put on, and the slides sealed with clear nail polish to prevent any drying out or damage to the specimen.
8. Slides were then examined under the compound microscope.

Slides were made for the isolates H88, T83, T88, Z87, K87 and K88. The pattern of branching and the number and position of cells which germinated were noted. Five samples were prepared for each inoculum type and each sampling time of 24 or 48 hours.

h) Modified Method for Making Monoconidial Isolates for Examination Under the Compound Microscope

This method was essentially the same as the original method except that instead of placing a piece of Cellophane on a slide, a piece of Cellophane was put on the surface of a plate of MEA+B (refer to Appendix 1 for recipe).

The modified method was easier to carry out, and there were no air bubbles formed, as in the original methods, where lack of adhesion between the cover slip and the piece of Cellophane resulted in air bubbles under the Cellophane which did not give a clear view.

1. Sterile Cellophane pieces were placed on the surface of MEA+B in a petri dish. About 15 pieces of Cellophane were placed in a petri dish.

2. A conidium was excised from the colony following the method in Making Monoconidial Isolates, and was placed on a piece of Cellophane on the MEA+B.
3. After the desired growth period of 24 and 48 hours, the piece of Cellophane with the germinating conidium was gently lifted off the surface of the MEA+B and placed on a drop of clear lactophenol on a slide. A further drop of the fixative was placed on the piece of Cellophane, and a coverslip was put on. The sides of the coverslip were sealed with clear nail polish to prevent drying out or damage to the specimen, before viewing under the compound microscope.

Slides were made for the isolates H88, T83, T88, Z87, K87 and K88. Each isolate was confined to its own plate and repeated on three different occasions. Five samples were made of each inoculum type and at each sampling time of 24 or 48 hours.

It was later pointed out that more than one isolate type should have been grown in the same petri dish as this would then have eliminated the possibility of any difference in growth due to varying humidity levels in different petri dishes. This was done in the fourth repeat of this experiment where all six inoculum types were grown in one petri dish.

i) Growth Rates of Fungal Colonies

The aim of this experiment was to compare the growth rates of the different inoculum types when grown on MEA+B (refer to Appendix 1 for recipe).

1. A 5 mm plug of sporulating fungal growth was randomly cut from a the edge of a colony growing on MEA+B and placed upside down in the centre of a new plate of MEA+B.
2. Five replicates were made for each inoculum type which had previously been examined for colony morphology. The inoculum types ANT148, H88, T83, T88, Z87, K87 and K88 were tested in this manner.
3. The plates were placed in an incubator set at 25°C, and measured once a day. Measurements were made to the nearest mm by taking the mean of two measurements of the colony diameter at right angles, on the reverse of the dish. Measurements were made and noted once every day until the growth reached the edge of the petri dish.

j) Cellulase Production (Cell-Wall Degrading Enzymes) in *D. teres*

In studying degradation of plant tissue, intracellular enzymes are important only so far as they later become extracellular. The conditions to which the parasite is exposed to, in the host or in culture, will affect the rate of synthesis of these enzymes, and possibly the rate at which they are secreted. There is some evidence that the types of enzymes which become extracellular are bound to surface layers of secreting cells before they are released into surrounding media - in such a position they are readily accessible to external factors.

A significant question concerned with the production of a lesion is how rapidly *D. teres* produces cell-wall degrading enzymes once the fungus has started to grow. This may be critical in determining whether or not they become established and multiply in host tissue.

1. About 15 pieces of sterile Cellophane (1.5 x 1.5 cm²) were placed on MEA+B medium in petri dishes.
2. Single conidia were excised and each one was placed on a Cellophane piece as described in Isolating *D. teres*.
3. Each isolate type was confined to a petri dish, and there was no mixing of the isolates types in the same petri dish.
4. Petri dishes were incubated at 25°C and the first 30 successfully germinated conidia were removed after 24 hours. The pieces of Cellophane were lifted off the MEA+B surface and carefully replaced in a fresh petri dish with MEA+B.
5. The used petri dish was incubated for a further 24 hours and then checked for any growth of *D. teres* on the surface of the MEA+B where a piece of Cellophane with a conidium had been. In lifting off the piece of Cellophane with a germinating conidium, any hyphae which had penetrated the Cellophane would be broken off, leaving it to grow in the agar. Examination with a dissecting microscope, of the agar surface where the piece of Cellophane had been, showed minute indentations where a hypha had grown into the agar. Otherwise the agar surface was smooth. It is possible that short germ tubes were lifted off with the Cellophane, but since all plates examined under a dissecting microscope which showed an indentation grew a small colony, any event of that happening was negligible. Penetration was identified by growth of *D. teres* on the surface where a piece of Cellophane had been. The numbers of germinated conidia which had penetrated the piece of Cellophane were noted each day.
6. The process of removing the Cellophane pieces and placing them on fresh MEA+B was carried out for 4 days only, as by this time all spores that were likely to germinate had already germinated and penetrated the Cellophane.

Studies of Barley Leaf Infection

a) Growing Barley

1. Barley seeds used were imbibed overnight in running water prior to sowing. This ensured that the seeds would all germinate at the same time.
2. Six pots of each of the five barley cultivars, H88, T89, Z87, K87 and K88 were sown in a peat-sand mixture (refer to Appendix 9).
3. Barley seeds were sown 20 per pot in 4 rows of 5.

4. The pots were placed in a growth room under 12 hour light - 12 hour dark regime at 20°C for germination and growth of the seedlings, where they were left for the duration of the experiment.
5. Barley plants were also grown in the glasshouse where the temperature and humidity varied more than in the growth room. A thermohygrograph in the glasshouse measured temperatures between 15-26°C, and the humidity varied between 50-95%.

b) Inoculating Barley Plants With *Drechslera teres*

1. The inoculum was taken from a *D. teres* inoculum type which had been growing for 7 days on MEA+B (refer to Appendix 1 for recipe). 15 ml of sterile distilled water plus a drop of Tween 80 was added to each dish. The mycelial growth and spores were scraped off the agar surface from a total of 5 petri dishes with a sterile loop into a 100 ml conical flask, yielding a total amount of about 75 ml inoculum suspension.
2. A spore/mycelium suspension was prepared from each of the 6 isolates, which were H88, T83, T88, Z87, K87 and K88.
3. Each suspension was then stirred using a magnetic stirrer for 15 minutes continuously, then filtered using a piece of No. 11 nylon mesh with 100µm x 100µm perforations, to remove any clumps of unfragmented hyphae.
4. The inoculum from one isolate was placed into an atomiser (Quickfit) and was applied to the both leaf surfaces of all the two week old barley plants growing in each pot for 10 seconds. After the application of each inoculum type the atomiser was rinsed once with 95% alcohol (ethanol) and then twice with sterile distilled water. The inoculum types ANT148, H88, T83, T88, Z87, K87 and K88 were applied to each of the cultivars H89, T89, Z87, K87 and K88 following the pattern shown in Table 4. The control pot of each barley cultivar was sprayed with a mixture of 75 ml sterile distilled water with a drop of Tween 80.

Table 4: Test grid showing the pattern of application of inoculum to cultivars

<div>Cultivar</div> <div>Inoculum</div>	H89	T89	K87	K88	Z87
ANT148 - ?					
H88 - net					
T83 - net					
T88 - net					
Z87 - net					
K87 - spot					
K88 - spot					
Control

Photograph 1: How the inoculated barley plants were grown in a glasshouse, with plastic bags on for the duration of the experiment

5. Each pot of inoculated seedlings was covered with a plastic bag after inoculation to keep the humidity level high and constant for the duration of the experiment. Refer to Photograph 1, to see how the plants were grown in a glasshouse. The pots

were kept in a growth chamber at 20°C on a 12 hours light-12 hours dark regime, or in the glasshouse where the temperature ranged from 15-28°C and were exposed to natural daylengths.

c) Examination of Fungal Penetration and Development of Hyphae

1. Two leaf samples of 4 cm segments were taken from each pot at intervals of 24 hours after inoculation for 7 days, then at two day intervals up to 21 days after inoculation.
2. The leaf segments were cleared in a 1:1 mixture of glacial acetic acid and glycerol (or glycerine).
3. The leaf segments were placed into small vials with 5 ml of glacial acetic acid and glycerol. The vials were placed into a water bath which was boiling and left there for about 45 minutes. The original method (Smedegard-Petersen, 1971) called for boiling the glacial acetic acid and glycerol until evaporation of the glacial acetic acid, but there seemed no way to do this without boiling the leaves to a pulp.
4. After 45 minutes or after the leaf segments had been cleared of chlorophyll, the sections were transferred to vials with lactophenol-cotton blue (100 ml lactophenol + 0.05 g aniline blue) and gently heated for 1 minute, then rinsed and mounted in clear lactophenol on a slide with a coverslip. The slides were then sealed with clear nail polish.
5. Each slide was examined for any noticeable lesions on the leaf segments, germinated conidia, the method of penetration through the leaf epidermis and the method of growth of invading hyphae inside the leaf.

Somatic Hybridisation Studies

The Crossing of a "Net" Type and a "Spot" Type

In this experiment the aim was to attempt to cross a *D. teres* "net" type and a "spot" type of which their levels of sensitivity or resistance had been previously determined. Here an attempted crossing was carried out between 13Y, a "net" type which was sensitive to both triadimenol and flutriafol, and KF₂, a "spot" type which was resistant to both triadimenol and flutriafol. Refer to Appendix 5 for further information on triadimenol and flutriafol.

Two methods were used in the attempt to cross 13Y and KF₂. The first method involved spraying both inoculum types on the same healthy barley plants. The second method involved growing both inoculum types together on WA+B barley leaf decoction media. It was observed in the course of experiments that many "connection tubes" formed between conidia, conidia and hyphae and between hyphae and hyphae, when grown on WA+B barley leaf decoction, and left under a NUV light under a 12 hour on-12 hour off regime. It was hoped that this might have been an indication of somatic crossing, and would occur between two different inoculum types.

Table 5: Information on the inoculum types 13Y and KF₂ used in the crossing experiment

Inoculum name	Type	Seed type	Source	Year
13Y	"Net"	Carlsberg	Wairarapa	1976
KF ₂	"Spot"	Kym	South Island	1986

a) Co-infection of barley plants:

1. Spore/mycelium suspensions were prepared from 7 day old culture of 13Y and KF₂ as described in Inoculating Barley Plants With *Drechslera teres*, in the methods section.
2. Both suspensions were then stirred using a magnetic stirrer for 15 minutes continuously, then filtered using a piece of No. 11 nylon mesh with 100µm x 100µm perforations, to remove any clumps of unfragmented hyphae.
3. The inoculum for 13Y was placed into an atomiser (Quickfit) and was applied to all the two week old plants in a pot for 10 seconds ensuring that both sides of the leaf were sprayed. Ten pots of barley plants were sprayed and left for half an hour to dry slightly. Then the inoculum type KF₂ was sprayed onto the same barley plants.
4. Separate pots of healthy barley plants were also sprayed with only 13Y, KF₂ or sterile distilled water + a drop of Tween 80 (control), respectively.

5. Each pot of inoculated seedlings was covered with a plastic bag for the duration of the experiment, to keep the humidity level high and constant. The pots were kept in a glasshouse, where the temperature ranged from 15-28°C and were exposed to natural daylengths.

b) Crossing on WA+B barley leaf decoction media:

1. Four plugs were cut randomly from the edge of 7 day old sporulating cultures of 13Y. The plugs were put right side up, in a straight line on a plate of WA+B barley leaf decoction (refer to Appendix 3). Four plugs of 7 day old sporulating cultures KF₂ were cut in the same way as 13Y and put in a straight line beside the plugs of 13Y. The distance between the two different plugs varied between 0.5 cm to 1.5 cm, to provide some variance for crossing to occur. Refer to Photograph 2 for plates after inoculation for crossing.
2. Ten replicate plates were left under a NUV light under a 12 hour on-12 hour off regime for 3 weeks for any somatic crossing to occur during co-culture.
3. After 3 weeks, a conidium or strand of hyphae from between the two different plugs was picked off under sterile conditions and plated onto a plate of MEA+B.
4. Forty replicate plates were made, one from each co-culture pair of plugs and were left under a NUV light (refer to Appendix 8 for emission and specifications) under a 12 hours on-12 hours off regime for a week to grow.
6. After a week, all successfully growing products of the crosses were numbered for identification.
7. The products of the crosses were tested by cutting a 5 mm plug of sporulating 7 day old fungal growth from the growing edge and placed upside down on a plate of MEA+B with fungicide. Each test was replicated. Therefore a plug of fungal growth was placed on a plate of 50 ppm triadimenol, 100 ppm triadimenol, 10 ppm flutriafol and 50 ppm flutriafol. One replicate was made for each test. Control plates, i.e. 0 ppm triadimenol and flutriafol were also set up.
8. The tests were carried out for all the viable cross progeny, as well as the two parental types 13Y and KF₂.
9. All plates were placed under a NUV light under a 12 hours on-12 hours off regime at room temperature, which varied between 18-23 °C. The growth diameter was measured at the appropriate time following the tables in Cereal Diseases 1986-1987, Report No. 24, pp 53 and 65.
10. Measurements were made by measuring the colony diameter to the nearest mm. Two measurements were made of the colonies at right angles on the reverse of the dish. Results were of the mean diameter after 5 mm had been subtracted to allow

for the inoculum plug. Growth diameter was compared to the tables and any similarities or differences were then analysed.

Photograph 2: The method of crossing 13Y and KF₂ on WA+B barley leaf decoction media

RESULTS

Comparison of isolates of *D. teres*

Examination of colony morphology

The inoculum types ANT148, H88, T83, T88, Z87, K87 and K88 were grown on MEA+B and their general appearance was noted. They were also examined for their ability to produce conidia, spermagonia and pseudopycnidia when grown on MEA+B. Spermagonia were defined as mycelium and conidia with thickened walls which formed into balls measuring up to approximately 1 mm in diameter. Pseudopycnidia are described in the Literature Review. Poor sporulators were also grown on WA+B barley leaf decoction medium, as this medium induced sporulation in poor sporulators. Perithecia did not form on MEA+B or WA+B barley leaf decoction medium. Pieces of Cellophane (regenerated cellulose) measuring 1 x 1 cm were added to the medium MEA+B as a cellulose source and left for more than a month to promote the formation of perithecia. The colour of the aerial hyphae was noted, as well as the colour the hyphal growth was when grown on the medium, MEA+B. The results of the observations are summarised in Table 6.

Table 6: Morphological description of colonies based on comparison of the overall appearance of a number of physical characteristics

Inoculum type	General appearance of colony in MEA+B*	Formation of conidia in MEA+B* or WA+B barley decoction [†]	Formation of spermagonia in MEA+B*	Formation of pseudopycnidia in MEA+B*	Aerial hyphae when grown on MEA+B*	Colour of hyphal growth in MEA+B*
ANT148 ?	white, no tufts	0 in MEA+B, 0 in WA+B barley leaf decoction	0	0	white	0
H88* net	white, tufts	+++ in MEA+B	+	+++	grey, white tufts	+++ black-green
T83 net	white, tufts	0 in MEA+B, +++ in WA+B barley decoction	+	+	white, grey tufts	++ black-green
T88 net	grey-white, tufts	0 in MEA+B, +++ in WA+B barley decoction	+	++	grey, white tufts	++ black-green
Z87 net	white, tufts	+++ MEA+B	+	+++	grey-white	++ black-green
K87 spot	white, coremia with conidia	+++ MEA+B	+++	++	white	+ black
K88 spot	white, coremia with conidia	+++ MEA+B	+++	++	white	+ black

For the complete data on the table above, please refer to Appendix 10.

Symbols:

- 0: no formation of structure, or no discernable colour of hyphal growth
- +: poor formation of structure, or poor colour intensity of hyphal growth
- ++: good formation of structure, or strong colour intensity of hyphal growth
- +++ : very good formation of structure, or very strong colour intensity of hyphal growth

* Refer to Appendix 1 for recipe.

[†] Refer to Appendix 3 for recipe.

■ H88 was the only isolate which formed perithecia in culture after 2 months growth in MEA+B*. None of the other isolate types could be persuaded to form perithecia even with the addition of Cellophane as a cellulose source.

The general appearance of the colonies were all different in appearance when grown in MEA+B. The inoculum type ANT148 stood out because it had fluffy, fine white hyphal growth which grew quickly, and never produced any conidia, pseudopycnidia or spermagonia on MEA+B (refer to Photograph 3). ANT148 also did not produce any conidia on WA+B barley decoction medium. This was unusual, as WA+B barley decoction stimulated the production of conidia in the poor sporulating forms like T83 and T88.

The "net" types, H88, T83, T88 and Z87 tended to have an overall tufty appearance, while the "spot" types tended to have an overall coremial appearance (refer to Photograph 3). Coremia, refers to the fan-like hyphal strands which form when grown in culture.

Photograph 3: The difference in morphology of the inoculum types studied, T83, T88, H88, Z87, K87, K88 and ANT148

The inoculum types H88, T83, T88, Z87, K87 and K88 produced conidia, spermatogonia, and pseudopycnidia in MEA+B, and had hyphal growth of varying colours and intensities when grown on MEA+B. All the inoculum types studied were different in their general morphology and ability to produce structures in MEA+B, but there was no correlation with whether it was a "net" or "spot" type. The two exceptions were, the isolates from the cultivar Triumph, T83 and T88, which were both poor sporulators, and the "spot" type isolates from the cultivar Kym, K87 and K88, were characteristic in that they both produced coremia, fan-like hyphal strands of growth, with conidia produced in the strands as well. However, the fact that the isolates T83 and T88 were poor sporulators may not be significant, as only two isolates from the cultivar Triumph were examined.

The SEM (Scanning Electron Microscope) micrographs showed that hyphae are made up of thicker strands which become the conidiophores, and thinner strands of hyphae. Refer to Photograph 4, for fungal growth comprising of hyphae and conidia.

Examination and measurement of conidia

A summary of the data from the measurement and examination of 100 conidia each from the inoculum types H88, T83, T88, Z87, K87 and K88 are shown in Tables 7 and 8 below. The data is also presented in graphical form in Graphs 1-4 for a visual reference showing the means and ranges of the data obtained on the length, width, estimated volume, number of cells per conidium and overall colour.

Photograph 4: SEM photo of seven day old fungal growth of H88 comprising of thick hyphae which make up the conidiophores, thin hyphae and conidia

Table 7: Mean and standard deviation (for a population based on a sample) for the lengths, widths, volumes, number of cells per conidium and predominant colour for each inoculum type

Inoculum Types	Lengths (μm)		Widths (μm)		Volumes (μm^3)		Number of Cells Per Conidium		Predominant Colour
	$\bar{x}$	σ_{n-1}	$\bar{x}$	σ_{n-1}	$\bar{x}$	σ_{n-1}	$\bar{x}$	σ_{n-1}	
H88 - net	51.213	13.172	17.775	2.9707	13822	7624.0	3.41	0.99590	green
T83 - net	54.031	10.446	15.056	2.2598	9913.5	3922.3	3.56	0.72919	olive
T88 - net	58.319	16.877	13.819	1.7563	9214.9	4379.8	3.78	0.95959	pale green
Z87 - net	57.2	15.728	16.913	2.5774	13911.3	7390.6	3.72	0.80503	yellow-green
K87 - spot	53.406	10.317	16.656	1.7165	11959	4072.3	3.63	0.59722	olive
K88 - spot	48.956	12.985	15.2	1.9133	9443.98	4564.2	3.19	0.80019	green

For the complete tables on the data above, please refer to Appendix 11.

Table 8: The ranges of the length, width, volume and number of cells per conidium

Inoculum types	Length (µm)		Width (µm)		Volume (µm)		Number of cells	
	Min	Max	Min	Max	Min	Max	Min	Max
H88 - net	26.25	92.5	12.5	26.25	3374.76	40978.76	1	6
T83 - net	30	75	11.88	21.25	3599.49	23939.30	2	5
T88 - net	23.75	120.5	10	18.13	2356.19	24353.48	2	6
Z87 - net	27.5	112.5	11.25	22.5	2982.06	36352.28	2	6
K87 - spot	22.5	76.25	12.5	22.5	3067.96	27832.55	2	5
K88 - spot	23.75	80	10	20	2159.84	24347.34	1	5

For the complete data on the table above, please refer to Appendix 11.

Graph 1: Mean lengths of 100 conidiospores

Testing the data

The data obtained was analysed using a one-way analysis of variance (ANOVA) to test if the means were equal. If they were not equal, then there was some difference in the

Widths of 100 Conidia

Mean, maximum and minimum values
of conidia width measurements

Graph 2: Mean widths of 100 conidiospores

means. That meant that the mean length, mean width, mean volume or mean number of cells per conidium were not all equal. An ANOVA was carried out separately on the length, width, volume and number of cells per conidium.

The data was further examined by checking the 95% confidence intervals for the means. Any confidence intervals which did not overlap, was identified as the mean (or means) which was not the same as the other means.

The hypothesis to be tested was, the means were equal

$$H_0: \mu_i = \mu_j \text{ for all values } i \text{ and } j = 1, 2, \dots, I, \text{ against}$$

$$H_1: \mu_i \neq \mu_j$$

the means were not all equal.

Decision rule:

To retain or reject the null hypothesis, H_0 , the test statistic, F , obtained from the ANOVA table, was compared with the F -value at the 5% significance level, in the F -distribution tables (Eton, 1982). If the test statistic, F exceeded the F -value on the same degrees of freedom (d.f.), then H_0 was rejected at the 5% level. The results were also significant at the 1% level if F exceeded the F -value at the 1% significance level. Otherwise, the null hypothesis, H_0 was retained, and it was assumed that the means tested were equal.

Volumes of 100 Conidia

Estimated by using lengths and widths

The mean, maximum and minimum values of estimated conidia volumes.

Graph 3: Estimated mean volumes of 100 conidiospores

Testing the lengths

The ANOVA results generated on Minitab to test the mean lengths of the conidia:

ANALYSIS OF VARIANCE				
SOURCE	DF	SS	MS	F
FACTOR	5	6233	1247	6.86
ERROR	594	107900	182	
TOTAL	599	114133		

LEVEL	TYPE	N	MEAN	STDEV
H88	net	100	51.21	13.17
T83	net	100	54.03	10.45
T88	net	100	58.32	16.88
Z87	net	100	57.20	15.73
K87	spot	100	53.41	10.32
K88	spot	100	48.96	12.98

POOLED STDEV = 13.48

The means of the lengths of the conidia were significantly different at the 5% and 1% levels when tested by an Analysis of Variance (ANOVA). K88 had the smallest mean length (48.96 µm), and T88 had the greatest mean length, (58.32 µm). There were no "outliers", and although the means were significantly different, there was no evidence to show that the "spot" type conidia were shorter than the "net" type conidia, or vice versa. Refer to Tables 7 and 8 and Graph 1 for a comparison of the lengths and range of lengths

Mean, maximum and minimum values for 100 conidia of each isolate type

The ANOVA results generated on Minitab to test the mean widths of the conidia:

The means of the widths of the conidia were also significantly different at the 5% and 1% level, tested by an Analysis of Variance (ANOVA). The test statistic, F, was 42.21, which showed that the widths were indeed very significantly different from each other. On examination of the 95% confidence intervals for the means of the widths, it showed that there was no overlap between any of the inoculum types except the inoculum types Z87 with K87 and T83 with K88. T88 which had the greatest mean length, had the smallest

mean width (13.82 μm), and H88 had the greatest mean width (17.78 μm). Refer to Tables 7 and 8 and Graph 2 for a comparison of the widths and range of widths of the inoculum types.

Testing the estimated volume

The ANOVA results generated on Minitab to test the mean estimated volumes of the conidia:

ANALYSIS OF VARIANCE					
SOURCE	DF	SS	MS	F	p
FACTOR	5	2.329E+09	465882432	15.13	0.000
ERROR	594	1.829E+10	30788174		
TOTAL	599	2.062E+10			

LEVEL	TYPE	N	MEAN	STDEV
H88	net	100	13822	7624
T83	net	100	9913	3922
T88	net	100	9215	4380
Z87	net	100	13911	7391
K87	spot	100	11959	4072
K88	spot	100	9444	4564

POOLED STDEV = 5549

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

The means of the estimated volumes of the conidia were also significantly different at the 5% and 1% level when tested using an Analysis of Variance (ANOVA). Examination of the 95% confidence interval for the means of the volumes, showed that there were many overlapping confidence intervals which fell into three groups, H88 and Z87, K87, and T83, T88 and

K88. The greatest mean estimated volume was Z87 with 13911 μm^3 and the smallest was T88 with 9215 μm^3 . The results did not show that the "spot" type inoculum produced smaller conidia than the "net" type, or vice versa. Refer to Tables 7 and 8 and Graph 3 for a comparison of the estimated volumes of the inoculum types.

Testing the number of cells per conidium

The ANOVA results generated on Minitab to test the mean number of cells per conidium:

ANALYSIS OF VARIANCE					
SOURCE	DF	SS	MS	F	p
FACTOR	5	23.748	4.750	6.97	0.000
ERROR	594	404.850	0.682		
TOTAL	599	428.598			

LEVEL	TYPE	N	MEAN	STDEV
H88	net	100	3.4100	0.9959
T83	net	100	3.5600	0.7292
T88	net	100	3.7800	0.9596
Z87	net	100	3.7200	0.8050
K87	spot	100	3.6300	0.5972
K88	spot	100	3.1900	0.8002

POOLED STDEV = 0.8256

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

The number of cells per conidium were also tested using an Analysis of Variance (ANOVA) because although the means were between 3.0 and 4.0, they were distinct

numbers and did indicate a difference. The means of the number of cells per conidium was significantly different at the 5% and 1% level. T88 had the greatest number of cells per conidium and K88 had the smallest number of cells. The only point to note here is that K88 also had the smallest mean length, while T88 had the greatest mean length. This could mean that the greater the length of a conidium, the greater the number of cells per conidium. Refer to Tables 7 and 8 and Graph 4 for a comparison of the number of cells per conidium in the isolate types.

Table 9: A summary of the decisions of whether the means were equal, based on the ANOVA tests for the conidia characteristics examined

Conidium characteristics tested	F-value (from Eton Statistical & Math Tables) on (5, 594) degrees of freedom		Test statistic F (from ANOVA table generated)	H ₀ decision and level of significance
	5%	1%		
Length	2.2141	3.0173	6.86	All means are not equal at the 5% and 1% level.
Width	2.2141	3.0173	42.21	All means are not equal at the 5% and 1% level.
Volume	2.2141	3.0173	15.13	All means are not equal at the 5% and 1% level.
Number of cells per conidium	2.2141	3.0173	6.97	All means are not equal at the 5% and 1% level.

Conidium colour

The conidia examined ranged between yellow-green to olive in colour. Refer to Table 7 for a comparison of the predominant colour of the inoculum types. The predominant colour of the conidia for each inoculum type examined also ranged between yellow-green to olive, and there was no way to distinguish the "net" types from the "spot" types based on the colour of the conidia. Different isolate types have a different predominant colour. The examination of conidia from older cultures from all isolate types approximately 2-3 weeks old showed that the walls of the conidia grew thicker and were a brown colour when they grew older.

Examination of germinated monoconidial isolates

The examination of germinated conidia under SEM did not prove to be as helpful as anticipated, due to the magnification having to be quite low to encompass the area covered by a germinated conidium. The following results are therefore based on germinated conidia examined under a light microscope.

Table 10: Single conidia, germinated on Cellophane placed on MEA+B, examined after 24 and 48 hours, using a compound microscope

Inoculum type and replicate	Number of germinated cells / total number of cells in a conidium after 24 or 48 hours growth		Position of germinated cells (1 denoting the top cell, and the largest number denoting the cell nearest the hilum)		Degree of branching of germinated hyphae and any other characteristics after 24 or 48 hours growth	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
H88 1	2/4	2/4	1 & 4	1 & 4	Poor branching. No other noticeable features.	Good branching. Some strands of hyphae appeared twisted together like rope.
2	2/4	2/3	1 & 4	1 & 3		
3	1/3	2/4	3	1 & 4		
4	2/4	2/4	1 & 4	1 & 4		
5	2/3	2/4	1 & 3	1 & 4		
T83 1	2/4	2/3	1 & 4	1 & 3	Good branching. No other noticeable features.	Good branching. Many "buds" of new branches, which had not developed after this time interval.
2	2/5	2/4	1 & 5	1 & 4		
3	2/5	1/3	1 & 5	3		
4	2/4	2/4	1 & 4	1 & 4		
5	1/3	2/4	4	1 & 4		
T88 1	2/5	2/4	1 & 5	1 & 4	Poor branching. No other noticeable features.	Poor branching. Some instances where strands of hyphae appeared twisted together like rope.
2	1/4	1/4	3	4		
3	2/4	2/5	1 & 4	3 & 5		
4	2/4	1/4	1 & 4	3		
5	2/4	2/5	1 & 4	1 & 5		
Z87 1	2/4	3/7	1 & 4	1, 4 & 7	Good branching. Formation of many enlarged sections in the middle and at the growing tip of hyphae. Could indicate appresoria.	Good branching. Formation of many enlarged sections in the middle and at the growing tip of hyphae. Could indicate appresoria.
2	2/4	2/3	1 & 4	1 & 3		
3	1/4	2/4	4	1 & 4		
4	2/5	2/4	1 & 5	1 & 4		
5	2/4	1/3	1 & 4	3		

K87	1	2/2	2/4	1 & 2	1 & 4	Extensive network of branching. Formation of enlarged sections of hyphae. Instances where strands of hyphae appeared twisted together like rope. Conidium no. 2 had 2 germ tubes from one cell.	Extensive network of branching. Formation of enlarged sections of hyphae. Many instances where strands of hyphae appeared twisted together like rope.
	2	1/3	1/4	3	2		
	3	2/4	2/3	1 & 4	1 & 3		
	4	2/4	2/4	1 & 4	1 & 4		
	5	2/5	2/4	3 & 5	1 & 4		
K88	1	2/3	3/3	1 & 3	1, 2 & 3	Poor branching. Formation of many enlarged sections of hyphae and appresoria. Conidium no. 1 had 2 germ tubes from cell 1.	Good branching. Formation of many enlarged sections of hyphae and appresoria. Conidium no. 1 had 2 germ tubes from cells 1 and 3. 3/3 cells germinated in Conidium no. 1.
	2	1/3	2/3	3	1 & 3		
	3	2/3	2/4	1 & 3	1 & 4		
	4	2/4	2/4	1 & 4	1 & 4		
	5	3/5	2/4	1, 3 & 5	1 & 4		

The inoculum type H88 did not branch well in the first 24 hours, but improved and all conidia examined had branched well by the next 24 hours. The isolates from the Triumph cultivar were not consistent in their branching. T83 had good branching ability (refer to Photograph 5), while T88 had poor branching. Z87 had good branching. K87 had the best branching pattern seen amongst all the inoculum types examined.

Photograph 5: T83 - 24 hours growth, shows the good branching pattern, and germ tubes emerging from opposite ends of the conidium

The inoculum types Z87, K87 and K88 all had enlarged middle sections of hyphae. The inoculum types Z87 and K88 had enlarged sections at the growing tips which could be appresoria.

All the isolates examined showed that any cells that germinated were separated by as many cells as was possible (refer to Photograph 5), and the first cell to germinate is probably the cell at the hilum. For instance, in a conidium which had 2 out of 4 cells germinated, the germinated cells would always be the first cell and the last cell. In a conidium with 4 cells which had only one germinated cell, the germinated cell was often the cell at the hilum. This indicates that the cell at the hilum is usually the first cell to germinate.

The "spot" type isolates from the cultivar Kym, K87 and K88, were unusual in that they had more than one germ tube emerge from one cell of a conidium. Refer to Photograph 6 showing two germ tubes emerge from the same cell.

Photograph 6: K87 - 24 hours growth, showing two germ tubes emerging from one cell

Growth rates of the different inoculum types

In this experiment plugs of sporulating 7 day old fungal growth were cut from the edge of a colony. The diameter of a colony was measured once a day at approximately the same time each day by taking the mean of the diameters of the colony at right angles to each other. Day 1 shows the measurement of the growth which was 5 mm, which was the diameter of the plug. The measurements on successive days are cumulative.

Growth Rate Slopes

Shown by diameters (mm) of
the different isolates

The mean of two measurements of the diameter were taken at right angles to each other once a day.

Graph 5: Growth rates of the different inoculum types over the 9 day period

Table 11: Mean diameters of colonies (mm) following inoculation (days)

Inoculum type	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
ANT148 - ?	5	21	30	49.4	64.8	76.2	82	82	82
H88 - net	5	16.6	23	37.2	50	60.8	72.6	81	82
T83 - net	5	14.2	22	32	45	55.4	67.2	78.6	82
T88 - net	5	15.8	24.6	36	49.6	59.8	71.2	80.8	82
Z87 - net	5	15.4	26	36	50.4	60	71.4	80.2	82
K87 - spot	5	19.6	30.8	43.8	55.4	66.6	74.6	82	82
K88 - spot	5	10.4	14	35.4	51.8	64.4	74.4	80.8	82

Note: 82 mm is the maximum diameter because that was the size of the petri dish. For complete details of the data, refer to Appendix 12.

In this experiment, five replicates of each inoculum type were grown in an incubator at 25°C. Any variation in growth would be due to the ability of the inoculum types to grow in MEA+B. Graph 5 shows the slopes of the means of the growth rates of the inoculum types, ANT148, H88, T83, T88, Z87, K87 and K88.

There did not seem to be any significant difference in the growth rates of the different inoculum types as seen in the very similar slopes of the inoculum types. ANT148 was clearly faster growing than any other inoculum types. This may not be comparable to the other inoculum types as ANT148 had changed in morphology and had aconidial, featureless growth, while the inoculum types H88, T83, T88, Z87, K87 and K88 had conidial growth with other structures such as, pseudopycnidia and spermagonia.

Cellulase Production

The ability of a germinating conidium to produce cellulase is important in the continued growth and invasion capabilities of a fungus in a host. In this experiment, 30 successfully germinated conidia of each inoculum type were grown on a piece of Cellophane (regenerated cellulose) on MEA+B and then the Cellophane was lifted off each day and placed on a fresh plate of MEA+B. The plate which had the pieces of Cellophane lifted off was incubated for a day and noted for any fungal growth. The number of conidia which had penetrated the Cellophane and grown on the MEA+B underneath were scored for each day. The experiment was carried out for four days, by which time all conidia had penetrated the Cellophane and grown on the MEA+B underneath.

Table 12: Demonstration of cellulase production - shown by the number out of germinated conidia, out of 30 tested, which successfully penetrated a piece of Cellophane after 1-4 days growth

Inoculum Type	No. of germinated conidia which successfully penetrated a piece of Cellophane after 1-4 days growth			
	Day 1	Day 2	Day 3	Day 4
H88 - net	0	2	18	10
T83 - net	0	0	12	18
T88 - net	0	0	3	27
Z87 - net	0	0	18	12
K87 - spot	0	2	19	9
K88 - spot	0	3	21	6

Note: No germinated conidia managed to penetrate the piece of Cellophane after one day, and all germinated conidia had penetrated the Cellophane after 4 days.

Cellulase Production

Shown by total germinated conidia which penetrated the Cellophane after each day

All 30 germinated conidia of each isolate had penetrated the Cellophane after 4 days growth.

Graph 6: Cellulase production by germinating conidiospores

Comparing the results from the branching abilities in the Examination of Germinated Conidia, it was seen that isolates which branched well like T83 and Z87 did not penetrate the Cellophane in two days (refer to Graph 6). There is no relation between the ability to branch well and the time taken (measured in days) to penetrate a piece of Cellophane.

Studies of Barley Leaf Infection

Pathogenicity Tests

Healthy two week old barley plants growing in pots of 20 plants each were sprayed with a spore/mycelium suspension. Each barley cultivar used, ANT86, H88, T83, T88, Z87, K87 and K88 was sprayed with each inoculum type, ANT148, H88, T83, T88, Z87, K87 and K88. The control plants of each cultivar were sprayed with suspension of sterile distilled water with a drop of Tween 80. The inoculated barley plants were observed on a daily basis for any lesions, i.e. brown discolouration, and any areas of water-soaking on the leaves. The results presented here are at one week and two weeks after inoculation.

Table 13: Degree of infection and type of symptoms on barley plants one week after inoculation

Cv Inoculum	ANT86	H89	T89	Z87	K87	K88
ANT148 - ?	1 Spot	0	0	0	0	2 Spot
H88 - net	1 Net	0	0	1 Net	0	1 Net/Spot
T83 - net	1 Net	2 Net	2 Net	2 Net	1 Net	2 Net/Spot
T88 - net	1 Net	2 Net	0	2 Net	1 Net	2 Net/Spot
Z87 - net	1 Net	2 Net	1 Net	0	2 Net	2 Net/Spot
K87 - spot	2 Spot	2 Spot	2 Spot	0	2 Spot	2 Spot
K88 - spot	1 Spot	1 Spot	0	0	0	0
Control	0	0	0	0	0	0

Infection rating scheme:

- 0 - Not infected (no noticeable lesions or areas of water-soaking)
- 1 - Lightly infected (just a few lesions and/or areas of water-soaking seen)
- 2 - Moderately infected (about half the leaves on the plant had noticeable lesions and/or areas of water soaking)
- 3 - Severely infected (more than half the leaves had lesions with yellow, water-soaked areas)

Table 14: Degree of infection and type of symptoms on barley plants two weeks after inoculation

Cv Inoculum	ANT86	H89	T89	Z87	K87	K88
ANT148 - ?	2 Spot	1 Spot	1 Spot	1 Spot	1 Spot	3 Spot
H88 - net	1 Net	2 Net	1 Net	2 Net	1 Net	2 Net/Spot
T83 - net	2 Net	3 Net	3 Net	3 Net	2 Net	3 Net/Spot
T88 - net	2 Net	3 Net	2 Net	3 Net	2 Net	3 Net/Spot
Z87 - net	1 Net	3 Net	2 Net	2 Net	3 Net	3 Net/Spot
K87 - spot	3 Spot	3 Spot	3 Spot	1 Spot	3 Spot	3 Spot
K88 - spot	1 Spot	1 Spot	1 Spot	1 Spot	1 Spot	3 Spot
Control	0	0	0	0	0	1 Spot

Infection rating scheme:

- 0 - Not infected (no noticeable lesions or areas of water-soaking)
- 1 - Lightly infected (just a few lesions and/or areas of water-soaking seen)
- 2 - Moderately infected (about half the leaves on the plant had noticeable lesions and/or areas of water soaking)
- 3 - Severely infected (more than half the leaves had lesions with yellow, water-soaked areas)

The pathogenicity tests showed that all isolate types examined were able to infect all the cultivar types used. Refer to Photographs 7 and 8 respectively, for examples of "net" and "spot" type symptoms on barley.

Photograph 7: An example of "net" type symptoms on barley

The cultivar K88 also developed "spot" symptoms on the control plants, and on plants inoculated with "net" type inoculum. This meant that the "spot" type inoculum on the infected seed was able to infect the barley plants. This is evidence of infected seed being the main source of primary inoculum in New Zealand. Refer to Photograph 9, for "net" and "spot" symptoms on K88 which had been inoculated with "net" type inoculum.

ANT148 which had previously been an unknown *D. teres* isolate, was confirmed to be a "spot" type of net blotch. Refer to Photograph 10 for the "spot" symptoms produced by ANT148.

Examination of fungal penetration and development of *D. teres* in the leaf

In this experiment 4 cm leaf segments were cut off the inoculated barley plants at 24 hour intervals after inoculation for 7 days and then at two day intervals for up to 21 days after inoculation. The leaf sections were cleared in a (1:1) glycerol: glacial acetic acid mixture and then stained in lactophenol cotton-blue. The results presented in Tables 15 and 16 show the extent of the invasion by the fungus in the leaf segments at 3 days and 21 days after inoculation respectively. The ratings are summarised after the tables and are followed by other observations not covered by the rating scheme.

Photograph 8: An example of "spot" type symptoms on barley

Photograph 9: K88 barley plants showing both "net" symptoms from the inoculation, and "spot" type symptoms which carried through from the infected seed.

Photograph 10: Barley plants inoculated with ANT148, which is a "spot" type of *D. teres*

Table 15: Examination of barley leaf segments harvested three days after inoculation, cleared and stained

Cv Inoculum	H89	T89	Z87	K87	K88
ANT 148 - spot	0	2	2	1	3
H88 - net	0	0	1	0	2
T83 - net	1	2	3	4	2
T88 - net	2	4	4	0	4
Z87 - net	0	2	0	0	0
K87 - spot	4	4	4	4	3
K88 - spot	0	0	0	0	0
Control	0	0	0	0	0

Infection rating scheme:

- 0 - No infection seen in leaf
- 1 - Very little infection, just one or two strands of hyphae in leaf
- 2 - Some infection, small areas of localised hyphal infection seen in leaf
- 3 - Good infection, large areas of hyphal infection seen in leaf
- 4 - Very good infection, almost entire leaf section riddled with hyphae

Other observations:

1. No point of entry from germinating conidia or growing hyphae from the inoculum sprayed on the barley plants was seen on any of the leaf sections. Either the leaf segments which were harvested did not have any germinating conidia on the surface, or the process of clearing and staining was not efficient in highlighting any structure which was not obvious. One or two shrivelled conidia were seen on the leaf surface but did not appear to have germinated. It is not certain whether these conidia were originally positioned there or had become lodged there in the clearing staining process.
2. Hyphae tended to grow intercellularly between cells in the leaf and travelled for quite long distances along the length of the leaf, without causing any cell disruption. The longest distance measured was the entire length of a cleared leaf segment which was approximately 4 cm.
3. The invading hyphae emerged from inside the leaf either straight through the epidermis or sometimes through the stomata. The hyphae were thin-walled and septate without any obvious colouration and the width was about 5 μm on average. These thin-walled hyphae grew on the leaf surface and were seen to penetrate the leaf again by going straight through the epidermis, or through stomata. Club-shaped appresoria were also seen at the end of some of the thin-walled hyphae. Refer to Photograph 11 for appresorium at the end of a thin-walled hypha.

Photograph 11: Appresorium at the end of an invading thin-walled hypha, growing on a barley leaf surface

4. No other forms of hyphae were seen at three days after inoculation, nor was any sporulation .

Table 16: Examination of barley leaf segments harvested three weeks after inoculation, cleared and stained

Cv Inoculum	H89	T89	Z87	K87	K88
ANT148 - spot	1	3	3	2	3
H88 - net	2	2	2	2	2
T83 - net	4	3	3	4	4
T88 - net	4	4	4	2	4
Z87 - net	2	3	2	2	2
K87 - net	4	4	4	4	4
K88 - net	4	4	2	4	3
Control	0	0	0	0	1

Infection rating scheme:

- 0 - No infection, no hyphae seen in leaf
- 1 - Very little infection, just one or two strands of hyphae in leaf
- 2 - Some infection, small areas of localised hyphal infection seen in leaf
- 3 - Good infection, large areas of hyphal infection seen in leaf
- 4 - Very good infection, almost entire leaf section riddled with hyphae

Other observations:

1. Again, no point of entry from germinating conidia or growing hyphae from the inoculum sprayed on the barley plants were seen on any of the leaf segments, although at three weeks after inoculation any germinating conidia would have grown far beyond the infection stage.
2. Hyphae still tended to grow intercellularly for long distances along the length of the leaf as in the leaf segments harvested three days after inoculation. There was also noticeable disruption of the cells in the leaf with the hyphae penetrating the cells instead of just travelling between the cells.
3. Two forms of hyphae emerged from the leaf at this stage. There was the thin-walled, septate, emergent hyphae as well as a thick-walled, septate form which was olivaceous in colour and measured about 8µm in width. They were shorter and seemed to stand up from the leaf instead of growing over and parallel to the leaf surface like the thin-walled hyphae. The thick-walled hyphae had a globose basal cell where it emerged from the leaf and these were not seen to penetrate the leaf again as was the case with the thin-walled hyphae.
4. Some conidia were seen on the infected leaf surfaces, but they were not attached to any hyphae. They were in good form, not shrivelled or damaged and always found with one end in a stoma. It is possible they fell off the thick-walled hyphae and lodged in the stomata in the clearing and staining process.

The inoculum type K87, produced good "spot" symptoms on the barley plants, and also showed up growing in the entire segment of the leaves, harvested and cleared, after three days and three weeks (refer to Tables 15 and 16). On the other hand, ANT148 and K88, the other "spot" types, did not show net blotch symptoms on barley plants as severe as K87 (refer to Tables 15 and 16). K88 did not show up well in the leaf sections harvested after three days, but riddled the entire section of leaves harvested after three weeks (refer to Tables 15 and 16).

Somatic Hybridisation Studies

The crossing of a triadimenol and flutriafol sensitive "net" type with a non-sensitive "spot" type of *D. teres* and testing the resultant progeny on fungicides

Two methods of crossing were attempted. 13Y, a sensitive "net" type of *D. teres*, was crossed with KF₂, a non-sensitive "spot" type. Both grew well on MEA+B, but growth was not measured in any way.

The first method involved spraying both inoculum types onto healthy barley plants. It was hoped that some somatic or parasexual recombination of the two types of *D. teres* would occur when sprayed onto barley plants as a result of cytoplasmic mixing as hyphae grew together either on the leaf surface or while growing internally in the leaf. Leaf segments would then be plated onto MEA+B (refer to Appendix 1 for recipe) and isolates made from single spores. The single spore cultures would be the resultant cross progeny.

The second method involved plates being inoculated with plugs of both isolates side by side. In the promotion of the formation of conidia, achieved by growing the fungus on MEA+B barley leaf decoction (refer to Appendix 3 for recipe), it was noticed that connection tubes formed between conidia, as well as between conidia and hyphae. Refer to Photographs 12 and 13. These connections might have been an indication of somatic or parasexual recombination. A single conidium or strand of hyphae, was then excised from between the plugs, and plated out on MEA+B (refer to Appendix 1 for recipe). Forty plates were made of the cross progeny.

The sensitivity of the cross type isolates to certain fungicides would then be tested using triadimenol and flutriafol. Refer to Appendix 5 for information on triadimenol and flutriafol. The results were compared with sensitivity tests carried out earlier by other researchers. Any intermediate growth would possibly indicate a hybrid between 13Y and KF₂.

Photograph 12: Connections that form between conidia, and between conidia and germ tubes

Photograph 13: Connections that form between conidia

Crossing on barley plants:

No visible lesions were seen in the barley plants inoculated with the cross or the parental types, 13Y and KF₂ in the first, second or third week after inoculation. The experiment was planned to be continued until some lesions were seen, but was abandoned after the third week when an aphid outbreak occurred. There reason why no infection occurred is not known, as the conditions were exactly the same as in the pathogenicity tests which were successful. The inoculum was also still viable as both 13Y and KF₂ grew well in MEA+B. The experiment was not repeated due to illness and time constraints.

Crossing on MEA+B barley decoction:

Of the 40 plates made from a strand of hypha or a conidium, only 15 grew successfully. It is possible that the process of separating conidia or hyphae with connection tubes, damaged them in some way, preventing them from growing. These 15 plates were used as the cross types. The plugs used in the sensitivity bioassay were cut from the growing edge of sporulating 7 day old plates of the parental and cross type isolates. The results of the sensitivity tests using triadimenol on the parental types, 13Y, KF₂ and the cross types, shown in Table 17, were compared with sensitivity tests using triadimenol carried out earlier by other researchers in 1986, on 13Y and KF₂, shown in Table 18, to determine if the cross type cultures were sensitive or resistant. Any intermediate results would indicate successful crosses.

Table 17: Mean colony diameters (mm)* of the "net" type and "spot" type of *D. teres* and the 15 isolates of their resultant crosses on 50 and 100 ppm triadimenol

Isolate type	Mean colony diameter (mm)*			
	50 ppm triadimenol (µg/ml)		100 ppm triadimenol (µg/ml)	
	Day 7	Day 10	Day 7	Day 10
13Y - net	0	0	0	0
KF ₂ - spot	12.5	15.5	4	6
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0

* Colony diameters were measured to the nearest mm (2 measurements/plate at right angles on reverse of dish) Results are for mean diameter after 5 mm has been subtracted to allow for the inoculum for 2 replicates.

Table 18: Sensitivity of 1973-1986 isolates of *D. teres* to triadimenol carried out in November 1986 (Sheridan and Nendick, 1987). Measurements show mean colony diameter (mm)*

Isolate type	Triadimenol Concentration (µg/ml)	0	5	10	50	100
13Y 1976 Carlsberg	Day 7	55	20	10	0	0
	Day 10	55	25	18	0	0
KF ₂ 1986 Kym	Day 7	69	60	50	25	19
	Day 10	75+	75	75	35	26

* Colony diameters were measured to the nearest mm (2 measurements/plate at right angles on reverse of dish) Results are for mean diameter after 5 mm has been subtracted to allow for the inoculum for 2 replicates.

The sensitivity bioassay showed that there was an increase in sensitivity, or a decrease in resistance to triadimenol, by the resistant isolate KF₂. There were no progeny which showed any levels of resistance to triadimenol.

Again, the same sensitivity tests were carried out using flutriafol. The results of the sensitivity tests using flutriafol are shown in Table 19, were compared with sensitivity tests using flutriafol carried out in 1987 shown in Table 20, to determine if the progeny cultures were sensitive or resistant. Any intermediate results would indicate successful crosses.

Table 19: Mean colony diameters (mm)* of the "net" type and "spot" type of *D. teres* and the 15 isolates of their resultant crosses on 10 and 50 ppm flutriafol

Isolate type	Mean colony diameter (mm)*					
	10 ppm flutriafol (µg/ml)			50 ppm flutriafol (µg/ml)		
	Day 7	Day 10	Day 11	Day 7	Day 10	Day 11
13Y - net	1	1	1	0	0	0
KF ₂ - spot	13	15.5	16	10	11	11.5
1	0	0	0	0	0	0
2	1	1	1	0	0	0
3	1	1	1	0	0	0
4	1	1	1	0	0	0
5	1	1	1	0	0	0
6	0	0	0	0	0	0
7	1	1	1	0	0	0
8	1	1	1	0	0	0
9	1	1	1	0	0	0
10	1	1	1	0	0	0
11	1	1	1	0	0	0
12	1	1	1	0	0	0
13	1	1	1	0	0	0
14	1	1	1	0	0	0
15	1	1	1	0	0	0

* Colony diameters were measured to the nearest mm (2 measurements/plate at right angles on reverse of dish) Results are for mean diameter after 5 mm has been subtracted to allow for the inoculum for 2 replicates.

Table 20: Sensitivity of isolates from farmers crops to flutriafol, 1986-1987 survey (Sheridan and Nendick, 1987). Measurements show mean colony diameter (mm)*

Isolate	Flutriafol concentration (µg/ml)	Mean colony diameter (mm)*			
		0	10	25	50
1976 Carlsberg (13Y)	Day 7	52	0	0	-
	Day 11	75	0	0	-
1986 Kym (KF₂)	Day 7	60	27	21	-
	Day 10[†]	72	51	-	23
	Day 11	75	40	34	-

* Colony diameters were measured to the nearest mm (2 measurements/plate at right angles on reverse of dish) Results are for mean diameter after 5 mm has been subtracted to allow for the inoculum for 2 replicates.

† Test carried out by DK Nendick, all others by JE Sheridan

Again, the sensitivity bioassay showed that there was an increase in sensitivity, or a decrease in resistance to flutriafol, of the isolate KF₂. None of the progeny tested showed any resistance to flutriafol. Although there was some growth in the progeny, this can be dismissed as the same growth occurred with 13Y, the sensitive isolate. Refer to Photograph 14, for the growth of the isolates 13Y and KF₂, on triadimenol and flutriafol.

Photograph 14: Growth of 13Y and KF_2 on triadimenol (50 and 100 ppm) and flutriafol (10 and 50 ppm), four weeks after inoculation

Note: The cross progeny had the same morphology and growth rate as 13Y, the sensitive "net" type

DISCUSSION

The results are discussed separately in this section, under the various experiments that were carried out.

Examination of colony morphology

The inoculum ANT148 was thought to be a "spot" type of *D. teres* because it was seen to be morphologically and behaviourally very similar to other "spot" type isolates from the Kym cultivars (Sheridan and Nendick, 1987). This was an interesting point to note, as ANT148 was morphologically different from both the "spot" and "net" types of *D. teres*. As the sensitivity tests were carried out in 1986, and at least three years had lapsed since the ANT148 isolate was subcultured from and grown in MEA+B. Some sort of change must occur in fungal cultures which are subcultured by cutting a plug of conidia and mycelium. This change in morphology to a featureless form of growth was also noticed in all the other inoculum types examined in the course of study (refer to Photograph 3), which were subcultured by cutting a plug of conidia and hyphae. For this reason, all subculturing was done by picking a conidium and plating it on agar.

Other morphological studies on *D. teres*, have backed up this observation. Studies have shown that the original phenotype of most isolates could be retained through many generations of monoconidial subculture, but was more difficult to recover after repeated mycelial transfer. Some lines, from both monoconidial isolates from infected material and single-ascospore progenies from controlled crosses, were stable, but others mutated readily to atypical variants that never reverted to the parental type. A white, non-sporulating, non-virulent mutant was isolated most frequently (McDonald, 1967). Studies by Karki and Sharp (1986) on pathogenic variation of the "spot" type on barley, also noticed that subculturing by transferring masses of mycelia containing conidiophores and conidia onto fresh agar, more than once reduced conidial production. Only monoconidial isolates should be made when subculturing from stock culture, as the identity of an isolate could be lost otherwise.

Another change which was noticed with older cultures, which had been subcultured by the transference of a plug of conidia and hyphae, was the change in colour from green-black to an orange-red colour, in some sectors in some plates (refer to Photograph 15). This was the reason new cultures were made from fresh inoculum from the seed and infected leaf sources for all the experiments. Cultures made this way were constant in their appearance and form.

The inoculum H88 was the only one which produced perithecia in MEA+B after two months growth, although no mature ascospores were ever found. None of the other isolate types could be persuaded to produce perithecia even with the addition of pieces of Cellophane or barley leaves as a cellulose source. Perithecia were not examined in the experiments, nor any crossing resulting in the formation of perithecia, because a long period of time was required to produce them in culture.

Smedegård-Petersen disagreed with the work of Ito and Kuribayashi (1931), on the claw-like or fan-like hyphal bands in culture as a basis of separating the "net" type and the "spot" type into two species, as his experiments showed that both the "net" type and "spot" type produced the fan-like and the claw-like hyphal bands. My experiments confirmed the observations of Ito and Kuribayashi. In this thesis, the fan-like hyphal bands have been termed coremia. The "net" types consistently formed tuft-like hyphal structures with the claw-like hyphal bands, mentioned by Ito and Kuribayashi, on the margin of the petri dish when the culture was older and the colony growth came in contact with it. The "spot" types also consistently formed the fan-like hyphal bands, in culture, when grown on nutrient rich media like MEA+B. ANT148 as stated previously did not form any particular growth pattern, as it seemed to have changed to a featureless, aconidial type with repeated subculturing. These growth forms were not seen when grown on a non-nutrient rich media like WA. More New Zealand "net" and "spot" type isolates should be studied to see if there is a consistency in this observation of the fan-like hyphal bands restricted to the "spot" types, and the claw-like hyphal bands on the edge of the petri dish, with hyphal tufts on the centre, restricted to the "net" types.

Photograph 15: A comparison of plates freshly subcultured from monoconidial isolates, with plates repeatedly subcultured by cutting a plug of hyphae and conidia

Examination and measurement of conidia

There was no correlation between the length and width of a conidium. The inoculum type which had the greatest mean length did not have the greatest mean width. In fact the reverse was the case with T88. Each inoculum type had a different mean length and mean width, but it bore no relation as to whether it was a "net" type or a "spot" type. Ito and

Kuribayashi (1931) based the separation of the "net" and the "spot" type into two species, on the conidia width. The results of my experiments on conidia measurements of the "net" and "spot" types of *D. teres*, do not support Ito and Kuribayashi and agree with Smedegård-Petersen's (1971) observations. It was then decided to calculate the volume of the conidia, as they are three-dimensional structures, and the length and width did not give the whole picture. Although the volumes were also significantly different, there was no relationship between the conidia size and whether it was a "net" type or a "spot" type.

The examination of the conidia showed that the conidia from different inoculum types had different lengths, widths, volumes, number of cells per conidium and colour. None of these features bore any relationship as to whether it was a "net" type or a "spot" type. The conidia with the greatest length had the greatest number of cells per conidium, and the reverse was also true. This probably means that the number of cells per conidium is a consequence of conidia length.

Examination of germinated monoconidial isolates

There seemed to be no difference in the branching pattern of the different inoculum types. Some were good branchers, and some were not. There did not seem to be any relationship between branching pattern and whether an inoculum type was "net" or "spot".

The presence of the enlarged middle sections of hyphae is not explainable. The function of these structures is uncertain. In slides, and SEM photos made from older cultures, these same structures were seen in all the inoculum types studied. The experiment showed that the enlarged middle sections of hyphae can form within 24 hours growth, and are not only produced in older cultures. The enlarged sections of hyphae in the growing tips, could be appresoria, which were club-shaped in structure. These observations agree with those of Van Caeseele and Grumbles (1979), who stated that club shaped germ tubes grew to various lengths before producing appresoria, and sometimes extended up to 0.5 cm before producing an appresorium.

The germination experiments showed that each germinated cell in a conidium, was separated by at least one cell, or the germinated cells were at opposite ends of the conidium. Although Van Caeseele and Grumbles (1979) stated that the conidia usually germinated from one of the end cells, these experiments showed that the first cell to germinate was usually the one at the hilum, followed by the cell at the opposite end. Their observations that germ tubes sometimes arose from as many as four cells simultaneously agree with my observations, although this was only seen in the "spot" type isolates, K87 and K88. More work needs to be done, using more "spot" isolates types, and many more replicates done to see if germination from all cells in a conidium is restricted to the "spot" type isolates.

The "spot" type isolates from the cultivar Kym, K87 and K88, were unusual in that they were the only conidia which had more than two germ tubes emerge from one cell. Refer to Photograph 6 for a conidium with two germ tubes from one cell. In spite of the isolate K88 having the smallest volume, and the smallest number of cells per conidium, it had good branching, probably achieved by more than one germ tube growing from each cell.

Strands of hyphae developed in the inoculum types H88, T88, K87 and K88, which were twisted together tightly. It is uncertain why this happens. Perhaps it is the early stages of the formation of tufts in the "net" type and coremia in the "spot" types of *D. teres*.

Growth rates of the different inoculum types

This experiment showed that the "spot" types, ANT148, K87 and K88 were faster growing than the "net" types, from Day 5 onwards. The literature does not appear to show any experiments on the growth rates of the different isolates of *D. teres*. Although the isolate K88 started off slowly, it grew faster and surpassed the "net" types from Day 5. The isolate ANT148 had become an aconidial, featureless isolate and perhaps should not have been considered in this experiment. More studies should be done on the growth rates of the "net" and "spot" types of *D. teres* using a greater number of isolates. This difference in growth rates could be an important factor in the spread of net blotch.

Cellulase production

An isolate type able to produce cellulase, is able to break down the cellulose component in Cellophane, which is regenerated cellulose (Tribe, 1987; The New Encyclopædia Britannica, 1988; The Encyclopedia Americana, 1985), to get through the Cellophane and grow on the MEA+B (refer to Appendix 1 for recipe) underneath. When the piece of Cellophane was pulled off, the strands of hyphae in the agar continued to grow and this was the indication that the isolate had broken down the cellulose component in the Cellophane. Refer to Photograph 16, showing the hyphae growing through and then underneath the Cellophane. Examination using a dissecting microscope, of the agar surface where a piece of Cellophane had been, showed small indentations on the surface if any hyphae had penetrated the Cellophane. The agar surface was smooth otherwise. This was clearly seen in the pieces of Cellophane lifted off after four days, where a hole measuring 2-3 mm had been broken down in the Cellophane by the germinating conidium.

There was no relation between the ability of an isolate to branch well and to produce cellulase. There were no differences between the "spot" and "net" types in their ability to produce cellulase, as all the isolates were able to produce cellulase. Again there is probable reason to conduct further research in this field using more "net" and "spot" isolates, as the "spot" isolates, K87 and K88, had more conidia which had penetrated the Cellophane piece by Day 3 (refer to Graph 6).

Pathogenicity tests

The pathogenicity tests were repeated three times. The first time to see if the method was successful, which it was, but needed minor alterations. Methods involved the inoculation of barley plants with a spore/mycelium suspension, and plastic bags to cover the barley plants for 48 hours after inoculation. Unfortunately, when this was tried, there was no spread of disease in the plants. Only the first three leaves which had been sprayed had developed any lesions. The other leaves were healthy. Obviously the conditions were too

Photograph 16: K87 - 24 hours growth, showing appresoria (club-shaped) penetrating Cellophane (regenerated cellulose) to grow underneath, on the MEA+B

dry for any disease to take hold, as *D. teres* grows well in humid conditions. Also, there was the fear of cross-contamination between pots as they were located quite close to each other, due to a lack of space. It was then decided to leave the plastic bags on for the duration of the experiment, as was done by Keon and Hargreaves (1983) when inoculating barley plants with *D. teres*. Photograph 1 shows the inoculated barley plants in pots covered with plastic bags..

The other problem encountered was that some seed cultivars like Triumph had a lowered viability. T87 was used initially, but had poor rates of germination. T89 which was used later, germinated well initially, but had a much lower germination rate eight months later. The cultivar Illia was not used in any experiments as it had a poor germination rate, and is reported to be resistant to net blotch (Sheridan, pers. comm.)

All inoculum types studied, ANT148, H88, T83, T88, Z87, K87 and K88, were able to infect all the cultivars used, ANT86, H89, T89, Z87, K87 and K88. Even barley plants grown from treated seed such as H89 and T89 were infected by *D. teres* inoculum types.

ANT148 was established to be a "spot" type of *D. teres* from the symptoms it caused on barley plants. (Refer to Photograph 10 for symptoms.) Perhaps this may have been the source of inoculum of the "spot" type of net blotch in New Zealand. The ANT seed was treated with mercury in Denmark, then treated with imazalil in New Zealand. No *D. teres* colonies grew from 400 seeds in laboratory tests (Sheridan, pers. comm.). However, there could have been a small amount of inoculum on some seeds which led to the presence of the "spot" type of net blotch, which was discovered in New Zealand in 1986, two years after the ANT seed was brought into New Zealand, in 1984.

It was thought ANT148 might not be pathogenic since it had changed in morphology from when it was described in 1987 (Sheridan and Nendick, 1987). However although its morphology in culture had changed, it still had the ability to infect barley plants although it did not produce any conidia. This showed that conidia were not necessary in inoculating barley plants with *D. teres*.

H88, T83, T88 and Z87 were proved to be "net" types of *D. teres*, while K87 and K88 were proved to be the "spot" type of *D. teres*. This was indicated by their characteristic symptoms. Narrow, dark brown, longitudinal and transverse streaks, which produce the characteristic net-like symptoms, in the "net" types, and elliptical spots or eye-spots in the "spot" types.

ANT148 and K88, the "spot" types, did not show any or much symptoms (depending on the cultivar it was on) in the first week, but did in the second week. Refer to Table 13 and Table 14.

All barley cultivars developed the symptoms of the inoculum type sprayed on them, with the exception of K88. The cultivar K88 developed "net" and "spot" symptoms even though "net" type inoculum, H88, T83, T88 and Z87, were applied to the leaves. Refer to Photograph 9, and Tables 13 and 14. The control for K88 also developed "spot" symptoms in the course of the experiment. This shows that the "spot" symptoms had arisen from the inoculum present within the seed coat.

Although pathogenic variation had been shown to exist in *D. teres* (Smedegård-Petersen, 1971; Karki and Sharp, 1986), no such pathogenic variation was seen to exist in the isolates tested on the barley cultivars. A greater range of New Zealand grown barley cultivars needs to be tested against more New Zealand *D. teres* "net" and "spot" type isolates. The resistance of any new barley cultivars introduced in New Zealand should be tested against the New Zealand "net" and "spot" type isolates. This could have an impact on the status of the "spot" type of net blotch which is still less common than the "net" type, but less sensitive to fungicides. The appearance and prevalence of the "spot" type of net blotch in the northern wheat belt of Western Australia has been shown to be related to the dominance of the barley cultivar "Clipper", which is resistant to the "net" type isolates of *D. teres* (Khan, 1982). Cultivar-induced changes such as these, demonstrate the plasticity of *D. teres*, and introduce a note of caution of regarding the long-term future of cultivars resistant to *D. teres*.

Examination of fungal penetration and development of *D. teres* in the leaf

The cultivar ANT was not used initially in the pathogenicity tests because it was assumed it would not germinate, as it dated from 1984. However, it was noticed that the ANT cultivar germinated quite well when it was plated onto MEA+B (refer to Appendix 1 for recipe) to see if there was any inoculum in the seed. Later it was discovered that the ANT seed was seed grown in the Wairarapa from the original ANT seed in 1986, hence its good germination capability, and its name, ANT86.

Unfortunately, when ANT86 was used in the last pathogenicity test, leaf samples were not obtained in the first two weeks after inoculation due to illness. In the third week, there was a problem with heavy infestation of aphids, and it was decided to abandon the pathogenicity tests at this point. However, the degree of infection was noted, at one and two weeks after inoculation.

Studies by Keon and Hargreaves (1983) on penetration and infection of barley plants with *D. teres*, showed that conidia germinated, formed appresoria and penetrated host epidermal cells within 24 hours of inoculation. Although leaf segments were harvested every 24 hours after inoculation in this experiment, no infection sites were ever seen in cleared and stained leaf material in the first two days after inoculation. This could be due to the fact that there were no infection sites on the harvested material, as no symptoms could be seen initially. Examination of leaf segments under the dissecting microscope did not show any germinated conidia in the first 48 hours after inoculation. Alternately the method of clearing and staining the harvested barley leaves could be less effective in highlighting less obvious structures in the leaf or disrupting any structures on the leaf.

The inoculum type K87, produced good "spot" symptoms on the barley plants, and also showed up growing in the entire section of the leaves, harvested and cleared, after three days and three weeks (refer to Tables 15 and 16). On the other hand, ANT148 and K88, the other "spot" types, did not show net blotch symptoms on barley plants as severe as K87 (refer to Tables 13 and 14). Hyphal growth of K88 did not show up well in the leaf segments harvested after three days, but riddled the entire segment of leaves harvested after three weeks (refer to Tables 15 and 16). Dr J.E. Sheridan has noted that the "net" type of net blotch spread rapidly, while the "spot" type spread slowly despite the higher seed borne inoculum (Sheridan and Nendick, 1987). These experiments show that although the "spot" type of *D. teres* is present inside the leaf, the symptoms are not manifested until later, compared to the "net" type where the symptoms are an indication of the amount of fungus present in the leaf. This backs up the claim that it is the type of toxin that is produced that causes the different symptoms of net blotch (Smedegard-Petersen, 1971).

It was noticed that there was little cell disruption in the leaf in advance of hyphal intercellular growth, in the early stages of infection. This was seen in both the "net" and "spot" inoculated barley leaves. This agrees with the studies by Van Caesele and Grumbles (1979), who stated that the most striking feature of the study was the lack of cell disruption in advance of intercellular growth. They claimed that two authors had reported cell death in advance of intercellular growth by *P. teres*, but had not elaborated on how cell death was assessed.

The claim by one author, Singh, S. could not be confirmed as it was from a PhD Thesis which was unavailable. However, I could find no mention of cell death in advance of intercellular growth by *P. teres* in work by Smedegård-Petersen. He did however do similar work on *P. graminea* which showed that branched hyphae proceeded intercellularly into mesophyll tissue, and advanced a distance of 3-4 cells, where hyphal growth ceased and was not resumed (Smedegård-Petersen, 1976). It was noted that the hyphae often ruptured, and did not exceed 100 µm in length. It could be possible that Van Caesele and Grumbles were confused between *P. graminea* and *P. teres*.

Although there was no cell disruption in advance of intercellular growth by *D. teres*, examination of leaf segments harvested three weeks after inoculation showed that there was massive cell disruption. Work carried out by Van Caesele and Grumbles (1979) showed that when hyphae occasionally penetrated a host cell, gross disruption of their contents was apparent. However, they did not state how long after inoculation this occurred, or whether it was independent of the period after inoculation.

These observations agree with those of Van Caesele and Grumbles (1979), who stated that club shaped germ tubes grew to various lengths before producing appresoria, and sometimes extended up to 0.5 cm before producing an appresorium.

Crossing of a "net" type with a "spot" type

In this experiment, a crossing was attempted between 13Y, a sensitive, "net" type of *D. teres* and KF₂, a resistant, "spot" type. This was done in two ways. It was realised that *D. teres* formed little connection tubes between conidia and hyphae, hyphae and hyphae and conidia and conidia, when grown in MEA+B barley leaf decoction (refer to Appendix 3). Since this did not happen when grown on WA+B or MEA+B (refer to recipes in Appendix 1 and 2), the key had to be the presence of barley leaves. Assuming this indicated some form of somatic crossing, experiments were carried out to cross the two types of inoculum on barley plants and WA+B barley leaf decoction (as explained in the Methods section).

The crossing of 13Y and KF₂ on barley plants did not succeed. Even after three weeks there was no sign of any net blotch symptoms on the plants which had both inoculum types sprayed on them. Even the control plants for 13Y and KF₂ did not show any symptoms. As the conditions the cross was carried out was the same as in the pathogenicity tests, environmental conditions were not the problem. The barley plants would have been left longer and monitored for symptoms, but an aphid outbreak prevented the experiment from going on unhindered. This experiment was not repeated due to illness and time constraints.

Crossing 13Y and KF₂ in petri dishes was more successful. Plugs of 13Y and KF₂ were cut and placed in MEA+B barley decoction (refer to Appendix 3 for recipe), and left to grow for three weeks. When plated onto MEA+B barley decoction, it was obvious that 13Y was green-black in culture and KF₂ was light coloured. Conidia and sections of hyphae which had formed connection tubes were separated and plated, one conidium or section of hyphae in a plate each of MEA+B (refer to Appendix 1 for recipe). Although 40 plates were made of the cross types, only 15 grew. The separation of hyphae and conidia from what they had formed connection tubes with, had possibly damaged the conidia or hyphae. Most of the cross types looked green-black when grown in MEA+B.

The sensitivity tests using the isolates 13Y, KF₂ and the cross types, on triadimenol and flutriafol were interesting. Comparing my results with the results obtained in 1986 on triadimenol, and 1987 on flutriafol, it was clear that KF₂ which had reduced sensitivity to both triadimenol and flutriafol, was now more sensitive to both the fungicides. Table 21 shows the difference in sensitivity, between the sensitivity results in 1986-87, compared to the sensitivity results of 1990.

Table 21: The increase in sensitivity (%), or decrease in resistance for triadimenol and flutriafol, in the period between 1986-87 and 1990

Fungicide and Concentration	Triadimenol 50 ppm	Triadimenol 100 ppm	Flutriafol 10 ppm	Flutriafol 50 ppm
Day 7	50	78.9	51.9	-
Day 10	55.7	76.9	69.9	52.2
Day 11	-	-	60	-

Note: Refer to Tables 17, 18, 19 and 20 in the Results section, for the full results from which the above percentages were calculated.

1986 and 1990 Triadimenol Sensitivity
 Increase in sensitivity of KF2 shown by
 comparing 1986 results with 1990 results

Diameters of colony on 50 and 100 ppm triadimenol measured on day 7 and 10

Graph 7: Comparing 1986 and 1990 triadimenol sensitivity tests

In the time between the tests, KF₂ had started to revert to the wild type when grown with no selection pressure, i.e., on MEA+B with no fungicide. This has been recorded in other cases where isolates of *Sphaerotheca fuliginea*, the cause of cucumber powdery mildew, reverted to the wild-type sensitivity within a period of two years, during which no selection pressure was present (Schepers, 1985). This may have been caused by genetic heterogeneity of the isolates, mutation, and contamination with wild-type conidia might

1987 and 1990 Flutriafol Sensitivity

Increase in sensitivity of KF₂ shown by
comparing 1987 results with 1990 results

Diameters of colony on 10 and 50 ppm
flutriafol measured on day 7 and 10

Graph 8: Comparing 1987 and 1990 flutriafol sensitivity tests

have caused this gradual shift towards the wild-type sensitivity.

These tests were conducted twice, the first time all the plates were put in an incubator in the dark for the duration of the experiment. KF₂, the non-sensitive isolate grew in a dense fashion and was pink, instead of the usual net blotch fashion. It was thought that it was contamination and lack of light that might have been responsible. However, when slides were made of the fungal growth, it looked like *D. teres*, with very dense growth. The test was repeated under a NUV light under 12 hours on-12 off regime, and although not dense looking and pink, gave the same diameters as the test from the incubators. So there is no doubt that KF₂ had increased in sensitivity, as both tests gave similar results.

The isolate 13Y and the other cross types did not grow on triadimenol or flutriafol. There was some growth of 1 mm in 10 ppm flutriafol by 13Y and all the cross types with the exception of two isolates. Refer to Table 19. This could be explained as an oligodynamic action, where some substances are toxic at high concentrations, but stimulate growth at lower concentrations (Frobisher, 1968).

It is difficult to prove that any somatic recombination occurred in this experiment. There was no evidence of any cross types which showed any level of resistance greater than 13Y. However, it is odd that out of the 15 surviving isolates, not one showed any intermediate characteristics. All isolates were dark in culture, like 13Y, and did not show any decrease

in sensitivity to triadimenol or flutriafol. Perhaps the "net" type is a dominant form like the wild-type, and the "spot" type is a recessive form. It is difficult to say so conclusively.

Smedegård-Petersen (1976) had reported that the "net" and "spot" types of *D. teres* readily crossed and produced progeny which segregated into "net" lesions (parental type), "spot" lesions (parental type), intermediate lesions (recombinant type) and fleck lesions (recombinant type) in a ratio of approximately 1:1:1:1. A segregation of parental types and recombinants in a ratio of 1:1, clearly indicates that the capacity of producing "net" and "spot" lesions is conditioned by two independent allelic pairs.

In the interspecific crosses between the "spot" type of *P. teres* (which produced "spot" lesions with *P. graminea* (which produced "gram" or leaf stripe lesions) by Smedegård-Petersen (1976), using ascospores, four distinct classes of lesions were distinguished. They were "spot" lesions (parental type), "gram" lesions (parental type), intermediate lesions (recombinant type) and chlorotic-necrotic flecks (recombinant type). The segregation for the lesion types were 166 parental : 33 recombinants. The far more frequent occurrence of parental types than recombinant types excludes independent inheritance and indicated close linkage of two loci for spot lesions and leaf stripe.

The interspecific crosses between the "net" type of *P. teres* and *P. graminea* yielded four distinct classes of lesions, which were "net" lesions (parental type), "gram" lesions (parental type), intermediate lesions (recombinant type) and chlorotic-necrotic lesions (recombinant type). The segregation for the lesion types was 23 parental : 41 recombinants. The large number of recombinants is difficult to explain on the basis of normal mechanisms of recombination. Smedegård-Petersen hypothesised that there was an unusually large number of asci containing less than 8 ascospores. The aberrant number of spores in the asci in addition to the relatively small number of spores isolated may have resulted in a certain selection of spores and a subsequent aberrant distribution among classes.

Smedegård-Petersen's work shows that there is need for research to be done on the crossing of the "net" and "spot" type isolates, and the progeny screened for their symptoms caused on a barley cultivar not resistant to net blotch. The progeny from the cross could also be tested for their sensitivity to fungicides and compared with the parental types. Interspecific crosses between the "net" and "spot" types of *D. teres* with *D. graminea* should also be carried out. When Smedegård-Petersen conducted his research, there was no mention of any resistance to fungicides.

Since the "spot" type of *D. teres* has developed resistance to certain groups of fungicides in New Zealand, it is very important to investigate the inheritance of resistance to fungicides in the crossing of the "net" and the "spot" types. The interspecific crossing of the "net" and "spot" types of *D. teres* with *D. graminea* should also be investigated, along with the sensitivity of the progeny to commercially used fungicides. Using New Zealand isolates for these studies is very important as no work of this sort has been done using New Zealand isolates. At present, the "spot" type of *D. teres*, although resistant to some groups of fungicides, is less common in the field and therefore not a problem. The consequences of hybrids arising from intraspecific crosses (between the "net" and "spot"

type of *D. teres*) or interspecific crosses (between *D. teres* and *D. graminea*) which are resistant to fungicides and fitter in the field could be of great economic importance in New Zealand.

A way to ensure somatic recombination, would be to fuse protoplasts after first removing the cell walls of the fungi to be crossed. Protoplast fusion is one way of ensuring somatic or parasexual recombination in fungi, and has been successfully carried out on *Rhynchosporium secalis*, which causes scald on barley (Newton, 1989). Somatic recombination should be looked into, as a method of crossing, in further work involving the crossing of *D. teres*.

The products of the cross between 13Y and KF₂, and the parental plates, were kept for some time after the they were measured. The plates were left where they were, i.e., in the dark in the incubator, and under the NUV light (refer to Appendix 8 for specifications). After six weeks (the plates under the NUV light), and eight weeks (the plates in the incubator) it was discovered that there was a range of varying growth, by 13Y and the cross types, on the 50 ppm triadimenol and 10 ppm flutriafol (refer to Photographs 17 and 18). There was not much difference in growth on 100 ppm triadimenol or 50 ppm flutriafol. This showed that 13Y, the "net" type and the cross types which appeared to be similar to 13Y, were able to grow on higher levels of fungicides, given time. It is unlikely the fungicides had dissipated as the petri dishes were sealed with Gladwrap.

Photograph 17: Growth of the cross types under NUV light, on 50 ppm triadimenol and 10 ppm flutriafol, six weeks after inoculation

Photograph 18: Growth of the cross types, in an incubator (dark), after eight weeks of growth on 50 and 100 ppm triadimenol, and 10 and 50 ppm flutriafol

It is possible to train plant pathogens which are not resistant to a particular fungicide, to grow on progressively higher levels of fungicides. However, often a higher resistance to fungicides means a lower fitness level in the wild. This shows that although 13Y and the other cross progeny were initially unable to grow on triadimenol and flutriafol, they can, given exposure to the fungicides, and time. This should serve as a warning that fungicides should be used sparingly and without regularity, as it was possible for 13Y, a sensitive "net" type to grow on high levels of triadimenol and flutriafol, given time.

Overview

The "spot" type and "net" type of *D. teres* are probably two types of the same fungus differing mainly in the symptoms produced on barley plants. There was not enough convincing evidence to prove that the two types of net blotch were sufficiently different to be separate species. The only differences documented in my research were the ability of the "spot" type to penetrate Cellophane faster and a difference in morphology when grown on MEA+B.

However, if more isolates of both the "net" and "spot" types were used in the experiments, the results may not have shown any differences, other than morphological differences. The recording of colony characteristics or growth rate of a fungus or an isolate grown on MEA (refer to Appendix 1) is probably not very useful. MEA and PDA (potato dextrose agar) for example are rich in sugars never normally encountered by fungi normally inhabiting wood, bark litter, straw or cellulosic residues (Tribe, 1987). Therefore, the growing of *D.*

teres on MEA which is high in maltose (an unusual sugar in the plant tissue context), together with dextrans, glucose and lesser amounts of other solubles, may not be the best medium to observe colony characteristics. Cellulose is a nutrient which makes up a major component of plant tissues and is a principle fungal nutrient (Tribe, 1987). Media incorporating soluble cellulose derivatives are much more satisfactory than sugar-based media. Cellophane (regenerated cellulose) is an excellent medium for growth and demonstration of many fungi.

The "spot" type of *D. teres* and *D. japonica* have been identified and documented in New Zealand. *D. japonica* was identified on wild barley grass (*Hordeum murinum* L.) in 1977-1978 growing season, and on barley in the 1975-76 growing seasons. The "spot" type of *D. teres* was discovered on barley (*Hordeum vulgare* L.) in January 1986. The question to be asked here is, whether the "spot" type of *D. teres* and *D. japonica* are the same fungal organism, since they were identified at such wide intervals apart? It is possible that *D. japonica* is a race of *D. teres* that normally grows on wild barley grass, that spread to barley, since *D. japonica* was found on both wild barley grass and barley in the same growing season.

To clarify this point, studies should be carried out on *D. japonica* isolates, the "spot" type of *D. teres* that was first identified in New Zealand and current "spot" type isolates of *D. teres*. The studies should include:

- a comparison of morphological features
- a comparison of conidia and conidiophore features
- the growth rates
- the rate and quantity of cellulase produced
- the ability of the isolates to infect different barley cultivars and wild barley grass
- the symptoms caused by the different isolates on the different barley cultivars and wild barley grass.

This should establish whether or not *D. japonica* and the "spot" type of *D. teres* are one and the same plant pathogen.

CONCLUSIONS

Examination of colony morphology

1. The inoculum types, ANT148, H88, T83, T88, Z87, K87 and K88, were morphologically distinctly different from each other.
2. The features of the different inoculum types were constant if made from the original inoculum source, i.e., the freeze dried infected barley leaves and infected seed, and subcultured by taking monoconidial isolates.
3. All the inoculum types studied had the ability to change from a normal green-black colour in culture, to an orange-red colour, even with monoconidial subculturing.
4. The noticeable difference between the "net" types, H88, T83, T88 and Z87, and the "spot" types, K87 and K88, was the "spot" types produced coremia, which were fan-like morphologically, with conidia in the coremial strands, while the "net" types produced tufts instead.
5. ANT148 had changed morphologically and structurally between 1986 and 1990 to produce white, fluffy aconidial growth, with no other structures. It had previously in 1987, been described as looking like typical "spot" type growth (Sheridan, 1987).

Examination and measurement of conidia

1. The mean conidial lengths of the isolate types H88, T83, T88, Z87, K87 and K88, were not all equal, i.e. they were significantly different. (Refer to ANOVA test on lengths, in Examination and Measurement of Conidia in the Results section.) However there was no correlation between the mean length of an isolate type and whether it was a "net" or "spot" type.
2. The mean conidial widths of the isolate types studied were not all equal, i.e. they were significantly different. (Refer to the ANOVA test on widths in Examination and Measurement of Conidia in the Results section.) Again there was no correlation between the mean width of an isolate type and whether it was a "net" or "spot" type.
3. There was no correlation between the length and width of a conidium. The isolate type with the greatest mean length did not have the greatest mean width. In fact T88 had the greatest mean length, but the smallest mean width.
4. The mean estimated volumes of conidia for each isolate type were not all equal, i.e. they were significantly different. (Refer to ANOVA test in Examination and Measurement of Conidia in the Results section.) There was no correlation between the mean volume of an isolate type and whether an isolate type was a "net" or "spot" type.

5. The number of cells per conidium of the isolate types studied were not all equal, i.e. they were significantly different. (Refer to the ANOVA test in Examination and Measurement of Conidia in the Results section.) There was no correlation between the mean number of cells per conidium and whether an isolate type was a "net" or "spot" type. There was evidence to prove that the greater the mean length, the greater the number of cells per conidium, and the reverse was also true.
6. All isolate types had their own distinct predominant conidial colours. However, there was no link between colour and whether an isolate was a "net" or "spot" type.

Examination of germinated monoconidial isolates

1. Branching patterns varied between the inoculum types. The "spot" types, K87 and K88, had good branching, with K87 being the best. The "net" types varied from poor to good branching. There was no evidence to prove that the "spot" types branch better than the "net" types, or vice-versa.
2. Enlarged sections of hyphae formed in the isolates Z87, K87 and K88, within 24 hours. These structures whose function is uncertain was seen in SEM photographs and slides made from 7 day old cultures of all isolates.
3. The inoculum types, Z87 and K88, had enlarged club-shaped formations at the growing tips, which could have been appresoria.
4. Germinated cells in a conidium were separated by as many cells as was possible, and the first cell to germinate was usually the cell at the hilum.
5. The "spot" type isolates, K87 and K88, were the only isolates which had more than one germ tube emerge from one cell of a conidium.
6. All cells in a conidium were capable of germinating.
7. The growth of strands of hyphae which twisted together tightly was seen in H88, T88, K87 and K88. It may be the early stages of the formation of "tufts" in the "net" types and coremia in the "spot" types.

Growth rates of the different inoculum types

1. The "net" and "spot" types had very similar growth rates and there was not enough evidence to suggest otherwise.

Cellulase production

1. The germinated conidia of both the "spot" type isolates, K87 and K88, managed to penetrate Cellophane pieces faster than the "net" types, and therefore could be assumed to produce cellulase faster as well.

Pathogenicity tests

1. All barley cultivars used, ANT86, H89, T87, T89, Z87, K87 and K88, were susceptible to varying degrees, to all the inoculum types tested, ANT148, H88, T83, T88, Z87, K87 and K88.
2. ANT148 was proved to be a "spot" type of *D. teres* by the symptoms it caused on barley plants.
3. Only hyphal growth was necessary to produce symptoms on barley plants, as ANT148 did not produce any conidia and was successful in producing net blotch symptoms when inoculated onto barley plants.

Examination of fungal penetration and development of *D. teres* in the leaf

1. The original point of entry from a germinating conidium was never located. However, hyphae growing on the leaf were seen to penetrate the leaf through the leaf epidermis, and occasionally through the stomata.
2. The thin form of hyphae growing inside the leaf emerged through the leaf epidermis and occasionally through the stomata. The thick form of hyphae, which are the conidiophores only emerged through the leaf epidermis and did not penetrate the epidermis again to re-enter the leaf. They also bore the conidia.
3. Although the "spot" type of *D. teres* is present inside the leaf, the symptoms are not manifested until later, compared to the "net" type, where the symptoms are an indication of the amount of fungus present in the leaf. This backs up the claim that it is the types of toxins produced by *D. teres* that causes the degree of symptoms (Smedegard-Petersen, 1971).

Crossing of a "net" type with a "spot" type

1. The isolates 13Y and KF₂ may not have been pathogenic, as experiments to cross the two isolates on barley plants did not result in any symptoms on the barley plants.
2. The lowered sensitivity of KF₂, the "spot" type, to triadimenol and flutriafol had dropped by 50%-78.9% between the years 1986-87 and 1990, when grown with no selection pressure, i.e. in a medium with no triadimenol or flutriafol.
3. It is hypothesised that 13Y, the sensitive "net" type is dominant, and KF₂, the resistant "spot" type is recessive, as the results of the cross yielded progeny isolates which were all sensitive to triadimenol and flutriafol, and were also similar morphologically to 13Y.

BIBLIOGRAPHY

- ARNST, B.J.; SHERIDAN, J.E. and GRBAVAC, N., 1978. Two important seed-borne diseases of barley in New Zealand: net blotch caused by *Drechslera teres* (Sacc.) Shoemaker and leaf stripe caused by *Drechslera graminea* (Rabenh. ex Schlecht.) Shoemaker. *NZ Journal of Agricultural Research*, 21:697-701.
- ARNST, B., 1976. Leaf stripe disease of barley. Proceedings of the 29th New Zealand Weed and Pest Control Conference, pp 228-230.
- COLE, G.T. and SAMSON R.A., 1979. Patterns of Development in Conidial Fungi. Pitman Publishing Limited, pp 128-130.
- ELLIS, M.B., 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, p 401-402.
- ELLIS, M.B. and ELLIS, J.P., 1985. Microfungi on Land Plants. Croom Helm, London & Sydney, p 502.
- The Encyclopædia Britannica, Vol. 3, 1988. Encyclopædia Britannica, Inc., p 15.
- The Encyclopedia Americana, International Edition, 1985. Grolier Incorporated, Danbury, Connecticut, p 139.
- Eton Statistical & Math Tables, Fourth Edition, 1982. Heinemann Publishers (NZ) Ltd, pp 50-52.
- FROBISCHER, M., 1968. Fundamentals of Microbiology (8th Ed.). W.B. Saunders Co., pp 261.
- HALL, D.M. and DONALDSON, L.A., 1962. *Nature, Lond.*, 194:1196.
- HANSEN, L.R. and MAGNUS, H.A., 1969. Bladflekksopper på byg i Norge. *Forskning og forsøk i landbruket*, 20: 95-105.
- ITO, S. and KURIBAYASHI, K., 1931. The ascigerous forms of some graminicolous species of *Helminthosporium* in Japan. *Jour. Facul. Agr.*, Hokkaido Imp. Univ. 29: 85-125.
- KARKI, C. B. and SHARP, E.L., 1986. Pathogenic Variation in Some Isolates of *Pyrenophora teres* f. sp. *maculata* on Barley, *Plant Disease*, 70(7): 684-687.
- KEON, J.P.R. AND HARGREAVES, J.A., 1983. A cytological study of the net blotch disease of barley caused by *Pyrenophora teres*. *Physiological Plant Pathology*, 22:321-329.

- KHAN, T.N. and TEKAUZ, A., 1982. Occurrence and Pathogenicity of *Drechslera teres* Isolates Causing Spot-Type Symptoms on Barley in Western Australia. *Plant disease*, 66: 423-425.
- KHAN, T.N., 1982. Changes in Pathogenicity of *Drechslera teres* Relating to Changes in Barley Cultivars Grown in Western Australia. *Plant disease*, 66: 655-656.
- KIRK, T.W., 1906. Principle fungus diseases of the year. New Zealand Department of Agriculture Report 5, pp 341-365.
- McDONALD, W.C., 1967. Variability and the inheritance of morphological mutants in *Pyrenophora teres*. *Phytopathology*, 57: 747-755.
- MÄKELÄ, K., 1972. Leaf spot fungi on barley in Finland. *Acta Agralia Fennica*, 124(3): 1-22.
- NEILL, J.C., 1928. Survey of diseases of cereals in New Zealand. *NZ Journal of Agriculture*, 40: 89-93.
- NEWTON, A.C., 1989. Somatic recombination in *Rhynchosporium secalis*. *Plant Pathology*, 38: 71-74.
- ROBERTS, E.A., SOUTHWICK, M.D. and PALMITER, D.H., 1948. *Plant Physiology*, 23:557.
- SCHEPERS, H.T.A.M., 1985. Fitness of isolates of *Sphaerotheca fuliginea* resistant or sensitive to fungicides which inhibit ergosterol biosynthesis. *Netherlands Journal of Plant Pathology*, 91: 65-76.
- SHERIDAN, J.E., 1975. *Helminthosporium* diseases of cereals and their control. In Cereal Seed Diseases and Their Control, Ed. R.C. Close, Proceedings of a seminar held at Lincoln College, 8 July 1975.
- SHERIDAN, J.E., 1977. *Drechslera* spp. and other seed-borne pathogenic fungi in New Zealand cereals. *NZ Journal of Agricultural Research*, 20:91-93.
- SHERIDAN, J.E., 1978. New Records of Plant Diseases and Pests. Mycology and Plant Pathology. Report No. 12, Botany Department, VUW, p 12.
- SHERIDAN, J.E., 1980. Laboratory Manual Containing Some Useful Information Relating to Microbiological, Mycological and Plant Pathological Studies. Mycology and Plant Pathology Report No. 4, Botany Dept, VUW, p 17.
- SHERIDAN, J.E., 1990. Personal communication.
- SHERIDAN, J.E., 1990. Cereal Diseases 1989-1990. Report No. 27, School of Biological Sciences, VUW, p iii.

- SHERIDAN, J.E. and GRBAVAC, N., 1979. Healthy barley crops demand constant surveillance. *N.Z. Journal of Agriculture*, 139(4): 25-29.
- SHERIDAN, J.E. and NENDICK, D.K., 1987. Cereal Diseases 1986-1987. Report No. 24, Botany Department, VUW, pp 51, 53 and 65.
- SHERIDAN, J.E. and NENDICK, D.K., 1988. I Cereal Diseases 1987-1988. Report No. 25, Botany Department, VUW, p 3.
- SHERIDAN, J.E. and NENDICK, D.K., 1989. Cereal Diseases 1988-1989. Report No. 26, School of Biological Sciences, VUW, p 3.
- SHERIDAN, J.E. and SHERIDAN M.H., 1986. Cereal Diseases 1985-1986. Report No. 23, Botany Dept, VUW, p ii.
- SHERIDAN, J.E. and SPENCER, M.M., 1975. Cereal diseases and their control. *N.Z. Journal of Agriculture* 131: 13, 15, 51, 53.
- SMEDEGÅRD-PETERSEN, V., 1971. *Pyrenophora teres* f. *maculata* f. nov and *Pyrenophora teres* f. *teres* on barley in Denmark. *Kgl. Vet. - og Landbohøjsk. Årsskr*, pp 124-144.
- SMEDEGÅRD-PETERSEN, V., 1976. Pathogenesis and Genetics of Net-Spot Blotch and Leaf Stripe of Barley Caused By *Pyrenophora teres* and *Pyrenophora graminea*. DSR FORLAG, pp 1-176.
- SMEDEGÅRD-PETERSEN, V. 1977. Isolation of two toxins produced by *Pyrenophora teres* and their significance in disease development of net-spot blotch of barley. *Physiological Plant Pathology*, 10:203-211.
- SMITH, I.M., DUNEZ, J., LELLIOT, R.A. and ARCHER, S.A. (editors), 1988. European Handbook of Plant Diseases. Blackwell Scientific Publications, pp 389-384.
- TARR, S.A.J., 1972. The Principles of Plant Pathology. MacMillan Publishers Ltd., pp 164-165.
- TIEDT, L.R.; JOOSTE, W.J. and HAMILTON-ATTWELL, V.L., 1987. Technique for preserving aerial fungus structures for scanning electron microscopy, *Transactions of British Mycological Society*, 88(3): 420-422.
- TRIBE, H.T., 1987. Do we think about our culture media? *The mycologist*, Vol. 21, Part 2, April 1987, pp 69-71.
- VAN CAESELEE, L. and GRUMBLES J., 1979. Ultrastructure of the interaction between *Pyrenophora teres* and a susceptible barley host. *Canadian Journal of Botany*, 57: 40-46.

GLOSSARY

Bioassay A measure of the amount of a biologically active substance by the extent of its action, under standard conditions, on a living organism.

Disease Involves harmful physiological changes in the plant and may be caused by non-parasitic agents such as adverse soil conditions, or it may be caused by a pathogen.

Fungicides Antifungal substances which kill fungal spores or mycelium.

Host The living plant from which plant pathogens derive the materials they need for growth.

Infection A parasitic relationship between a pathogen and a susceptible plant.

Leaf blotch A fungal disease of cereals caused by *Rhynchosporium secalis*, and is also known as scald.

Leaf stripe A fungal disease of cereals caused by *Drechslera graminea*.

Net blotch A fungal disease of cereals caused by *Drechslera teres*.

Pathogen Any agent which causes damage, but the term is generally used to denote living organisms, especially fungi and bacteria, which attack plants.

Pathogenicity The ability to cause disease.

Resistance The extent to which the plant is damaged by the pathogen, or the extent to which the plant prevents the entry or subsequent growth of the pathogen within its tissues. It also refers to the extent to which a fungus is able to grow in the presence of a fungicide.

Spot blotch A fungal disease of cereals caused by *Drechslera sorokiniana*, which also causes foot rot in cereals.

Virulence A measure or degree of pathogenicity, in the sense that a pathogen may comprise of several strains (races) of varying virulence.

ACKNOWLEDGEMENTS

I wish to thank the following people:

Dr J.E. Sheridan, for suggesting this topic and for help and advice throughout the project,

Prof. J.K. Heyes, for help and advice on the project,

all the technicians, who have been helpful with their advice and technical expertise,

Dave, Alan and Helen for their company and support in the lab,

Graeme Hart, for technical expertise concerning computer work and the loan of computer equipment,

Gerry Keating for help with enlarging some of my photos and making sure they were printed properly,

my parents, for continually asking me when I was going to complete my MSc,

the people concerned from the School of Biological Sciences, the Marketing Group and the Management Group, for providing me with employment during the course of the MSc,

my cats, Retard and Blackie, for typing little pearls of wisdom like, "]]]]]uikkktt" and "222qwwaq", when I wasn't looking,

and anyone else I have not mentioned here, but who have been of help in some way.

APPENDICES

Appendix 1: Preparation of Malt Extract Agar + Benomyl (MEA + B)

Ingredients:

- 20 g Davis's Bacteriological Agar
- 20 g Wilson's Maltexo malt
- 1 litre tap water
- 10 mg (10 µg/g) Benomyl

Method:

1. Agar was put into a 2 l conical flask and 1 l tap water was added.
2. Solution was heated in a steamer or a pressure cooker without the top on for 45 minutes for the agar to dissolve.
3. Malt was added to the agar solution and stirred.
4. The agar solution was poured into two 500 ml Duran bottles and sterilised in an autoclave or pressure cooker for 15 minutes at 103 kPa, at 121°C.
5. Benomyl was suspended in 20 ml sterile distilled water and added aseptically after sterilisation if it was to be poured immediately after sterilisation.
6. Sterilised agar was left to cool and poured into petri dishes, or stored for future use.

Appendix 2: Preparation of Water Agar + Benomyl (WA + B)

Ingredients:

- 20 g Davis's Bacteriological Agar
- 1 litre tap water
- 10 mg (10µg/g) Benomyl

Method:

1. Agar was put into a 2 l conical flask and 1 litre tap water was added.
2. Agar solution was heated in a steamer or pressure cooker without the top on for 45 minutes for the agar to dissolve.
3. The agar solution was poured into two 500 ml Duran bottles and sterilised in an autoclave or pressure cooker for 15 minutes at 103kPa at 121°C
4. Benomyl was suspended in 20 ml sterile distilled water and added aseptically after sterilisation if agar was to be poured immediately after sterilisation.
5. Sterilised agar was left to cool and poured, or stored for future use.

Appendix 3: Preparation of Water Agar + Benomyl (WA + B) barley leaf decoction

Ingredients:

- 20 g Davis's Bacteriological Agar
- 1 litre tap water
- 10 mg (10 µg/g) Benomyl
- 8 g fresh barley leaves (4 g for each 500 ml of agar)

Method:

1. Agar was put into a 2 l conical flask and 1 litre tap water was added.
2. Agar solution was heated in a steamer or pressure cooker without the top on for 45 minutes for the agar to dissolve.
3. Two lots of 4 g of barley leaves were washed and cut into 2-3 cm sections.
4. The agar solution was poured into two 500 ml Duran bottles. The barley leaf sections were added to each Duran bottle and sterilised in an autoclave or pressure cooker for 15 minutes at 103 kPa at 121°C
5. Benomyl was suspended in 20 ml sterile distilled water and added aseptically after sterilisation if agar was to be poured immediately after sterilisation.
6. Sterilised agar was left to cool and poured, or stored for future use.

Appendix 4: Preparation of MEA + B with fungicide

Ingredients:

- 20 g Davis's Bacteriological Agar
- 20 g Wilson's Maltex malt
- 1 litre tap water
- 10 mg (10 µg/g) Benomyl
- triadimenol (powder)
- flutriafol (liquid)

Method:

1. MEA + B was made in the same way as in Preparation of MEA + B.
2. For 50 ppm triadimenol, 0.334 g of triadimenol was weighed and added to the 1 litre melted MEA and stirred thoroughly.
3. The agar was then poured into two 500 ml Duran bottles and sterilised.
4. Benomyl was suspended in 20 ml sterile distilled water and added aseptically after sterilisation if agar was to be poured immediately after sterilisation.
5. Sterilised agar was left to cool and poured or stored for future use.

6. For the quantities of fungicides for the different levels of concentration to make 1 litre of MEA + B, please refer to the table below.

Table 22: The quantities of fungicide required to make up the different concentrations of triadimenol and flutriafol used

Fungicide type	Concentration (ppm)	Quantity for 1 l
triadimenol	50	0.334 g
triadimenol	100	0.667 g
flutriafol	10	80 µl
flutriafol	50	400 µl

Appendix 5: Source of EBI fungicides

Table 23: Details on triadimenol and flutriafol

Common name and year introduced	Chemical name	EBI class	Product name, formulation and supplier
flutriafol 1983	(R,S)-2,4'-difloro- α -(1H-1,2,4-triazol-1-ylmethyl)benzhydryl alcohol	triazole	IMPACT 12.5% EC. ICI
triadimenol + imazalil 1977	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-butan-2-ol	triazole + imidazole	BAYTAN IM 15% + 15% WP. BAYER

(Sheridan and Nendick, 1987)

Appendix 6: Preparation of Clear Lactophenol

Ingredients:

- 100 ml glycerine (or glycerol)
- 100 ml lactic acid
- 100 ml distilled water
- 100 g phenol crystals

Method:

1. Glycerine, lactic acid and distilled water were combined in a beaker.
2. Phenol crystals were added and stirred until all crystals had dissolved.

3. The clear lactophenol was poured into an airtight bottle and stored away from direct sunlight.

Appendix 7: Preparation of Lactophenol-Cotton Blue

Materials:

- 100 ml clear lactophenol (refer to Appendix 6 for recipe)
- 0.5 g aniline blue (crystals)

Method:

1. 0.5 g aniline blue crystals were weighed out and added to 100 ml clear lactophenol. The solution was stirred for approximately half an hour on a magnetic stirrer, or until all crystals had dissolved.
2. The lactophenol-cotton blue was poured into an airtight bottle and stored away from direct sunlight.

Appendix 8: NUV (Near Ultra Violet) Light

The light source for the NUV light came from two THORN, 20 watt, 600 mm, non-filtered ultra violet florescent tubes with an emission of 300-400 nm, which were suspended about 20 cm above the petri dishes.

Appendix 9: Peat-Sand Pot Mix

Materials:

- 20 l peat/sand in a 3/2 proportion
- 18 g potassium nitrate
- 24 g super phosphate
- 30 g limestone
- 02 g trace elements
- 60 g osmocote

Method:

1. Peat and sand were sterilised separately at 15 lbs pressure in an autoclave for 30 minutes.
2. Peat, fertilisers and sand were spread on a plastic sheet in three layers, then the pile was turned over three times, ensuring that the fertilisers were mixed in thoroughly.

Appendix 10: Details on the morphological description of *D. teres* isolates

Table 24: Morphological description of colonies based on comparison of the overall appearance of a number of physical characteristics

Description	Overall appearance of colony in MEA+B*	Formation of conidia	Formation of spermagonia in MEA+B*	Formation of pseudopycnidia	Type of fungal growth on MEA+B*	Aerial hyphae
ANT148	White, without being fluffy.	Did not sporulate on MEA+B or WA barley leaf decoction [†] (which promoted sporulation in cultures which did not sporulate readily).	Did not form any.	Did not form pseudopycnidia.	Did not turn MEA+B a darker colour.	Dense white cover.
H88 [■]	White and fluffy.	Sporulated readily in MEA+B.	Formed spermagonia, but to a small degree only.	Formed pseudopycnidia in MEA+B readily.	Turned MEA+B black-green to a great degree.	Largely grey, with white tufts.
T83	White and fluffy.	Did not sporulate readily on MEA+B, but did on WA barley leaf decoction [†] .	Formed spermagonia, but to a small degree only.	Formed pseudopycnidia in agar, to a small degree.	Turned MEA+B black-green to some degree.	Largely white, with grey tufts.
T88	Grey-white and fluffy.	Did not sporulate readily on MEA+B, but did on WA barley leaf decoction [†] .	Formed spermagonia, but to a small degree only.	Formed pseudopycnidia in agar to a greater degree than T83.	Turned MEA+B black-green to some degree (similar to T83).	Largely grey with white tufts.
Z87	White and fluffy.	Sporulated readily in MEA+B.	Did not form spermagonia very readily.	Formed pseudopycnidia in agar very readily.	Turned agar black-green.	Grey-white.
K87	White with coremial strands.	Sporulated easily in agar, with conidia also found in the coremial strands.	Formed spermagonia very readily and in large quantities.	Formed pseudopycnidia in agar.	Turned agar slightly black.	Largely white.
K88	White with coremial strands.	Sporulated easily in MEA+B, with conidia also found in the coremial strands.	Formed spermagonia very readily and in large quantities.	Formed pseudopycnidia in agar.	Turned agar slightly black.	Largely white.

* Refer to Appendix 1 for recipe.

[†] Refer to Appendix 3 for recipe.

[■] H88 was the only isolate which formed perithecia in culture after 2 months growth in MEA+B*. None of the other isolate types could be persuaded to form perithecia even with the addition of Cellophane as a cellulose source.

Appendix 11: Complete data on the length, width, number of cells per conidiospore and colour of all inoculum types examined

Table 25: H88 leaf - dimensions, number of cells and colour of conidiospores

CONIDIUM #	LENGTH (e.p.u. x 2.5)	LENGTH (µm)	WIDTH (e.p.u. x 2.5)	WIDTH (µm)	VOLUME (µm ³)	# OF CELLS PER CONIDIUM	COLOUR
1	29	72.5	9	22.5	28826.567	3	green
2	29	72.5	6.5	16.25	15036.08	3	green
3	17	42.5	6	15	7510.3699	2	green
4	29.5	73.75	10	25	36201.947	5	green
5	15.5	38.75	7	17.5	9320.4673	3	green
6	22	55	10.5	26.25	29765.363	3	green
7	16	40	7	17.5	9621.1275	3	green
8	18	45	8	20	14137.167	3	green
9	25	62.5	8	20	19634.954	3	green
10	11	27.5	5	12.5	3374.7577	2	green
11	16	40	6	15	7068.5835	3	green
12	29	72.5	8.5	21.25	25712.586	4	green
13	14	35	7	17.5	8418.4866	2	green
14	22	55	8	20	17278.76	4	green
15	32.5	81.25	9	22.5	32305.635	4	green
16	23	57.5	10	25	28225.247	3	green
17	21	52.5	7.5	18.75	14496.118	5	green
18	26	65	7.5	18.75	17947.575	5	green
19	21	52.5	8	20	16493.361	4	green
20	30	75	9.5	23.75	33226.024	5	green
21	25	62.5	8.5	21.25	22166.022	5	green
22	18	45	8	20	14137.167	3	green
23	21	52.5	7.5	18.75	14496.118	4	green
24	20	50	7	17.5	12026.409	3	green
25	21	52.5	8	20	16493.361	4	green
26	22.5	56.25	7.5	18.75	15531.555	4	green
27	13	32.5	6	15	5743.2241	2	green
28	20	50	6	15	8835.7293	4	green
29	22	55	7.5	18.75	15186.41	4	green
30	15	37.5	7	17.5	9019.807	3	green
31	25.5	63.75	9.5	23.75	28242.12	4	green

32	22.5	56.25	6	15	9940.1955	4	green
33	13	32.5	6.5	16.25	6740.3116	2	green
34	24.5	61.25	7.5	18.75	16912.138	4	green
35	27	67.5	8	20	21205.75	5	green
36	20.5	51.25	7	17.5	12327.07	4	yellow-green
37	15.5	38.75	5.5	13.75	5753.9619	3	green
38	25	62.5	7	17.5	15033.012	5	green
39	14	35	7	17.5	8418.4866	3	green
40	25.5	63.75	5.5	13.75	9466.1954	3	green
41	22.5	56.25	7	17.5	13529.711	4	green
42	19	47.5	7.5	18.75	13115.536	3	green
43	21.5	53.75	5.5	13.75	7981.302	4	green
44	23	57.5	5.5	13.75	8538.1371	2	green
45	24	60	8	20	18849.556	5	olive
46	26	65	7.5	18.75	17947.575	4	olive
47	25	62.5	6	15	11044.662	5	olive
48	15	37.5	5.5	13.75	5568.3503	2	olive
49	11.5	28.75	6	15	5080.5444	2	olive
50	12	30	6	15	5301.4376	2	olive
51	18.5	46.25	6	15	8173.0496	3	yellow-green
52	19	47.5	7.5	18.75	13115.536	3	olive
53	17	42.5	6	15	7510.3699	4	olive
54	16	40	6	15	7068.5835	1	green
55	21	52.5	6.5	16.25	10888.196	3	olive
56	24	60	8	20	18849.556	4	olive
57	31	77.5	8	20	24347.343	6	olive
58	15.5	38.75	5	12.5	4755.3404	2	green
59	13	32.5	5	12.5	3988.35	1	green
60	19.5	48.75	7	17.5	11725.749	3	green
61	19	47.5	6.5	16.25	9851.2246	3	green
62	37	92.5	9.5	23.75	40978.763	6	green
63	20	50	7	17.5	12026.409	3	green
64	18	45	6	15	7952.1564	3	green
65	17	42.5	6	15	7510.3699	4	green
66	18	45	7	17.5	10823.768	3	green
67	11.5	28.75	5.5	13.75	4269.0685	2	green
68	21	52.5	7	17.5	12627.73	4	green

69	11.5	28.75	5.5	13.75	4269.0685	3	green
70	24	60	8	20	18849.556	4	green
71	26	65	8	20	20420.352	5	green
72	17	42.5	6	15	7510.3699	3	green
73	22	55	8	20	17278.76	3	green
74	14.5	36.25	5.5	13.75	5382.7386	3	yellow-green
75	17	42.5	7	17.5	10222.448	3	green
76	22	55	8	20	17278.76	3	green
77	16.5	41.25	6	15	7289.4767	3	green
78	25.5	63.75	8	20	20027.653	5	green
79	24.5	61.25	8.5	21.25	21722.702	4	yellow-green
80	24	60	7.5	18.75	16566.993	3	green
81	19.5	48.75	8	20	15315.264	3	green
82	20.5	51.25	7	17.5	12327.07	3	green
83	16	40	6.5	16.25	8295.7681	3	yellow-green
84	12.5	31.25	6	15	5522.3308	2	green
85	22.5	56.25	9	22.5	22365.44	4	olive
86	14	35	6	15	6185.0105	3	green
87	19	47.5	7.5	18.75	13115.536	4	green
88	21	52.5	7	17.5	12627.73	3	green
89	20	50	7	17.5	12026.409	3	olive
90	10.5	26.25	5.5	13.75	3897.8452	2	green
91	26	65	8	20	20420.352	4	green
92	17	42.5	7	17.5	10222.448	3	green
93	21	52.5	7	17.5	12627.73	3	green
94	22.5	56.25	7.5	18.75	15531.555	4	green
95	14	35	6.5	16.25	7258.7971	3	green
96	25.5	63.75	8	20	20027.653	5	green
97	17.5	43.75	6	15	7731.2632	3	green
98	28	70	7.5	18.75	19328.158	4	olive
99	22	55	7	17.5	13229.05	3	green
100	22.5	56.25	6.5	16.25	11665.924	4	green

Minimum size	26.25		12.5	3374.7577	1	
Maximum size	92.5		26.25	40978.763	6	
Mean	51.2125		17.775	13822.348	3.41	
Std. dev.	13.17191		2.9707114	7623.9658	0.9959007	
Variance	173.49921		8.8251263	58124855	0.9918182	
Predominant colour						green

Table 26: T83 seed - dimensions, number of cells and colour of conidiospores

CONIDIUM #	LENGTH (e.p.u. x 2.5)	LENGTH (µm)	WIDTH (e.p.u. x 2.5)	WIDTH (µm)	VOLUME (µm ³)	# OF CELLS PER CONIDIUM	COLOUR
1	20	50	7	17.5	12026.409	3	pale green
2	16	40	7	17.5	9621.1275	3	pale green
3	16.5	41.25	6.5	16.25	8555.0109	3	pale green
4	22.5	56.25	8	20	17671.459	3	pale green
5	13.5	33.75	6.5	16.25	6999.5543	3	pale green
6	23.5	58.75	6.5	16.25	12184.409	4	pale green
7	16.5	41.25	7.5	18.75	11389.807	4	pale green
8	21.5	53.75	7.5	18.75	14841.264	4	pale green
9	16	40	8	20	12566.371	3	pale green
10	18	45	8	20	14137.167	3	pale green
11	30	75	6.5	16.25	15554.565	4	pale green
12	21	52.5	6.5	16.25	10888.196	4	olive
13	28.5	71.25	7	17.5	17137.633	4	olive
14	29	72.5	8	20	22776.547	4	pale green
15	27	67.5	8.5	21.25	23939.304	5	pale green
16	12	30	7	17.5	7215.8456	2	pale green
17	13	32.5	8	20	10210.176	3	pale green
18	20	50	6.5	16.25	10369.71	3	pale green
19	22	55	8	20	17278.76	3	pale green
20	13	32.5	5	12.5	3988.35	3	pale green
21	23	57.5	6.5	16.25	11925.167	4	pale green
22	22	55	5.5	13.75	8166.9137	4	pale green
23	21	52.5	5.5	13.75	7795.6904	4	pale green
24	21.5	53.75	7	17.5	12928.39	3	pale green
25	19	47.5	7.5	18.75	13115.536	3	pale green
26	28	70	7	17.5	16836.973	4	pale green

27	26	65	7.5	18.75	17947.575	4	pale green
28	23.5	58.75	6.5	16.25	12184.409	4	pale green
29	19.5	48.75	5.5	13.75	7238.8553	3	pale green
30	17.5	43.75	6.5	16.25	9073.4964	3	pale green
31	22	55	6.5	16.25	11406.681	4	pale green
32	28	70	7	17.5	16836.973	4	pale green
33	26	65	5.75	14.375	10549.186	5	pale green
34	18.25	45.625	5.25	13.125	6172.9304	3	olive
35	23.5	58.75	5.5	13.75	8723.7487	4	olive
36	25	62.5	5	12.5	7669.9039	4	pale green
37	27.5	68.75	6.5	16.25	14258.351	4	olive
38	25	62.5	5.5	13.75	9280.5838	4	pale green
39	23.5	58.75	5.5	13.75	8723.7487	4	olive
40	22	55	5	12.5	6749.5155	4	olive
41	29	72.5	7	17.5	17438.294	5	olive
42	22	55	5	12.5	6749.5155	4	olive
43	26.5	66.25	7	17.5	15934.992	5	olive
44	24	60	7.25	18.125	15480.934	4	olive
45	25	62.5	6	15	11044.662	4	olive
46	18	45	5.5	13.75	6682.0203	3	olive
47	17	42.5	6	15	7510.3699	3	pale green
48	21	52.5	5	12.5	6442.7193	3	olive
49	18	45	5.5	13.75	6682.0203	3	olive
50	23	57.5	5.5	13.75	8538.1371	3	olive
51	26.5	66.25	6	15	11707.341	4	olive
52	17	42.5	5.5	13.75	6310.797	3	pale green
53	21	52.5	5	12.5	6442.7193	3	olive
54	25	62.5	6	15	11044.662	5	pale green
55	26	65	6	15	11486.448	4	olive
56	22	55	6	15	9719.3023	3	olive
57	22.5	56.25	5	12.5	6902.9135	3	olive
58	28	70	5.75	14.375	11360.662	5	olive
59	21	52.5	5.5	13.75	7795.6904	3	olive
60	22.5	56.25	5.5	13.75	8352.5254	4	olive
61	24.5	61.25	6	15	10823.768	5	olive
62	23	57.5	5.25	13.125	7779.5836	4	olive
63	20	50	5.5	13.75	7424.467	3	olive

64	17	42.5	5	12.5	5215.5347	3	olive
65	22	55	5.25	13.125	7441.3408	4	olive
66	12	30	5.5	13.75	4454.6802	2	pale green
67	21.5	53.75	5.75	14.375	8723.3652	3	olive
68	22	55	5	12.5	6749.5155	3	olive
69	20	50	5	12.5	6135.9232	3	olive
70	19.5	48.75	5.5	13.75	7238.8553	3	olive
71	22	55	5.5	13.75	8166.9137	4	olive
72	17.5	43.75	5.5	13.75	6496.4086	3	olive
73	21	52.5	4.75	11.875	5814.5542	3	olive
74	23.5	58.75	5	12.5	7209.7097	3	olive
75	27.5	68.75	6	15	12149.128	4	olive
76	21	52.5	6	15	9277.5158	3	olive
77	26	65	6.5	16.25	13480.623	4	olive
78	13	32.5	4.75	11.875	3599.4859	2	olive
79	26	65	6	15	11486.448	4	olive
80	25	62.5	6	15	11044.662	4	pale green
81	26	65	6	15	11486.448	5	olive
82	20	50	5	12.5	6135.9232	3	olive
83	12	30	5	12.5	3681.5539	2	pale green
84	22	55	5.5	13.75	8166.9137	4	olive
85	19	47.5	5	12.5	5829.127	3	olive
86	25.5	63.75	6	15	11265.555	5	olive
87	20	50	5.5	13.75	7424.467	3	olive
88	20	50	5.5	13.75	7424.467	3	olive
89	24.5	61.25	5.75	14.375	9940.579	4	olive
90	18	45	6	15	7952.1564	3	olive
91	24.5	61.25	5.5	13.75	9094.9721	5	olive
92	26	65	5.25	13.125	8794.3119	4	olive
93	19.5	48.75	5.5	13.75	7238.8553	3	olive
94	22.5	56.25	5	12.5	6902.9135	4	olive
95	21	52.5	5.5	13.75	7795.6904	3	olive
96	16	40	5.5	13.75	5939.5736	3	olive
97	19	47.5	6	15	8393.9429	3	olive
98	23	57.5	6.5	16.25	11925.167	4	olive
99	25.5	63.75	5.5	13.75	9466.1954	4	olive
100	17	42.5	5	12.5	5215.5347	3	olive

Minimum size		30		11.875	3599.4859	2	
Maximum size		75		21.25	23939.304	5	
Mean		54.03125		15.05625	9913.4985	3.56	
Std. dev.		10.446312		2.2597554	3922.3191	0.7291894	
Variance		109.12543		5.1064946	15384587	0.5317172	
Predominant colour							olive

Table 27: T88 leaf - Dimensions, number of cells and colour of conidiospores

CONIDIUM #	LENGTH (e.p.u. x 2.5)	LENGTH (µm)	WIDTH (e.p.u. x 2.5)	WIDTH (µm)	VOLUME (µm ³)	# OF CELLS PER CONIDIUM	COLOUR
1	27	67.5	6	15	11928.235	3	olive
2	13	32.5	5	12.5	3988.35	3	olive
3	15	37.5	6	15	6626.797	3	olive
4	16	40	5.5	13.75	5939.5736	3	olive
5	16	40	6	15	7068.5835	3	olive
6	23	57.5	5.75	14.375	9331.9721	4	olive
7	22	55	6	15	9719.3023	3	olive
8	17.5	43.75	5	12.5	5368.9328	3	olive
9	25.5	63.75	6	15	11265.555	4	olive
10	18	45	7	17.5	10823.768	3	olive
11	29.5	73.75	6	15	13032.701	4	pale green
12	27	67.5	5.5	13.75	10023.03	4	olive
13	26	65	5	12.5	7976.7001	4	green
14	32	80	6	15	14137.167	4	green
15	22	55	5	12.5	6749.5155	4	pale green
16	19.5	48.75	5.5	13.75	7238.8553	3	pale green
17	30	75	5.5	13.75	11136.701	4	pale green
18	18	45	6	15	7952.1564	2	olive
19	15.5	38.75	4.75	11.875	4291.6947	3	pale green
20	20	50	5	12.5	6135.9232	4	pale green
21	22	55	5	12.5	6749.5155	4	pale green
22	17	42.5	6	15	7510.3699	3	pale green
23	20	50	5	12.5	6135.9232	4	pale green
24	12	30	4	10	2356.1945	2	pale green
25	19.5	48.75	4.75	11.875	5399.2289	4	pale green

26	16	40	5	12.5	4908.7385	3	pale green
27	24.5	61.25	5	12.5	7516.5059	4	pale green
28	17	42.5	4.5	11.25	4224.5831	3	olive
29	36	90	6.5	16.25	18665.478	5	olive
30	27	67.5	5.25	13.125	9132.5546	5	olive
31	30	75	6	15	13253.594	4	olive
32	22	55	5	12.5	6749.5155	4	olive
33	20	50	5	12.5	6135.9232	3	olive
34	17	42.5	4.25	10.625	3768.2238	3	olive
35	17.5	43.75	5	12.5	5368.9328	3	olive
36	27	67.5	6	15	11928.235	5	pale green
37	11	27.5	5	12.5	3374.7577	2	pale green
38	27	67.5	5.5	13.75	10023.03	6	pale green
39	40.5	101.25	6.5	16.25	20998.663	6	olive
40	26	65	5.5	13.75	9651.8071	4	olive
41	25	62.5	4.75	11.875	6922.0883	4	pale green
42	10	25	5	12.5	3067.9616	2	pale green
43	17	42.5	5.5	13.75	6310.797	3	olive
44	24	60	6	15	10602.875	4	olive
45	24.5	61.25	5.5	13.75	9094.9721	4	olive
46	27	67.5	5.5	13.75	10023.03	4	pale green

63	21.5	53.75	5.75	14.375	8723.3652	4	olive
64	22	55	5	12.5	6749.5155	4	olive
65	20	50	5.5	13.75	7424.467	4	olive
66	40.5	101.25	7	17.5	24353.479	6	olive
67	15	37.5	4.5	11.25	3727.5733	3	pale green
68	29	72.5	4.75	11.875	8029.6224	3	pale green
69	21	52.5	5	12.5	6442.7193	4	pale green
70	26	65	5.25	13.125	8794.3119	5	pale green
71	22.5	56.25	5.5	13.75	8352.5254	5	pale green
72	20	50	5	12.5	6135.9232	3	olive
73	21	52.5	4.5	11.25	5218.6026	4	olive
74	9.5	23.75	5	12.5	2914.5635	2	pale green
75	23.5	58.75	5	12.5	7209.7097	4	pale green
76	35	87.5	5.5	13.75	12992.817	6	pale green
77	34	85	6	15	15020.74	4	pale green
78	12.5	31.25	4.5	11.25	3106.3111	2	pale green
79	15	37.5	4	10	2945.2431	3	pale green
80	22.5	56.25	5.5	13.75	8352.5254	4	pale green
81	16.5	41.25	5	12.5	5062.1366	3	pale green
82	23.5	58.75	5.5	13.75	8723.7487	4	olive
83	20	50	6	15	8835.7293	4	pale green
84	19	47.5	5	12.5	5829.127	3	pale green
85	24	60	7	17.5	14431.691	4	olive
86	25	62.5	6	15	11044.662	4	olive
87	32	80	7.25	18.125	20641.245	5	olive
88	30	75	6	15	13253.594	4	pale green
89	33	82.5	7	17.5	19843.575	6	olive
90	21	52.5	7	17.5	12627.73	3	pale green
91	18.75	46.875	5.75	14.375	7607.586	3	olive
92	22	55	7	17.5	13229.05	3	olive
93	31.5	78.75	6.5	16.25	16332.293	5	olive
94	22	55	6.5	16.25	11406.681	3	olive
95	30.5	76.25	6.5	16.25	15813.808	4	olive
96	32	80	6.5	16.25	16591.536	5	olive
97	27	67.5	5.5	13.75	10023.03	4	olive
98	26	65	6	15	11486.448	4	olive
99	21	52.5	5.75	14.375	8520.4963	3	olive

100	23.5	58.75	5.75	14.375	9534.8411	4	olive
Minimum size		23.75		10	2356.1945	2	
Maximum size		102.5		18.125	24353.479	6	
Mean		58.31875		13.81875	9214.8968	3.78	
Std. dev.		16.87715		1.7563355	4379.7606	0.9595875	
Variance		284.83819		3.0847143	19182303	0.9208081	
Predominant colour							pale green

Table 28: Z87 seed - Dimensions, number of cells and colour of conidiospores

CONIDIUM #	LENGTH (e.p.u. x 2.5)	LENGTH (µm)	WIDTH (e.p.u. x 2.5)	WIDTH (µm)	VOLUME (µm ³)	# OF CELLS PER CONIDIUM	COLOUR
1	22	55	5.5	13.75	8166.9137	4	yellow-green
2	19	47.5	5.5	13.75	7053.2437	4	yellow-green
3	19.5	48.75	4.5	11.25	4845.8453	3	yellow-green
4	21.5	53.75	7	17.5	12928.39	4	yellow-green
5	12	30	4.5	11.25	2982.0587	2	yellow-green
6	23	57.5	6.5	16.25	11925.167	4	yellow-green
7	20	50	6	15	8835.7293	3	yellow-green
8	15	37.5	6	15	6626.797	3	green
9	23.5	58.75	6.5	16.25	12184.409	3	yellow-green
10	23	57.5	6.5	16.25	11925.167	4	yellow-green
11	23.5	58.75	8	20	18456.857	4	yellow-green
12	21	52.5	7	17.5	12627.73	4	yellow-green
13	11	27.5	5	12.5	3374.7577	2	yellow-green
14	24	60	7	17.5	14431.691	4	yellow-green
15	16	40	5.5	13.75	5939.5736	3	yellow-green
16	22.5	56.25	6.5	16.25	11665.924	4	yellow-green
17	25	62.5	8	20	19634.954	4	yellow-green
18	20	50	6	15	8835.7293	3	yellow-green
19	20	50	6.5	16.25	10369.71	3	yellow-green
20	22	55	8	20	17278.76	4	yellow-green
21	25	62.5	8	20	19634.954	4	yellow-green
22	21	52.5	7	17.5	12627.73	3	yellow-green
23	21	52.5	7	17.5	12627.73	3	yellow-green
24	21	52.5	6.5	16.25	10888.196	3	yellow-green

25	41	102.5	8.5	21.25	36352.277	5	yellow-green
26	19	47.5	6.5	16.25	9851.2246	3	yellow-green
27	25	62.5	7.5	18.75	17257.284	4	yellow-green
28	19	47.5	6	15	8393.9429	3	yellow-green
29	26	65	8	20	20420.352	5	yellow-green
30	23	57.5	7	17.5	13830.371	4	yellow-green
31	15	37.5	7	17.5	9019.807	3	yellow-green
32	28	70	7	17.5	16836.973	4	yellow-green
33	26	65	9	22.5	25844.508	4	yellow-green
34	24	60	7	17.5	14431.691	4	yellow-green
35	29	72.5	8.5	21.25	25712.586	4	yellow-green
36	20.5	51.25	7	17.5	12327.07	3	yellow-green
37	21.5	53.75	7	17.5	12928.39	4	yellow-green
38	21	52.5	6	15	9277.5158	4	yellow-green
39	20	50	6	15	8835.7293	3	yellow-green
40	12.5	31.25	6	15	5522.3308	2	yellow-green
41	23	57.5	7.5	18.75	15876.701	4	yellow-green
42	23	57.5	7.5	18.75	15876.701	4	yellow-green
43	28.5	71.25	9	22.5	28329.557	4	yellow-green
44	27	67.5	8	20	21205.75	4	yellow-green
45	34	85	8	20	26703.538	6	yellow-green
46	22.5	56.25	6.5	16.25	11665.924	4	yellow-green
47	15	37.5	6.5	16.25	7777.2826	2	yellow-green
48	26	65	8.5	21.25	23052.663	4	yellow-green
49	29.5	73.75	8.5	21.25	26155.906	4	yellow-green
50	25	62.5	7.5	18.75	17257.284	4	yellow-green
51	18	45	7	17.5	10823.768	3	yellow-green
52	21	52.5	6	15	9277.5158	4	yellow-green
53	23	57.5	6.5	16.25	11925.167	4	yellow-green
54	23	57.5	8	20	18064.158	4	yellow-green
55	23	57.5	7	17.5	13830.371	4	yellow-green
56	20.5	51.25	7	17.5	12327.07	3	yellow-green
57	25	62.5	7	17.5	15033.012	4	yellow-green
58	20.5	51.25	5.5	13.75	7610.0787	4	yellow-green
59	21	52.5	6.5	16.25	10888.196	4	yellow-green
60	15.5	38.75	5	12.5	4755.3404	3	yellow-green
60	21	52.5	7	17.5	12627.73	4	yellow-green

62	17	42.5	6	15	7510.3699	3	yellow-green
63	27	67.5	8	20	21205.75	4	yellow-green
64	17.5	43.75	6	15	7731.2632	3	yellow-green
65	20	50	6	15	8835.7293	3	yellow-green
66	14	35	5	12.5	4295.1462	3	yellow-green
67	17	42.5	5	12.5	5215.5347	3	yellow-green
68	19.5	48.75	6	15	8614.8361	3	yellow-green
69	13.5	33.75	5	12.5	4141.7481	3	yellow-green
70	21	52.5	7	17.5	12627.73	3	yellow-green
71	41	102.5	8	20	32201.325	5	yellow-green
72	14	35	5	12.5	4295.1462	3	yellow-green
73	29	72.5	5.5	13.75	10765.477	4	yellow-green
74	36	90	8.5	21.25	31919.072	5	yellow-green
75	25	62.5	7	17.5	15033.012	4	yellow-green
76	39	97.5	8.5	21.25	34578.995	6	yellow-green
77	45	112.5	7.5	18.75	31063.111	6	yellow-green
78	24	60	6.5	16.25	12443.652	4	yellow-green
79	20	50	6.5	16.25	10369.71	4	yellow-green
80	24	60	7	17.5	14431.691	4	yellow-green
81	26	65	8	20	20420.352	4	yellow-green
82	23.5	58.75	7	17.5	14131.031	4	yellow-green
83	21.5	53.75	6.5	16.25	11147.438	4	yellow-green
84	24.5	61.25	7.5	18.75	16912.138	4	yellow-green
85	15	37.5	6	15	6626.797	3	yellow-green
86	17	42.5	5.5	13.75	6310.797	3	yellow-green
87	35.5	88.75	7	17.5	21346.877	6	yellow-green
88	17	42.5	6	15	7510.3699	3	yellow-green
89	20.5	51.25	6.5	16.25	10628.953	3	yellow-green
90	25.5	63.75	7	17.5	15333.672	4	yellow-green
91	21	52.5	6	15	9277.5158	3	yellow-green
92	23	57.5	6.5	16.25	11925.167	4	yellow-green
93	27	67.5	7	17.5	16235.653	4	yellow-green
94	32	80	8	20	25132.741	5	yellow-green
95	19	47.5	6	15	8393.9429	3	yellow-green
96	28	70	6.5	16.25	14517.594	4	yellow-green
97	14.5	36.25	5	12.5	4448.5443	3	yellow-green
98	35	87.5	8	20	27488.936	5	yellow-green

99	21	52.5	6.5	16.25	10888.196	4	yellow-green
100	31	77.5	7.5	18.75	21399.032	4	yellow-green
Minimum size		27.5		11.25	2982.0587	2	
Maximum size		112.5		22.5	36352.277	6	
Mean		57.2		16.9125	13911.288	3.72	
Std. dev.		15.72844		2.5774309	7390.6392	0.8050347	
Variance		247.38384		6.6431503	54621548	0.6480808	
Predominant colour							yellow-green

Table 29: K87 seed - Dimensions, number of cells and colour of conidiospores

CONIDIUM #	LENGTH (e.p.u. x 2.5)	LENGTH (µm)	WIDTH (e.p.u. x 2.5)	WIDTH (µm)	VOLUME (µm ³)	# OF CELLS PER CONIDIUM	COLOUR
1	21	52.5	5.5	13.75	7795.6904	3	olive
2	18.5	46.25	5.5	13.75	6867.632	3	olive
3	21	52.5	6	15	9277.5158	4	olive
4	21.5	53.75	6.25	15.625	10306.433	4	yellow-green
5	26	65	6	15	11486.448	4	olive
6	23.5	58.75	6.5	16.25	12184.409	3	yellow-green
7	22	55	5.5	13.75	8166.9137	4	yellow-green
8	21	52.5	5.5	13.75	7795.6904	4	yellow-green
9	28	70	6.5	16.25	14517.594	5	olive
10	19	47.5	6.5	16.25	9851.2246	4	olive
11	23.5	58.75	6.5	16.25	12184.409	4	olive
12	26	65	7.75	19.375	19164.022	4	olive
13	28.5	71.25	6.5	16.25	14776.837	3	olive
14	26	65	7.25	18.125	16771.012	4	olive
15	20	50	6	15	8835.7293	4	olive
16	20	50	6.75	16.875	11182.72	4	olive
17	9	22.5	6	15	3976.0782	3	olive
18	19	47.5	6.5	16.25	9851.2246	3	olive
19	25	62.5	7	17.5	15033.012	4	olive
20	19.75	49.375	6.5	16.25	10240.089	4	olive
21	25.5	63.75	6.5	16.25	13221.38	4	olive
22	23	57.5	7.5	18.75	15876.701	4	olive
23	12.5	31.25	6	15	5522.3308	3	olive

24	16	40	7	17.5	9621.1275	3	olive
25	22	55	7	17.5	13229.05	4	olive
26	26	65	7.5	18.75	17947.575	4	olive
27	18	45	6.25	15.625	8628.6419	3	olive
28	17	42.5	6.5	16.25	8814.2536	3	olive
29	25	62.5	7.25	18.125	16125.973	4	dark olive
30	24	60	7	17.5	14431.691	4	dark olive
31	18	45	6.5	16.25	9332.7391	3	olive
32	22	55	8	20	17278.76	4	olive
33	20	50	6.5	16.25	10369.71	3	olive
34	27	67.5	8	20	21205.75	4	olive
35	15	37.5	6.5	16.25	7777.2826	2	olive
36	19	47.5	7.5	18.75	13115.536	3	brownish
37	20.5	51.25	7.5	18.75	14150.973	4	olive
38	22	55	7.25	18.125	14190.856	4	olive
39	28	70	9	22.5	27832.547	4	olive
40	12.5	31.25	6.5	16.25	6481.0688	2	olive
41	20	50	6.5	16.25	10369.71	4	olive
42	25.5	63.75	7	17.5	15333.672	4	olive
43	22.5	56.25	6.75	16.875	12580.56	4	olive
44	19.5	48.75	7	17.5	11725.749	4	olive
45	20	50	6.5	16.25	10369.71	4	olive
46	23	57.5	7	17.5	13830.371	4	olive
47	22.5	56.25	6.5	16.25	11665.924	4	olive
48	23	57.5	7.5	18.75	15876.701	4	olive
49	22.5	56.25	7	17.5	13529.711	4	olive
50	20.5	51.25	6.5	16.25	10628.953	4	olive
51	20	50	6.5	16.25	10369.71	3	olive
52	22.5	56.25	7.25	18.125	14513.376	4	olive
53	21	52.5	7	17.5	12627.73	3	olive
54	24	60	7.5	18.75	16566.993	4	olive
55	22	55	6.5	16.25	11406.681	4	olive
56	24	60	7	17.5	14431.691	4	olive
57	23	57.5	6.75	16.875	12860.128	4	olive
58	19.5	48.75	6.5	16.25	10110.467	4	olive
59	19.5	48.75	6.5	16.25	10110.467	4	olive
60	19.5	48.75	7	17.5	11725.749	4	olive

61	23.5	58.75	7	17.5	14131.031	4	olive
62	18.5	46.25	7.25	18.125	11933.22	3	olive
63	18	45	6.5	16.25	9332.7391	3	olive
64	15	37.5	6.5	16.25	7777.2826	3	olive
65	27	67.5	8.5	21.25	23939.304	4	brownish
66	26.5	66.25	8	20	20813.051	4	olive
67	16.5	41.25	6	15	7289.4767	3	olive
68	18	45	6.5	16.25	9332.7391	3	olive
69	20.5	51.25	7	17.5	12327.07	4	olive
70	28.5	71.25	7	17.5	17137.633	5	olive
71	23	57.5	7	17.5	13830.371	4	olive
72	24.5	61.25	7	17.5	14732.351	4	olive
73	21.5	53.75	6.5	16.25	11147.438	4	olive
74	23	57.5	7	17.5	13830.371	4	olive
75	10	25	5	12.5	3067.9616	2	olive
76	22.5	56.25	6	15	9940.1955	4	olive
77	19	47.5	7	17.5	11425.089	3	olive
78	19.5	48.75	7.25	18.125	12578.259	3	brownish
79	22	55	6.75	16.875	12300.992	3	brownish
80	28.5	71.25	7.5	18.75	19673.304	4	olive
81	20	50	7.5	18.75	13805.827	3	olive
82	14	35	5.5	13.75	5197.1269	3	olive
83	18	45	6	15	7952.1564	3	olive
84	23	57.5	6.75	16.875	12860.128	4	olive
85	21	52.5	6	15	9277.5158	3	olive
86	21.5	53.75	6.75	16.875	12021.424	4	olive
87	25	62.5	5.5	13.75	9280.5838	3	olive
88	29	72.5	7	17.5	17438.294	3	olive
89	15	37.5	6	15	6626.797	3	olive
90	29.5	73.75	5.5	13.75	10951.089	3	olive
91	30.5	76.25	6.25	15.625	14620.754	5	olive
92	14.5	36.25	5.75	14.375	5883.1998	3	olive
93	21.5	53.75	6	15	9498.409	4	olive
94	21	52.5	6	15	9277.5158	4	olive
95	20.5	51.25	6	15	9056.6226	4	olive
96	19.5	48.75	6	15	8614.8361	4	olive
97	24	60	6	15	10602.875	4	olive

98	16.5	41.25	6	15	7289.4767	3	olive
99	23.5	58.75	6.5	16.25	12184.409	4	olive
100	18	45	7	17.5	10823.768	3	olive
Minimum size		22.5		12.5	3067.9616	2	
Maximum size		76.25		22.5	27832.547	5	
Mean		53.40625		16.65625	11958.554	3.63	
Std. dev.		10.317473		1.7165246	4072.2819	0.5972158	
Variance		106.45024		2.9464568	16583480	0.3566667	
Predominant colour							olive

Table 30: K88 seed - dimensions, number of cells and colour of conidiospores

CONIDIUM #	LENGTH (e.p.u. x 2.5)	LENGTH (µm)	WIDTH (e.p.u. x 2.5)	WIDTH (µm)	VOLUME (µm ³)	NUMBER OF CELLS PER CONIDIUM	COLOUR
1	15	37.5	5.5	13.75	5568.3503	2	green
2	18.5	46.25	6	15	8173.0496	3	green
3	15.5	38.75	6	15	6847.6902	3	green
4	20	50	6.5	16.25	10369.71	3	green
5	31	77.5	6	15	13695.38	4	green
6	28.5	71.25	6	15	12590.914	4	green
7	19.5	48.75	6	15	8614.8361	4	green
8	13.5	33.75	5	12.5	4141.7481	3	green
9	24	60	6.5	16.25	12443.652	4	yellow-green
10	27.5	68.75	6.5	16.25	14258.351	3	green
11	18	45	6.5	16.25	9332.7391	3	yellow-green
12	26.5	66.25	6	15	11707.341	4	green
13	17	42.5	6	15	7510.3699	3	green
14	22.5	56.25	6.5	16.25	11665.924	4	green
15	18.5	46.25	6	15	8173.0496	3	green
16	10	25	6	15	4417.8647	2	green
17	31.5	78.75	7.5	18.75	21744.178	5	green
18	16	40	6	15	7068.5835	2	green
19	19	47.5	7	17.5	11425.089	3	green
20	11	27.5	4	10	2159.8449	1	green
21	13.5	33.75	5	12.5	4141.7481	2	green
22	21.5	53.75	6	15	9498.409	3	green

23	16	40	6	15	7068.5835	3	green
24	21	52.5	6	15	9277.5158	4	green
25	17	42.5	5.5	13.75	6310.797	3	green
26	20.5	51.25	6	15	9056.6226	4	green
27	17.5	43.75	5.5	13.75	6496.4086	3	green
28	19	47.5	6.5	16.25	9851.2246	3	green
29	16	40	6	15	7068.5835	3	green
30	16	40	6	15	7068.5835	3	green
31	20	50	6.25	15.625	9587.3799	3	green
32	12.5	31.25	5.5	13.75	4640.2919	2	green
33	22	55	6.5	16.25	11406.681	4	green
34	13	32.5	5	12.5	3988.35	2	green
35	14	35	5	12.5	4295.1462	2	green
36	21	52.5	7	17.5	12627.73	3	olive
37	18.5	46.25	6.5	16.25	9591.9819	3	olive
38	17	42.5	5.5	13.75	6310.797	3	olive
39	21.25	53.125	6	15	9387.9624	4	olive
40	11	27.5	4.5	11.25	2733.5538	3	olive
41	29.5	73.75	6.5	16.25	15295.322	5	olive
42	22	55	6	15	9719.3023	3	olive
43	16.5	41.25	6	15	7289.4767	3	olive
44	21.5	53.75	6	15	9498.409	3	olive
45	29.5	73.75	5.5	13.75	10951.089	4	olive
46	13	32.5	5	12.5	3988.35	2	olive
47	17.5	43.75	6	15	7731.2632	3	green
48	15.5	38.75	5	12.5	4755.3404	3	green
49	24	60	6.25	15.625	11504.856	4	yellow-green
50	20.5	51.25	6	15	9056.6226	3	green
51	13	32.5	6	15	5743.2241	1	green
52	12	30	5	12.5	3681.5539	2	green
53	25	62.5	7	17.5	15033.012	4	green
54	14.5	36.25	6.5	16.25	7518.0398	3	green
55	18.5	46.25	6	15	8173.0496	3	green
56	24	60	6.5	16.25	12443.652	4	green
57	15	37.5	6	15	6626.797	3	yellow-green
58	13.5	33.75	5	12.5	4141.7481	3	green
59	14.5	36.25	4.5	11.25	3603.3209	2	green

60	17	42.5	6	15	7510.3699	3	green
61	20	50	5.5	13.75	7424.467	3	green
62	14.5	36.25	6.5	16.25	7518.0398	3	green
63	32	80	7.5	18.75	22089.323	5	olive
64	16	40	5.5	13.75	5939.5736	3	pale green
65	29	72.5	8	20	22776.547	4	yellow-green
66	16	40	5	12.5	4908.7385	3	green
67	17.5	43.75	5.5	13.75	6496.4086	3	green
68	16	40	5.5	13.75	5939.5736	3	green
69	23.5	58.75	6	15	10381.982	4	green
70	19.5	48.75	7	17.5	11725.749	3	pale green
71	24.5	61.25	6	15	10823.768	3	yellow-green
72	21.5	53.75	6.5	16.25	11147.438	4	yellow-green
73	23.5	58.75	7	17.5	14131.031	4	yellow-green
74	20	50	7	17.5	12026.409	3	olive
75	19.5	48.75	6.25	15.625	9347.6954	3	yellow-green
76	25	62.5	7.5	18.75	17257.284	4	yellow-green
77	18	45	5.5	13.75	6682.0203	3	green
78	21.5	53.75	6	15	9498.409	3	green
79	22	55	7	17.5	13229.05	4	olive
80	20	50	5.5	13.75	7424.467	4	green
81	22	55	6.5	16.25	11406.681	3	yellow-green
82	21	52.5	6.5	16.25	10888.196	3	green
83	9.5	23.75	4.5	11.25	2360.7964	2	green
84	22.5	56.25	6	15	9940.1955	4	olive
85	14	35	5.5	13.75	5197.1269	2	green
86	21	52.5	7	17.5	12627.73	3	yellow-green
87	25	62.5	7.25	18.125	16125.973	4	yellow-green
88	15	37.5	6	15	6626.797	2	green
89	22	55	6.5	16.25	11406.681	4	yellow-green
90	17.5	43.75	6	15	7731.2632	3	green
91	17.5	43.75	6.25	15.625	8388.9574	3	green
92	25	62.5	7	17.5	15033.012	4	yellow-green
93	16	40	6	15	7068.5835	3	green
94	16	40	6	15	7068.5835	3	green
95	16	40	5.5	13.75	5939.5736	3	green
96	29.5	73.75	7	17.5	17738.954	4	yellow-green

97	31	77.5	8	20	24347.343	5	green
98	24	60	6.5	16.25	12443.652	3	yellow-green
99	17.5	43.75	5.25	13.125	5919.2484	3	green
100	29	72.5	8	20	22776.547	5	yellow-green
Minimum size		23.75		10	2159.8449	1	
Maximum size		80		20	24347.343	5	
Mean		48.95625		15.2	9443.8568	3.19	
Std. dev.		12.984625		1.9132875	4564.2373	0.8001894	
Variance		168.6005		3.6606692	20832262	0.640303	
Predominant colour							green

Appendix 12: Complete data on the measurement of the diameters of all the inoculum types, following inoculation

Table 31: ANT148 - growth rates

Inoculum type		Diameter of colony (mm) following inoculation (days)								
		1	2	3	4	5	6	7	8	9
ANT148	1	5	21	30	50	65	76	82	82	8
	2	5	21	29	49	65	76	82	82	82
	3	5	21	30	49	64	78	82	82	82
	4	5	22	31	51	65	77	82	82	82
	5	5	20	30	48	65	74	82	82	82
Mean		5	21	30	49.4	64.8	76.2	82	82	82

Table 32: H88 - growth rates

Inoculum type		Diameter of colony (mm) following inoculation (days)								
		1	2	3	4	5	6	7	8	9
H88	1	5	15	23	34	46	58	69	78	82
	2	5	16	21	40	54	65	75	82	82
	3	5	16	22	38	50	60	74	82	82
	4	5	18	25	36	49	60	72	82	82
	5	5	18	24	38	51	61	73	81	82
Mean		5	16.6	23	37.2	50	60.8	72.6	81	82

Table 33: T83 - growth rates

Inoculum type		Diameter of colony (mm) following inoculation (days)								
		1	2	3	4	5	6	7	8	9
T83	1	5	16	25	36	48	57	70	82	82
	2	5	15	24	36	46	56	66	77	82
	3	5	9	22	35	41	55	66	78	82
	4	5	12	14	16	40	49	59	74	82
	5	5	19	25	37	50	60	75	82	82
Mean		5	14.2	22	32	45	55.4	67.2	78.6	82

Table 34: T88 - growth rates

Inoculum type		Diameter of colony (mm) following inoculation (days)								
		1	2	3	4	5	6	7	8	9
T88	1	5	15	26	36	50	60	73	82	82
	2	5	15	24	33	48	57	69	78	82
	3	5	18	23	39	52	63	75	81	82
	4	5	16	25	37	50	61	71	82	82
	5	5	15	25	35	48	58	68	81	82
Mean		5	15.8	24.6	36	49.6	59.8	71.2	80.8	82

Table 35: Z87 - growth rates

Inoculum type		Diameter of colony (mm) following inoculation (days)								
		1	2	3	4	5	6	7	8	9
Z87	1	5	15	26	35	49	59	70	78	82
	2	5	15	25	36	50	59	70	80	82
	3	5	16	26	36	50	59	70	80	82
	4	5	16	27	37	53	62	75	82	82
	5	5	15	26	36	50	61	72	81	82
Mean		5	15.4	26	36	50.4	60	71.4	80.2	82

Table 36: K87 - growth rates

Inoculum type		Diameter of colony (mm) following inoculation (days)								
		1	2	3	4	5	6	7	8	9
K87	1	5	19	30	45	56	68	78	82	82
	2	5	20	32	45	58	69	76	82	82
	3	5	21	31	45	58	69	75	82	82
	4	5	19	32	42	53	63	72	82	82
	5	5	19	29	42	52	64	72	82	82
Mean		5	19.6	30.8	43.8	55.4	66.6	74.6	82	82

Table 37: K88 - growth rates

Inoculum type		Diameter of colony (mm) following inoculation (days)								
		1	2	3	4	5	6	7	8	9
K88	1	5	9	12	32	50	66	72	82	82
	2	5	14	16	42	59	71	80	82	82
	3	5	9	14	35	52	61	74	82	82
	4	5	9	13	32	48	64	71	76	82
	5	5	11	15	36	50	60	75	82	82
Mean		5	10.4	14	35.4	51.8	64.4	74.4	80.8	82