THE PATHOGENICITY OF *PYRENOPHORA TERES*

by

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ABBREVIATIONS

c	circa (about)
C.T.R.	constant temperature room
cv	cultivar(s)
cm	centimeter
diam	diameter
g	gramme(s)
h	hour(s)
L	litre(s)
LBA	lima bean agar
min	minute(s)
ml	millilitre(s)
mg	milligramme(s)
mm	millimeter
m	meter(s)
µℓ	microlitre(s)
ppm	part per million
PCA	potato carrot agar
rpm	revolutions per minute
sec	second(s)
UV	ultra violet

ABSTRACT

The pathogenicity of Pyrenophora teres Drechs., the causal agent of net blotch disease of barley (Hordeum vulgare L.), has been investigated. The cultural characteristics (mycelial growth, colour, sporulation) of 32 isolates collected from England and Wales were determined. The reactions of winter barley cv. Sonja to 18 isolates of P. teres using a mycelial disk inoculation method on leaf segments were studied as well as the reactions of 15 barley cultivars (spring and winter) to P. teres, isolate PC 215, by inoculating whole plants with spore suspensions. The virulence of 10 isolates of P. teres (provided by Dr. B.C. Clifford, The Welsh Plant Breeding Station, Aberystwyth in November 1984) on barley cv. Sonja and the reactions of non-host plants to a virulent isolate of P. teres (Pt 80-12) from Aberystwyth have also been studied. The non-host plants included two species of wild barley (Hordeum murinum, Hordeum jubatum), 41 lines of Hordeum spontaneum, three species of wheat, Triticum aestivum (spring cv. Maris Dove), Triticum monococcum, Triticum turgidum, three species of oat, Avena sativa cv. Milo, cv. Peniarth, Avena fatua, Avena strigosa, rye (Secale cereale cv. Rheidol), and 17 species of grasses belong to 11 genera of Gramineae, including Agrostis, Arrhenatherum, Alopecurus, Agropyron, Cynosurus, Dactylis, Festuca, Holcus, Lolium, Phleum and Poa.

The pathogenicity tests were done in the laboratory by inoculating leaf segments with a droplet of a spore suspension at 21 ± 1°C, in the constant temperature room at 21 ± 1°C using whole plants inoculated with spore suspensions by spraying, and in the field by spraying whole plants with spore suspensions. Pathogen/host and pathogen/non-host interactions were studied in inoculated leaf segments by microscopic examinations for spore germination, method of penetration and growth of the pathogen in leaves. The relationship between leaf age and susceptibility to net blotch was examined. Induced resistance and susceptibility using virulent and less virulent isolates on leaf segments in the laboratory and on whole plants in the constant temperature room at 21 ± 1°C were also investigated.

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INTRODUCTION

Pyrenophora teres (Died.) Drechs.), the causal organism of net blotch disease of barley (Hordeum vulgare L.), was first described in its conidial form (Helminthosporium teres Sacc.) in 1881 (Butler and Jones, 1955).

Net blotch is a common disease of barley wherever the crop is grown in the temperate, humid regions of the world (Dickson, 1956; CMI map No. 364, Ed. 2, 1968). The disease has assumed increasing importance as a destructive pathogen in the United Kingdom during the past few years, particularly in the south and west of England, (Clifford and Jones, 1981; Jordan, 1981). In the U.K. net blotch disease was reported in epidemic form in 1930, and in 1932 it was prevalent in East Anglia and the Isle of Wight (Moore et al., 1934).

The disease was again apparent in 1933 and 1941 (Moore, 1943). James (1967) did not record net blotch in his survey of foliar diseases of spring barley in England and Wales in 1967. But Evans (1969) reported the disease to be prevalent in West Sussex in 1968 on some crops of spring barley. King (1972, 1977) reported for the years 1967-1970, 1972-1975, that net blotch was infrequent and generally not severe, and that the disease was most common in 1972 and 1973 when the months of May and June were wetter than in 1974 or 1975. According to Jordan (1982), net blotch was seen frequently on early sown winter barley in the Cotswolds; in 1978, it was commonly found in southern England. In 1979, it was

widespread throughout the U.K. and locally severe in 1980, and by 1981 it had become epidemic in all regions of the U.K. Data from the 1981 ADAS National Winter Barley Diseases Survey (King, 1982) have shown that net blotch is the most common and severe disease on winter barley in the U.K. In 1981, yield losses of up to 77% were demonstrated in fungicide field trials, (Jordan, 1982). There have been attempts to explain the great increase in net blotch disease and why it has become a major problem on winter barley.

Jordan, in collaboration with Evans and Locke (1982) and Hessayon (1982), attributed the great increase to the expansion in winter barley acreage by adoption of new cultivars such as Sonja and Igri which are more susceptible to net blotch than the more widely grown older cultivar Maris Otter, to favourable weather conditions, continuous cropping, decreased ploughing, and possibly to a change in the fungus itself. There is also the recent discovery that carbendazim and triadimefon widely used for control of certain foliar diseases of cereals were ineffective against net blotch and exacerbate the disease by increasing sporulation twofold (Jordan and Best, 1981). Nitrogen fertilizers used on winter barley stimulate growth of the pathogen and there appears to be a close link between the amount of nitrogen used and the severity of the disease (Hessayon, 1982). The initial sources of infection are barley seed, debris from diseased crops and infected volunteer barley

plants (Locke, 1981; Jordan, 1981). The research described in this thesis was initiated with the objective of examining the reactions of host and non-host plants to different isolates of Pyrenophora teres. In the first section, the cultural characteristics of 32 isolates of P. teres collected from England and Wales were determined. In Section 2, the reactions of winter barley cv. Sonja to 18 isolates of P. teres using a mycelial disk inoculation method on leaf segments were studied. In Section 3, the reactions of 15 barley cultivars (spring and winter) to P. teres isolate PC 215, by inoculating whole plants with suspensions of spores were examined. In Section 4, the virulences of 10 isolates of P. teres (provided by Dr. B.C. Clifford, The Welsh Plant Breeding Station, Aberystwyth, in November 1984) on a highly susceptible barley cv. Sonja were studied. In Section 5, the reaction of host and non-host plants to infection by P. teres was studied, by inoculating leaf segments with droplets of suspension of spores; by inoculating whole plants (three leaf growth stage seedlings) by spraying with spore suspension in a C.T. room at 21 ± 1°C and in the field. The sporulation of P. teres on host and non-host plants was estimated in the C.T. room and in the field. The non-host plants included two species of wild barley (Hordeum murinum, H. jubatum); 41 lines of Hordeum spontaneum; three species of wheat (Triticum aestivum spring cultivar Maris Dove, T. monococcum, T. turgidum); three species of oat (Avena sativa cv. Milo, cv. Peniarth, A. fatua and A. strigosa); rye (Secale cereale cv. Rheidol); and 17

species of grasses belonging to 11 genera of Gramineae including: Agrostis, Arrhenatherum, Alopecurus, Agropyron, Cynosurus, Dactylis, Festuca, Holcus, Lolium, Phleum and Poa.

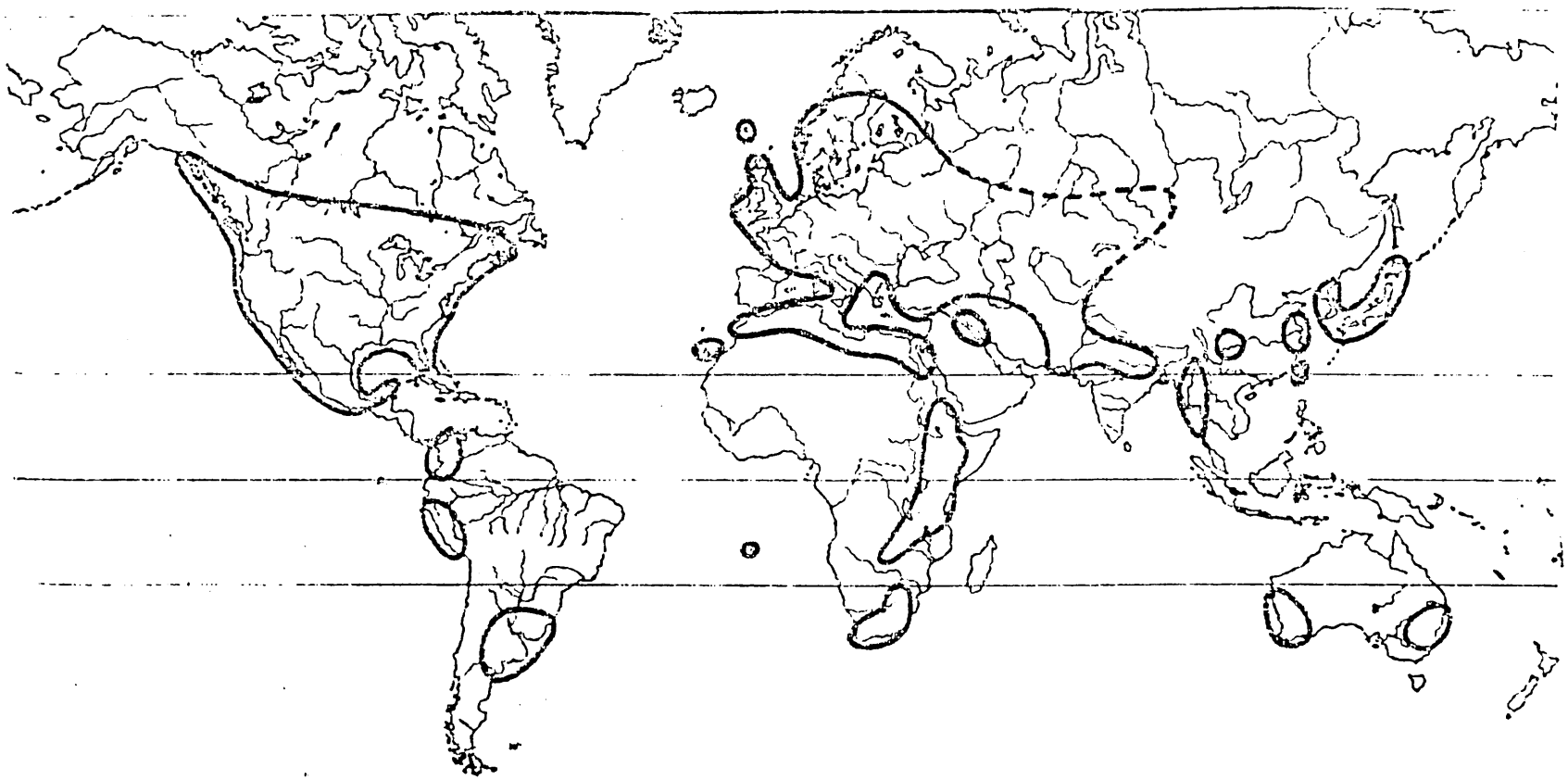
Pathogenicity was studied by inoculating leaf segments with droplets of spore suspension; spraying whole plants (three leaf growth stage seedlings) with suspension of spores in the C.T. room at 21 ± 1°C and similarly in the field. Section 6, gives the results of experiments aimed at establishing whether non-host plants serve as alternative hosts for the fungus in the absence of the normal host (cultivated barley). Section 7 describes experiments on induced resistance and susceptibility (systemic and local), by using virulent and less virulent isolates of the pathogen. In Section 8, pathogen/host and pathogen/non-host interactions are described on inoculated leaf segments in terms of spore germination, penetration and colonization of leaves. In Section 9, a possible role of pseudothecia in the epidemiology of net blotch was studied. Section 10 is on the relationship between leaf age and susceptibility of barley (cv. Sonja) to net blotch. Section 11, deals with the viability of P. teres conidia at low temperatures. These investigations are focussed therefore on the following:

1. The cultural characteristics of a range of isolates of P. teres.
2. The development of methods for studying pathogenicity on leaf segments and on whole plants.

3. The pathogenicity of selected isolates to cultivars of barley, to other cereals and to grasses, representing host and non-host plants.
4. The behaviour of isolates on host and non-host plants
5. Effect of leaf age on susceptibility.
6. Induction of resistance and of susceptibility.

DISTRIBUTION MAP of Pyrenophora teres Drech.

COMMONWEALTH MYCOLOGICAL INSTITUTE. Map No. 364 (edition 2, Issued 1. vii. 1968).



List of countries in which net blotch disease has been recorded.

(COMMONWEALTH MYCOLOGICAL INSTITUTE, Appendix to Map No. 364).

AFRICA
- Egypt [xxxvii, 279]
- Kenya [xxxv,358]
- South Africa [xxxiv,324]
- Tanzania [Wallace (xxviii,472)p.3]
- Tunisia [xiv,429]

ASIA
- Afghanistan [xx,234]
- China (Nanking, Hangchow) [Wei (xxii,327)p.42]
- India (U.P., Pusa) [xv.86; xiv,80]
- Iraq (Mesopotamia) [i,54]
- Israel [xxxvi,8]
- Japan [Fujioka (xxxii,278)p.19]
- Nepal [xv,86]
- Turkey [xxxvi,576]
- U.S.S.R. (Tashkent) [Zaprometoff (xi.123)p.34]

EUROPE
- Austria [xxxvii,405]
- Bulgaria [Atanasoff (x,210)p.23]
- Cyprus [xxxvi,95]
- Denmark [xxxiv,711]
- England [vi,714]
- ?France [xiv,424]
- Germany [xxviii,268]
- Holland [Westerdijk & van Luijk, Ned. kruidk. Arch. 1016,p.111]
- Italy [xxxvi,18]
- Malta [Herb. I.M.I. No. 70477]
- Norway [Jorstad (xxv,184)p.25]
- Romania [Herb. I.M.I. No. 9807]

EUROPE (Cont.)
- Scotland [xxxvii,223]
- Sweden [xxiii,127]
- U.S.S.R. (European, Latvia) [viii.165; Smarods (x,223)p.7]

NORTH AMERICA
- Canada (Alta, B.C., Man., N.B., N.S., Ont., P.E.I., Que., Sask [Rep. Canad. Plant Dis. Surv. 1920-57]
- United States (widespread) [Weiss (xxx,112) p.446]

SOUTH AMERICA
- Argentine [xxxiv,775]
- Uruguay [De Brotos & Boasso (xxxv,751)p.13]

Syn. Helminthosporium teres Sacc.

LITERATURE REVIEW

1. The host plant

A- Origin and distribution

Barley (Hordeum vulgare L.) is among the most important cereals; its origin remains largely unresolved. It has been dated as far back as 8000 BC. Barley is a self-pollinating diploid (2n = 2x7 = 14) which probably originated from the two-rowed, wild type Hordeum spontaneum. The six-rowed, naked grain types appeared by about 6000 BC and are believed to have originated from Hordeum agriocrithon which is found wild in Tibet (Jones and Clifford, 1978). Barley is grown in most temperate regions of the world, but it grows best where the ripening season is long and cool, rainfall is moderate, and soils are well drained (Mathre, 1982).

B- Classification

Barley (Hordeum vulgare L. emend. Bowden), belongs to the grass family Gramineae, tribe Hordeae. Most cultivated barleys have either a winter or a spring growth habit. The spring cultivars do not require a cold dormant period before they will head (Mathre, 1982).

C- Economic importance

Barley grains are very nutritious, having on average more protein than maize, considerably less oil and a high fibre content. The largest use, over half of world production, is for animal and poultry feed. It is also used

for production of malt for brewing and in many parts of the world, barley is consumed in soups and dressings and as a milled product in bread or breadlike foods (Jones and Clifford, 1978; Mathre, 1982). Hessayon (1982) reported that the total area of barley cultivated in England and Wales in 1980/81 was 1.844 million hectares; 1.044 million hectares for spring barley and 0.799 million hectares for winter barley, which represented 30.5% and 23.4% of the total cereal acreage respectively. The estimated yield of barley in the U.K. ranges between 4.3 - 4.5 tonnes per hectare.

2. The pathogen and the disease

A- Origin and names

The generic name of *Helminthosporium* was given by Link (1809) as proposed initially by Persoon (1801). The genus *Helminthosporium* is now represented by many species widely distributed, and including many species parasitic on Gramineae. The species which causes net blotch disease of barley was first described in 1881 by Saccardo in its conidial state and named *Helminthosporium teres* (Ellis, 1971). The perithecial stage was not discovered until 1919 by Drechsler at Madison, Wisconsin; U.S.A., on straw, stubble and infected spikelets of barley and later recognized as the ascigerous stage of *H. teres* and named *Pyrenophora teres* (Drechsler, 1923). Later, *Pyrenophora teres* was shown to be heterothallic because mature perithecia are formed only when opposite mating

types are crossed, (Mathre, 1982; Western, 1971). Perithecia have been reported on culms, straw, stubble and spikes of barley in many countries. In the U.K., it has been reported by Smith (1925); and in Canada in Quebec by Crowell (1941); in Alberta by Piening (1961); and in Denmark by Smedegard-Petersen, (1972). P. teres produces conidia profusely on infected debris of barley after harvest; they are considered to be important sources of primary infection in the autumn (Jordan, 1981; 1983). Webster (1951), reported that conidia are also produced from sclerotia on barley stubble. Berdugo, (1968), has reported synonymous names for the fungus as follows:-

Helminthosporium teres	Saccardo,	1882
Helminthosporium hordei	Eidam,	1891
Pleospora teres	Diedicke,	1903
Pyrenophora teres	Drechsler,	1923
Drechslera teres	Ito,	1930

B- Sources of inocula and main features of the pathogen

The initial sources of inocula are barley seed and straw debris from previously diseased crops, and infected barley volunteer plants (Mathre, 1982; Hessayon, 1982; Jordan, 1981). Infection of barley seedlings from seed-borne inoculum is greatest at low temperatures (10 - 15°C) and rarely occurs at 20°C and above (Machacek and Wallace, 1942; Mathre, 1982). Webster (1961) believed that seed-borne inoculum was probably the most frequent source of infection in the U.K., and Piening (1968) stated that even 1 percent of infected seed could be significant in the

development of disease in the field. After sowing infected seeds the primary phase of attack may develop on the first leaf as streak symptoms which usually are not found on later leaves. The first leaf becomes infected from the fungus in the coleoptile (Western, 1971; Jordan, 1983). The severe autumn net blotch symptoms that developed in 1979 on a field observation site in Cornwall, U.K., in which barley had not been grown for at least four years was attributed to seed-borne infection (Jordan, 1981). Seed-borne inoculum probably serves to introduce the pathogen into areas previously free of net blotch.

Pycnidia have been found under natural conditions on plant tissues such as straw debris, chaff, senescent leaves, culms, stubble, spikes and seed of barley (Eriksson, 1914; Smith and Putterill, 1932; Western, 1971). They are globose to pear-shaped, yellow to brown in colour, 64-172µm in diameter with a thin fragile wall. They have also been produced in cultures (Mathre, 1982). Kenneth (1962) found that spores from pycnidia were unable to infect barley leaves and considered them to be spermatia. Other attempts by Berdugo (1968) and Jordan (1981), to infect barley leaves with pycnidiospores were unsuccessful. Helminthosporium teres produces sclerotium-like bodies on straw and dead leaves during the winter. They are 1-2mm in diameter and develop beneath the epidermis, bursting through as elongate black bodies. Two months at 10-15°C are required for maturation. Fertile sclerotium-like bodies develop

only when pairs of thalli of P. teres of compatible mating type come together. At maturity they produce protoperithecia which later develops to perithecia in late summer. Non-fertile sclerotium-like bodies remain immature and later become functional sclerotia, sometimes called pseudoperithecia, which may produce conidiophores bearing conidia in the spring (Butler and Jones, 1955; Webster, 1961; Berdugo, 1968). Perithecia which form in late summer release their ascospores in autumn/winter and are dispersed to infect newly-emerging barley seedlings (Jordan, 1983). The conidia which are produced profusely on straw debris after harvest, are dispersed throughout the autumn and are of major importance as primary inocula for infection of leaves of crop seedlings or volunteer plants, which then become further reservoirs of inocula (Jordan, 1983).

Conidia produced on the primary lesions then cause secondary infection of the developing foliage (Putterill, 1954). Net blotch secondary infection occurs at 8 to 33°C, with an optimum at 25°C, declining sharply at 33°C and with none at 35°C in artificially inoculated plants. Infection occurred with a minimum moist period of 5 hours. Moist period requirements for optimum infection were found to differ and ranged between 10 to 30 hours depending on the isolate of the pathogen.

Pre-inoculation wetting of leaves resulted in increased net blotch symptoms. Susceptibility depended on cultivar,

age of plant at infection, and isolate of the pathogen. It also depended on age of the culture producing the inocula with maximum infection from 10 day old inoculum and very much less infection from 20, 25 and 35 day old inocula (Singh, 1963). The rate at which net blotch develops in crops is influenced by the amount and source of inocula, the crop structure and density, and the weather. Although most severe disease develops in wet weather, net blotch can also develop in periods without rain when occasional new lesions ensure the continued availability of inoculum (Jordan and Royle, 1983).

C- Economic importance

Net blotch disease is considered to be one of the most important factors influencing barley production especially in areas of high humidity and rainfall. The severity of the disease is related to the susceptibility of barley cultivars, and yield losses can approach 100% in severely affected fields of highly susceptible cultivars. More typically, losses range between 10 and 40% (Mathre, 1982). Shipton (1973), showed that reduction in yield depended on the nutrient status of the soil; losses were less than c. 13% for fertile soils compared to losses of c. 43% in a poor soil. Grain yield and weight (1,000 kernel weight) are reduced when barley plants are severely infected with net blotch whereas number of grains seems to be little affected (Smedegard-Petersen, 1976; Mathre, 1982). But Jordan (1981) reported that the severe disease of winter

barley during tillering decreased both the number of grains per ear and grain weight. Other responses to disease include premature ripening and shrivelling of the grain. In severe attacks, infected leaves wither from the tip leading to early death of leaves which can seriously reduce the yield (Hessayon, 1982).

Many countries have reported much greater incidence of net blotch in the past two decades or so. In the U.S.A., Gray (1966) has estimated an annual reduction in barley yield of up to 20 million bushels. In Europe, net blotch is known to cause grain yield losses of 10 to 40% in some years (Hessayon, 1982). In Denmark, the disease may reduce grain yield by up to 11% (Smedegard-Petersen, 1976). In Germany, Rintelen (1969), reported that infection of spring barley which occurred before tillering caused 30 to 40% yield loss on diseased shoots, and 20% on entire plants, whereas infection between tillering and flowering decreased whole plant yield by c. 10%. In Egypt, net blotch disease caused 5 - 10% reduction in the grain yield (Selim, 1973). A serious outbreak of net blotch in Iraq was reported in December 1979 in the Khalas area (c. 75 Km. from Baghdad) by Wilson et al., (1981); over 60% infection was recorded on the barley cultivar Arivat which is grown extensively in this area.

Net blotch has only recently become a serious threat to winter barley in the U.K. The reasons for the sudden outbreak of the disease in 1979 are discussed above. King

(1982), in the National Leaf Disease Levels Survey of winter barley in England and Wales in 1980/1981 reported that the average loss of leaf area due to net blotch disease was 14.2% on leaf 2 and 6.7% on the flag leaf at GS 71-73. In the Regional Leaf Disease Levels Survey of winter barley, net blotch was the most severe disease at GS 71-73 in all regions of England and Wales, occurring at the highest mean levels of 27.2% in the south-west and 22% in the south-east and diminishing towards the north at less than 5%, leaf 2 area affected. In the Varietal Leaf Disease Levels Survey, net blotch was most severe on Sonja (22.1% average percentage leaf 2 area affected) in all regions of England and Wales except the east of England where cv. Maris Otter contributed most of the samples, and on which disease was least severe (2.9% Average Percentage leaf 2 area affected).

3. Host range of Pyrenophora teres

Knowledge of the host range of any pathogen is important from an epidemiological standpoint. Butler and Jones (1955) mentioned that graminaceous hosts may play an important role in the epidemiology of net blotch in one region and not in another. Butler and Jones (1955) and Kenneth (1962), reported that the net blotch pathogen is commonly found on the wild barley grass (Hordeum murinum). Sprague (1955), reported that specimens of wild barley (Hordeum brachyantherum) collected from Alaska in 1952 were infected by P. teres. The wild barley grass (Hordeum

distichon) has also been found infected by P. teres (Sprague, 1950; Putterill, 1954; Shoemaker, 1962). Kenneth (1962), reported that wild barley grass (Hordeum murinum) was susceptible to infection by P. teres. Braverman (1960); Kenneth (1962), and Kenneth et al. (1967), found that under the conditions of natural infection, most evidence suggests that P. teres is specifically pathogenic on Hordeum species. Kenneth (1962) examined the reaction of numerous species of Hordeum to infection of P. teres and reported the infectivity of isolates from wild species including Hordeum leporinum on their cultivated relatives and vice versa. Kenneth (1967), concluded that Hordeum spontaneum (an ancestral progenitor of cultivated barley) serves as a host reservoir of different pathogenic forms of P. teres and hence plays an important role in the epidemiology of net blotch in Palestine. Khan and Boyd (1968), reported that Hordeum hystrix and Bromus diandrus were susceptible to infection by P. teres. Hosts other than Hordeum spp. harbouring P. teres include oat (Avena sativa) in Finland (Talvia, 1966) and Bromus gussonii, in western Australia (Khan and Boyd, 1968).

Winter (1977), reported that out of 24 rye (Secale cereale) seed samples collected from different regions of Argentina, 21 showed infection by P. teres. Singh (1962), examined the reaction of twenty two species belonging to 16 genera of Gramineae to P. teres, and classified them in groups according to the degree of susceptibility.

A- light infection on:

Alopecurus pratensis	,	Avena sativa
Festuca elatior	,	Hordeum jubatum
Lolium perenne	,	Panicum miliaceum
Phalaris arundinacea	,	Secale cereale
Sorghum vulgare	,	Triticum durum
Triticum vulgare		

B- moderate infection on:

Agropyron repens	,	Bromus inermis
Triticum diococcum		

C- Heavy infection on:

Avena fatua	,	Hordeum vulgare
Triticum monococcum		

D- No infection on:

Dactylis glomerata	,	Phleum pratense
Poa pratensis	,	Zea mays

Kenneth (1962) and Khan and Boyd (1968) have suggested that the limited growth of P. teres on many graminaceous hosts must not be overlooked in the epidemiology of the disease. Keeling and Banttari (1965) indicated that in detached leaf culture, the fungus may sporulate on leaves of field resistant barley. Therefore it is possible that limited sporulation may occur on the surface of lesions on resistant grasses in nature, so that the grasses could be a significant source of primary inoculum.

4. The symptoms

Net blotch is named for the well-known net-like symptoms produced on barley leaves and leaf sheaths, flowers and even on the hulls. They are most common on leaves (Jordan, 1981; Mathre, 1982). Butler (1955), reported that symptoms of net blotch on barley are not easily discernible in the earlier parts of the season because the primary lesions on the first leaves of the seedling almost always appear as small spots or as streaks not more than 1mm in length.

Jordan (1981), has described the development of symptoms on different parts of barley plants. Leaves of young shoots of barley are infected by conidia or ascospores during winter or early spring to produce small brown lesions within two days. As the lesion expands, a dark brown reticulation develops on a light brown background. A zone of chlorosis usually surrounds the lesion. The symptoms of seedling infection, also seen on volunteer barley plants, first appear either as a single, water-soaked strip extending up the first seedling leaf, or as a pale, greyish white lesion in the centre of the lamina. Both types of lesion later turn brown and develop a central, reticulate pattern. Infection of flag leaves and the second youngest leaves of mature plants appear as short, narrow, brown lesions arranged in interrupted parallel stripes. The spot symptoms of this disease were first recorded by McDonald in Canada in 1967. Later Smedegard-Petersen (1971),

described two forms of P. teres which differ only in the symptoms caused on barley. They have been identified as P. teres Drechs. forma teres Smedeg. which produces dark brown, longitudinal and transverse necrotic streaks which usually form a characteristic, net-like pattern on leaves of infected barley, and P. teres Drechs. forma maculata Smedeg., which produces well defined dark brown circular or elliptical lesions without netting. Common to both is that affected tissue becomes chlorotic often with areas of water-soaked tissue.

Smedegard-Petersen (1976) has obtained four different classes of lesion types in the progeny from crosses between the spot and net form of P. teres. The four classes were designated and defined as:

1. Spot lesions which are well defined, dark brown, elliptical or circular lesions identical to those induced by the spot form of P. teres (parental type).
2. Net lesions which are dark brown, longitudinal and transverse streaks of varying length, typically forming a net-like pattern (parental type).
3. Intermediate lesions which are dark brown elongated spots or short necrotic streaks but with no net pattern, in lesions that are intermediate between spot and net lesions (recombinant type).
4. Fleck lesions which are limited dark brown necrotic flecks which do not enlarge, often few in number and hardly recognizable (recombinant type).

Al-Ali (1978) described six different types of lesions caused by P. teres on non-identified volunteer barley plants in south-west France (Toulouse region). The lesion types are of net, oval, pin-head, rectangular, linear and irregular form. Each lesion type comprises a necrotic zone surrounded by a chlorotic halo zone. Smedegard-Petersen (1977) reported that chlorosis and water-soaking are important in the symptom complex of net-spot blotch of barley. Thus, certain highly pathogenic isolates cause a blight-like reaction characterized by chlorosis and a diffuse general water-soaking appearing three to four days after inoculation and followed by rapid necrosis of affected tissue but often with no or weak expression of the usual necrotic lesions. The symptoms caused by highly pathogenic isolates of P. teres indicate that a toxin might be involved in pathogenesis. This assumption was supported by histological investigations of barley leaves inoculated with such isolates.

After infection, chlorosis and water-soaking developed rapidly in advance of the hyphae, indicating the effects of diffusible toxic substances. Furthermore, excised barley leaves immersed in a desalted liquid medium in which the pathogen had grown, developed symptoms similar to those caused by the pathogen itself whereas no symptoms developed on leaves immersed in a desalted medium in which the fungus had not been grown.

Similar necrotic lesions were obtained by Al-Ali (1978) when seedlings of barley (cv. Monlon) at the three leaf stage were treated with 25 µℓ droplets of filtrates from cultures of seven isolates of P. teres. The results indicated that there were differences between the isolates in toxin production. The most virulent of the seven isolates which cause net-like lesions when conidial suspensions are sprayed on seedlings, produced necrotic spot lesions in 36 hours. The moderately virulent isolates produced necrotic spot lesions in 42 hours and the less virulent isolates in 65 hours.

MATERIALS AND METHODS

1. Plants and their growth

1.1 Host plants

Seeds of winter and spring barley were obtained from Dr. P. Hewitt (National Institute of Agricultural Botany, Cambridge) in 1983, including 10 cultivars of spring barley (Carnival, Atem, Golf, Goldmaker, Tasman, Egmont, Koru, Kym, Midas and Triumph), and 5 cultivars of winter barley (Tipper, Athene, Maris otter, Igri and Sonja). Two spring barley cultivars (Zephyr and Vada) were provided by Dr. S.G. Evans (National Agricultural Advisory Service, Kent) in 1984. A set of 13 differential cultivars (lines) of barley were provided by Dr. B.C. Clifford (Welsh Plant Breeding Station, Aberystwyth) in 1985, including 10 spring lines (C.I. 5401; C.I. 6311; C.I. 9820; C.I. 739; C.I. 1243; C.I. 4795; C.I. 4502; C.I. 4979; C.I. 9214; Proctor) and 3 winter lines (C.I. 9518; CODE 65; TENN 61-119). These 13 differential cultivars (lines) were used in the 1980, 1981, 1982 and 1983 UK cereal pathogen virulence surveys at The Welsh Plant Breeding Station. ND 3112 spring barley cultivar which has good resistance to strains of *P. teres* in North Dakota State University (USA) was provided by Professor Vernyl Pederson in 1985.

1.2 Non-host plants

The non-host plants which were used in the study of the host range of *P. teres* (isolate 80-12) included wild barley, wild oat, cultivated oat, rye, wheat, corn, rice and wild grasses.

1.2.1 Wild barley

A) Wall barley (Hordeum murinum)

Wall barley (H. murinum) was collected from Silwood Park, Ascot, and at Sunninghill and Sunningdale during July and August. It resembles cultivated barley so closely that it is often called wild barley.

B) Hordeum jubatum

H. jubatum was provided by Dr. B.C. Clifford, in 1985.

C) Hordeum spontaneum

Forty one samples (lines) of Hordeum spontaneum were provided by Dr. J.G. Robin (Genetic Resources Unit, Plant Breeding Institute, Cambridge) in 1985. They were arranged in accession order with an identity code as follows:

Accession number	Identity Code
01517	063-0-5-002
01520	063-0-7-004
01536	064-0-4-001
05732	063-0-1-005
05733	063-0-1-006
05735	963-0-2-006
05738	063-0-5-003
05739	063-0-6-009
05742	063-0-6-012
05743	063-0-7-005
05744	063-0-7-006
05745	063-0-8-007

Accession number	Identity Code
05747	063-0-8-009
05750	064-0-1-013
05753	064-0-1-015
05761	064-0-3-010
06200	090-0-1-016
06206	090-0-2-013
06207	090-0-2-014
06210	090-0-3-013
08330	005-1-2-003
09242	063-0-7-003
13786	005-1-1-004
13787	005-1-3-004
13789	005-2-3-004
14293	063-0-2-007
14296	063-0-6-013
14298	063-0-8-010
14301	064-0-1-017
14302	064-0-1-018
14303	064-0-1-019
14304	064-0-2-012
14305	064-0-2-013
14309	064-0-4-005
14411	005-1-3-005
14586	090-0-1-021
14589	090-0-2-015
14590	090-0-2-016
14591	090-0-3-014
14593	090-0-4-005
14595	090-0-5-012

D) Wheat

The cultivated wheat (Triticum aestivum) spring cv. Maris Dove, was obtained from the Plant Breeding Institute, Cambridge. T. monococcum and T. turgidum were provided by Dr. B.C. Clifford in 1985.

E) Oat

Avena sativa cv. Milo (Spring Oat), Avena sativa cv. Peniarth (Winter Oat), and Wild Oat Avena strigosa, were provided by Dr. B.C. Clifford in 1985. Wild Oat, Avena fatua was collected from Silwood Park in 1985.

F) Rye

Forage rye (Secale cereale) cv. Rheidol, was provided by Dr. B.C. Clifford in 1985.

G) Grasses

Seventeen species of grasses belonging to 11 genera of Gramineae were provided by B and S Weed Seed suppliers - Nottingham; and The University of London, Botanic Garden, Egham, Surrey, and Plant Ecology Section, Silwood Park. The 17 species are:

Agrostis gigantea

Agrostis stolonifera

Agrostis tenuis

Agropyron repens

Cynosurus cristatus

Dactylis glomerata

Festuca pratensis

Festuca rubra

Holcus lanatus

Lolium perenne

Phleum pratense

Phleum bertolonii

Poa annua

Poa trivialis

Poa pratensis

Arrhenatherum elatius

H) Rice

Five cultivars of rice (Oryza sativa), Malaysian origin, were provided by Dr. D. Mills, ICI Ltd., Jealott's Hill Research Station, Bracknell in 1986. The cultivars are:

BAHAPIE line 0/81/978; TNI, SMII, 81979; and MR35 (2 Kepol).

I) Sweet Corn

F_1 hybrid of sweet corn (Zea mays) was bought from Suttons Seeds Ltd. (Torquay-England) in 1986.

1.3 Growing of seedlings

Stocks of seed of barley, wheat, oat, rye, corn, rice and wild grasses were kept in an incubator at c. 5°C. Samples were taken immediately before sowing. Seeds were washed for 3 minutes in 10% sodium hypochlorite, then washed twice in sterile distilled water and kept on sterile

moist filter paper on a germination tray (37 x 25cm) at 20°C until emergence of roots, generally after 3 days for cultivated barley, wheat, oat, rye and corn and after 3 to 7 days for wild grasses and rice. Germinated seeds were then sown at c. 2cm deep in plastic pots (11cm diameter) containing John Innes No. 1 compost or UC-type soil mixes. Germinated seed of wild grasses were sown c. 1cm deep in a plastic tray (36 x 22 c 5cm). Pots and trays were kept inside filtered-air cabinets designed to exclude fungal spores (Finney, 1973).

The cabinets were placed on a self-watering sand bench in a glasshouse at 18 ± 2°C and 70 - 90%RH. Cabinets were illuminated to a light intensity of 8000 lux at plant level provided from fluorescent tubes with a photoperiod of 16 hours daily. Seedlings with abnormal or mis-shapen leaves were removed to give a uniform stand, usually 5 to 8 seedlings per pot.

1.4 Soils

a) UC-type soil mixes

A UC-type soil mix (Matkin and Chandler, 1957) was used for growing barley, wheat, oat, rye, rice, corn and wild grasses for glasshouse experiments. The composition of the mix was:

Peat	75L
Sand	25L
KNO_3	15g
K_2SO_4	15g

Superphosphate	120g
Magnesium limestone	295g
Calcium carbonate	235g
Frit	37g

b) John Innes No. 1 Compost

2. Fungi and their culture

2.1 Origin of *P. teres* isolates

Sources of isolates of *P. teres* were:

A) Silwood Park isolates

Two isolates were obtained from an infected, unidentified cultivar of barley (H.t.SP.) with net form symptoms and from wild barley (*H. murinum*) (H.t.WB.) with spot form symptoms in Silwood Park, in 1983 (See Table 3). The cultivated and wild barley were growing close to each other.

B) Bristol isolates

Twenty isolates of *P. teres* were provided by Dr. J. Hargreaves (Long Ashton Research Station, Bristol) in 1983. The isolates had been kept under oil for two years in Bristol and carried the following code numbers: PC215, PC724, PP50C, PP36, PP50G, PC673, LARS, PC719, PP27, PP32, PP23, PP33, PP44, PP50C, PC216, PP29, PC1067, PP40-41, PP46, PP26.

C) Aberystwyth isolates

Ten isolates of *P. teres* in the form of diseased barley leaf fragments, were provided by Dr. B.C. Clifford (Welsh Plant Breeding Station at Aberystwyth),

in 1983. The isolates were numbered by Dr. B.C. Clifford as follows:

BNS-82-58, BNS-82-59, BNS-82-88, BNS-82-19, BNS-82-25, BNS-82-70, BNS-82-103, BNS-82-5, BNS-82-63, BNS-82-209.

The highly virulent isolate (Pt.80-12) provided by Dr. B.C. Clifford in November 1984, was used in studies of the host range of the pathogen and of induced resistance and susceptibility.

D) Kent isolates

Seven isolates of P. teres were provided by Dr. J.M. Davies, Mrs. Angela Berrie and Miss Rachael Farley, (MAFF, ADAS, Kent) in October 1984. They had been collected from barley fields in West and East Sussex. The samples were from winter barley volunteer plants (cv. Igri) with net blotch symptoms and they were numbered as follows:

PC3152, PC3153, PC3154, PC3155, PC3170, PC3199, PC3135.

2.2 Culture media

A) Potato Carrot agar (PCA)

The medium as recommended by Langeron and Vanbreuseghem (1952) and by the Commonwealth Mycological Institute, was as follows:

Grated potato	20g
Grated carrot	20g
Agar	20g
Distilled water	1 L

The grated potato and carrot were boiled for one hour in distilled water, filtered through muslin and agar

was dissolved in the extract before autoclaving at 120°C for 20 minutes. The pH was adjusted to 5 with 10% sodium hydroxide before autoclaving.

B) V-8 agar

V-8 agar medium as recommended by Miller (1955) contained:

V-8 (vegetable juice)	200 ml
Agar	20 g
Distilled water	800 ml

Agar was dissolved in distilled water, V-8 vegetable juice was added and the pH adjusted to 5 with 10% sodium hydroxide before autoclaving for 20 minutes at 120°C.

C) Water agar medium

The medium was:

Agar	20 g
Distilled water	1 L

The agar was dissolved in distilled water, the pH was adjusted to 5 with 10% sodium hydroxide, before autoclaving for 20 minutes at 120°C. This medium was used to obtain monospore, pure cultures.

D) Lima bean agar (LBA)

The medium comprised:

Lima bean agar (Difco)	23 g
Distilled water	1 L

The lima bean agar was dissolved in distilled water, pH was adjusted to 5 with 10% sodium hydroxide, then medium autoclaved at 120°C for 20 minutes.

2.3 Isolation of pure cultures

A spore dilution method was used to obtain monospore culture isolates from infected barley leaves.

2.3.1 Van Tieghem humid chamber method

Samples of infected barley leaves were cut into small segments (c. 1 cm long), immersed in 90% ethanol for 10 seconds then in 1% sodium hypochlorite solution for 90 seconds and rinsed finally in sterile distilled water (Clifford and Jones, 1980). The segments were placed in Petri dishes containing PCA and incubated at 21 ± 1°C under near-ultra violet (black) light with a 12 hour photoperiod. Suspensions of spores were obtained from 10 day old cultures by flooding the agar with 25 ml sterile distilled water, and gently rubbing the agar with a sterilized paint brush. The suspension of spores was then filtered through muslin. The concentration of the suspension was diluted to give 10^3 spore/ml using a haemocytometer. One 5µl droplet of this suspension from an Agla micrometer syringe contained one or two conidia. Three separate droplets were placed in the centre of a sterile cover slip which was gently inverted on to the previously sterilized humid chamber van Tieghem. The droplets were examined under the microscope, and one containing a single conidium was directly absorbed with a sterilized piece of filter paper which was placed on PCA in a Petri dish and incubated at 21 ± 1°C.

2.3.2 Dummy objective method

A few drops of the suspension (10^3 spore/ml) were spread over plates of 2% water agar which were incubated for 2-3 hours at 21 ± 1°C. An isolated germinating spore was removed by cutting the agar around the spore with a sterilized dummy objective fitted on a microscope. The agar block with the single spore was transferred with a sterile needle to a plate of PCA and then incubated in 21 ± 1°C to give a single spore culture.

2.4 Induction of sporulation

Four methods were used to increase sporulation of P. teres isolates.

2.4.1 Pellet formation method

Lukens'(1960) technique was used. Mycelial discs, 5mm diam. from growing margins of 10 day old colonies of P. teres on PCA were placed in 100 ml, 20% V-8 juice liquid medium containing small sterilized glassbeads, in 250 ml. conical flasks and then were shaken using a Griffin Flask Shaker continuously for 8 days at 21 ± 1°C. Mycelial pellets from the cultures were then distributed over a dry sterilized filter paper in Petri dishes. Some Petri dishes were then incubated at 12 hour photoperiod (cool light) and 12 hour dark cycle. Others were placed under 12 hour near ultra violet and 12 hour dark cycle.

A third group were incubated in full darkness. All

plates were incubated at 21 ± 1°C for 7 days.

2.4.2 Autoclaved barley leaves method

Srinivasan et al. (1971)technique for seed borne fungi was used. Young leaves of barley seedlings (cv. Sonja) were cut into 2 cm long segments which were suspended in distilled water and autoclaved at 120°C for 20 minutes. Single segments were transferred to the surface of 2% water agar in Petri dishes. A mycelial disk, 5 mm diam. from the growing margin of a 10 day old culture of P. teres on PCA, was placed on the surface of each segment. Plates were then incubated as above.

2.4.3 Moistened filter paper method

A moistened filter paper technique (Kilpatrick, 1966) was used. A mycelial disk, 5 mm diam. from the growing margin of a 10 day old colony of P. teres on PCA, was placed on the surface of sterilized moist filter paper, and then incubated as above.

2.4.4 12-h near ultra violet and 12-h dark cycle method

A mycelial disk, 5 mm in diam. from the growing margin of a 10 day old culture of P. teres on PCA was placed on a plate of PCA. Some plates were incubated in darkness at 21 ± 1°C for 7 days, others were exposed to 12-h. near ultra violet and 12-h darkness at 21 ± 1°C for 7 days.

2.5 Preservation of cultures

Three methods were used.

2.5.1 Preservation on agar

Isolates were grown on PCA and also on 20% V-8A slants in test tubes and then stored at 5°C. They were transferred to fresh agar every 3 months.

2.5.2 Preservation under mineral oil

Isolates were grown on PCA slants in vials and covered with sterile mineral oil to a depth of 1 cm above the top of the slant. Cultures were stored at 5°C.

2.5.3 Preservation on infected leaf segments of barley

Three leaf growth stage seedlings of barley cv. Sonja were infected with P. teres (virulent isolate, 80-12). Leaves with good net form symptoms were cut into 1 cm long segments which were immersed in 90% ethanol for 10 seconds and then in 1% sodium hypochlorite for 90 seconds before washing twice in sterile distilled water. The segments were then placed on sterilized moist filter papers in Petri dishes (30 segments per plate).

The plates were incubated for 3 days at 21 ± 1°C in darkness, then transferred to a near ultra violet (black) light cabinet set to give a 12-h. photoperiod at 21 ± 1°C. Seven days later, spores were produced abundantly over the upper surface of the segments. Plates were then stored at 1°C for up to one year with little loss of spore viability.

2.6 Preparation of suspensions of spores

Monospore isolates of P. teres were cultured on plates

of LBA or PCA at 21 ± 1°C under 12 h. near ultra violet and 12 h. dark cycle for 10 days. Spores were produced abundantly and harvested by adding 25 ml. sterile distilled water to the surface of the cultures and brushing the surfaces gently with a sterile smooth paint brush. Suspensions of spores were then filtered through muslin and concentrated by centrifuging in a refrigerated centrifuge for 6 minutes at 5920 rpm at 2-5°C (Thomas, 1984).

Spore pellets were resuspended in the required volume of sterile distilled water, and a few drops of 0.05% Tween 20 were added to disperse the spores into homogeneous suspensions. Concentrations of the suspensions were adjusted by using a haemocytometer. The same procedure was followed for preparing suspensions of spores from barley leaves (cv. Sonja) infected by P. teres and with net form symptoms after exposure for 10 days under a 12 h. near ultra violet and 12 h. dark cycle at 21 ± 1°C.

3. Inoculation of plants

3.1 By mycelial disks

Leaf segments 5 cm long were taken from first leaves of 3 leaf growth stage seedlings of winter barley (cv. Sonja), highly susceptible to net blotch (King, 1982). Segments were placed floating on 4 ml. of a 50 ppm benzimidazole solution inside plastic boxes (5.5 x 3.5 x 2 cm). Three small wounds were made equidistantly, 1.5 cm. from each other by means of a sterilized needle on the ventral surface of the segments.

Each wound was then inoculated by a mycelial disk, 1 mm diam. taken from the growing margins of 7 day old cultures of isolates of P. teres. Three segments were used for each isolate. Wounds were left uninoculated in the controls. Boxes were kept closed by their lids for the first 24 h., and incubated at 21 ± 1°C. Lesions were measured 7 days after inoculation.

3.2 By spore suspension droplets

Suspensions of 5×10^4 spore/ml were prepared (see 2.6). Segments 5 cm. long from fully expanded first leaves, taken from 3 leaf growth stage seedlings were placed floating on 4 ml. of a 50 ppm benzimidazole solution in small plastic boxes (5.5 x 3.5 x 2 cm). Droplets of spore suspension from an Agla micrometer syringe were placed on the ventral surface of the segment 1.5 cm. from each other. The boxes were then treated as above.

3.3 By spraying whole plants in a C.T. room with suspensions of spores

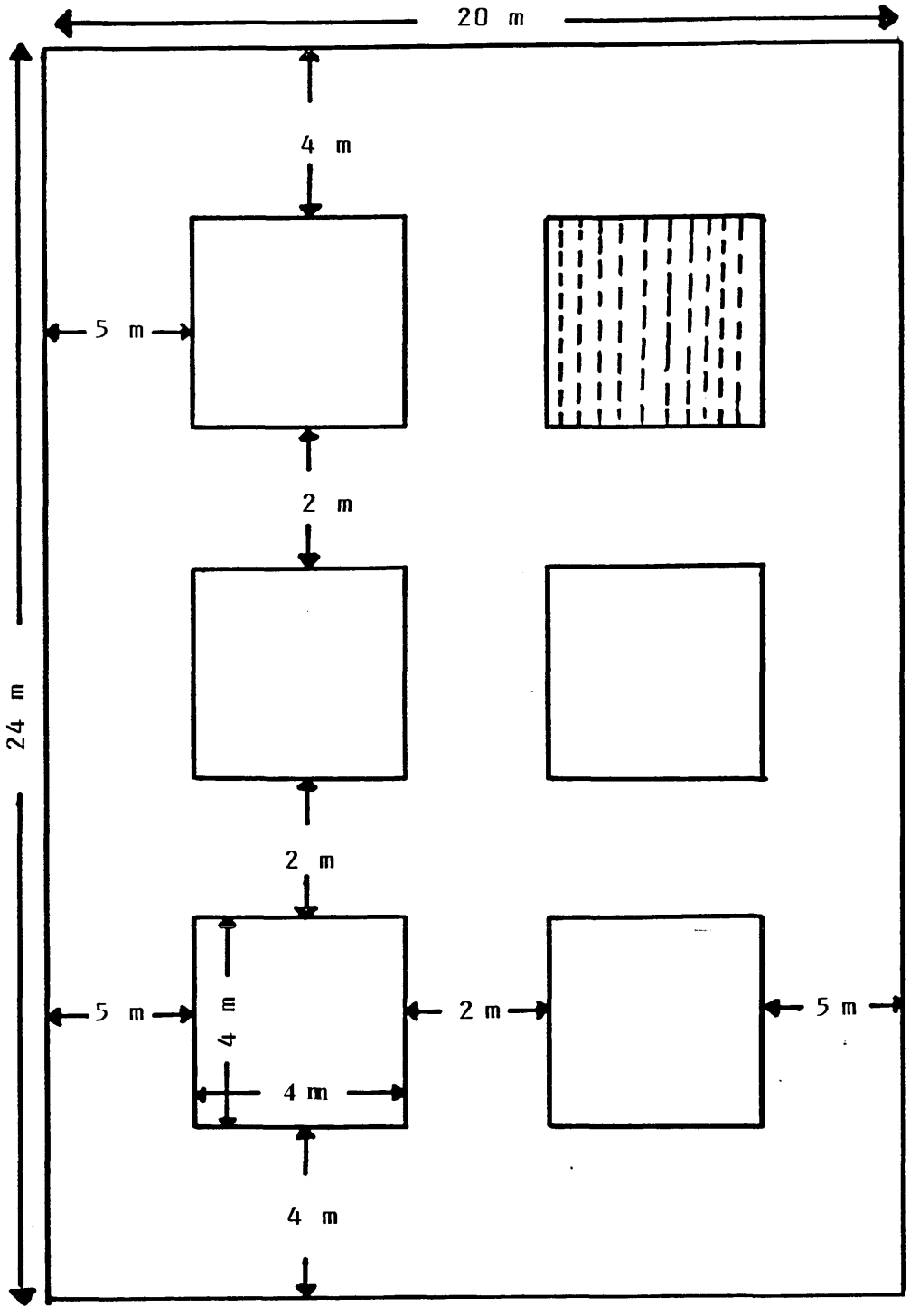
Seedlings with three fully expanded leaves of host and non-host plants were grown in a glasshouse as described in 1.3. The plants were: cultivated barley, wheat, rye, oat, corn and rice; 41 lines of wild barley (Hordeum spontaneum), wall barley (Hordeum murinum) and eighteen species of grasses belong to eleven genera of Gramineae. They were sown in a plastic pot (11 cm. diam.), containing a UC-type soil mix (1.4,a). Seedlings were transfered into plastic boxes (46 x 25 x 26 cm)

with tap water c. 2 cm. deep. The seedlings were sprayed with a suspension 5 x 10^4 spore/ml of P. teres - virulent isolate 80-12 using a sprayer (Crown Industrial Products Company) attached to an aerosol propellant (SIGMA spray; SIGMA Chemical Company, USA). The boxes were then covered by their lids and kept at 21 ± 1°C in a controlled temperature room, with fluorescent tubes (cool light) and a photoperiod of 16 h. daily. The lids of the boxes were removed 24 h. after spraying.

3.4 By spraying whole plants in the field with suspensions of spores

Seeds of host and non-host plants were sown in a field plot at Silwood Park (see Table 1). The main plot (20 x 24 m) was divided into six plots (4 x 4m). Each plot contained 10 rows which were protected with a net after sowing the seed. Seedlings with three fully expanded leaves were sprayed with suspensions (5 x 10^4 spore/ml) of spores from 10 day old cultures of P. teres virulent isolate 80-12. They were enclosed in polythene bags containing a wad of wet cotton wool for 24 h. When the bags were removed, the temperature at the time of the inoculation was c. 16°C. Leaves were assessed for disease 15 days after the inoculation.

Table 1: Diagram of field plots containing host and non-host plants sprayed with suspensions of spores of *P. teres*. Sixty rows of seedlings.



4. Assessment of infection

4.1 On leaf segments

4.1.1 Pre-penetration stage: spore germination and growth of germ tubes

Segments were treated as in 3.2 with three (5 µl) droplets of spore suspension (5 x 10^4 spore/ml). The segments were removed 16 h. after inoculation and placed on glass slides. A piece of a clear doubled face adhesive cellulose tape was pressed firmly on the inoculated surface of the segments, peeled off and then fixed on a glass slide. A drop of 0.1% lactophenol-cotton blue was added to the surface of the tape. Germinated spores in which germ tubes was longer than the spore were counted. Growth of germ tubes was measured 16 h. after inoculation. Ten microscopic fields of view (40 x 15X) were examined for estimating the mean of the number of germinated spores and the length of the germ tubes on host and non-host plants.

4.1.2 Post-penetration stage: appressorial formation and penetration, using a whole mount method.

Five cm. long segments were inoculated with three (5 µl) droplets of spore suspension (5 x 10^4 spore/ml) as described above, then were incubated in closed boxes at 21 ± 1°C. The lids were removed 16 h. after the inoculation. Segments were taken 24 h. after inoculation, and fixed on a glass slide, a drop of 0.1% lactophenol-cotton blue was added. Ten microscopic fields of view (40 x 15X) were examined for estimating the percentages of appressorial formation and the percentages penetrations (the penetrated spores).

4.1.3 Assessment of dead and browning cells of leaf segments

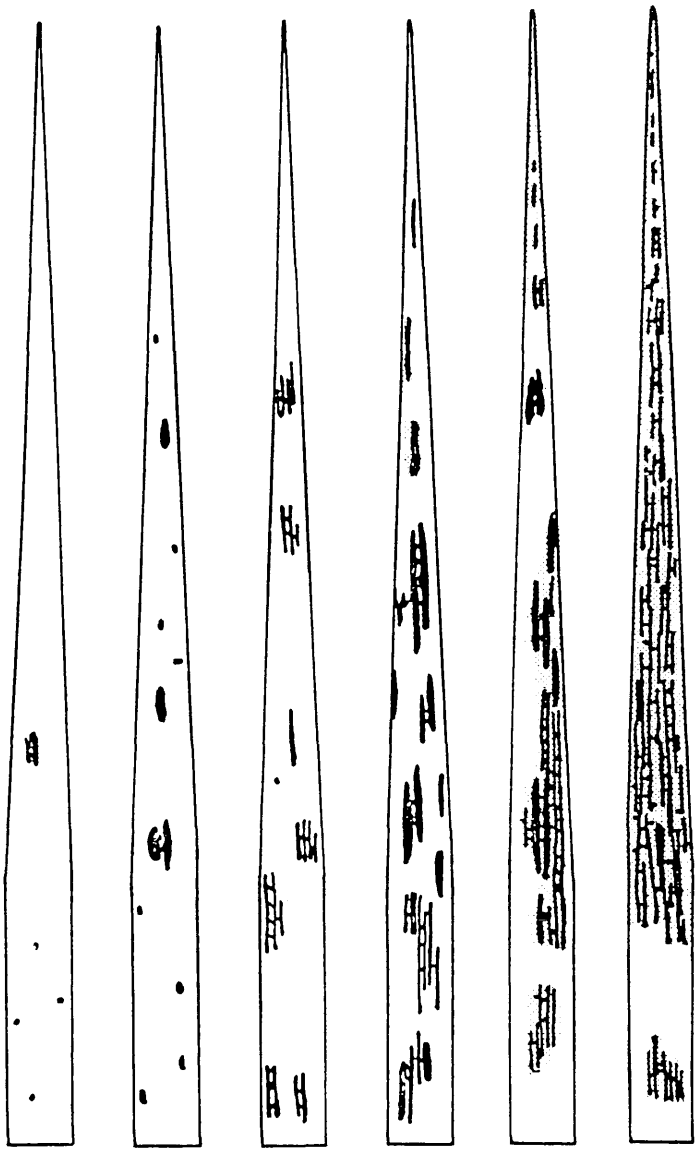
Segments were taken 120 h. after inoculation with 5 µl. droplets of spore suspension (5×10^4 spore/ml), then were cut into 2 cm. long sections and cleared, then stained by a method based on that of Shipton and Brown (1962). Alcoholic lactophenol-cotton blue was used for staining. The leaf sections were boiled in the stain in a test tube for 90 sec. then left for 48 h., then removed, washed twice in water and transferred to chloral hydrate solution (5g/2 ml.) and left for 2-3 days. Sections were then removed, washed in distilled water and mounted in 50% glycerine on a glass slide and examined microscopically. Number of dead, browning and unaffected cells per microscopic field of view (40 x 15X) were assessed. The mean of the percentages in ten fields of view of dead and browning cells was determined. As regards the lesions produced on leaf segments of rye grass (Lolium perenne), oat (Avena sativa cv. Peniarth) and rye (Secale cereale cv. Rheidol), the cells were not obviously dead or brown, but were dark green in colour (darker than normal). The mean of percentages in 10 fields of view of the dark green cells were determined.

4.2 Assessment of infection on whole plant leaves

4.2.1 Assessment of net form lesions

Seedlings with three fully expanded leaves of plants with net form symptoms after spraying with a spore suspension, were taken from the field and from the glasshouse and assessed in the laboratory by the MAFF (1984) method

Figure 1. Disease assessment key of barley net blotch, showing the percentage of leaf area affected (MAFF, 1984).


1
5
10
25
50
75

as shown in Figure 1.

4.2.2 Determination of the correction factor for lesions (Table 2).

1. Twenty five lesions were taken randomly from the group of lesions produced by 18 isolates of P. teres after inoculating with mycelial disks, first leaf segments of barley cv. Sonja. For leaf segments of host and non-host plants inoculated with spore suspension droplets of Pt.80-12, ten lesions were taken randomly. Inoculation methods and lesions development were described in (3.1, 3.2).
2. The greatest lengths (L_1, L_2) of lesion parallel and at right angles to the leaf veins were measured. The mean of two lengths was determined, then the diametrical area (AD) was calculated from this mean. Mean of ten diametrical areas was determined.
3. The outline of each lesion was drawn on tracing paper, then copied on to graph paper from which areas were measured (AM). Mean of ten areas by measurement was determined.
4. The mean of ten areas by measurement (AM) was divided over the mean of ten diametrical area (AD), to give the correction factor to be applied to (AD), (Table 2).
5. Diametrical area (AD) was then multiplied by the correction factor to give the area of lesion similar to that obtained by measurement (AM).
6. This method was used for estimating the areas of lesions from their lengths and widths.

Table 2: Calculation of the correction factor for lesions developed on host and non-host plants inoculated by mycelial disks, or suspension of spores of different isolates of P. teres.

Host and non-host plants	Inoculation method	Number of lesions measured	Length (L_1) (mm) Mean ± SD	Length (L_2) (mm) Mean ± SD	Diametrical area (AD) (mm^2) Mean ± SD	Area by measure-ment Mean ±SD	Correction factor (C.F.)
Barley cv. Sonja first leaf segments	Mycelial disks	25 *	9.3±2	3.7±1	35±13	27.6±12	0.79
Barley cv. Sonja first leaf segments	Droplets of suspension of spores	10 *	11.4±1.5	6.6±1	64±14.8	48.6±13.5	0.76
Wild barley Hordeum murinum first leaf segments	Droplets of suspension of spores	10 *	4.9±0.6	2.5±0.34	10.45±2.1	8.6±1.84	0.82
Wheat. cv. Maris Dove first leaf segments	Droplets of suspension of spores	10 *	3.8±0.46	2.1±0.28	6.9±1.34	5.6±0.9	0.81
Common rye grass Lolium perenne first leaf segments	Droplets of suspension of spores	10 **	2.6±0.73	1.4±0.3	3.3±1.6	2.9±1.3	0.88

* Spot form necrotic lesions
**dark green lesions

4.2.3 Assessment of spot form lesions

Areas were calculated from linear measurements, and the correction factor was applied to give estimated total areas of lesions. This was expressed as a percentage of leaf area which was measured by drawing the outline of the leaf on a tracing paper and then on to copying graph paper.

5. Assessment of sporulation

5.1 Assessment of sporulation on lesions on leaf segments

Leaf segments 5 cm long from the first leaf of seedlings were inoculated with 20 µl droplets of spore suspensions as in 4.2. Segments with lesions were placed aseptically on sterilized moist filter papers in sterile Petri dishes and were kept at 21 ± 1°C under a 12h near ultraviolet and 12h dark cycle for 10 days. Spores were removed from the lesions with an artist's brush into 25 ml sterile water. A few droplets of Tween 20 were added to the suspension to disperse the spores. Numbers in 5 µl droplets of the suspension were counted microscopically on a glass slide.

5.2 Assessment of sporulation *Pyrenophora teres* isolates cultured on different media and exposed to different sources of light

Isolates of *P. teres* in the mycelial forms were cultured on: sterilized filter paper, autoclaved barley leaf segments, sterilized moist filter paper, and on PCA

(2.4.1-4). The cultures were then incubated at 21 ± 1°C for five days in continuous darkness, then transferred and exposed for 10 days at 21 ± 1°C to, 24h of continuous darkness, 12h of cool light followed by 12h darkness, or 12h of near UV light followed by 12h darkness. Spores from 3 replicates (3 Petri dish cultures) were suspended in 25 ml sterile distilled water and a few droplets of Tween 20 were added. Droplets of 5µℓ of suspension of spores were taken in an Agla micrometer syringe and placed on a glass slide and then examined microscopically. For each isolate, numbers of spores were counted from five separate 5µℓ droplets (replicates) taken from the cultures incubated at 24h continuous darkness, 12h cool light followed by 12h darkness, 12h near UV light followed by 12h darkness. The mean of the number of spores per 5µℓ was determined, then multiplied by two hundred to obtain the number of spores per ml. The isolates were then classified into four groups according to the number of spores per ml, namely, no sporulation (-); sparse (+), 500-1000 spores; moderate (++), 1000-5,000 spores; and dense sporulation (+++), 5000-10,000.

EXPERIMENTAL

SECTION 1

CHARACTERS OF 32 ISOLATES OF P. TERES COLLECTED FROM ENGLAND AND WALES

Helminthosporium teres Sacc. shows morphological variability in culture expressed as amount of mycelial growth, zonation, colour, amount of sporulation, sclerotial and non-sclerotial and in optimum temperature for growth (Pon, 1949; Sheodhan, 1962). The maintenance and successive transfer of P. teres cultures on media such as PDA, Czapek agar or malt extract agar, resulted in cultures which lacked conidia and had lost their pathogenicity (Piening, 1963). McDonald (1967) considered that the differences in cultural appearance in wild-type isolates of P. teres from infected barley were within the range normally expected in a heterothallic species of a fungus. Clifford and Jones (1980) examined seventy two isolates of P. teres which were found to vary considerably in their cultural characteristics and aggressiveness. Some isolates failed to sporulate on agar, others failed to produce symptoms or produced only limited symptoms on the differential cultivars. They found that the cultures which were isolated directly from barley leaf infected samples in 1980 sporulated better and were more aggressive than cultures obtained from the field in 1979 and subsequently maintained on PDA. The following reports results of similar experiments.

Experiment 1: Mycelial growth of 32 isolates of P. teres.

Of the thirty two isolates of P. teres, used in this

study, twenty were obtained as mycelial cultures kept under oil from Long Ashton Research Station, Bristol (2.1.B); ten were received as diseased barley leaf fragments from The Welsh Plant Breeding Station, Aberystwyth (2.1.C), and two were isolated from naturally infected leaves of barley and wild barley (Hordeum murinum) at Silwood Park, Ascot, (2.1.A). The isolates were subcultured on PCA, and from a 7 day old colony, mycelial disks, 7 mm diam. were taken and cultured on PCA in Petri dishes, and incubated in continuous darkness at 21 ± 1°C. Nine days after inoculation, the diameters of colonies were measured. Results are shown in Table 3.

Experiment 2: Colour of cultures of 32 isolates of P. teres

Mycelial disks 7 mm in diam. from 7 day old cultures of thirty two isolates of P. teres were placed on PCA in Petri dishes and incubated in continuous darkness at 21 ± 1°C. The colours and zonation were recorded 9 days after inoculation (Table 4).

Experiment 3: Sporulation of 32 isolates of P. teres

The mycelial pellets or disks 5 mm in diam. of thirty two isolates of P. teres obtained from Bristol, Aberystwyth and Silwood Park at Ascot, (see 2.1.A,B,C), were distributed on sterile dry filter paper (see 2.4.1) cultured on autoclaved barley (cv. Sonja) leaf segments (see 2.4.2) distributed on sterile moist filter paper (see 2.4.3), or cultured on PCA (see 2.4.4) in Petri dishes, then were

Table 3: Mycelial growth of isolates of *Pyrenophora teres* after 9 days incubation at 21 ± 1°C on PCA

Isolates		Diameter of colony (mm) *	Mean
Origin	Code number		
Bristol	PC 215	64 ± 1.7	54.4
	PC 724	62 ± 1.7	
	PP 50C	55 ± 2	
	PP 36	28 ± 2.6	
	PP 50G	63 ± 1.7	
	PC 673	52 ± 1	
	LARS	64 ± 1.7	
	PC 719	56 ± 1	
	PP 27	31 ± 1	
	PP 32	65 ± 1.7	
	PP 23	44 ± 3	
	PP 33	74 ± 2	
	PP 44	55 ± 0.6	
	PP 50e	63 ± 1.7	
	PC 216	60 ± 2.6	
	PP 29	43 ± 2	
	PC 1067	66 ± 2.6	
	PP 40-41	48 ± 3	
	PP 46	64 ± 0.6	
	PP 26	30 ± 2	
Aberystwyth	NB-82-5	68 ± 1.2	74.9
	NB-82-19	70 ± 1	
	NB-82-25	84 ± 0.6	
	NB-82-58	78 ± 2.6	
	NB-82-59	75 ± 1	
	NB-82-63	81 ± 1	
	NB-82-70	80 ± 1.7	
	NB-82-88	67 ± 1	
	NB-82-103	74 ± 1.7	
	NB-82-209	72 ± 2	
Silwood Park	H.t. SP	69 ± 1.2	62.5
	H.t. WB	56 ± 1	

* Mean ± SD of three replicates

Table 4: Colours of cultures of isolates of Pyrenophora teres.

Colour	Zonation	Cultures
Dark green	+	PC 215, PC 724, PC 216, PC 1067, PP 46, (B-5)
Dark green	-	PP 50C, LARS, NB-82-19, NB-82-25, NB-82-58, NB-82-59, NB-82-63, NB-82-103, NB-82-209, H.t.sp, H.t.WB (B-2, A-7, SP-2)
Light green	+	NB-82-5, NB-82-70, NB-82-88, (A-3)
Light green	-	PP 36, PP 50G, PC 673, PC 719, PP 27, PP 23, PP 33, PP 44, PP 50e, PP 29, PP 40-41, PP 26 (B-12)
Light brown	-	PP 27 (B-1)
B - Bristol, A - Aberystwyth, SP - Silwood Park.		

exposed differently to light at 21 ± 1°C (see 5.2). Estimation of sporulation after 10 days was as described in 5.2. The results are shown in Table 5.

Discussion

The result in Table 3 shows that the rates of mycelial growth of Bristol isolates (see 2.1.B), were less than of Aberystwyth (see 2.1.C) and Silwood Park isolates (see 2.1.A), probably because the isolates had been kept under oil for two years (letter from Dr. John Hargreaves, 3rd February, 1983), whereas the Aberystwyth and Silwood Park isolates had been obtained recently from infected barley leaf samples. Also the long period of Bristol isolates

Table 5: Sporulation of 32 isolates of P. teres cultured on 4 media (A,B,C and D) after 10 days at 21 ± 1°C under different light conditions.

	P. teres isolates	Continuous cool light				Continuous darkness				12h cool light / 12h darkness				12h near UV light / 12h darkness				Continuous near UV light			
	Medium	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	PC 215	-	+	-	-	-	+	-	+	-	+	+	++	++	+++	++	+++	+	++	+	+
	PC 724	-	-	-	-	-	+	-	+	-	+	+	+	+	++	+	+++	+	+	+	+
	PP 50c	-	-	-	-	-	+	-	-	-	+	+	+	+	+++	+	+++	+	+	+	+
	PP 36	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+
	PP 50G	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+
	PC673	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LARS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PC 719	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PP 27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PP 32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PP 23	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	+	-	+
	PP 33	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	+	-	+
	PP 44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PP 50e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PC 216	-	-	-	-	-	-	-	-	-	+	-	+	+	++	-	++	+	+	-	+
	PP 29	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+
	PC 1067	-	-	-	-	-	-	-	-	-	+	-	+	+	++	-	++	-	+	-	+
	PP 40-41	-	-	-	-	-	-	-	-	-	+	-	-	-	++	-	++	-	+	-	+
	PP 46	-	-	-	-	-	-	-	-	-	+	-	+	-	++	-	+	-	+	-	+
	PP 26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	NB-82-5	-	+	-	-	-	+	-	+	+	++	+	+	++	+++	+	+++	+	++	+	++
	NB-82-19	-	-	-	-	-	-	-	-	+	+	+	+	+	++	+	++	+	+	+	+
	NB-82-25	-	-	-	-	-	-	-	-	+	++	+	+	+	+++	+	+++	+	++	-	++
	NB-82-58	-	+	-	-	-	-	-	-	++	++	+	+	++	+++	+	+++	+	++	+	++
	NB-82-59	-	-	-	-	-	-	-	-	+	++	+	++	+	+++	+	+++	+	++	+	++
	NB-82-63	-	-	-	-	-	-	-	-	+	+	-	+	+	+++	+	++	+	+	+	+
	NB-82-70	-	+	-	-	-	+	-	-	-	+	-	+	+	+++	+	+++	+	++	+	++
	NB-82-88	-	-	-	-	-	-	-	-	+	+	+	+	+	++	+	++	+	+	+	+
	NB-82-103	-	-	-	-	-	-	-	-	-	+	-	+	+	++	+	++	+	+	+	+
	NB-82-209	-	-	-	-	-	-	-	-	-	+	-	-	+	++	+	++	+	+	+	+
3	H.t.sp	-	+	-	-	-	+	-	-	-	++	+	+	++	+++	+	+++	+	++	+	++
	H.t.WB	-	+	-	-	-	+	-	-	+	++	-	+	+	+++	+	+++	+	++	+	++

* A - mycelial pellets distributed on sterilized dry filter paper
* B - mycelial disks cultured on autoclaved barley (cv. Sonja) leaf segments.
* C - mycelial disks distributed on sterilized moist filter paper.
* D - mycelial disks cultured on PCA.

1. Bristol isolates
2. Aberystwyth isolates
3. Silwood Park isolates

Scale of sporulation

- No sporulation
+ sparse sporulation (500-1000 spore per ml)
++ moderate sporulation (1000-5000 spore //)
+++ dense sporulation (5000-10,000 spore //)

under oil, probably decreased the ability of some isolates to sporulate and caused others to lose the ability to sporulate. Table 5 shows that continuous cool light or, continuous darkness, had little effect on induction to sporulate; only a few isolates sporulated sparsely. Twelve hours cool light followed by 12h of darkness had more effect on sporulation of Aberystwyth isolates but had less or no effect on Bristol isolates. Twelve hours of near UV light followed by 12h of darkness induced good sporulation of many isolates. Only a few Bristol isolates did not sporulate under these conditions. The continuous near UV light had little effect on twelve Bristol isolates but had more effect on all Aberystwyth and Silwood Park isolates. In summary, the 12h of near UV light followed by 12h of darkness, increased greatly sporulation by many isolates and sporulation was best on autoclaved barley leaf segments, followed closely by sporulation on PCA.

Table 4 shows the colours with or without zonation of 32 isolates of P. teres cultured on PCA. Most of the Bristol isolates (2.1.B) had produced light green cultures whereas most Aberystwyth isolates (2.1.C) and Silwood Park isolates (2.1.A) had produced dark green cultures. In general it is concluded that, the isolates obtained recently from host plants at Aberystwyth and Silwood Park, were darker green in colour than were those kept for long periods under oil as had been the Bristol isolates. Also it was observed during this research that continuous sub-culturing

of the dark green fresh isolates on PCA for a long period without passing through the normal host plant, caused the cultures to fade in colour.

SECTION 2

PATHOGENICITY OF 18 ISOLATES OF P. TERES; MYCELIAL DISK INOCULA ON A HIGHLY SUSCEPTIBLE CULTIVAR OF BARLEY (SONJA) LEAF SEGMENTS.

P. teres can persist from one growing season to the next as seed-borne mycelium which probably serves to introduce the pathogen into fields previously free of net blotch (Mathre, 1982). The inoculation of barley leaf segments by mycelial inocula was used by Rapilly and Ponchet in 1962. Piening (1963), used mycelial disks of P. teres as inocula; he found that, the mycelial inocula induced a fleck-type lesion on Olli and Gateway barley. This method was used for testing the pathogenicity of the isolates which either sporulated poorly or not at all. This method gave rapid tests of the pathogenicity of the isolates 4 to 7 days after inoculation.

Experiment: Pathogenicity of 18 isolates of P. teres.

The pathogenicity of 18 isolates of P. teres was tested by inoculating leaf segments of a susceptible cultivar of barley (Sonja) with mycelial disks. Five segments were left uninoculated as a control. The technique for inoculation and incubation is described in 3.1.

The assessment of the size of the lesions and the percentage of the necrotic area per segment were determined by the method described in 4.1.3. The mean of nine replicates

of necrotic lesions for each isolate was estimated 120h after inoculation (Table 6). The mean of the percentages of segments with lesions for the 18 isolates were also determined, (Table 7, Figure 2).

Results

Dark brown necrotic lesions, surrounded by a chlorotic zone developed in all the inoculated segments, 120h after inoculation. Generally, the lesions were oval in shape (Plate 1).

According to the size of the necrotic lesions, the isolates were classified into four groups (see Table 6).

Table 6: Estimation of lesion size on leaf segments of barley cv. Sonja, inoculated with mycelial disks of 18 isolates of P. teres

Groups	Number of lesions	Size of lesions (mm^2)	Isolates
1	9	32 - 34.7	BNS-82-5, BNS-82-70 BNS-82-63. (A-3)
2	9	21.6-29.7	BNS-82-58, BNS-82-59, BNS-82-103, BNS-82-209 BNS-82-19, BNS-82-25, PP 33 (B-1, A-6)
3	9	17.0-19.9	PC 724, BNS-82-88, PP 46, PC 1067, PC 215 (B-4, A-1)
4	9	9.6-12.3	PP 50e, PP 23, PP 50c (B-3)

B - Bristol, A - Aberystwyth

Table 7: Estimation of the percentage of the necrotic lesions on barley cv. Sonja leaf segments inoculated by mycelial disks of 18 isolates of *P. teres*

Isolates	Segments	Diametrical area of lesions (mm^2) Mean ± SD	Estimated area of lesions (mm^2) Mean ± SD	Total area of lesions (mm^2)	% of segment with lesions	Mean % of segment with lesions Mean ± SD
PC 215	1	21.9±5.3	17.2±4.2	51.7	21.1	20.8±3.8
	2	25.4±5.5	20.1±4.4	60.1	24.5	
	3	17.4±5.7	14.7±4.5	41.1	16.8	
PC 724	1	29.1±3.7	22.9±2.9	68.9	28.1	24.3±3.9
	2	21 ±2.4	16.6±1.8	49.6	20.2	
	3	25 ±6.9	20.1±5.4	60.3	24.6	
PC 50c	1	10.8±4.6	8 ±3.5	25.7	10.5	11.7±1.7
	2	14.2±6.5	11.2±5	33.6	13.7	
	3	11.2±3.7	8.9±2.9	26.7	10.9	
PP 23	1	10 ±4.7	7.9±3.6	23.7	9.7	12.5±2.6
	2	15.4±2.6	12.1±3	36.4	14.9	
	3	13.2±3.2	10.4±2.6	31.3	12.8	
PP 33	1	26.9±4.6	21.2±3.7	63.5	25.9	26.5±2.3
	2	30 ±5.1	23.7±4	71	29	
	3	25.4±4.8	20 ±3.7	60	24.5	
PP 50e	1	9.7±2.8	7.7±2.1	23.1	9.4	15.1±4.9
	2	19 ±2.9	15 ±2.3	45	18.4	
	3	18 ±5.6	14.2±4.4	42.6	17.4	
PC 1067	1	23.1±3.2	18.2±2.5	54.7	22.3	21.7±1.7
	2	20.5±5	16.2±4	48.5	19.8	
	3	23.9±4.3	18.8±3.4	56.5	23.1	
PP 46	1	25.6±6.9	20.2±5.4	60.5	24.7	22.8±2.4
	2	22 ±7.6	17.4± 6	52.2	21.3	
	3	23.1±3.2	18.2±2.5	54.7	22.3	

*Area of each segment 245 mm^2.
*Correction factor for area of lesion 0.79.

Table continued

Table 7 (continued)

Isolates	Segments	Diametrical area of lesions (mm^2) Mean ± SD	Estimated area of lesions (mm^2) Mean ± SD	Total area of lesions (mm^2)	% of segment with lesions	Mean % of segment with lesions Mean ± SD
BNS-82-25	1	26.7±1.3	21.1±1	63.3	25.8	28.8±2.6
	2	31.5±3.8	24.9±2.9	74.7	30.5	
	3	31.1±8.5	24.5±6.8	73.5	30	
BNS-82-19	1	37.8±2.8	29.8±5.3	89.3	36.4	33.2±3.4
	2	30.7±2.4	24.2±2	72.7	29.7	
	3	34.8±4.1	27.5±2.7	82.4	33.6	
BNS-82-5	1	44.2±3	34.8±2.4	104.5	42.6	42.4±0.5
	2	43.4±4.3	34.2±5.7	102.6	41.9	
	3	44.4±3.3	35 ±2.6	105	42.8	
BNS-82-58	1	36.7±1.5	28.9±1.2	86.7	35.4	36.4±3
	2	35.1±4.7	27.6±5.1	83	33.9	
	3	41.3±2.8	32.5±2.2	97.6	39.8	
BNS-82-59	1	37 ±4.6	29.2±2.6	87.7	35.8	33.9±2.3
	2	32.4±3.8	25.5±3	76.6	31.3	
	3	35.8±3.6	28.1±2.1	84.5	34.5	
BNS-82-63	1	41.4±5	32.6±4	97.8	39.9	39.1±0.9
	2	40.8±2.7	32.1±3.6	96.4	39.3	
	3	39.5±5.7	31.1±4.5	93.4	38.1	
BNS-82-70	1	46.6±2.4	32 ±2.3	96.1	39.2	40.6±1.3
	2	42.3±4.4	33.3±3.5	100	40.8	
	3	43.3±3.4	34 ±2.7	102.2	41.7	
BNS-82-88	1	27 ±2.9	21.2±4.6	63.7	26	23.3±2.5
	2	21.8±4.1	17.2±4.2	51.5	21	
	3	23.8±2.2	18.7±1.7	56.2	22.9	

Table continued

Table 7: (continued)

Isolates	Segments	Diametrical area of lesions (mm^2) Mean ± SD	Estimated area of lesions (mm^2) Mean ± SD	Total area of lesions (mm^2)	% of segment with lesions	Mean % of segment with lesions Mean ± SD
BNS-82-103	1	38 ±3.5	29.8±3.2	89.6	36.6	33.7±6.2
	2	39.5±4.3	31.1±3.4	93.3	38	
	3	27.7±4.9	21.8±3.9	65.3	26.6	
BNS-82-209	1	35.9±5.3	28.3±4.2	84.9	34.6	35.8±1.3
	2	36.9±6.8	29 ±5.3	87.2	35.6	
	3	38.5±2.7	30.3±2.2	91	37.1	

Figure 2. Mean % of segments with lesions, 120h after inoculation with mycelial disks of 18 isolates of *P. teres*, on first leaf segments of barley cv. Sonja.

Number	Isolate	
9	BNS 82-5	- A
15	BNS-82-70	- A
14	BNS-82-63	- A
12	BNS 82-58	- A
18	BNS-82-209	- A
13	BNS-82-59	- A
17	BNS-82-103	- A
10	BNS-82-19	- A
11	BNS-82-25	- A
5	PP 33	- B
2	PC 724	- B
16	BNS-82-88	- A
8	PP 46	- B
7	PC 1067	- B
1	PC 215	- B
6	PP 50e	- B
4	PP 23	- B
3	PC 50c	- B

B - Bristol, A - Aberystwyth

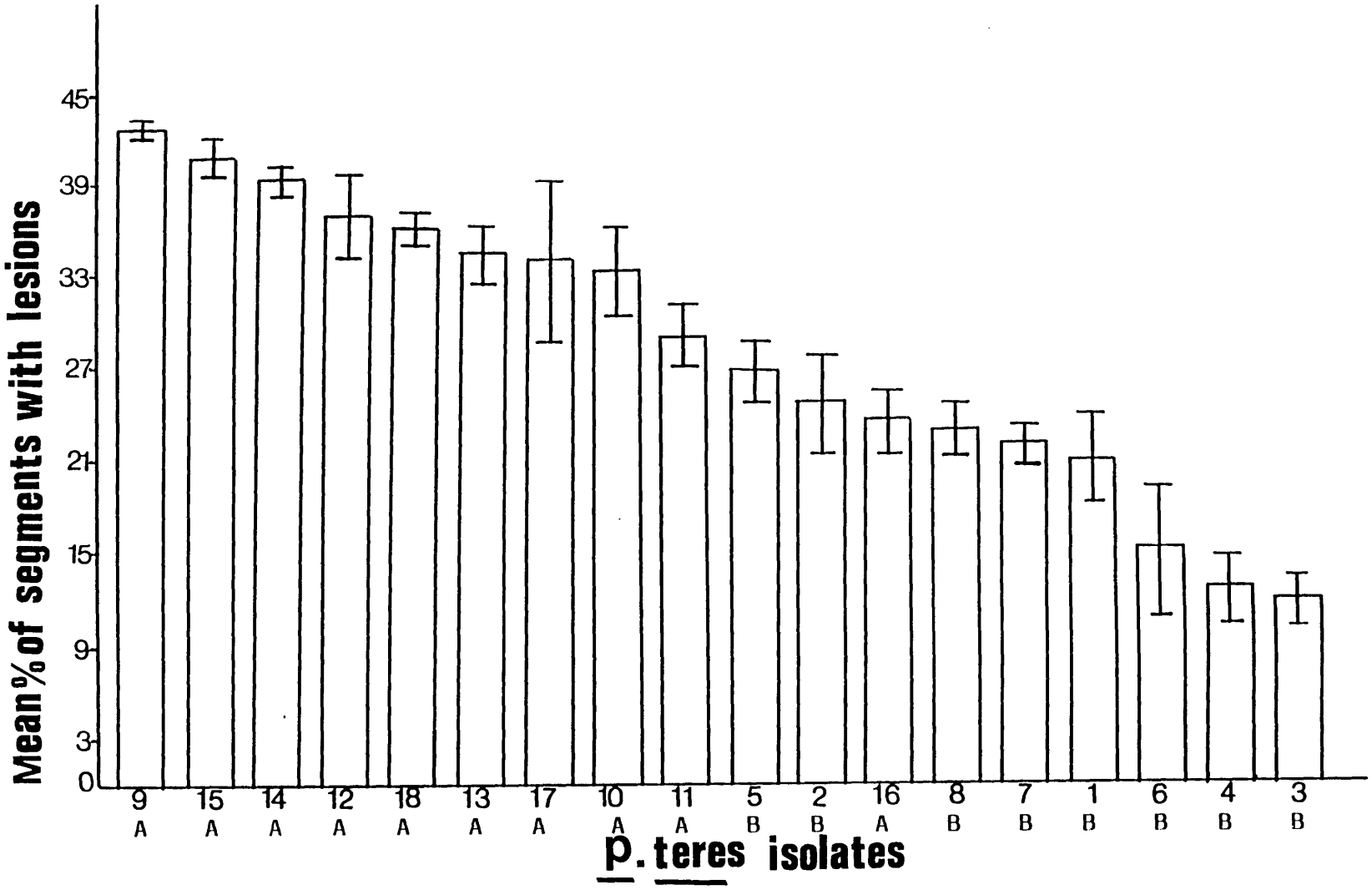
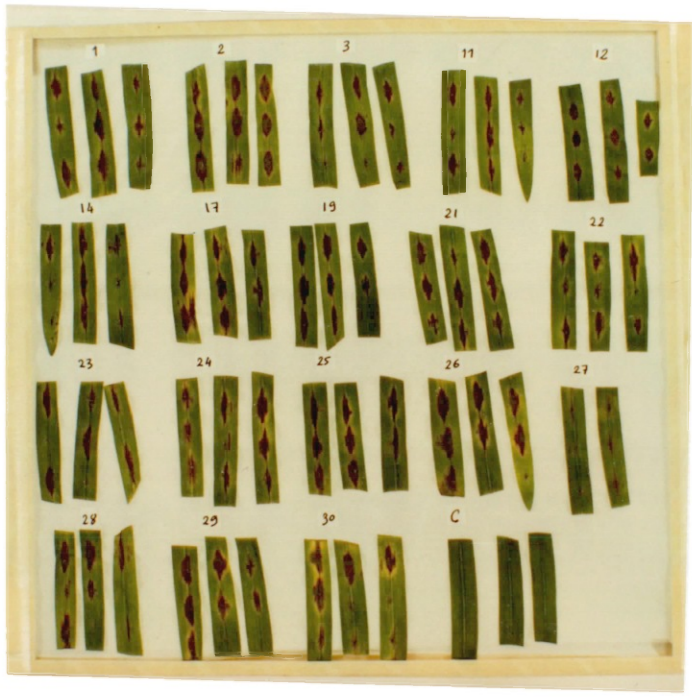

Mean% of segments with lesions
45
39
33
27
21
15
9
3
0
9 A
15 A
14 A
12 A
18 A
13 A
17 A
10 A
11 A
5 B
2 B
16 A
8 B
7 B
1 B
6 B
4 B
3 B
P. teres isolates

Plate 1. Development of necrotic lesions on barley (cv. Sonja) segments, infected with mycelial disks of 18 isolates of *Pyrenophora teres*


1
2
3
11
12
14
17
19
21
22
23
24
25
26
27
28
29
30
C

Discussion

Each of the 18 isolates of *P. teres* was able to infect barley (cv. Sonja) leaf segments, inducing necrotic, oval lesions. Almost always the lesions induced by the Aberystwyth isolates were bigger than the lesions induced by the Bristol isolates, possibly because of the two years of preservation continuously under oil with partial loss of virulence, whereas the Aberystwyth isolates were used much sooner after isolation from diseased barley leaf fragments. Nevertheless, it is surprising that the differences in virulence were so consistent.

SECTION 3

REACTION OF 15 CULTIVARS OF BARLEY TO SELECTED ISOLATES OF *PYRENOPHORA TERES*

Caddel and Wilcoxson (1975) examined the reaction of 2608 cultivars of barley (World Barley Collection), to *P. teres*. They found that most cultivars reported to be resistant to net blotch in other parts of the world were susceptible in Morocco.

The evaluation of 140 cultivars of barley obtained from the Middle-East (Iraq, Syria, Lebanon, and Turkey), for net blotch reaction with Canadian isolates indicates that Canada could be a good source of resistance to *P. teres* (Metcalfe *et al.*, 1977). Al-Ali *et al.* (1979) examined the reaction of 20 cultivars of barley obtained from France, Iraq, Syria and Jordan, to a French isolate of *P. teres* (RS2), and found that two cultivars from Syria and one from Iraq were moderately resistant whereas they had been reported to be susceptible in the countries of origin. Clifford *et al.* (1984) examined the response of 17 cultivars of spring barley and 16 cultivars of winter barley to two isolates of *P. teres*, BNS-82-5, a virulent isolate and BNS-82-38, a less virulent isolate. The cultivars showed a similar order of response to the two isolates but there were exceptions notably with cultivar Athene which was highly susceptible to isolate BNS-82-5 but was much less susceptible to isolate BNS-82-38. Winter cultivar

Sonja and spring cultivars, Tasman and Carnival were highly susceptible to both isolates. A wide range of susceptibility was observed in both the winter and spring cultivars.

Experiment: Reaction of 15 cultivars of barley to P. teres isolate PC 215

Five winter and ten spring cultivars of barley, were grown in pots in a glasshouse (see 1.3). The first leaves of the ten replicates of the three leaf stage seedlings (21 day old) of each cultivar, were inoculated by the spray method with a suspension of spores (5×10^4 spore/ml) of P. teres, isolate PC 215 (see 3.3). The percentage of leaf damage was estimated 10 days after inoculation by the method described in 4.2.3.

Results

Spot form lesions surrounded by chlorotic haloes developed in all winter and spring cultivars, 96h after inoculation. Generally, the spot lesions on winter cultivars were bigger than those in spring cultivars, so that the total of necrotic areas in the first leaves of the winter cultivars was greater than in spring cultivars (Table 8).

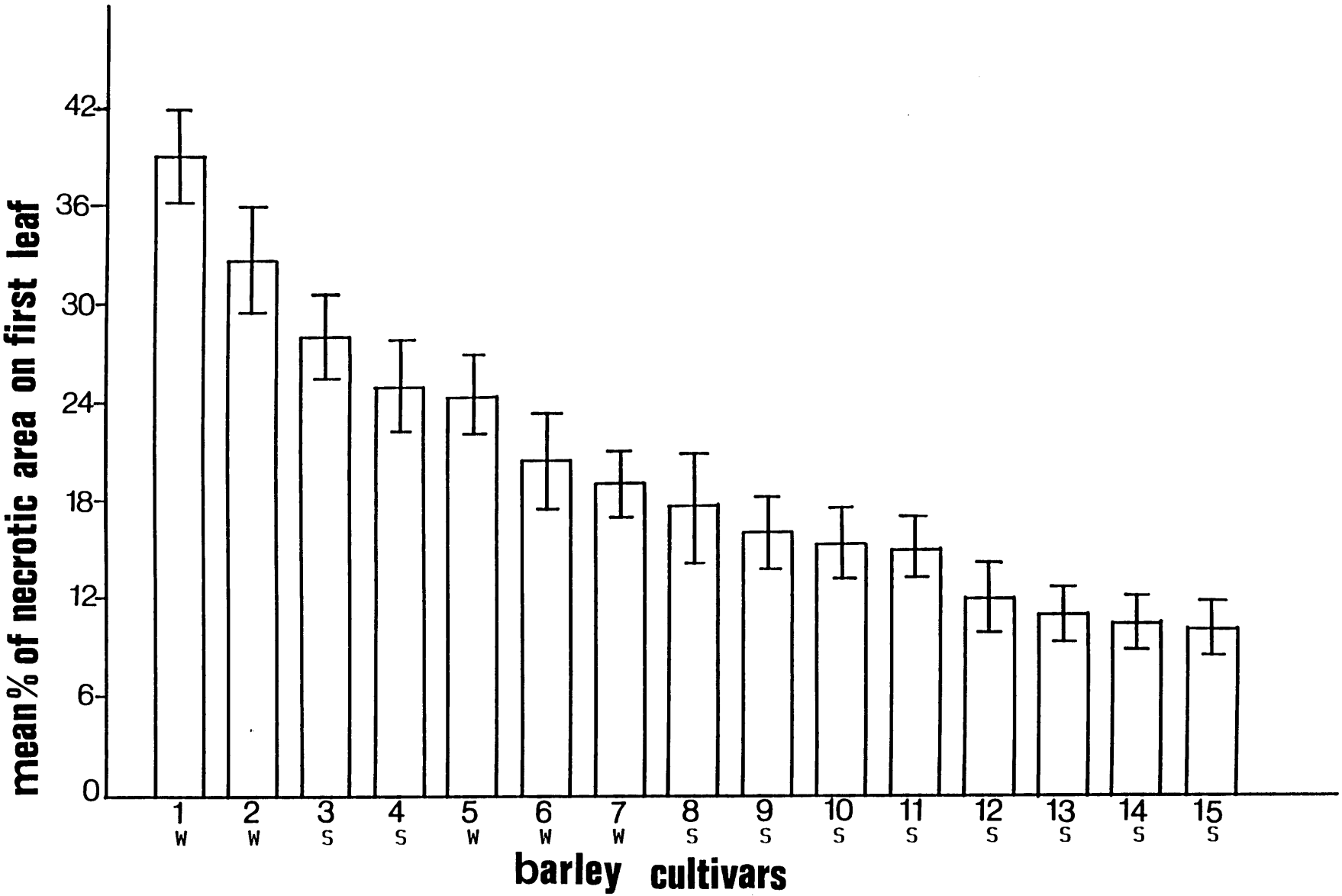
Spring cultivars were less susceptible than winter cultivars except for two cultivars, Carnival and Tasman which were similar to the winter cultivars in their reactions. The spring cultivars, Goldmarker, Koru and Atem were much more resistant than the other spring

Figure 3: Reaction of 15 cultivars of barley to isolate PC 215 of *P. teres*

1.	Sonja	(W)
2.	Athene	(W)
3.	Carnival	(S)
4.	Tasman	(S)
5.	Igri	(W)
6.	Maris Otter	(W)
7.	Tipper	(W)
8.	Triumph	(S)
9.	Midas	(S)
10.	Egmont	(S)
11.	Kym	(S)
12.	Golf	(S)
13.	Goldmarker	(S)
14.	Koru	(S)
15.	Atem	(S)

W - Winter cultivar

S - Spring cultivar


mean% of necrotic area on first leaf
42
36
30
24
18
12
6
0
1 W
2 W
3 S
4 S
5 W
6 W
7 W
8 S
9 S
10 S
11 S
12 S
13 S
14 S
15 S
barley cultivars

cultivars. The winter cultivars, Sonja, Athene and the spring cultivars Carnival and Tasman were the most susceptible. Igri, Maris Otter and Tipper, were the most resistant among the other winter cultivars (Table 8; Figure 3).

Table 8: Reaction of cultivars of barley to isolate PC 215 of Pyrenophora teres

Barley cultivars		% of necrotic area per leaf * (Mean ± SD)
Sonja	(W)*	39.0 ± 2.6
Athene	(W)	32.5 ± 3.4
Carnival	(S)*	28.0 ± 2.3
Tasman	(S)	25.0 ± 2.6
Igri	(W)	24.4 ± 2.4
Maris Otter	(W)	20.5 ± 2.9
Tipper	(W)	19.0 ± 1.7
Triumph	(S)	17.5 ± 3.2
Midas	(S)	16.0 ± 2
Egmont	(S)	15.2 ± 2.2
Kym	(S)	15.0 ± 1.8
Golf	(S)	12.0 ± 2
Goldmarker	(S)	11.0 ± 1.5
Koru	(S)	10.5 ± 1.5
Atem	(S)	10.3 ± 1.6

* first leaf
(W) Winter cultivar
(S) Spring cultivar

Discussion

The spot form lesions which developed in the first leaves of winter and spring cultivars inoculated by PC 215, indicate that PC 215 is a low virulent isolate compared with the P.t.80-12 isolate recorded in the next experiments and which induced net form symptoms on susceptible cultivars such as Sonja and Athene.

SECTION 4

VIRULENCE OF P. TERES ISOLATES ON BARLEY

Isolates of P. teres differ sharply in their pathogenicity on barley varieties (Pon, 1949; Singh, 1962; Gray, 1966). Certain isolates fail to induce the typical net blotch effect, (McDonald, 1967; Smedegard-Petersen, 1971). Clifford and Jones (1980), examined the virulence of 72 isolates of P. teres; 40 isolates were obtained directly from infected leaves in 1980, and 32 isolates were received as plate cultures from an A.D.A.S. Field Survey in 1979. The 72 isolates varied considerably in their aggressiveness. Some isolates failed to produce symptoms and others produced only limited symptoms on the differential cultivars. The isolates obtained directly from 1980 infected leaves were more aggressive than were the cultures from A.D.A.S. obtained from the field in 1979 and subsequently maintained on PDA. The experiment recorded below investigated the virulence of a range of isolates on barley cv. Sonja.

Experiment: Virulence of 10 Aberystwyth isolates of P. teres on barley cv. Sonja

Segments 5 cm long from first leaf stage seedlings of ten day old barley cv. Sonja, were inoculated with 10 µl of suspension of spores (5×10^4 spore/ml), taken from ten day old cultures of 10 isolates of P. teres. The leaf segments were placed floating on 4 ml of a 50 ppm benzimidazole solution in small plastic boxes and incubated

at 21 ± 1°C (see 3.2, Plate 2). Each segment was inoculated with three separated droplets and 5 segments were used as replicates for each isolate. Five segments treated with 10 µℓ droplets of sterile distilled water were used as a control. Size of lesions was measured six days after inoculation by copying the outline of the lesions over a tracing paper and then measuring areas with the use of graph paper.

Results

The ten isolates of P. teres showed differences in their virulence. Isolates BNS-82-5 and BNS-82-70, showed a high level of virulence whereas the isolates BNS-82-63, BNS-82-58, BNS-82-209, BNS-82-59, BNS-82-103, BNS-82-19 and BNS-82-25, were moderately virulent.

Isolate BNS-82-88 showed a low level of virulence (Table 9). Generally, the lesions were oval in shape (Figure 4). The inner areas were dark brown in colour and were surrounded by a narrow chlorotic zone.

Plate 2. Agla micrometer syringe used for inoculating leaf segments with droplets of spore suspensions.

A. Agla micrometer syringe

B. Barley leaf segments and plastic boxes.

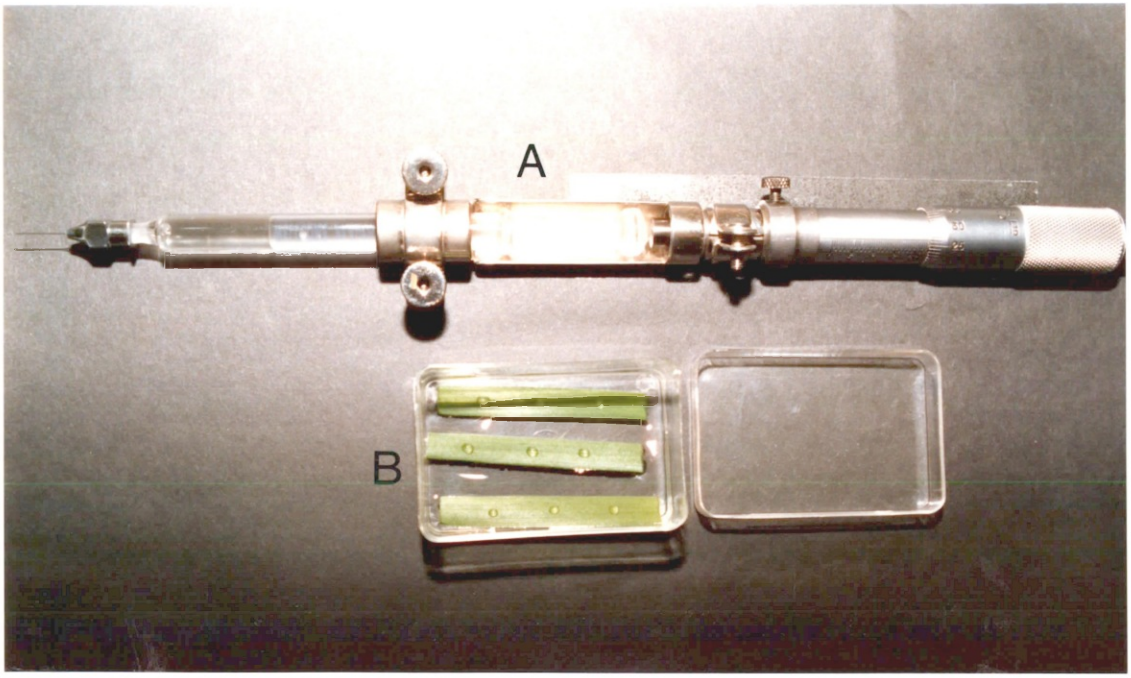

A
B

Figure 4: Tracings of lesions caused by 10 isolates of *Pyrenophora teres*, on leaf segments of barley cv. Sonja, 6 days after inoculation with 10 µl droplets of suspension (5×10^4 spore/ml) of spores. All Aberystwyth isolates.

1. BNS-82-5
2. BNS-82-70
3. BNS-82-63
4. BNS-82-58
5. BNS-82-209
6. BNS-82-59
7. BNS-82-103
8. BNS-82-19
9. BNS-82-25
10. BNS-82-88

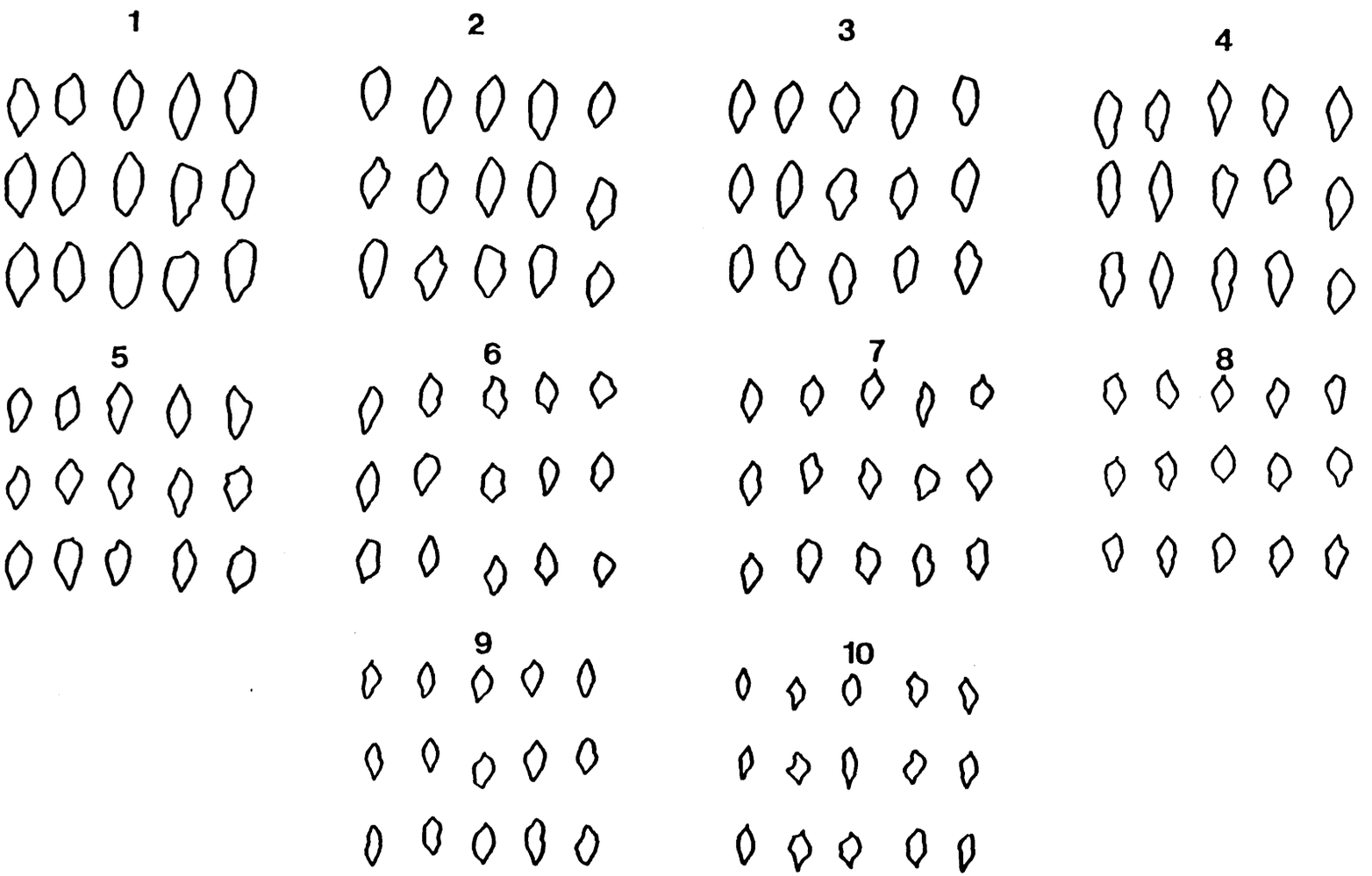

1
2
3
4
5
6
7
8
9
10

Table 9: Virulence of 10 isolates of *Pyrenophora teres* on barley cv. Sonja. All Aberystwyth isolates.

P. teres Isolates	Segments	Total area of 3 lesions per segment	% of segment with lesions	Mean % of segment with lesions
BNS-82-5	1	151	61.6	57 ± 3.5
	2	141	57.5	
	3	138	56.4	
	4	126	51.4	
	5	142	58.0	
BNS-82-70	1	97	39.6	42.5±5.8
	2	88	35.9	
	3	114	46.5	
	4	123	50.2	
	5	98	40.0	
BNS-82-63	1	80	32.6	34.9±1.8
	2	83	33.9	
	3	85	34.7	
	4	89	36.3	
	5	91	37.1	
BNS-82-58	1	90	36.7	34.1±1.6
	2	80	32.6	
	3	81	33.0	
	4	84	34.3	
	5	83	33.9	
BNS-82-209	1	67	27.3	30 ± 1.5
	2	76	31.0	
	3	74	30.2	
	4	75	30.6	
	5	76	31.0	
BNS-82-59	1	79	32.2	28.3±2.9
	2	67	27.3	
	3	73	29.8	
	4	68	27.7	
	5	60	24.5	
BNS-82-103	1	64	26.1	27.9±1.9
	2	74	30.2	
	3	73	29.8	
	4	66	26.9	
	5	65	26.5	
BNS-82-19	1	63	25.7	26.9±1.1
	2	70	28.6	
	3	64	26.1	
	4	66	26.9	
	5	67	27.3	

Table 9: (Continued)

P. teres Isolates	Segments	Total area of 3 lesions per segment	% of segment with lesions	Mean % of segment with lesions
BNS-82-25	1	45	18.4	24.3±4.3
	2	53	21.6	
	3	71	28.9	
	4	63	25.7	
	5	66	26.9	
BNS-82-88	1	39	15.9	17.7±2
	2	43	17.5	
	3	42	17.1	
	4	52	21.2	
	5	41	16.7	

Discussion

The ten isolates of *P. teres* caused lesions of different sizes on segments of leaves of barley cv. Sonja. These differences were presumably related to different levels of virulence of the isolates. Singh (1962), attributed such differences in virulence to the multinucleate condition of conidia of *P. teres*, so that numerous variants and races of varying virulence arise following germination of and growth of hyphae from conidia.

SECTION 5

REACTION OF HOST AND NON-HOST PLANTS TO *PYRENOPHORA TERES*

According to the literature, *P. teres* may infect a wide range of graminaceous hosts. Seven species of wild barley belonging to genus *Hordeum* were found naturally infected by *P. teres* in the field by Shipton *et al.* (1973).

The pathogen was found causing a light, moderate or heavy infection of 18 species belonging to 16 genera of Gramineae by Singh (1962). Hosts other than *Hordeum* spp. harbouring *P. teres* have also been recorded, including oats (Talvia, 1966), and two species of *Bromus*; *B. gussonii* and *B. diandrus* (Khan and Boyd, 1968). Knowledge about the host range of *P. teres* is very important from the standpoint of the epidemiology of the disease it causes and the life cycle of the pathogen. With this in mind, a study was made in a controlled temperature room and in the field of the reactions to *P. teres* of 17 species of grasses found growing naturally within or around fields of barley, and of other graminaceous crops, three species of wheat, three species of oat, one of rye, three of wild barley and of rice and corn. Susceptible grasses and certain crops could serve as reservoirs of the pathogen and as continuous sources of inoculum in the field throughout the year could play an important role in the epidemiology of net blotch.

Experiment 1: Reaction of host and non-host plants to P. teres using leaf segments.

Segments 5 cm. long were taken from the first leaf of 22 day old seedlings of barley cv. Sonja, barley cv. ND B112, wheat cv. Maris Dove, Oat (Avena sativa cv. Peniarth), rye (Secale cereale cv. Rheidol), wall barley (Hordeum murinum), common rye grass (Lolium perenne) and Cock's foot grass (Dactylis glomerata). The seedlings were grown in the glasshouse (1.3). The segments were placed floating on 4 ml of a 50 ppm benzimidazole solution in small boxes (3.2). Each segment was inoculated by three separate droplets of a suspension of spores (5×10^4 spore/ml), of P. teres virulent isolate Pt.80-12. Five segments were used as replicates for each host plant. The inoculated segments were then incubated at 21 ± 1°C. Lesions were measured 120 h. after inoculation by the method described in (4.2.3). Five segments for each host and non-host plant had been treated with 5 µl of sterile distilled water as controls.

Results

The reactions of the host and non-host plants to the virulent isolate (Pt.80-12) of P. teres are given in Table 10 and Figure 5. Barley cv. Sonja and cv. ND B112 showed a high level of susceptibility. Wild barley (Hordeum murinum), was susceptible, whereas, wheat cv. Maris Dove showed a moderate level of susceptibility (Plate 3). Rye (Secale cereale cv. Rheidol) and common rye grass (Lolium perenne) were resistant.

Table 10: Mean percentages of segments with lesions on host and non-host plants inoculated with suspensions of spores of *P. teres*, virulent isolate (Pt.80-12). Lesions were measured five days after inoculation.

Plants Host	Mean % of segment with lesions	Symptoms
Cultivated barley cv. Sonja	49 ± 2.4	Oval-irregular lesions with chlorotic haloes
Cultivated barley cv. ND B112	42 ± 2.5	Oval-irregular lesions with chlorotic haloes
Wild barley *Hordeum murinum*	32 ± 2.7	Oval necrotic lesions with narrow chlorotic zones
Non-Host Cultivated wheat cv. Maris Dove	23 ± 2.5	Oval necrotic lesions with narrow chlorotic zones
Cultivated rye *Secale cereale* cv. Rheidol	14.3 ± 1.7	Dark green spots (darker than normal) at points of inoculations
Common rye grass *Lolium perenne*	12.4 ± 1.8	Dark green spots (darker than normal) at points of inoculations
Cultivated oat *Avena sativa* cv. Peniarth	9.3 ± 1.3	Dark green spots (darker than normal) at points of inoculation
Cock's foot grass *Dactylis glomerata*	Light chlorosis	Lighter green than normal at points of inoculations

Figure 5: Susceptibility of host and non-host plants to *P. teres* (virulent isolate Pt.80-12) by inoculation of leaf segments with droplets of spore suspension.

1. Barley cv. Sonja
2. Barley cv. ND B112
3. Wild barley (*Hordeum murinum*)
4. Wheat cv. Maris Dove
5. Rye cv. Rheidol
6. Rye grass (*Lolium perenne*)
7. Oat cv. Peniarth

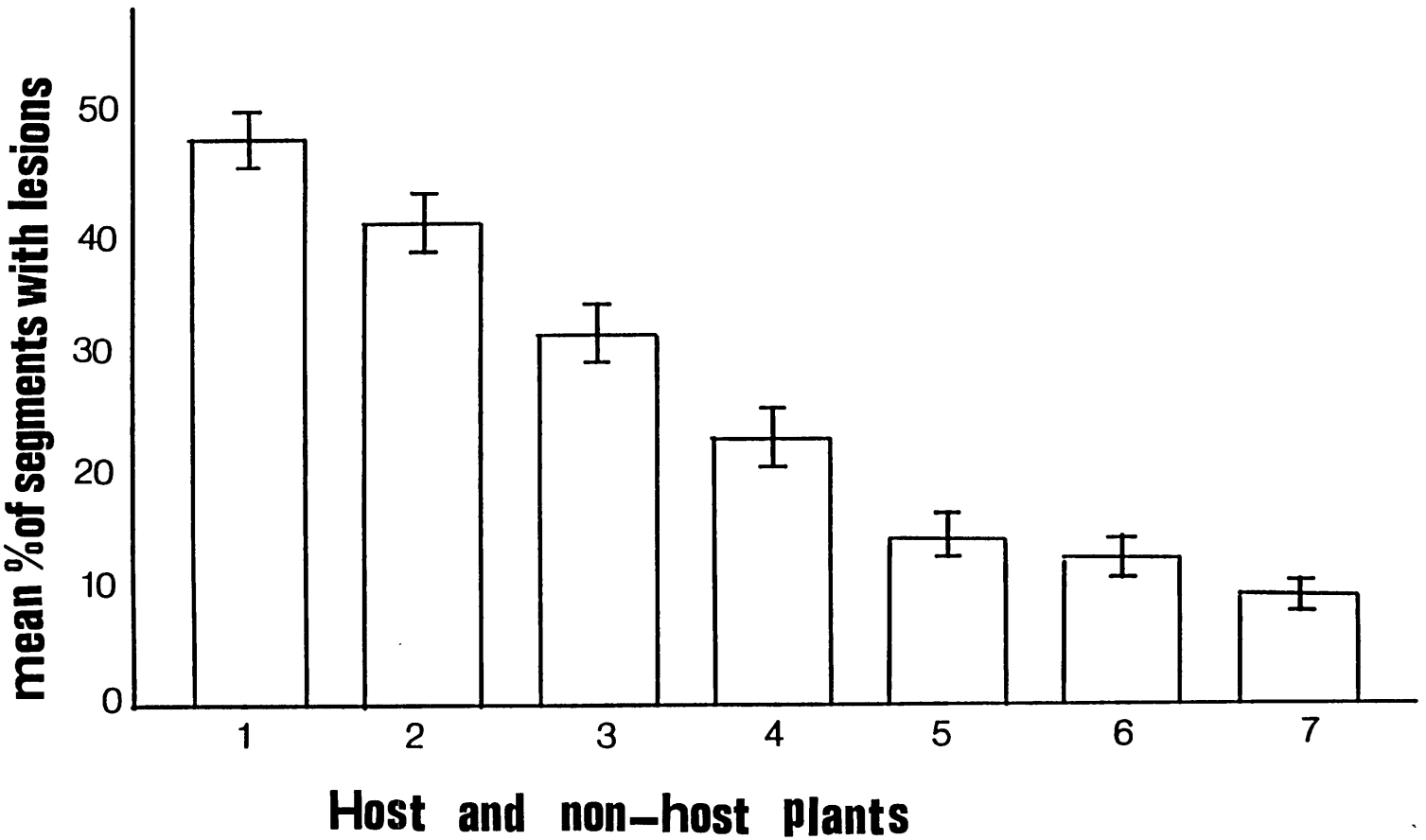
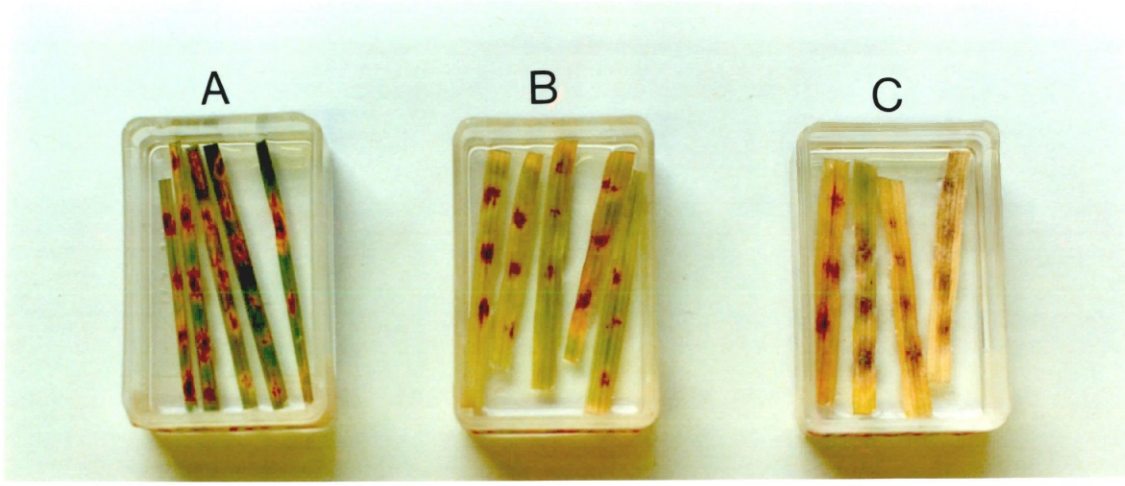

mean %of segments with lesions
50
40
30
20
10
0
1
2
3
4
5
6
7
Host and non–host plants

Plate 3. Leaf segments of host and non-host plants, inoculated with 5 µℓ of suspension of spores of *P. teres*, virulent isolate (Pt.80-12). Photograph taken 5 days after inoculation.

A. Wheat cv. Maris Dove

B. Wild barley (*H. murinum*)

C. Barley


A
B
C

Oat (Avena sativa cv. Peniarth) showed a high level of resistance, whereas Cock's foot grass (Dactylis glomerata) showed only a light chlorisis (lighter green than normal) at points of inoculations. The corresponding symptoms which developed on the segments, 120 h. after inoculation were oval to irregular necrotic lesions surrounded by a chlorotic halo on the barley cultivars (Sonja, ND B112); small necrotic spots surrounded by a narrow chlorotic zone on wild barley and wheat, small dark green spots (darker than normal) at points of inoculations on rye, common rye grass and oat and lightly chlorotic spots (lighter green than normal), on Cock's foot grass. No lesions or chlorosis appeared on the uninoculated segments.

Experiment 2: Response of host and non-host plants to infection with P. teres in a controlled temperature room at 21 ± 1°C.

Two cultivars of barley, three species of wheat, three species of oat, two species of wild barley, 41 lines of wild barley (Hordeum spontaneum), rye, five cultivars of rice, sweet corn, and 17 species of grasses belonging to 11 genera of Gramineae (1.2), were grown in a glasshouse at 15-18°C except for rice which was grown in high humidity in a glasshouse at 25°C (1.3). Three leaf growth stage seedlings were transferred to large plastic boxes containing a 2 cm. deep layer of water (3.3). The seedlings were sprayed with a suspension (5×10^4 spore/ml) of spores of P. teres,

virulent isolate (Pt.80-12) so that each leaf received a similar volume of suspension. There were ten seedlings in each treatment. The boxes were closed by their lids to maintain a humid atmosphere during incubation at 21 ± 1°C in a controlled temperature room with a photoperiod of 12 h. daily for the first 24 h. Then the lids were removed. Sterile distilled water was used in the controls. Lesions on host and non-host plants were recorded and measured to give the mean percentages of the leaf with lesions, ten days after inoculation (4.2.1, 4.2.3). Degree of susceptibility and the resistance in C.T. room and in the field, of host and non-host plants were rated by using the scale of disease assessment given in Table 11.

Results

These are summarized in Tables 12, 13, 14. The reactions of host and non-host plants to infection by P. teres (virulent isolate, Pt.80-12) in the constant temperature room at 21 ± 1°C, showed that the cultivated barley cultivars Sonja, ND B112, were highly susceptible and developed a net form symptom (Table 12). Two species of wild barley (Hordeum murinum, H. jubatum) were moderately susceptible, developing oval-irregular, necrotic lesions with chlorotic haloes (Table 12, Plate 4).

Three species of wheat (Triticum aestivum cv. Maris Dove, T. monococcum, T. turgidum) were resistant, developing

Table 11:

Disease Assessment Scale

The following scale was used in Section 5 for the assessment of the disease on host and non-host plants and was based on the percentage of the leaf with necrotic lesions (net, spot forms); dark green spots (darker than normal); pronounced chlorosis and light chlorosis.

1.	Highly susceptible (HS)	=	% of leaf with lesions (50-75%)
2.	Susceptible (S)	=	% of leaf with lesions (25-50%)
3.	Moderately susceptible (MS)	=	% of leaf with lesions (15-25%)
4.	Resistant (R)	=	% of leaf with lesions (10-15%)
5.	Highly resistant (HR)	=	% of leaf with lesions (1-10%)
6.	Resistant chlorotic (RC)	=	Pronounced chlorosis (++)
7.	Highly resistant chlorotic HRC)	=	Light chlorosis (somewhat lighter green than normal)
8.	No symptoms (NS)	=	leaves apparently healthy (-)

Table 12 Mean percentages of leaves with lesions on seedlings of host and non-host plants, sprayed with suspensions of spores of P. teres, virulent isolate (Pt.80-12) in a C.T. room at 21 ± 1°C. Assessment 10 days after inoculation.

Plants	Mean % of leaf with lesions			Symptoms	Reaction *		
	1st leaf	2nd leaf	3rd leaf		1st leaf	2nd leaf	3rd leaf
Hordeum vulgare cv. Sonja	74±5	55±4	38±3.2	Net form necrotic lesions	HS	HS	S
Hordeum vulgare cv. ND 8112	62±3.8	44±3.3	30±2.5	Net form necrotic lesions	HS	S	S
Triticum aestivum cv. Maris Dove	15±2	8±1.1	3±0.7	Spot form necrotic lesions	MS	HR	HR
Triticum monococcum	13±1.7	9±1.5	4.5±0.6	Spot form necrotic lesions	R	HR	HR
Triticum turgidum	10±1.4	5±0.9	+ +	Spot necrotic lesions on 1st,2nd leaf, pronounced chlorosis on 3rd leaf	R	HR	RC
Avena sativa cv. Milo	+ +	+	+	Pronounced chlorosis on 1st leaf, light on 2nd and 3rd leaf	RC	HRC	HRC
Avena fatua	+ +	+	+	Pronounced chlorosis on 1st leaf, light on 2nd and 3rd leaf	RC	HRC	HRC
Avena strigosa	8±1	4±0.4	+ +	Dark green spots on 1st, 2nd leaf, pronounced chlorosis on 3rd leaf	HR	HR	RC
Avena sativa cv. Peniarth	5±0.6	3.5±0.5	+	Dark green spots on 1st 2nd leaf, light chlorosis on 3rd leaf	HR	HR	HRC
Secale cereale cv. Rheidol	7±0.9	4.5±0.7	+ +	Dark green spots on 1st, 2nd leaf, pronounced chlorosis on 3rd leaf	HR	HR	RC
Hordeum murinum	24±2.5	13.5±1.7	7.5±1.2	Spot necrotic lesions	MS	R	HR
Hordeum jubatum	17±2	11±1.3	5±0.8	Spot necrotic lesions	MS	HR	HR
Oryza sativa cv. Bahapie	+	+	+	Light chlorosis on 1st, 2nd and 3rd leaves	HRC	HRC	HRC
Oryza sativa cv. TN1	+ +	+	+	Pronounced chlorosis on 1st leaf, light chlorosis on 2nd and 3rd leaves	RC	HRC	HRC
Oryza sativa cv. SM2	+	+	+	Light chlorosis on 1st 2nd and 3rd leaves	HRC	HRC	HRC
Oryza sativa cv. 81979	+	+	+	Light chlorosis on 1st . 2nd and 3rd leaves	HRC	HRC	HRC
Oryza sativa cv. MR 35	+ +	+	+	Pronounced chlorosis on 1st leaf, light chlorosis on 2nd and 3rd leaves	RC	HRC	HRC
Zea mays	+	+	+	Light chlorosis on 1st 2nd and 3rd leaves.	HRC	HRC	HRC

*see disease assessment scale (Table 11)

Table 13: Mean percentages of leaves with lesions in three leaves growth stage seedlings of 17 species of grasses sprayed with suspensions of spores of P. teres, virulent isolate (Pt.80-12) in a C.T. room at 21 ± 1°C. Assessment 10 days after inoculation

Plants	Mean % of leaf with lesions			Symptoms	Reaction *		
	1st leaf	2nd leaf	3rd leaf		1st leaf	2nd leaf	3rd leaf
Agrostis gigantea	-	-	-	No symptoms	-	-	-
Agrostis tenuis	+	+	+	Light chlorosis	HRC	HRC	HRC
Agrostis stolonifera	-	-	-	No symptoms	-	-	-
Agropyron repens	+	+	+	Light chlorosis	HRC	HRC	HRC
Alopecurus myosuroides	-	-	-	No symptoms	-	-	-
Cynosurus cristatus	-	-	-	No symptoms	-	-	-
Dactylis glomerata	-	-	-	No symptoms	-	-	-
Festuca pratensis	5.5±0.8	++	++	Dark green lesions on 1st leaf, pronounced chlorosis on 2nd and 3rd leaves	HR	RC	RC
Festuca rubra	4±0.5	++	+	Dark green lesions on 1st leaf, pronounced and light chlorosis on 2nd and 3rd leaves	HR	RC	HRC
Holcus lanatus	-	-	-	No symptoms	-	-	-
Lolium perenne	13±2	10±1.5	4±0.6	Dark green lesions on 1st 2nd and 3rd leaves	R	R	HR
Phleum pratense	-	-	-	No symptoms	-	-	-
Phleum bertolonii	-	-	-	No symptoms	-	-	-
Poa annua	4±0.4	++	+	Dark green lesions on 1st leaf, pronounced and light chlorosis on 2nd, 3rd leaves	HR	RC	HRC
Poa trivialis	-	-	-	No symptoms	-	-	-
Poa pratensis	-	-	-	No symptoms	-	-	-
Arrhenatherum elatius	+	+	+	Light chlorosis on 1st, 2nd and 3rd leaves	HRC	HRC	HRC

* See disease assessment scale (Table 11)

Table 14: Mean percentages of leaves with lesions on three leaf growth stage seedlings of 41 lines of H. spontaneum sprayed with suspension of spores of P. teres (virulent isolate Pt.80-12), in a C.T. room at 21 ± 1°C. Assessment 10 days after inoculation.

H. spontaneum lines	Mean % of leaf with lesions			Symptoms	Reaction *		
	1st leaf	2nd leaf	3rd leaf		1st leaf	2nd leaf	3rd leaf
HS.1517	45±3	36±4	25±4	net form	S	S	S
HS.1520	34±4	28±3	22±3	net form	S	S	MS
HS.1536	42±6	32±4	24±2	net form	S	S	MS
HS.5732	42±4	30±3	23±3	net form	S	S	MS
HS.5733	40±3	35±5	28±3	net form	S	S	S
HS.5735	54±6	41±5	33±3	net form	HS	S	S
HS.5738	44±5	40±4	32±3	net form	S	S	S
HS.5739	43±4	32±3	24±2	net form	S	S	MS
HS.5742	32±3	27±3	22±3	net form	S	S	MS
HS.5743	48±4	39±5	27±3	net form	S	S	S
HS.5744	19±4	14±3	11±2	spot form	MS	R	R
HS.5745	54±5	42±3	29±5	net form	HS	S	S
HS.5747	20±3	15±3	11±2	spot form	MS	MS	R
HS.5750	22±3	14±2	8±2	spot form	MS	R	HR
HS.5753	27±3	19±4	15±2	spot form	S	MS	MS
HS.5761	53±4	39±4	25±3	net form	HS	S	S
HS.6200	29±4	22±3	16±2	net form	S	MS	MS
HS.6206	23±2	17±3	10±2	spot form	MS	MS	R
HS.6207	37±2	30±4	26±4	net form	S	S	S
HS.6210	41±4	28±3	24±4	net form	S	S	MS
HS.8330	35±3	27±2	22±3	net form	S	S	MS
HS.9242	20±4	15±3	12±2	spot form	MS	MS	R

Continued

Table 14: (continued)

H. spontaneum lines	Mean % of leaf with lesions			Symptoms	Reaction *		
	1st leaf	2nd leaf	3rd leaf		1st leaf	2nd leaf	3rd leaf
HS.13786	37±3	31±5	23±2	net form	S	S	MS
HS.13787	44±5	36±3	26±2	net form	S	S	S
HS.13789	23±2	16±2	12±3	spot form	MS	MS	R
HS.14293	48±3	41±4	30±3	net form	S	S	S
HS.14296	22±3	20±4	13±2	spot form	MS	MS	R
HS.14298	43±4	40±2	31±3	net form	S	S	S
HS.14301	47±3	40±3	30±3	net form	S	S	S
HS.14302	46±4	37±3	28±4	net form	S	S	S
HS.14303	52±4	36±4	27±3	net form	HS	S	S
HS.14304	56±5	44±4	29±2	net form	HS	S	S
HS.14305	19±3	12±2	8±1	spot form	MS	R	HR
HS.14309	17±3	13±2	8±1	spot form	MS	R	HR
HS.14411	33±3	22±3	13±2	net form	S	MS	R
HS.14586	50±5	42±3	29±4	net form	HS	S	S
HS.14589	27±3	20±2	15±2	spot form	S	MS	MS
HS.14590	43±4	36±3	28±2	net form	S	S	S
HS.14591	56±5	45±4	37±3	net form	HS	S	S
HS.14593	54±5	37±6	30±3	net form	HS	S	S
HS.14595	48±3	39±3	30±4	net form	S	S	S

* See disease assessment scale (Table 11).

spot necrotic lesions (Table 12, Plate 5). Two species of oat (Avena strigosa, A. sativa cv. Peniarth), and rye (Secale cereale cv. Rheidol), were highly resistant, developing dark green spots (darker than normal), at points of inoculation (Table 12, Plate 6). Two species of oat (Avena fatua and A. sativa cv. Milo) showed resistant chlorotic reactions developing only a pronounced or light chlorosis, (Table 12). Five cultivars of rice (Oryza sativa) and corn (Zea mays), were highly resistant chlorotic (HRC), developing light chlorosis (Table 11., Disease assessment scale). The reactions of 17 species of grasses showed that two species of Festuca, (Festuca pratensis, F. rubra), and Poa annua, were highly resistant, developing dark green spots (darker than normal) on the first leaf, pronounced chlorosis on the second leaf and light chlorosis on the third leaf (Table 13). Rye grass (Lolium perenne), was resistant, developing dark green lesions (darker than normal) on the first, second, and third leaves (Table 13, Plate 7).

Agrostis tenuis, Agropyron repens and Arrhenatherum elatius showed a highly resistant chlorotic reaction, developing only a light chlorosis (Table 13). Ten species of grasses (Agrostis gigantea, Agrostis stolonifera, Alopecurus myosuroides, Cynosurus cristatus, Dactylis glomerata, Holcus lanatus, Phleum pratense, Phleum bertolonii, Poa trivialis and Poa pratensis), did not show any symptoms (Table 13). As regards the reaction of 41 lines of wild barley (Hordeum

Plate 4. Symptoms on first leaves (21 day old) of wild barley (*Hordeum murinum*) inoculated by spraying in a CT room at 21 ± 1°C with a suspension (5×10^4 spore/ml) of spores of *P. teres*, virulent isolate (Pt.80-12).
Photograph taken 10 days after inoculation.

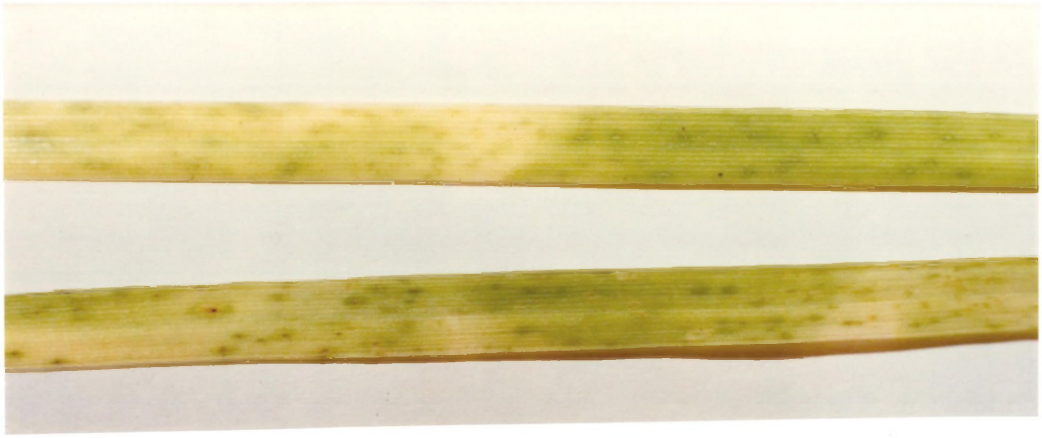
Plate 5. Symptoms on first leaves (21 day old) of wheat cv. Maris Dove, inoculated by spraying in a CT room at 21 ± 1°C with a suspension (5×10^4 spore/ml^{-}) of spores of *P. teres*, virulent isolate (Pt.80-12). Photograph taken 10 days after inoculation.





Plate 6. Symptoms on first leaves (21 day old) of rye (*Secale cereale* cv. Rheidol), inoculated by spraying in a CT room at 21 ± 1°C, with a suspension (5×10^4 spore/ml$^-$) of spores of *P. teres*, virulent isolate (Pt.80-12).
Photograph taken 10 days after inoculation.

Plate 7. Symptoms on first leaves (21 day old) of rye grass (*Lolium perenne*) inoculated by spraying in a CT room at 21 ± 1°C, with a suspension (5×10^4 spore/ml$^-$) of spores of *P. teres*, virulent isolate (Pt.80-12).
Photograph taken 10 days after inoculation.





spontaneum), 30 lines were susceptible, developing net form symptoms on their first, second and third leaves, whereas 11 lines showed moderate levels of susceptibility on the first, and second leaf, and a resistant reaction on the third leaf, developing spot necrotic lesions (Table 14).

Experiment 3: Reaction of host and non-host plants to Pyrenophora teres, isolate (Pt.80-12) in the field

Seventeen species of host and non-host plants belonging to eleven genera of Gramineae were selected to examine their reactions in the field to inoculation with P. teres, virulent isolate (Pt.80-12). Host and non-host plants were selected on the results of their responses to P. teres in earlier experiments (Table 12,13 and 14).

The seeds were sown in plots (Table 1), in mid-March 1987. Seedlings at the three leaf growth stage were sprayed with suspensions (5×10^4 spore/ml) of spores at 10 am so that leaves received similar volumes of suspensions. Ten seedlings were used as replicates for each host and non-host plant After inoculation, seedlings were enclosed by polythene bags containing wads of wet cotton wool for 24 h. when the bags were removed (3.4). The temperature at the time of the inoculation was c. 16 ^{o}C. Control seedlngs of each host and non-host plant were sprayed with sterile distilled water. Disease on the leaves was asssessed 10 days

after inoculation by the method described in 4.2.3 and 4.2.1. (Table 11).

Results

These are given in Table 15. Barley cultivars Sonja, ND B112 and wild barley (Hordeum spontaneum line 14591), were susceptible but reactions were less susceptible than corresponding seedlings in a C.T. room at 21 ± 1°C (Table 12, 14). Hordeum spontaneum lines 6200, 5753 and 14309; Hordeum murinum and H. jubatum, were moderately susceptible; net form lesions developed on H. spontaneum line 6200 and spot necrotic lesions developed on H. spontaneum line 5753 and 14309, H. murinum and H. jubatum (Table 15). Two species of wheat (T. aestivum cv. Maris Dove, T. monococcum), Avena strigosa, Lolium perenne and Secale cereale, were moderately resistant. Spot form lesions developed on the wheat species and dark green lesions (darker than normal) developed on Avena strigosa, Lolium perenne and Secale cereale. Avena fatua and A. sativa cv. Milo exhibited a high level of resistance developing only a light chlorosis (lighter than normal) on their first, second and third leaves, whereas Poa annua and Festuca pratensis developed heavy chlorosis on their first leaves and light chlorosis on their second and third leaves. No symptoms were detected on Dactylis glomerata, Arrhenatherum elatius, Phleum bertolonii and Cynosurus cristatus (Table 15).

Table 15: Mean percentages of first, second and third leaves with lesions on host and non-host plants, sprayed in the field with suspension of spores (5×10^4 spore/ml^{-}) of P. teres, virulent isolate (Pt.80-12). Assessment 10 days after inoculation.

Host and non-host plants	Mean % of leaf with lesions			Symptoms	Reaction *		
	1st leaf	2nd leaf	3rd leaf		1st leaf	2nd leaf	3rd leaf
Hordeum vulgare c.v. Sonja	63±4.6	42±3.8	27±4.3	Net form necrotic lesions	HS	S	S
c.v. ND B112	52±4	29±3	21±2.7	Net form necrotic lesions	HS	S	MS
Hordeum murinum	15.5±2	8±0.7	4.5±0.8	Small, spot form necrotic lesions	MS	HR	HR
Hordeum jubatum	11.5±1.7	5.7±0.8	3.2±0.5	Small, spot form necrotic lesions	R	HR	HR
Hordeum spontaneum line 14309	13±2.2	8±0.9	4.7±0.6	Small, spot form necrotic lesions	R	HR	HR
line 6200	25±3	20±2.2	12±2.4	Net form necrotic lesions	S	MS	R
line 5753	17±2.5	14±1	9±0.9	Small, spot form necrotic lesions	MS	R	HR
line 14591	43±3.5	28±2.5	22±2	Net form necrotic lesions	S	S	MS
Triticum aestivum c.v. Maris Dove	9.5±1.4	5.5±0.5	3±0.4	Small, spot form necrotic lesions	HR	HR	HR
Triticum monococcum	8±1.1	5±0.6	3±0.35	Small, spot form necrotic lesions	HR	HR	HR
Avena strigosa	4.6±0.8	2.4±0.4	+	Small, dark green spots (darker than normal) light chlorosis (3rd leaf)	HR	HR	HRC
Avena fatua	+	+	+	Light chlorosis (lighter green than normal)	HRC	HRC	HRC
Avena sativa c.v. Milo	+	+	+	Light chlorosis (lighter green than normal)	HRC	HRC	HRC
Secale cereale c.v. Rheidol	5.5±0.6	+	+	Dark green spots on 1st leaf, light chlorosis on 2nd and 3rd leaf.	HR	HRC	HRC
Lolium perenne	9±1.6	++	+	Dark green spots on 1st leaf, pronounced chlorosis on 2nd, and light chlorosis on 3rd.	HR	RC	HRC
Poa annua	++	+	+	Pronounced chlorosis on 1st leaf, light chlorosis on 2nd and 3rd.	RC	HRC	HRC
Festuca pratensis	++	+	+	Pronounced chlorosis on 1st leaf, light chlorosis on 2nd and 3rd.	RC	HRC	HRC
Dactylis glomerata	-	-	-	No symptoms	NS	NS	NS
Arrhenatherum elatius	-	-	-	No symptoms	NS	NS	NS
Phleum bertolonii	-	-	-	No symptoms	NS	NS	NS
Cynosurus cristatus	-	-	-	No symptoms	NS	NS	NS

* See disease assessment scale (Table 11)

Experiment 4: Sporulation of P. teres virulent isolate (Pt.80-12) on host and non-host plants, in the field and in a constant temperature room.

Infected first leaves of host and non-host plants were collected from seedlings in the field or in a constant temperature room, four weeks after inoculation. Three leaves were cut into small segments and suspensions of spores from them were prepared. Spore number per leaf were estimated as described earlier.

Results

These are shown in Table 16. P. teres sporulated to different degrees on host, non-host plants, both in the field and in a constant temperature room. Moderate to dense sporulation was obtained on barley cv. Sonja in a C.T. room whereas it was moderate in the field. Sporulation on barley cv. ND B112 was moderate in the C.T. room and sparse in the field. As regards sporulation on wild barley (Hordeum murinum) it was sparse to moderate in the C.T. room and sparse in the field. Sparse sporulation in the C.T. room, and very little in the field was observed on leaves of Hordeum jubatum. Moderate sporulation was estimated in the C.T. room for Hordeum spontaneum line 14591 whereas sparse to moderate was estimated in the field. For H. spontaneum line 5753, sparse to moderate sporulation were estimated in the C.T. room, with sparse sporulation in the field. Two species of wheat(T. aestivum cv. Maris Dove, T. monococcum), one

Table 16: Estimation of sporulation on first leaves of host and non-host plants, inoculated in a controlled temperature room at 21 ± 1°C, and in the field. Sporulation estimated 4 weeks after inoculation.

Plants	Sporulation[1] in C.T. room 12h photo-period at 21 ± 1°C	Reaction[2] in C.T. room (Tables 9, 10, 11)			Sporulation in the field	Reaction in[2] the field (Table 12)		
		1st leaf	2nd leaf	3rd leaf		1st leaf	2nd leaf	3rd leaf
Hordeum vulgare cv. Sonja	++/+++	HS	HS	S	++	HS	S	S
Hordeum vulgare cv. ND B112	++	HS	S	S	+	HS	S	MS
Hordeum murinum	+/++	MS	R	HR	+	MS	HR	HR
Hordeum jubatum	+	MS	HR	HR	+ -	R	HR	HR
Hordeum spontaneum line 14591	++	HS	S	S	+/++	S	S	MS
Hordeum spontaneum line 5753	+/++	S	MS	MS	+	MS	R	HR
Triticum aestivum cv. Maris Dove	+	MS	HR	HR	+ -	HR	HR	HR
Triticum monococcum	+	R	HR	HR	+ -	HR	HR	HR
Avena strigosa	+	HR	HR	RC	+ -	HR	HR	HRC
Lolium perenne	+	R	R	HR	+ -	HR	RC	HRC
Festuca pratensis	-	HR	RC	RC	-	RC	HRC	HRC
Poa annua	-	HR	RC	HRC	-	RC	HRC	HRC

(1) - No
+- Few - Fewer than 100 spores per leaf
++ - Moderate - 10,000-25,000 spores per leaf
++/+++ - Moderate to dense - 25,000-40,000 spores per leaf
+ Sparse - 1,000-5,000 spores per leaf
+/++ Sparse to moderate - 5,000-10,000 spores per leaf

(2) See disease assessment scale (Table 11).

species of oat (Avena strigosa) and rye grass (Lolium perenne), sporulated sparsely in the C.T. room and only a few spores were obtained in the field. No spores were obtained on leaves of Festuca pratensis and Poa annua either in the C.T. room or in the field (Table 16).

Discussion

One purpose of these experiments was to compare reactions, following inoculation with a virulent isolate, of segments and of leaves of seedlings grown in the artificial conditions of a constant temperature room and in the field. These comparisons are summarized in Table 17 and 18.

Highly susceptible barley showed more severe symptoms in the C.T. room than in segments (Table 17) but the reverse occurred in the less susceptible and resistant plants. But in both groups the differences were not pronounced in terms of areas colonized. Symptoms differed in character in barley in that net blotch features in the C.T. room did not develop in segments. They were more similar in the other plants.

Table 18 shows that reactions in the C.T. room and in the field were very similar in terms of colonization of leaves, type of symptom and in the general assessment of reaction varying from highly susceptible to highly resistant. But almost invariably and for the first, second and third

Table 17: Comparison of reactions of leaf segments inoculated in the C.T. room (Tables 10, 12, 13)

Plants	Mean % of segment with lesions			Symptoms
	1st leaf	2nd leaf	3rd leaf	
Hordeum vulgare c.v. Sonja	49 (74)*	- (55)	- (38)	Oval - irregular necrotic lesions (net form necrotic lesions)*
Hordeum vulgare c.v. ND B112	42 (62)	- (44)	- (30)	Oval - irregular necrotic lesions (net form necrotic lesions)
Hordeum murinum	32 (24)	- (13.5)	- (7.5)	Oval - irregular necrotic lesions (spot necrotic lesions)
Triticum aestivum c.v. Maris Dove	23 (15)	- (8)	- (3)	Oval necrotic lesions (spot necrotic lesions)
Secale cereale c.v. Rheidol	14.3 (7)	- (4.5)	- (++)	Dark green spots. (dark green spots on 1st, 2nd leaves, pronounced chlorosis on 3rd leaf.)
Lolium perenne	12.4 (13)	- (10)	- (4)	Dark green spots (dark green spots)
Avena sativa c.v. Peniarth	9.3 (5)	- (3.5)	- (+)	Dark green spots (dark green spots on 1st, 2nd leaves, light chlorosis on 3rd leaf.)
Dactylis glomerata	+ (-)	+ (-)	+ (-)	Light chlorosis (No symptoms)

* Leaves inoculated in the C.T. room at 21 ± 1°C.

host and non-host plants

Table 18: Comparison of reactions of inoculated in a C.T. room and in the field (Tables 9,10,11,13).

Plants	Mean % of leaf with lesions			Symptoms	Reaction****		
	1st leaf	2nd leaf	3rd leaf		1st leaf	2nd leaf	3rd leaf
H. vulage cv. Sonja	74(63)*	55(42)	38(27)	net (net)*	HS(HS)	HS(S)	S(S)
H. vulgare cv. ND B112	62(52)	44(29)	30(21)	net (net)	HS(HS)	S(S)	S(MS)
H. murinum	24(15.5)	13.5(8)	7.5(4.5)	spot (small spot)	MS(MS)	R(HR)	HR(HR)
H. jubatum	17(11.5)	11(5.7)	5(3.2)	spot (small spot)	MS(R)	HR(HR)	HR(HR)
H. spontaneum line 14309	17(13)	13(8)	8(4.7)	spot (small spot)	MS(R)	R(HR)	HR(HR)
H. spontaneum line 6200	29(25)	22(20)	16(12)	net (net)	S(S)	MS(MS)	MS(R)
H. spontaneum line 5753	27(17)	19(14)	15(9)	spot (small spot)	S(MS)	MS(R)	MS(HR)
H. spontaneum line 14591	56(43)	45(28)	27(22)	net (net)	HS(S)	S(S)	S(MS)
T. aestivum cv. Maris Dove	15(9.5)	8(5.5)	3(3)	spot (small spot)	MS(HR)	HR(HR)	HR(HR)
T. monococcum	13(8)	9(5)	4.5(3)	spot (small spot)	R(HR)	HR(HR)	HR(HR)
Avena strigosa	8(4.6)	4(2.4)	++(+)	Dark green 1,2,PC3 ** (dark green 1,2, LC3)***	HR(HR)	HR(HR)	RC(HRC)
Secale cereale cv. Rheidol	7(5.5)	4.5(+)	++(+)	Dark green 1,2, PC3 (dark green 1, LC 2,3)	HR(HR)	HR(HRC)	RC(HRC)
Poa annua	4(++)	++(+)	+(+)	Dark green 1, PC2, LC3 (PC1, LC2, 3)	HR(RC)	RC(HRC)	HRC(HRC)
Festuca pratensis	5.5(++)	++(+)	++(+)	Dark green 1, PC2,3 (PC1, LC2,3)	HR(RC)	RC(HRC)	RC(HRC)

* ( ) - leaves inoculated in field. ** PC - pronounced chlorosis. *** LC - Light chlorosis
**** See disease assessment scale (Table 11).

leaves, more of the leaves were colonized in the C.T. room than in the field. But the differences again were not pronounced and did not result in substantial differences in the general assessments of disease reaction, and did not alter the rankings in order of susceptibility.

A feature which emerges from almost all the data in Tables 13, 14, and 15 is the difference in susceptibility of first, second and third leaves. This is well shown by Table 17 (based on Table 14) for reactions of 41 lines of H. spontaneum. Differences were similar for net blotch and spot lesions and for both together were 100, 78 and 58 for first, second and third leaves.

Almost invariably there were similar differences for other plants both in the C.T. room and in the field. This clear cut result led to another experiment described later.

Table 19 also shows that net blotch lesions always were associated with considerably more colonization. For first, second and third leaves the ratios were 204, 221 and 236 where 100 represents spot lesions. However, the main purpose of experiments 1, 2 and 3 was to investigate the reactions of a wide range of graminaceous plants to inoculation with P. teres and regarding cultivated barley (H. vulgare) and related species of Hordeum as normal hosts and other species as non-hosts. The following summarizes the more important findings based on results in Tables 12,13, 14 and 15.

Table 19: Mean of means of percentages of leaf with lesions of 30 lines (net blotch lesions) and 11 lines (spot lesions) of Hordeum spontaneum (Table 14).

Hordeum spontaneum lines	Mean of means percentages of leaf with lesions		
	1st leaf	2nd leaf	3rd leaf
Net blotch lesions (30 lines)	44.3±7.5 (100)	35.1±6.7 (79)	26.4±4.8 (60)
Spot lesions (11 lines)	21.7±3.2 (100)	15.9±2.8 (73)	11.2±2.6 (52)
Both forms (41 lines)	38.3±12 (100)	30.0±10.2 (78)	22.3±8 (58)

1. Net blotch lesions were characteristic of susceptible reactions and the most extensive colonization. Spot lesions were associated with much less colonization and of less susceptible to moderately resistant reactions. Still more resistant reactions were characterized by cells that were darker green than normal. This is an unusual form of resistant response and is superficially similar to green island responses in many other diseases. But here the island is green in a background of less green or chlorotic tissue and is a late and not an early response to a pathogen. Finally, the most resistant responses exhibit a pronounced or light chlorosis similar to that of plants highly resistant to other pathogens, especially biotrophs (and also to viruses); or exhibit no symptoms visible to the naked eye.

2. The ranking of susceptibility was approximately as follows:

Reaction	Plant
HS	*Hordeum vulgare* (two cultivars)
HS	*H. spontaneum* (certain lines)
MS	*H. spontaneum* (certain lines)
MS	*H. murinum*
MS	*H. jubatum*
MS	*Triticum aestivum* (Maris Dove)
R	*T. monococcum*

Reaction	Plant
R	*T. turgidum*
R	*Lolium perenne*
HR	*Avena strigosa*
HR	*Secale cereale* (Rheidol)
HR	*Avena sativa* (Milo)
HR	*Festuca pratensis*
HR	*Festuca rubra*
HR	*Poa annua*
RC	*Avena sativa* (Peniarth)
RC	*Avena fatua*
RC	*Oryza sativa* (TNI)
RC	*O. sativa* (MR35)
HRC	*O. sativa* (Bahapie, SMII,81979)
HRC	*Zea mays*
HRC	*Agrostis tenuis*
HRC	*Agropyron repens*
HRC	*Arrhenatherum elatius*
-	*Agrostis stolonifera*
-	*Alopecurus myosuroides*
-	*Cynosurus cristatus*
-	*Dactylis glomerata*
-	*Holcus lanatus*
-	*Phleum pratense*
-	*P. bertolonii*
-	*Poa trivialis*
-	*P. pratensis*

HS - highly susceptible, MS - moderately susceptible, R - resistant, HR - highly resistant, RC - resistant chlorotic, HRC - highly resistant chlorotic, - - no symptoms. Of the 33 plants tested, 24 reacted with visible symptoms. Of the 24, 15 almost have reacted with visible colonization of leaves ranging from 4 to 74% in the CT room; 13 reacted similarly in the field. Eight of the 33 plants reacted with colonization of 10% or more in the CT room and in the field. The question now arises as to the use of host and non-host in relation to parasitism by P. teres. Certainly it seems that Triticum aestivum, T. monococcum, T. turgidum, Avena strigosa, Secale cereale and Lolium perenne could be regarded as hosts, albeit highly resistant, of P. teres.

In relation to the species of Triticum it is surprising, therefore, that Agropyron repens reacted with no more than a light chlorosis. These results are of interest in relation to specificity of parasitism by P. teres which commonly is regarded as a rather specific pathogen of cultivated barley and related species of Hordeum. Their significance in the epidemiology of net blotch in the field would depend on the extent to which the pathogen sporulated on lesions of resistant and highly resistant plants growing close to crops of barley. But it does seem possible that they could be significant in overwintering because restricted colonization and little or no sporulation in growing plants could be followed by further colonization and more or less extensive

sporulation in moribund or dead tissues. The preliminary results given in Table 16 do show that although there was very much more sporulation on leaves of infected, highly susceptible plants taken from the C.T. room and from the field, sporulation on leaves from resistant and highly resistant plants was still on a scale that could be significant.

SECTION 6

ROLE OF CERTAIN GRAMINACEOUS PLANTS AS ALTERNATIVE HOSTS FOR PYRENOPHORA TERES

It has been reported that certain graminaceous plants other than barley (Hordeum vulgare), can harbour P. teres (Sprague, 1955; Khan, 1973). Winter (1977) obtained leaf spots, several centimeters long on certain susceptible cultivars of rye after artificial inoculation. In the light of the results obtained in Section 5, on the reaction of many graminaceous plants to inoculation with an isolate Pt.80-12 of P. teres virulent on cultivated barley, three species of wild barley and three species of wheat, one moderately susceptible and two resistant, and one species of oat which was highly resistant, and rye grass which was resistant, were further studied for their role as alternative hosts.

Experiment: Host and alternative host plants for Pyrenophora teres

Two groups of five seedlings of barley cv. Sonja at the three leaf growth stage were inoculated by spraying with a suspension of spores from the normal host and from nine non-host plants as in earlier experiments (Table 15). Controls were sprayed with similar volumes of sterile distilled water. The mean percentages of first, second and third leaves with net form lesions in plants grown in a

controlled temperature room or in the field were assessed ten days after inoculation (4.2.1). The means were obtained from five replicates each of one leaf.

Results

Typical net form symptoms developed on all leaves of barley cv. Sonja inoculated with spores from each of the nine non-host plants (Table 20). There were no significant changes in the virulence of isolate (Pt.80-12) of P. teres after passage through the non-host plants. The results show clearly the decrease in susceptibility from first to second to third leaves both in plants grown in the constant temperature room and in the field. They also show that plants grown in the field were somewhat more resistant than were plants grown in the constant temperature room.

Discussion

The results summarized in Sections 5 and 6 confirmed that P. teres can infect a considerable number of non-host plants, (plants not usually regarded as hosts) but in which it causes symptoms different from those produced in barley, its common host. They also showed that P. teres can sporulate on leaves of these non-host plants and on some in amounts that could be significant in the epidemiology of the disease in barley crops. Results also confirm that spores from infected non-host plants readily infect barley producing typical net form symptoms both in the constant temperature

Table 20: Mean percentages of leaves with net form lesions on barley cv. Sonja, inoculated in constant temperature room and in the field by spraying with a suspension of spores of P. teres from host and non-host plants. Disease assessment 10 days after inoculation.

Source of spores	Mean % of leaves with net form lesions					
	In Constant temperature room			In the field		
	1st leaf	2nd leaf	3rd leaf	1st leaf	2nd leaf	3rd leaf
Hordeum vulgare cv. Sonja	77±4.5	60±4.3	52±2.7	65±4.2	48±4.7	43±4.1
Hordeum murinum	74±3.9	63±2.6	45±3.6	59±4.7	45±4.5	40±3.6
Hordeum jubatum	71±4	62±2.7	47±3.2	62±5.3	51±4	42±3
Hordeum spontaneum line 14591	79±3.5	68±4.5	56±4.2	70±4	58±3.7	48±2.7
Hordeum spontaneum line 5753	75±3	65±2.6	50±2.8	68±2.8	60±2	50±5
Triticum aestivum cv. Maris Dove	73±2.9	59±5.4	45±3	65±4.1	54±4.3	42±4.4
Triticum monococcum	80±5.6	70±5	55±3.8	72±4.8	60±3	47±2.5
Triticum turgidum	77±3.2	67±4	58±3.4	65±3.6	50±4	41±3
Avena strigosa	72±3.9	63±3	54±2.5	60±4	48±3.7	40±3.4
Lolium perenne	76±4	70±4.3	58±2.8	62±3	50±3.5	43±3
Mean of means	75.4	64.7	52	64.8	52.4	43.6

Difference of mean of mean in *C.T. Room and in the field (1st, 2nd and 3rd leaves)	1st leaf	2nd leaf	3rd leaf
	85.9	81.0	83.8

*Mean in C.T. Room = 100

room and in the field experiments. Again these results may be significant in the epidemiology of net blotch disease because these non-host plants could be important sources of primary infection of barley crops in that some could occur naturally near fields of barley. Further research is required to assess the importance of non-host plants as alternative host or as overwintering hosts for P. teres in the absence of barley and especially species of wild barley, wild oats and rye grass which are common and widely distributed where barley is grown in the U.K. and elsewhere.

SECTION 7

INDUCED RESISTANCE AND SUSCEPTIBILITY TO *PYRENOPHORA TERES*

A number of investigators working with powdery mildews clearly have demonstrated that pre-inoculation with a compatible pathogen rendered the plants susceptible to a previously incompatible pathogen. Tsuchiya and Hirata (1973) showed that preliminary infection of barley with a compatible race of *Erysiphe graminis* made the leaves susceptible to 42 species (out of 45 species tested) of powdery mildew fungi pathogenic to dicotyledons.

Ouchi and Oku (1981) reported that when the abaxial surface of barley leaves was inoculated with a compatible race of *E. graminis hordei*, susceptibility in the adaxial epidermis to incompatible races of the same pathogen was established within three days and had reached a maximum within five days after the primary inoculation. Ouchi *et al.* (1974, 1982) extensively studied induced susceptibility and resistance using the barley and wheat - *E. graminis* systems and made the following interesting observations:

1. Susceptibility to an originally avirulent race of *E. graminis* or resistance to an originally virulent race was induced in barley leaves by prior inoculation of a virulent or an avirulent race respectively.
2. Susceptibility of barley leaves induced by prior inoculation with a virulent race of *E. graminis* was

effective in promoting additional development not only of avirulent races of that fungus but also of other powdery mildew fungi and of necrotrophic non-pathogenic fungi.

As regards induction of resistance in plant by pathogen, Littlefield (1969) found that infection of flax by Melamspora lini was reduced by pre-inoculating the leaves with an avirulent race of the same fungus. Elliston et al. (1971) reported similar induction of resistance in leaves of bean Phaseolus vulgaris by a cultivar non-pathogenic race of Colletotrichum lindemuthianum against a cultivar pathogenic race of the same fungus.

There are many other examples in the literature of induced resistance but many fewer examples of induced susceptibility. In both cases the effects do not extend far beyond the point of first inoculation that is, the effects are local; systemic effects, at a distance from the first inoculation have been reported much less frequently. The outstanding work on induced systemic resistance is that of Kuć and his co-workers with Colletotrichum lagenarïum infecting Cucumis spp, and Peronospora tabacina infecting tobacco (Kuć, 1982).

In the light of the above-mentioned investigations, induced resistance and susceptibility both local and systemic in barley using virulent and less virulent isolates of Pyrenophora teres were studied.

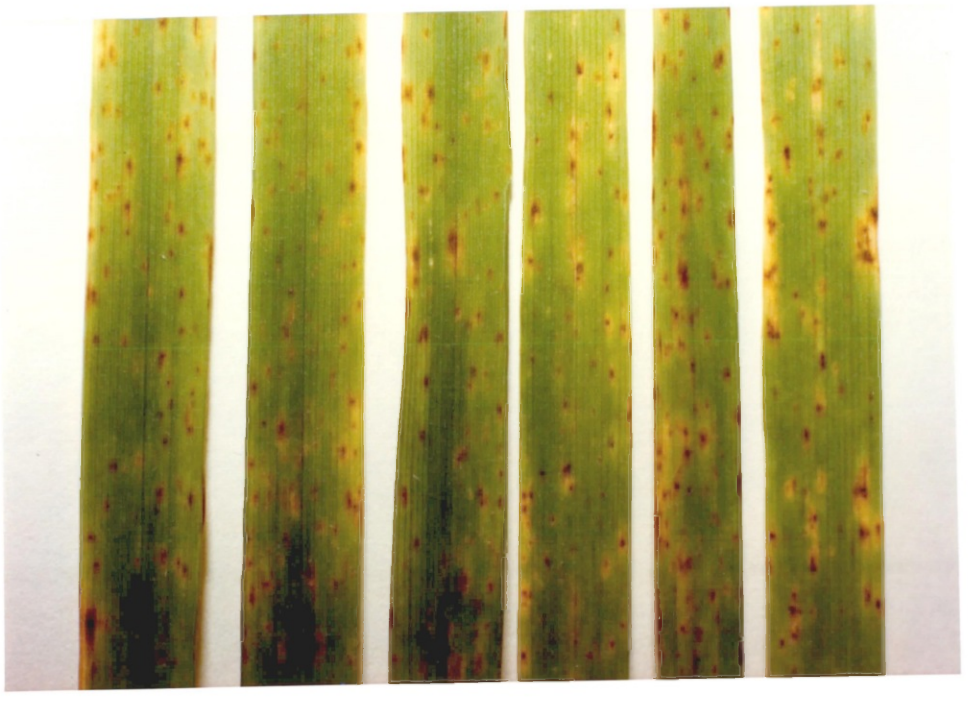
Experiment 1: Systemic induction of resistance and susceptibility in leaves of barley cv. Sonja.

Three leaf stage seedlings (22 days old) of barley cv. Sonja were used in this experiment with two isolates of Pyrenophora teres. A less virulent isolate (BNS-82-70) which causes a spot lesion on barley cv. Sonja (Plate 8) was used to induce resistance, and a virulent isolate (Pt.80-12) of P. teres which cause net form symptoms on barley cv. Sonja (Plate 9), was used to induce susceptibility. Inoculation and incubation of the seedlings (see 3.3), were done in a constant temperature room at 21 ± 1°C, provided with a 12 h. cool light photoperiod daily cycle. Each treatment comprised 10 seedlings. In the first group of seedlings (Figure 6), first leaves were pre-inoculated with suspensions (5 x 10^4 spore/ml) of spores of a less virulent isolate. The second and third leaves were enclosed by polythene bags during the pre-inoculation. In the second group of seedlings first leaves were sprayed with sterile distilled water containing Tween 20, and were used as controls. Five days later the second and third leaves of the twenty seedlings were inoculated with a suspension (5 x 10^4 spore/ml) of spores of the virulent isolate (Pt.80-12) of P. teres. In the third group of seedlings first, second and third leaves were inoculated simultaneously.

The first leaves were inoculated with the less virulent isolate and the second and third leaves were inoculated with

Plate 8. Spot form symptoms on first leaves (21 day old) of barley cv. Sonja, inoculated by spraying in a CT room at 21 ± 1°C, with a suspension (5×10^4 spore/ml) of spores of *P. teres*, less virulent isolate (BNS-82-70). Photograph taken 10 days after inoculation.

Plate 9. Net form symptoms on first leaves (21 day old) of barley cv. Sonja, inoculated by spraying in a CT room at 21 ± 1°C, with a suspension (5×10^4 spore/ml) of spores of *P. teres*, virulent isolate (Pt.80-12). Photograph taken 10 days after inoculation.



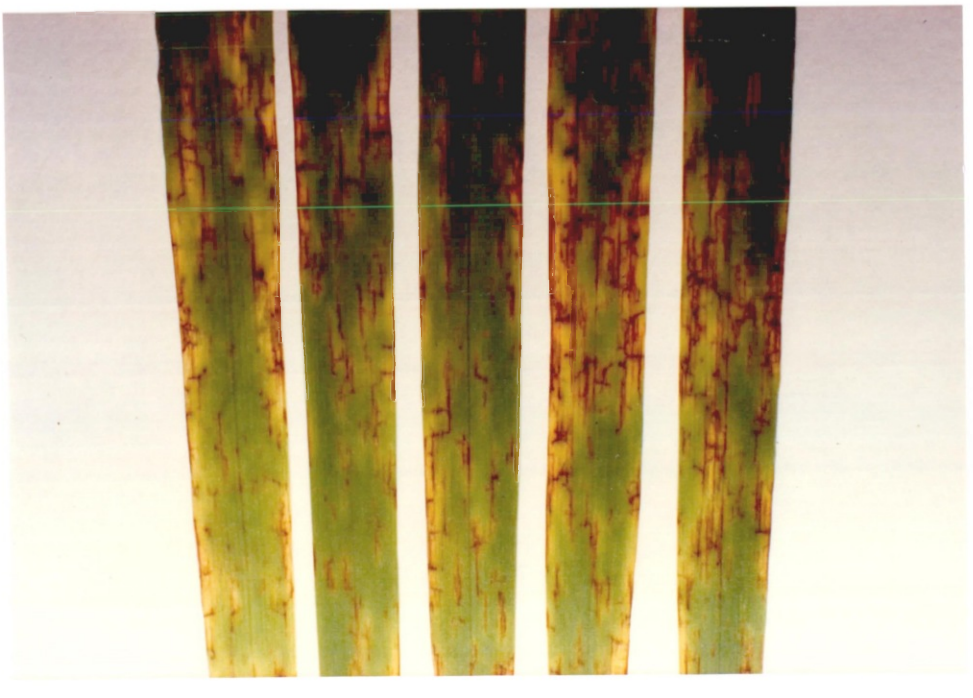
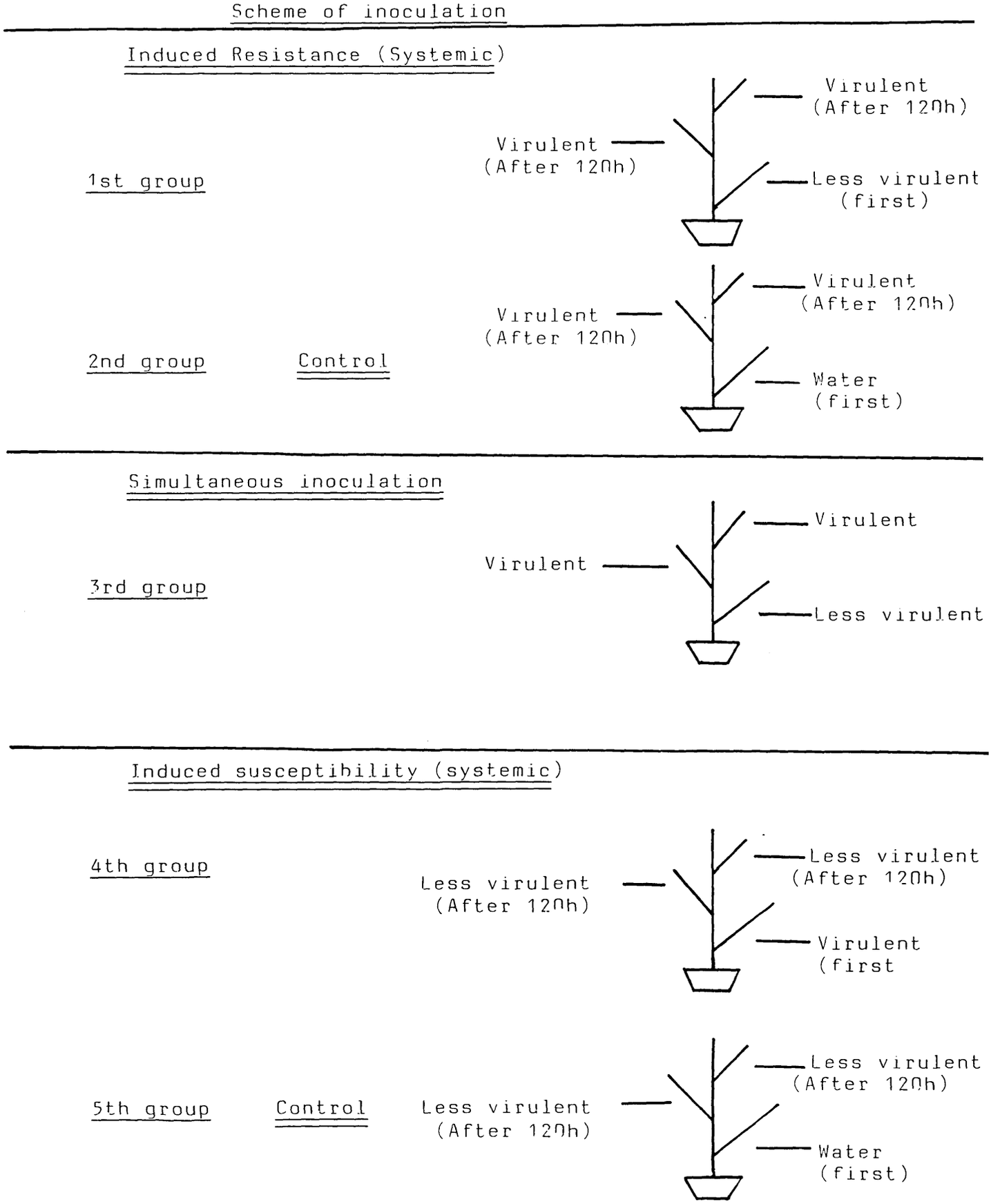


Figure 6: Induced Systemic Resistance and Susceptibility in Seedlings

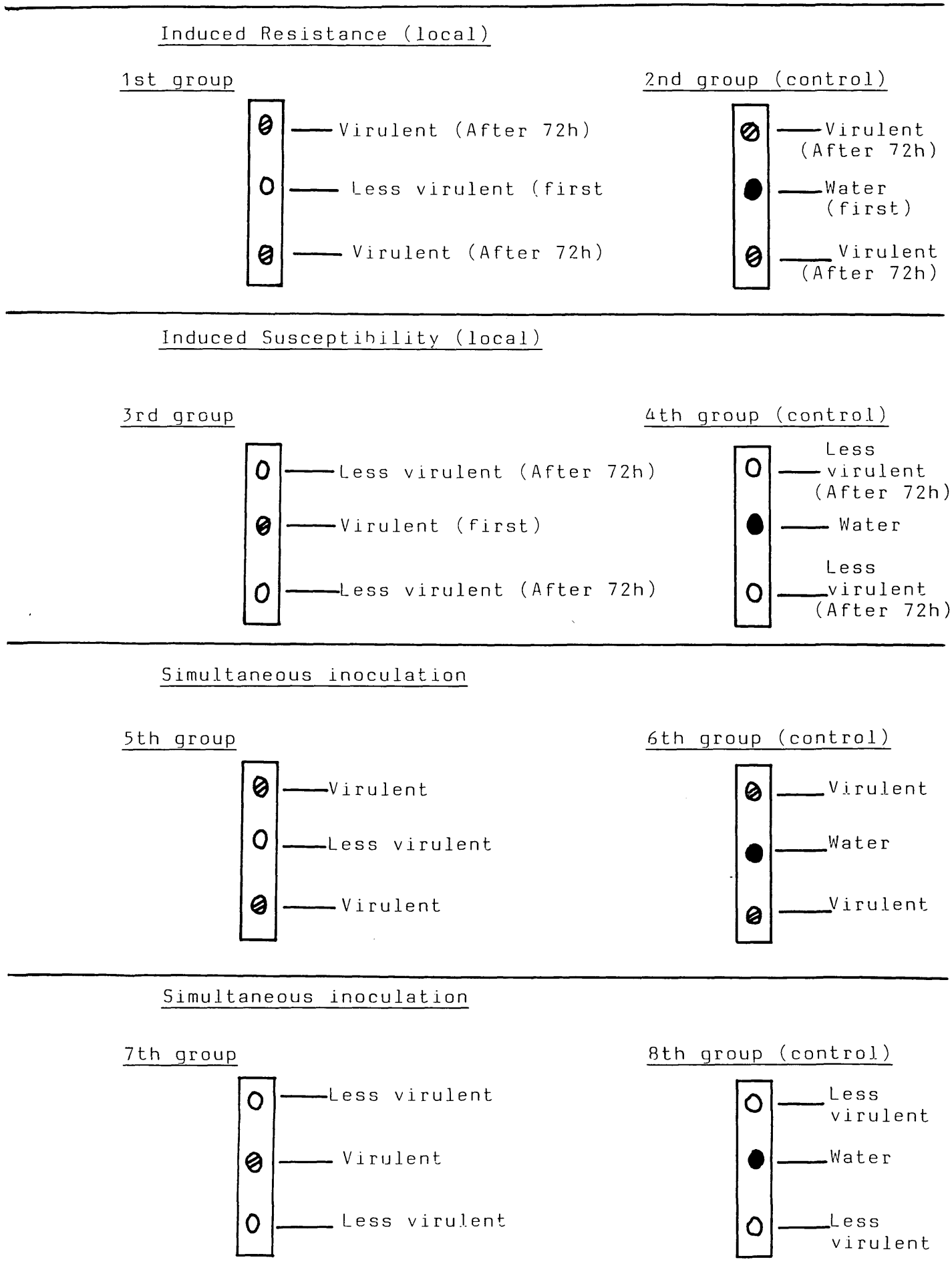


virulent isolate. The leaves received similar volumes of suspension of spores and the same concentration of spores in all inoculations with the two isolates of P. teres. In the fourth group of seedlings, first leaves were pre-inoculated with the virulent isolate and the second and third leaves were inoculated later with the less virulent isolate. In the fifth group of seedlings, first leaves were sprayed with sterile distilled water containing Tween 20; five days later the second and third leaves were inoculated with suspension of spores of the less virulent isolate. The mean percentages of leaves with net or spot lesions were assessed ten days after inoculation, using the method described in 4.2.1 and 4.2.3.

Experiment 2: Local induction of resistance and susceptibility in leaf segments of barley cv. Sonja.

Eighty segments each 5 cm long, were taken from the first leaves of three leaf stage (22 days old) seedlings of barley cv. Sonja. Each treatment comprised ten segments. In the first group, segments were inoculated at the centres with a droplet (10 µl) of suspensions (5×10^4 spore/ml) spores of the less virulent isolate. Sterile distilled water containing Tween 20, was used in the second group as controls (Figure 7). The segments were floated on 4 ml. 50 ppm benzimidazole solution in plastic boxes (see 3.2), and incubated at 21 ± 1 °C in the incubator.

Figure 7: Induced Local Resistance and Susceptibility in Segments



Seventy two hours later, the segments were inoculated separately 1.5 cm. from the first inoculation with two droplets (10 µℓ each) of suspensions (5×10^4 spore/ml) of spores of the virulent isolate.

In the third group of segments, the virulent isolate was used first and the less virulent isolate was used 72 h. later and the fourth group of segments were the controls in which water was used first. The fifth and sixth group of segments corresponded to the first and second groups, and the seventh and eighth group corresponded to the third and fourth groups but with simultaneous inoculation. Areas of lesions were assessed 72 h. after inoculation by the method described in 4.2.1 and 4.2.3.

Results

These are given in Tables 21 and 22 and are summarised in Table 23. In seedlings, pre-inoculation with the less virulent isolate induced 17% and 9% resistance to the virulent isolate in second and third leaves respectively. Pre-inoculation with the virulent isolate induced 24% and 18% susceptibility to the less virulent isolate in second and third leaves respectively. There was little or no induction of resistance of of susceptibility when the less virulent and virulent races were inoculated together. Induction of resistance or of susceptibility did not change the spot or net form of the lesions caused respectively

Table 21: Induction of resistance and susceptibility in seedling of barley cv. Sonja with isolates of P. teres

Leaves	Inoculation								Inoculation			
	1	2	1	2	1	2	1	2	1	1	1	1
	LV[3]	V[4]	Water	V	V	LV	Water	LV	LV	V	V	LV
1st leaf	37±2	-	0		77±4	-	0		35±2.4	-	80±5	
2nd leaf	-	50±3.5	-	60±3.3	-	31±3	-	25±2.5	-	62±3.8	-	27±2.4
3rd leaf	-	32±2	-	35±2.5	-	20±2.1	-	17±1.7	-	35±3.2	-	18±1.5

1,2- First, second inoculation 120 h. later

V- virulent

LV- less virulent

3- Mean percentage of 10 leaves with spot lesions

4- Mean percentage of 10 leaves with net lesions

Table 22: Induction of resistance and susceptibility in leaf segments of barley cv. Sonja with isolates of *P. teres*

Leaves	Inoculation								Inoculation			
	1	2	1	2	1	2	1	2	1	1	1	1
	LV[3]	V	Water	V	V	LV	Water	LV	LV	V	V	LV
Centre	13 ± 0.9	-	0	-	27.8 ± 2.9	-	0	-	13.1 ± 0.3	-	28.7 ± 1.5	-
Top	-	20 ± 1.4	-	28 ± 1.5	-	17 ± 1.1	-	13.3 ± 0.9	-	29.4 ± 3	-	13.8 ± 0.6
Bottom	-	18.6 ± 1.8	-	26.8 ± 1.2	-	16.5 ± 1.2	-	12.9 ± 1.1	-	29.2 ± 1.3	-	13.2 ± 0.4

1, 2 - first, second inoculation 72h later.

3 - Mean area (mm^2) of 10 lesions.

Table 23: Percentage of induced resistance and susceptibility systemically and locally by inoculation after an interval and simultaneously of leaves and leaf segments of barley cv. Sonja with isolates of P. teres. Summary of results from Tables 21, 22.

	% of induced resistance and susceptibility on 2nd and 3rd leaves of seedlings.				% of induced resistance and susceptibility on lesions/segments	
	Inoculation after interval 120h		Inoculation simultaneously		Inoculation after after interval 72h	Inoculation simultaneously
	2nd leaf	3rd leaf	2nd leaf	3rd leaf	Lesion size	Lesion size
Induced Resistance	-17	-9	-3	0	-30	7
Induced Susceptibility	24	18	8	6	28	3

following inoculation with the less virulent or the virulent isolate. In leaf segments, pre-inoculation with the less virulent isolate decreased the size of lesions caused by the virulent isolate by about 30%. Pre-inoculation with the virulent isolate increased by about the same figure the size of lesions caused by the less virulent isolate. When the isolates were inoculated together there was little effect on susceptibility to the less virulent isolate and a slight and anomalous increase and not the expected decrease of about 7% in size of lesions caused by the virulent isolate.

Discussion

Pre-inoculation of first leaves of seedlings and on the centres of leaf segments with the less virulent or the virulent isolate had quite detectable effects in increased resistance or susceptibility to the virulent and less virulent isolate. These results agree with those obtained with other diseases but the increases are not nearly so striking as are many that have been reported. It seems unlikely that the increases are sufficiently large to be important epidemiologically for example in crops of multilines or mixed cultivars. The effects are also likely to be less significant because they were not obtained when the less virulent and virulent isolate were inoculated together.

SECTION 8

PATHOGEN/HOST AND NON-HOST INTERACTIONS

Interactions between susceptible and resistant cultivars of barley and Pyrenophora teres have been studied by a number of investigators aiming to characterize cellular response to the pathogen. Conidia of P. teres were found to germinate, form appressoria and then penetrate host epidermal cells within 24 h. after inoculation (Keon and Hargreaves, 1983). Entry of the pathogen into the host (cv. Parkland), rarely occurred through stomata. Normal entry was by penetration into epidermal cells. After penetration, the fungus produced a large infection vesicle which gave rise to one or sometimes two intracellular hyphae which spread through the leaf tissue intracellularly for one or two cell layers and intercellularly thereafter (Van Caeseele and Grumbles, 1977). Spore germination, germ tube growth and numbers of penetrations of leaves of resistant and susceptible cultivars of barley were found not to be significantly different (Keeling, 1965). Recently, Hargreaves (1982), found that resistance of oat (Avena sativa cv. Maris Osprey) to P. teres could be due to the formation of papillae and cell wall appositions deposited on the inner surfaces of the cell walls adjacent to appressoria.

In the light of these observations, reactions of host

and non-host plants to infection by P. teres (virulent isolate, Pt.80-12) were studied using detached leaf segments. Spore germination, germ tube growth and number of penetrations were examined on barley (susceptible and resistant cultivars), wild barley, oat, rye, wheat and rye grass (Lolium perenne). Percentages of dark green, browning and dead cells were also estimated microscopically.

Experiment: Pathogen/host and non-host interactions.

Segments 5 cm. long were taken from the first leaves of three leaf growth stage seedlings of host and non-host plants (Table 21). Segments were inoculated with 5 µl droplets of suspensions (5 x 10^4 spore/ml) of spores of P. teres, virulent isolate (Pt.80-12); segments were incubated on surfaces of 4 ml. 50 ppm benzimidazole solution in plastic boxes at 21 ± 1°C (3.2). Germinated spores and lengths of germ tubes were estimated per ten microscopic fields (40 x 15X), 16 h. after inoculation (4.1.1.).

Numbers of appressoria and penetrations were estimated 24 h. after inoculation (4.1.2). The percentages of dead, browning and dark green cells were estimated per ten microscopic fields (40 x 15X), 120 h. after inoculation (4.1.3). Sporulation on certain host and non-host plants were estimated after incubating leaf segments on sterile moist filter paper in a Petri dish under 12 h. near UV light at 21 ± 1°C for ten days (5.1).

Results

On inoculated segments of host and non-host plants, most conidia had germinated from one to three cells of the conidium including in most observations the end cells of the conidium (Plate 10). The germ tubes had grown to various lengths before producing appressoria. Usually, penetration occurred 24 h. after inoculation, and mostly occurred through the epidermal cell wall but some penetrations occurred through stomata (Plate 11). The percentage of spore germination, length of germ tubes and appressorial formation on host and non-host plants leaf segments were not significantly different except in rye and rye grass where they were less (Table 24). As regards the percentage of penetration 24 h. after inoculation, barley and wild barley (H. spontaneum lines 14591, 5753) showed higher percentages than did wild barley (H. murinum), wheat, oat, rye and rye grass (Table 24).

Papillae were observed frequently in oat and wheat, on the inner surface of the cell walls adjacent to the appressoria (Plate 12), and rarely on barley and wild barley. Dark green cells were observed in the sub-epidermal tissue of rye, rye grass and oat as a response to the entrance of the pathogen.

Dead and browning invaded cells of barley, wild barley and wheat were disrupted and disordered with a complete loss

Table 24: Percentages of germinated spores, lengths of germ tubes, appressoria and penetrations from appressoria on detached leaf segments of host and non-host plants inoculated with *P. teres* virulent isolate (Pt-80-12). Estimations for 10 fields of view

Plants	1 Mean % germinated spores at 16h	2 Mean length of germ tubes (µ) at 16h	3 Mean % appressoria at 24h	4 Mean % of penetrations from appressoria at 24h	5 Number of penetrations as % of spores
Barley cv. Sonja	81 ± 3.7	1125 ± 13.5	69 ± 3.2	58 ± 4	40
Barley cv. Maris otter	77 ± 2.3	1137 ± 17	60 ± 4	47 ± 3.3	28
Barley line CI 9820	74 ± 3.8	1073 ± 11.2	63 ± 3.4	50 ± 3	32
Barley line CI 4795	72 ± 3.5	1081 ± 14	56 ± 3	44 ± 3.5	25
Hordeum spontaneum line 14591	76 ± 3	1093 ± 13.3	61 ± 3.9	56 ± 4.1	34
Hordeum spontaneum line 5753	73 ± 2.2	1087 ± 16	59 ± 4.1	51 ± 3.7	30
Hordeum murinum	70 ± 3.9	1079 ± 12.4	54 ± 3.2	33 ± 3.6	18
Wheat cv. Maris Dove	68 ± 4.2	1082 ± 14.7	49 ± 3	27 ± 2.1	13
Oat cv. Peniarth	72 ± 3.3	1072 ± 15	55 ± 2.6	20 ± 2.8	11
Rye cv. Rheidol	64 ± 5.7	1067 ± 11.8	37 ± 2.5	12 ± 2.3	4
Rye grass (*Lolium perenne*)	61 ± 3.1	1061 ± 10.5	36 ± 2.3	17 ± 1.2	6

1. Germinated as % total spores.
2. Length of germ tubes from germinated spores.
3. Spores with appressoria as % total spores.
4. Penetration as % appressoria.
5. Number of penetrations as % of spores.

Plate 10. Formation of appressoria on surface of leaf segment of wild barley (Hordeum murinum), 24h after inoculation with a droplet (5 µℓ) of suspension (5×10^4 spore/ml$^-$) of spores of P. teres, virulent isolate (Pt.80-12). Magnification X600.

A. Conidium

B. Germ tube

C. Appressorium

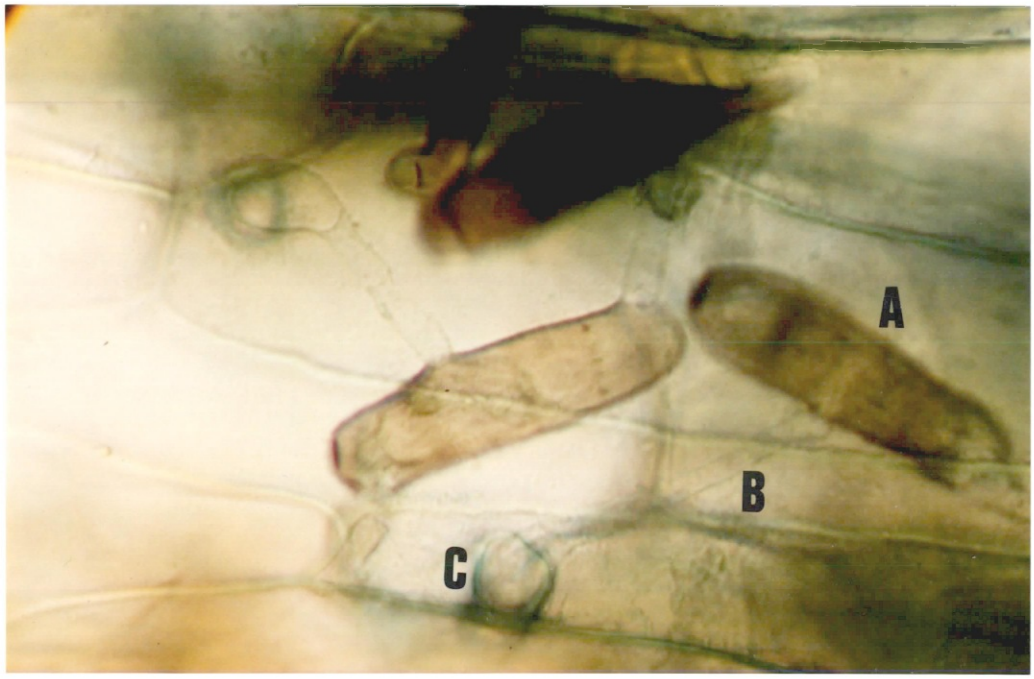

A
B
C

Plate 11. Penetration from spores through a stoma on a leaf segment of barley cv. Sonja, 24 h. after inoculation with *P. teres*, virulent isolate (Pt.80-12). Magnification X400.

A. Conidium

B. Germ tube

C. Appressorium

D. Penetration through stoma

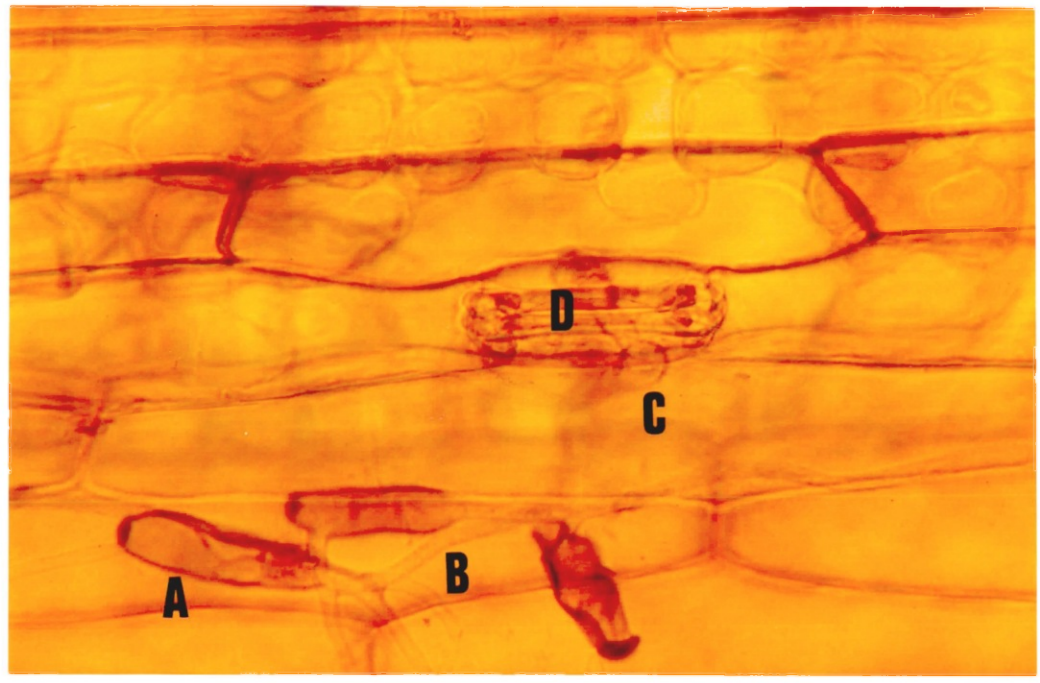

D
C
A
B

Plate 12. Formation of papilla on the inner surface of the cell wall adjacent to an appressorium on leaf segment of wheat cv. Maris Dove, 24 h. after inoculation with *P. teres*, virulent isolate (Pt.80-12).

Magnification X400

A. Conidium

B. Germ tube

C. Appressorium

D. Papilla

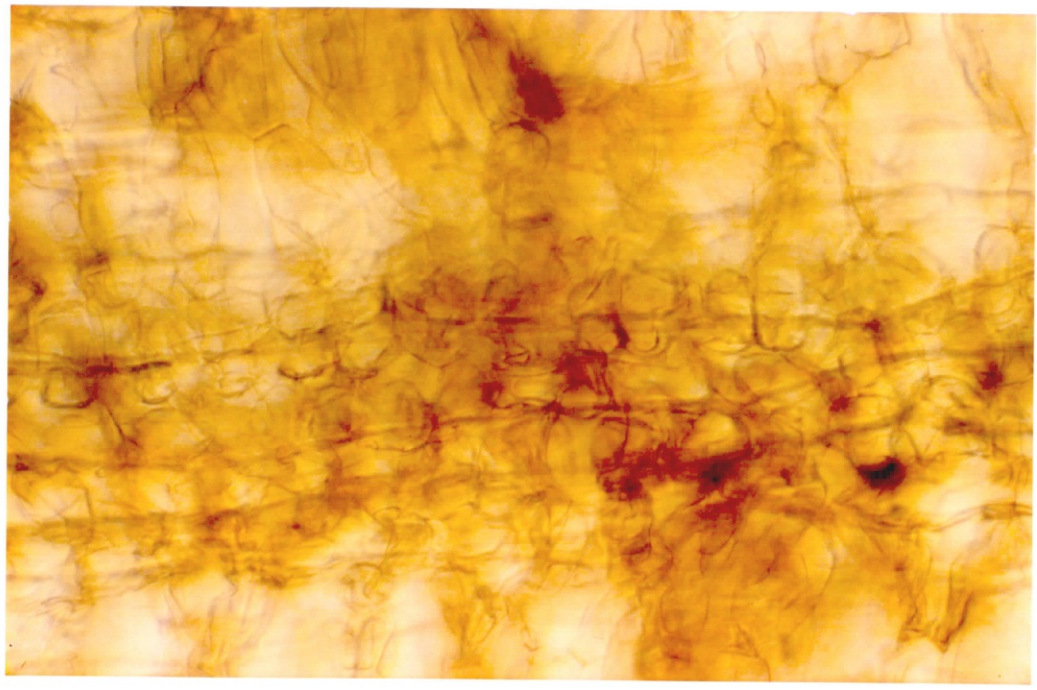

A
B
C
D

of membrane integrity, 120 h. after inoculation (Plates 13, 14). The percentage of dead and browning cells were higher in barley than in wild barley and wheat (Table 25).

P. teres, virulent isolate 80-12 was observed to sporulate abundantly on leaf segments of barley, moderately on wild barley and wheat and poorly on rye grass, when the inoculated segments were incubated under 12 h. near UV light followed by 12 h. of darkness at 21 ± 1°C for 10 days (Table 26).

Plate 13. Dead and browning invaded cells of barley cv. Sonja, 120 h. after inoculation with P. teres, virulent isolate (Pt.80-12). Magnification X400.

Plate 14. Healthy cells of barley cv. Sonja (control), 120 h. after treatment with a droplet of sterile distilled water. Magnification X400.



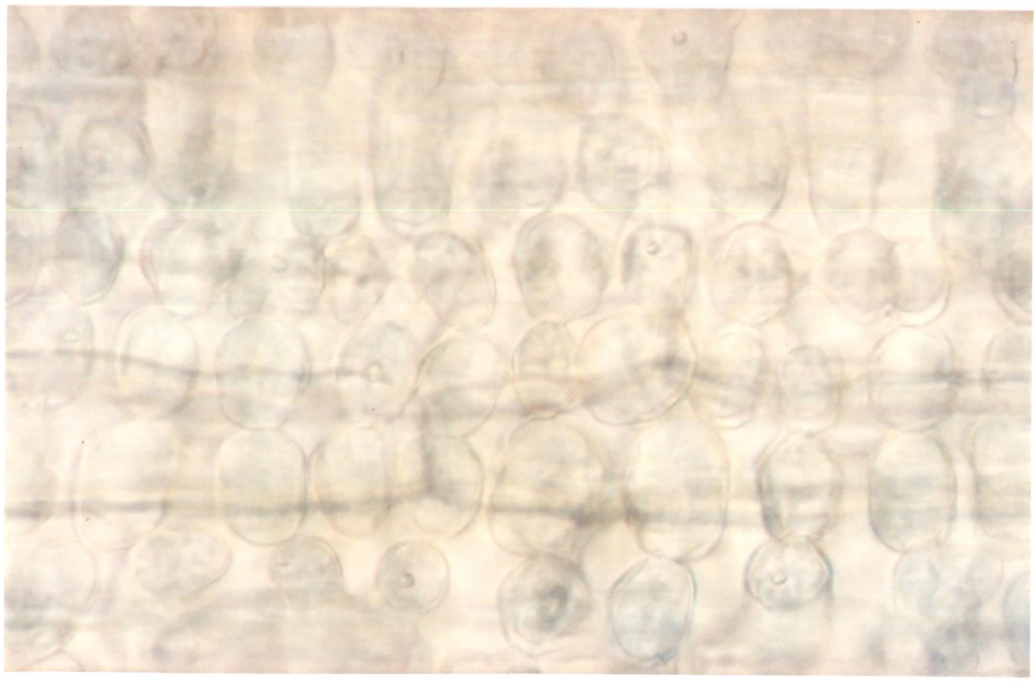


Table 25: Percentages of dead, browning and dark green cells in host and non-host plants inoculated with spores of virulent isolate (Pt.80-12) of *P. teres*.

Observations and estimations done 120h after inoculation for 10 fields of view.

Plants	Mean of dead cells	Mean of browning cells	Mean of dark green cells	Mean of healthy cells	Mean % of dead and browning cells	Mean % of dark green cells
Barley cv. Sonja	32 ± 4	8 ± 2	none	5 ± 2	89 ± 5	none
Barley cv. Maris Dove	20 ± 2.8	10 ± 1.6	none	12 ± 2.2	71 ± 4.6	none
Barley line CI. 9820	14 ± 2.6	11 ± 1.9	none	24 ± 3	51 ± 5.5	none
Barley line CI. 4795	10 ± 2.1	8 ± 1.7	none	28 ± 2.5	39 ± 3.7	none
Hordeum spontaneum line 14591	15 ± 1.8	13 ± 2.3	none	26 ± 3.8	52 ± 4.7	none
Hordeum spontaneum line 5753	12 ± 2	10 ± 1.5	none	33 ± 2.9	40 ± 4.5	none
Hordeum murinum	9 ± 1.6	12 ± 1.4	none	27 ± 2.1	44 ± 2.3	none
Wheat cv. Maris Dove	7 ± 1.4	10 ± 1.7	none	31 ± 3.2	35 ± 3.7	none
Oat cv. Peniarth	none	none	19 ± 2.2	37 ± 3.2	none	34 ± 4.4
Rye cv. Rheidol	none	none	15 ± 1.3	44 ± 4	none	25 ± 3.5
Rye grass *Lolium perenne*	none	none	11 ± 1.4	45 ± 3	none	20 ± 4

Table 26: Sporulation on leaf segments of host and non-host plants inoculated with *P. teres* virulent isolate (Pt.80-12), and incubated at 21 ± 1°C under 12h near U.V. light photoperiod for 10 days.

Plants	Number of spores[2] per 5µl droplets	Number of spores per 25 ml	Number of spores per lesion
Barley cv. Sonja [1]	98 ± 7	49×10^4	12564
Barley cv. ND B112	75 ± 7	37×10^4	9487
Wild barley (*H. murinum*)	46 ± 3	23×10^4	5897
Wheat cv. Maris Dove	33 ± 4	16×10^4	4102
Rye grass *L. perenne*	8 ± 2	4×10^4	1025

(1) Spores from 39 lesions suspended in 25 ml. water.

(2) Mean of 10 replicates.

Discussion

No significant differences were observed for spore germination and growth of germ tubes between host and non-host plants, so it is clear that, viability of spores was not affected by the host.

As regards appressorial formation, the spores behaved somewhat differently on both host and non-host plants. Some spores produced only one germ tube ending with or without appressorium whereas other spores were observed producing 2-3 germ tubes ending with 1 to 3 appressoria.

Papillae were observed frequently in leaf segments of oat and wheat but were rarely observed in barley and wild barley. Some observations showed failure of spores to penetrate through the papillae, so it is possible that the papillae might have a role in the mechanical resistance of these non-host plants against entrance of the pathogen.

Another type of cellular reaction was observed on oat, rye and rye grass. In these, the response of the cells to entry of the pathogen was to become darker green in colour in what could be a hypersensitive reaction. The cells adjacent to the dark green cells were lighter green healthy cells in colour. The growth of the penetrating hyphae was restricted to the darker green area.

SECTION 9

POSSIBLE ROLE OF PSEUDOTHECIA IN THE EPIDEMIOLOGY OF NET BLOTCH

Sclerotium-like bodies as named by Webster (1951) and Butler (1955), or pseudothecia by Mathre (1982), which are small black bodies (1-2 mm. diameter) covered with dark setae and have been observed on residues of host tissue infected by P. teres and occasionally in cultures of P. teres (Mathre, 1982). Webster (1951) has distinguished between two kinds of sclerotial-like bodies found on pieces of barley stubble incubated on damp sand in Petri dishes, one kind produced numerous conidiophores bearing conidia whereas the second gave rise after a few days to beak-like processes surrounded by black hairs and containing asci with three to eight ascospores. In view of these observations, a short study was made of pseudothecia produced by the virulent isolate (Pt.80-12) of P. teres used in this research.

Experiment 1: Development of pseudothecia on PCA, straw of barley and on leaf segments of host and non-host plants

The following were used: 10 day cultures of P. teres, virulent isolate (Pt.80-12) on PCA, infected segments (4-6 cm. long) of leaves of host and non-host plants, and straw (8 cm. long) of infected barley. The leaf segments and the

barley straw were well washed with sterile distilled water, and then placed on sterile moist filter paper in Petri dishes and incubated in a 12 h. (cool light) photoperiod at 21 ± 1°C for thirty days. Numbers of pseudothecia in five Petri dishes were counted as were numbers on fifty leaf segments and on fifty straws of barley. The dimensions of the ten biggest and the ten smallest pseudothecia on PCA, leaf segments and on straw, were measured (Table 27).

Experiment 2: Pathogenicity of pseudothecia on barley cv. Sonja.

Pseudothecia were taken from leaf segments of barley (cv. Sonja), wheat (cv. Maris Dove), wild barley (Hordeum murinum), rye grass (Lolium perenne) and straw of barley (cv. Sonja), and placed on PCA and incubated at 18 ± 1°C for five days during which they germinated and produced colonies. The cultures then were incubated in 12 h. of cool light photoperiod at 21 ± 1°C for ten days. Conidia were produced abundantly by all colonies. Suspensions (5×10^4 spore/ml) of spores were prepared (see 2.6).from these cultures. Three leaf growth stage seedlings of barley cv. Sonja were inoculated with these suspensions in a constant temperature room (see 3.3). For controls, seedlings at the same growth stage were sprayed with suspension (5×10^4 spore/ml) of spores taken from ten day old cultures of the original P. teres, virulent isolate (Pt.80-12). Ten days after inoculation, symptoms and damage on leaves were recorded.

Table 27: Numbers and dimensions of pseudothecia of *P. teres* virulent isolate (Pt.80-12) on PCA, leaf segments of host and non-host plants and on barley straw after 30 days incubation at 21 ± 1°C on moist sterile filter paper

Substrate	Mean number of pseudothecia	Mean range of dimensions of pseudothecia (mm)
PCA	168 ± 24.5(1)	(1.25 x 0.95)(4) -(0.73x0.56)(5)
Leaf segments of barley cv. Sonja	27 ± 8.6(2)	(0.92 x 0.81) -(0.47x0.41)
Leaf segments of Wheat cv. Maris Dove	21 ± 7.5	(0.82 x 0.76) -(0.42x0.30)
Leaf segments of Wild barley (*H. murinum*)	25 ± 9.1	(0.75 x 0.68) -(0.39x0.32)
Leaf segments of rye grass (*Lolium perenne*)	31 ± 8.2	(0.77 x 0.69) -(0.35x0.26)
Straw of barley cv. Sonja	29 ± 5.6(3)	(0.74 x 0.70) -(0.42x0.35)

(1) Mean of 5 replicates (5 Petri dishes)
(2) Mean of 50 leaf segments (4-6 cm. long)
(3) Mean of 50 barley straws (8 cm. long)
(4) Mean of 10 largest pseudothecia
(5) Mean of 10 smallest pseudothecia

Results

Pseudothecia of P. teres, virulent isolate (Pt.80-12), developed on PCA (Plate 15), leaf segments of barley cv. Sonja (Plate 16), wild barley (Plate 17), wheat cv. Maris Dove (Plate 18), rye grass (Plate 19) and on straw of barley cv. Sonja (Plate 20), after thirty days incubation on sterile moist filter paper at 21 ± 1 °C. All pseudothecia were black, hard and spherical in shape, variable in size, bearing stiff, black hairs all over their surface. There were no pronounced differences in the numbers and dimensions of pseudothecia that developed on leaf segments of host and non-host plants and on barley straw (Table 27). Numbers decreased in the following order: rye grass, barley straw, barley, wild barley (H. murinum) and wheat. The largest pseudothecia were formed on PCA. They were smaller on the leaf segments and the straw (Table 27). Inoculation of barley seedlings with spores of the original isolate and from cultures from pseudothecia caused similar and typical net blotch symptoms to develop.

Plate 15. Formation of pseudothecia of P. teres, virulent isolate (Pt.80-12), on potato carrot agar, after 30 days incubation at 21 ± 1°C.

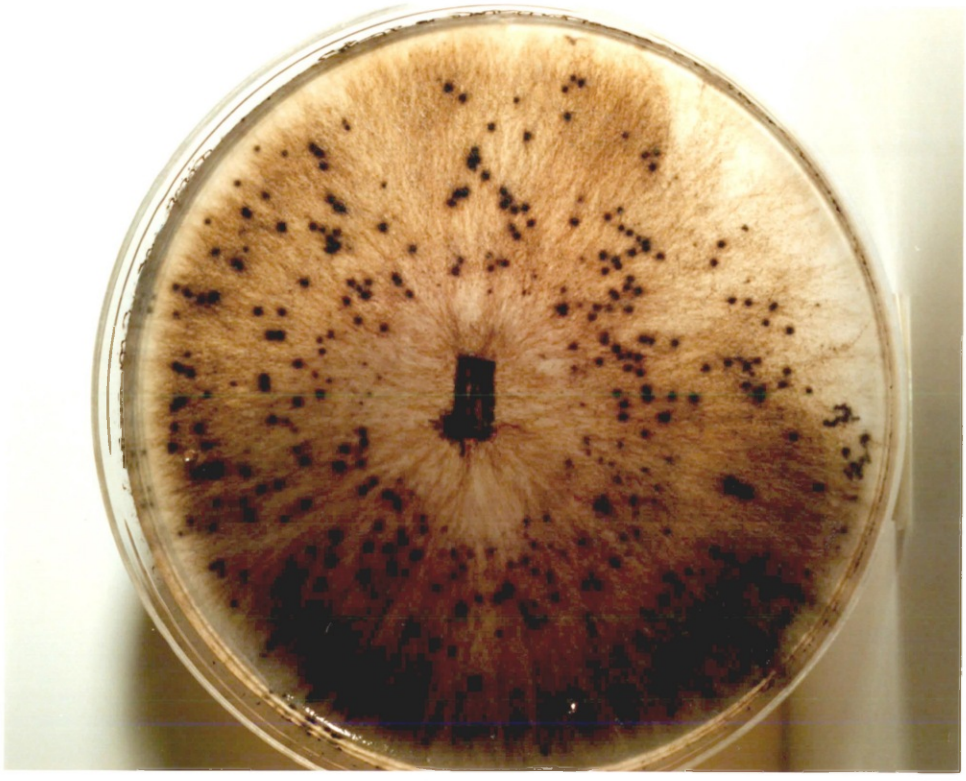


Plate 16. Pseudothecia of *P. teres*, virulent isolate (Pt.80-12), on leaf segments of barley cv. Sonja, after 30 days incubation on sterile filter paper at 21 ± 1°C.

Plate 17. Pseudothecia of *P. teres*, virulent isolate (Pt.80-12), on leaf segments of wild barley (*Hordeum murinum*) after 30 days incubation on sterile filter paper at 21 ± 1°C.

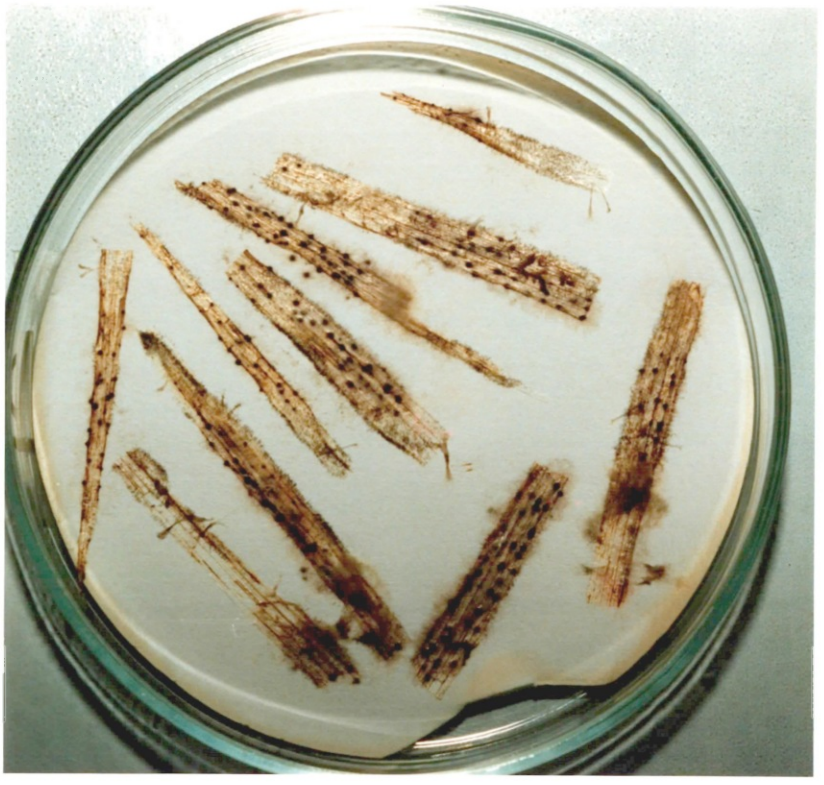




Plate 18. Pseudothecia of *P. teres*, virulent isolate (Pt.80-12), on leaf segments of wheat cv. Maris Dove, after 30 days incubation on sterile filter paper at 21 ± 1°C.

Plate 19. Pseudothecia of *P. teres*, virulent isolate (Pt.80-12), on leaf segments of rye grass (*Lolium perenne*), after 30 days incubation on sterile filter paper at 21 ± 1°C.

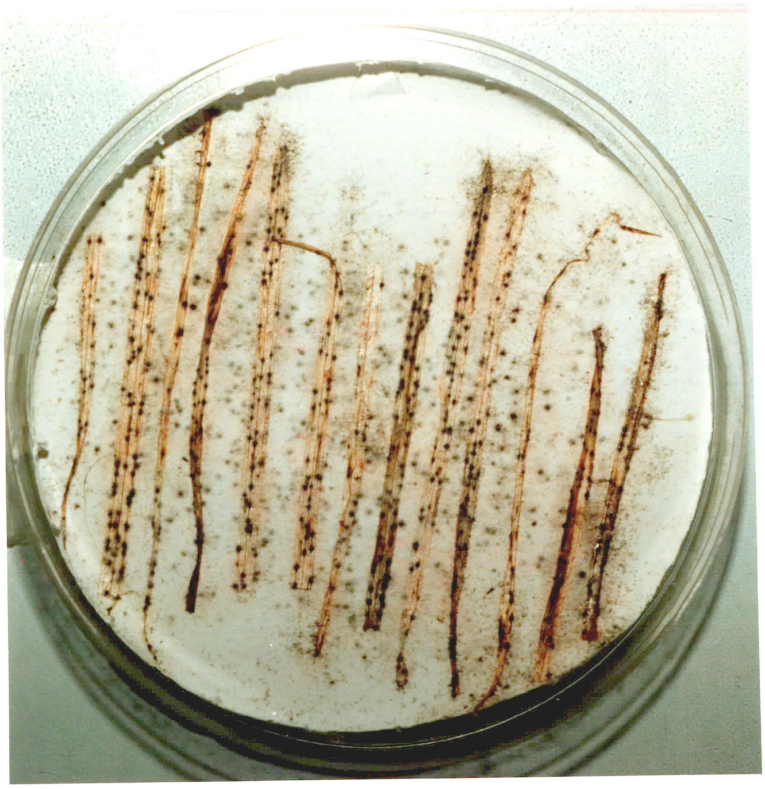




Plate 20. Pseudothecia of *P. teres*, virulent isolate (Pt.80-12), on straw of barley cv. Sonja after 30 days incubation on sterile filter paper at 21 ± 1°C.



Discussion

Infected leaf segments of host and non-host plants and straw of infected barley produced pseudothecia as freely as did cultures of *P. teres* on PCA. Pseudothecia germinated readily on PCA and produced conidia, which were as infective as conidia from the original isolate. This indicates the potential for abundant pseudothecial production in the field on leaf fragments and on straw of host and non-host plants. Pseudothecia on stubble of barley on the soil surface have been reported by Webster (1951) and by Mathre (1982), and recently Jordan *et al.* (1984) showed that when straw from a previously diseased crop was incorporated into the soil by conventional ploughing, the incidence and severity of net blotch in the early stage of crop development was greatly decreased. Further research on the role of pseudothecia in the epidemiology of net blotch seems to be justified.

SECTION 10

EFFECT OF AGE OF BARLEY LEAVES ON SUSCEPTIBILITY TO NET BLOTCH

Age of barley plants has been found to affect the incidence and severity of net blotch. Ravn (1900) reported that susceptibility to net blotch increases with the age of the host. Ibrahim and El-Fahl (1977) examined the reactions of cultivars of barley at different ages to infection by P. teres and found that some cultivars showed a transition from susceptibility to resistance with age, whereas other cultivars became more susceptible with age. In a third group of cultivars, both young and old plants were resistant. However, Sheodhan(1963) found that the age of the inoculum was a more important factor than the age of the host in determining virulence and susceptibility. He showed that ten day old inoculum irrespective of the age of the host caused severe infection and disease and that severity decreased considerably with older inocula. These conflicting results led to the following experiments on the susceptibility of leaves of barley of different ages following inoculation with spores taken from 10 day old cultures of P. teres, virulent isolate (Pt.80-12).

Experiment: Susceptibility of barley leaves to infection by P. teres

Three groups of seedlings of barley cv. Sonja were grown

in pots in a glasshouse at intervals in order to obtain a seedling with each of three leaves at the same age (8 days old). The fourth group of plants comprised seedlings with three leaves, the first, second and third leaf being 22, 15 and 8 day old (Figure 8). These four groups of seedlings were inoculated by spraying with a suspension (5×10^4 spore/ml) of spores of P. teres, virulent isolate (Pt.80-12), taken from 10 day old cultures on PCA. Leaves received similar volumes of spore suspensions and there were fifteen leaves for each treatment. Inoculation and incubation of seedlings were as described in 3.3. The percentages of areas of leaves with net blotch lesions were estimated ten days after inoculation using the disease scale given in 4.2.1.

Results

The first, second and third leaves of barley cv. Sonja, at the same age (8 days), reacted very similarly to infection by P. teres. Differences between mean percentages of areas with net blotch symptoms were not significant (Table 28). First, second and third leaves at different ages (22, 15 and 8 day old), differed in susceptibility as was expected from the results of many earlier experiments. The differences were statistically highly significant. The order of decreasing susceptibility was 22 day old- 100, 15 day old-80, 8 day old-47 (Table 28).

Figure 8: Leaf Age of Barley and Susceptibility to Net Blotch. Diagram of Plants.

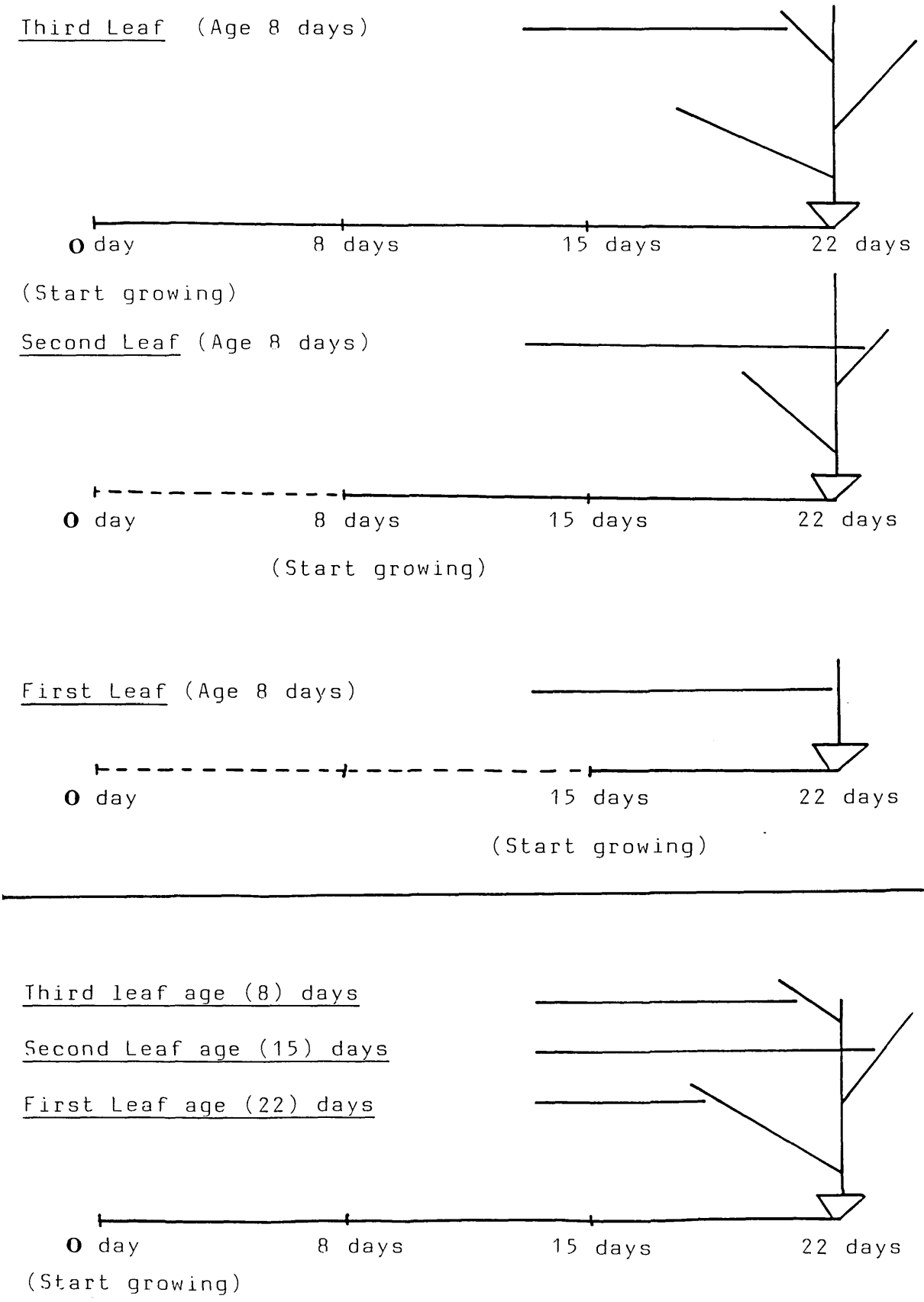


Table 28: Percentage of first, second and third leaves with net blotch lesions in seedlings of barley cv. Sonja inoculated with P. teres, virulent isolate (Pt.80-12). Assessment 10 days after inoculation.

Leaves	Mean % of leaf with net blotch lesions		
	1st leaf	2nd leaf	3rd leaf
Same age (8 days old)	35 ± 3.2 (100)	33.5 ± 2.7 (96)	33 ± 2.4 (94)
Different ages (22, 15 and 8 days old)	75 ± 4.5(1) (100)	60 ± 3.6(2) (80)	35.5 ± 3(3) (47)

(1), (2), (3) - 22, 15 and 8 days old.

Discussion

The susceptibility of the young leaves (8 days old) of barley regardless of their position on seedlings, to infection by *P. teres* was very similar. Young leaves (8 days old), were much more resistant than older leaves (15 and 22 days old). Therefore, differences in resistance of leaves depended on their age and not on their position in the plant. It is, perhaps, surprising that differences in susceptibility were so large for leaves differing in age by 15 days old and when the older leaves were still only 22 days old.

SECTION 11

VIABILITY OF CONIDIA OF P. TERES AFTER LONG TERM PRESERVATION

The long term storage of P. teres is complicated by the fact that cultures lose their sporulating ability with age, and because frequent mycelial transfers result in reversion of cultures to non-sporulating forms (Piening, 1963). The preservation of cultures under paraffin oil did not maintain the sporing ability of the cultures used by Khan and Boyd (1968). But Khan and Boyd (1969), successfully maintained conidia of P. teres, after storage at 5°C in the lyophilized state for six months. When the conidia were then tested on differential cultivars of barley, their virulence was found to be unchanged. Another more convenient method for preserving conidia of P. teres, long term storage at low temperature on leaf segments of barley was therefore examined.

Experiment: Viability of conidia of P. teres after six months of incubation at 2°C on leaf segments

Leaf segments c. 1 cm. long of barley cv. Sonja with typical net form symptoms were taken five days after inoculation of three leaf stage seedlings in a C.T. room with P. teres, virulent isolate (Pt.80-12). The segments were washed with sterile distilled water, then distributed on the

surface of a sterile moist filter paper in disposable Petri dishes (Plate 21). They then were incubated at 18°C until the emergence of visible mycelia on the surface of the segments (3 to 4 days). The segments were then exposed to a 12 h. near UV light photoperiod at 21 ± 1°C for ten days. During this period conidia developed abundantly over the surfaces of the segments. The Petri dishes were then sealed by adhesive cellulose tape and incubated at 2°C for six months. Percentage decreases in spore germination after six months incubation at 2°C, were estimated 16 h. after inoculation of segments (5 cm. long) taken from the first leaf of three leaf growth stage seedlings of barley cv. Sonja (see 3.2, 4.1.1). Conidia 10 days old taken from freshly infected segments of barley cv. Sonja were used as controls.

A conidial suspension (5×10^4 spore/ml) of spores from six month old conidia was prepared (see 2.6), and then sprayed on to three leaf growth stage seedlings of barley cv. Sonja, in a C.T. room at 21 ± 1°C. (see 3.3). The mean percentages of first, second and third leaves with net blotch lesions were assessed ten days after inoculation. Ten replicate seedlings were used. For controls, ten seedlings of barley cv. Sonja were sprayed with a 5×10^4 spore/ml suspension of spores obtained from 10 day old leaf segment cultures. Each leaf of the seedlings received similar volumes of suspension of spores. Percentage reduction in virulence on first, second and third leaves was estimated ten

Plate 21. Leaf segments of barley cv. Sonja with abundant conidia of *P. teres*, virulent isolate (Pt.80-12), on sterile filter paper. Segments had been stored for 6 months at 2°C then kept at 21 ± 1°C for 7 days.



days after inoculation.

Results

The reduction in virulence of P. teres, virulent isolate (Pt.80-12), after six months incubation at 2°C, was 7, 10 and 14% on the first, second and third leaves of barley cv. Sonja, respectively (Table 29). The mean percentages of first, second and third leaves with net blotch lesions were 65, 45 and 30 respectively ten days after inoculation with suspension of spores obtained from six month old inocula, whereas the corresponding mean percentages for controls inoculated with 10 day old spores were 70, 50 and 35 (Table 29). Incubation of spore inocula for six months at 2°C. decreased germination of spores by no more than c. 7%.

Table 29: Estimation of reduction in virulence and in spore germination of *P. teres* (Pt.80-12), after six months of incubation on barley (cv. Sonja) leaf segments at 2°C, in comparison with inocula of 10 day old, incubated at 21 ± 1°C on barley (cv. Sonja) leaf segments. Assessment of mean % of first, second and third leaves with net blotch lesions 10 days after inoculation.

Age of inocula (spores)	Mean % with net blotch lesions						Mean % spore germination	
	1st leaf	% reduction	2nd leaf	% reduction	3rd leaf	% reduction	After 16 h.	% reduction
6 months	65±4.5	7	45±4	10	30±3.7	14	73±2.1	7
10 days	70±4	-	50±4.3	-	35±3.3	-	78±3	-

% reduction in net blotch lesions, spore germination = $100 - \left(\frac{\text{6 month value}}{\text{10 days value}} \times 100\right)$

Discussion

The results in Table 29 showed clearly that virulence of P. teres after six months of incubation at 2°C. decreased very little and that the pathogen continued to cause typical net blotch lesions on first, second and third leaves of barley. Also the decrease in germination of 6 months compared with 10 day old spores was only c. 7%. From these results it is concluded that the pathogen can be conveniently and reliably preserved on leaf segments at low temperatures for at least six months. Further experiments are needed to see whether spores could be stored in this way for much longer periods with no significant loss of viability or change in virulence. But even for six months, storage as infected leaf segments is a more natural and convenient method than storage under oil and is worth studying further for these and for other reasons.

DISCUSSION

Isolates of Pyrenophora teres from infected plants that had been stored in agar culture under mineral oil for about two years were found as a group to differ considerably from a group of isolates from Aberystwyth obtained from debris of recently infected plants, or directly from infected plants at Silwood Park. They grew more slowly on agar plates and under various conditions most of them sporulated much less following growth in culture. A much larger proportion of them were lighter green in colour than were most of the other isolates which were darker green in culture on a nutrient agar. Also, in terms of colonization of detached leaf segments and size of lesions, they were as a group significantly less aggressive than were isolates much more recently isolated from plants. Similar results have been recorded by Singh (1962), Piening (1963), Gray (1966) and Clifford and Jones (1980). The capacity to sporulate and aggressiveness of a few of the stored isolates that were examined increased substantially after passage through susceptible barley plants. Loss of vigour and of virulence of pathogens after prolonged growth in culture is well known, and so is recovery after passage through host plants. But the genetical and physiological basis of these changes are not known and have been little studied.

Interesting results were obtained by exposing cultures

to a variety of light and dark regimes. There was little or no sporulation in continuous cool light or in continuous darkness. Sporulation of recently obtained isolates increased substantially in 12 h. cool light and 12 h. darkness. This treatment had little effect on stored isolates but about half of these and all the other isolates sporulated abundantly in 12 h. near UV light and 12 h. darkness. Even under these favourable conditions, there was little or no sporulation of the other isolates which seemed to have lost the capacity to sporulate which is surprising in view of the good sporulation by other members of the same group. Continuous near UV light had much less dramatic effects in being comparable to 12 h. cool light and 12 h. darkness.

Similar effects for a variety of fungi have been reported by Barnett and Lilly (1950), Leach (1961, 1967), Vargas and Wilcoxson (1967) and by Onesirosan and Banttari (1969). Again, the physiological basis of the effects is not known.

These experiments also demonstrated the striking effects of substrate on sporulation even under the most favourable conditions of 12 h. near UV light and 12 h. darkness. By far the most favourable substrates were autoclaved barley straw and a potato carrot agar, media which provided only limited amounts of readily available energy sources but amounts which

would have been much greater than those available from sterilized filter paper.

Early tests with young seedlings of a range of barley cultivars and with one of the more vigorous of the stored isolates showed that winter cultivars were generally more susceptible than spring cultivars but the number of cultivars tested was too low and the range of conditions was too narrow for this to be regarded as little more than an indication of general differences between the two forms of cultivar.

One of the main purposes of the research was to study the range of parasitism by P. teres within members of the Gramineae and as investigated earlier by Singh (1962), Talvia (1966), Khan and Boyd (1968) and Shipton et al. (1973). A further purpose was to compare results on parasitism of a wide range of plants following inoculation of segments of detached leaves, of leaves of 3 leaf stage seedlings, both in reasonably well controlled conditions in a constant temperature room, and following inoculation of leaves of 3 leaf stage seedlings in the field.

A number of interesting results emerged from these experiments and observations on sizes and types of lesions and on proportions of host tissue occupied by lesions. The first point is that although there were differences in severity of disease recorded under the three conditions, the

differences were not substantial and did not vary with condition. This meant that rankings for severity of parasitism of plants under the three sets of conditions were very similar so that the somewhat unnatural but simple rapid and convenient test of pathogenicity using segments of detached leaves in controlled conditions gave results that compared reliably with those produced by the other tests. However, lesions that developed on leaf segments were different in character from those that developed on seedlings. Typical net blotch lesions did not develop but lesions and symptoms were very similar in seedlings inoculated and infected in a constant temperature room and in the field. This suggests that young seedlings grown in controlled conditions could be used with reasonable confidence in studies of parasitism by P. teres.

A wide range of responses to P. teres was obtained in the many plants that were inoculated with an isolate from Aberystwyth that was highly virulent on barley cultivar Sonja. The range was as follows in terms of decreasing virulence: rapidly spreading net blotch necrotic lesions occupying much of the leaf; less rapidly spreading spot necrotic lesions occupying less of the leaf; limited lesions containing around points of inoculation cells darker green in colour than surrounding cells; limited lesions around points of inoculation containing chlorotic, light or pronounced cells; no symptoms visible to the naked eye. These five

categories related to the proportions of leaf tissue carrying lesions and, therefore, to susceptibility and resistance with the rapidly spreading net blotch necrotic lesion typical of highly susceptible responses, the light chlorotic lesions typical of highly resistant responses, and the absence of visible symptoms corresponding to what is often called an immune response of very high resistance.

A general feature which emerged from each group of experiments was the difference in susceptibility of leaves of different ages although in fact the differences in leaf age were only a few days. The older leaves were significantly more susceptible than were the younger leaves as also reported by Ravn (1900) and Ibrahim and El-Fahl (1977). In many other diseases there would be a tendency to assume, possibly on inadequate evidence, that younger tissues are the more susceptible.

A later experiment designed to reinvestigate the susceptibility of first, second and third leaves of seedlings apparent in experiments on host range showed quite clearly that the effect was one of age and not one that depended on position of a leaf on a plant. Again, it is surprising that differences in susceptibility were so large as found bearing in mind that the ages of successive leaves differed by only a few days. Once again very little is known about the mechanisms affecting susceptibility of leaves of different ages in any disease.

At first, and for convenience, cultivated barley and closely related species of Hordeum were regarded as host plants and other members of the Gramineae, and other plants were regarded as non-hosts. The results of the extensive series of inoculations of many members of the Gramineae showed that this distinction was too arbitrary because P. teres grew quite significantly and sporulated reasonably well on many of the non-host plants. The more important points about the range of parasitism by P. teres were as follows. Many lines of H. spontaneum were as susceptible as were two cultivars of barley ranked as highly susceptible with net blotch lesions. The remaining lines, H. murinum, and H. jubatum were considerably less susceptible producing spot form lesions but they still colonized up to 20% of available leaf tissue. Triticum aestivum, T. turgidum and T. monococcum exhibited the same order of susceptibility with spot form necrotic lesions and could well be regarded as hosts of P. teres, albeit with some resistance.

The next group comprised Avena strigosa, A. sativa, Secale cereale, Festuca pratensis, F. rubra, Lolium perenne and Poa annua. Percentage colonization ranged from 13 for L. perenne to 4 for F. rubra and P. annua in reactions that can be regarded as highly resistant. Lesions were unusual in being a group of cells that were darker green than cells of the surrounding tissue and thus in this respect resembled "green islands" of many other diseases, particularly those

caused by rusts and powdery mildews. However, the cells of green islands are usually normal green in colour and thus contrast with the chlorotic tissue around them. Also, green islands develop towards the end of parasitism whereas the darker green cells in P. teres lesions are an early response to the pathogen. Still more resistant responses comprised a group of cells in which chlorosis was light or pronounced. Within this group was Agropyron repens which is surprising in view of the much higher susceptibility of Triticum spp. and Secale cereale. Also surprising were the responses of Oryza spp. and of Zea mays.

A point of interest therefore is the pronounced difference in the resistant reactions which produce darker green and chlorotic cells.

A final group contained species with no visible response to inoculation. It included Poa trivialis and P. pratensis which, therefore were still more resistant than P. annua.

Clearly from the above results, it would be difficult to decide the species that are to be regarded as hosts or non-hosts of P. teres despite the fact that P. teres is commonly regarded as a relatively specialized parasite of barley and closely related species. The question now arises as to the importance of these other hosts in the infection of barley by P. teres and particularly in the epidemiology of

disease on barley crops. It is clear that a wide range of graminaceous plants other than barley, some of which occur widely in the U.K., could become infected especially from barley crops. While colonization of these plants would usually be limited, as would sporulation on living plants, it is likely that the pathogen would sporulate more or less freely on lesions as infected leaves become moribund and then die.

Experiments did, in fact, show that P. teres sporulated when leaves with limited lesions characteristic of resistant or highly resistant responses were incubated in favourable conditions; sporulation was on a scale that could be significant in the field. It was also shown that spores from lesions on resistant, "non-host" plants were as vigorous in culture and as virulent on barley as were spores from the original virulent strain; both types caused typical, rapidly spreading net blotch lesions. However, the significance of parasitism of non-host plants in the epidemiology of net blotch in the U.K. is problematical and remains to be assessed particularly in the light of the scale and continuity with which barley is grown and the probable abundance of volunteer barley near barley crops. But this parasitism may be more significant in other barley growing countries where there are long periods between successive barley crops.

Induction of susceptibility and of resistance by pre-inoculation with virulent and avirulent races of a pathogen is now well known for a variety of diseases (Tsuchiya and Hirata, 1973; Ouchi et al. 1974, 1982; Kuć, 1982). Effects induced locally have been reported much more frequently than systemic effects which are confined to a few diseases. Experiments with a virulent and less virulent strain of P. teres showed that pre-inoculation of the first leaf with either the virulent or the less virulent strain did make second and third leaves more susceptible or less susceptible respectively to the less virulent and virulent strain inoculated a few days later. The effects were relatively small compared with those reported for other diseases and they did not alter the type of lesion produced. Larger effects were caused locally following pre-inoculation of leaf segments. Neither systemic nor local effects were obtained when the virulent and less virulent strain were applied to leaves together. This again is a result similar to that reported for many other diseases.

Induction of resistance and of susceptibility are factors that could be significant in the epidemiology of any disease in which strains of the pathogen differ markedly in virulence and cultivars of the host plant differ markedly in susceptibility. But in no disease has their significance in epidemiology been assessed critically. Considerable reserch would be needed to do so for net blotch of barley; the size

of the effects and the biology of the disease suggest that neither induced susceptibility nor induced resistance are likely to be important in the epidemiology compared with many other factors.

Analysis of the behaviour of the pathogen before and during infection of host and non-host plants produced results similar to those obtained by Keeling (1965), and to those reported for many other diseases in that there were no substantial differences in germination and growth of germ tubes on leaves. But with increasing Leaf resistance, numbers of appressoria decreased to a minimum of c. 50% compared with numbers on susceptible barley, and so did numbers of penetrations from appressoria to a minimum of about a third compared with numbers on susceptible barley.

In combination, therefore, as proportions of spores applied to leaves, penetrations ranged from 40 for susceptible barley to 6 for resistant rye grass. These differences were, perhaps, greater than expected in the light of results reported for other diseases particularly those caused by rusts and mildews. But much more research would be required to assess their significance in the development of disease in the field. Behaviour of the pathogen after penetration was studied only to a limited extent mainly because of technical difficulties in observing growth of hyphae in whole leaves. Papillae as described by Hargreaves

(1982) were observed on resistant oat and wheat plants but not on susceptible barley and wild barley. Whether papillae in this and in other diseases are the causes of resistance, or the results of resistant responses occurring earlier remains largely to be established.

As mentioned earlier, there were striking differences in certain highly resistant responses as between host cells that became darker green in colour and cells that became chlorotic. Both have the characters of hypersensitive responses in that there was little of any growth of the pathogen from the tissue comprising these cells but so far as could be detected few of the cells were necrotic or discoloured in other ways. Further studies of these responses would seem to be justified particularly by transmission electron microscopy.

Reverting to the biology of the disease, pseudothecia, sclerotium-like bodies (Webster, 1951), were found abundantly in culture on potato carrot agar, (PCA), on infected segments of leaves of host and non-host plants, and on barley straw. They germinated readily on PCA producing conidia that were fully virulent on barley irrespective of the substrates (host and non-host) on which they had developed.

They have been recorded from barley stubble in the field (Webster, 1951; Butler, 1955; Mathre, 1982). This and their

development on debris of non-host plants suggests that they may be of some significance in the epidemiology of net blotch in barley crops.

Finally, and for practical, experimental purposes, there are the simple experiments on storage of the pathogen for long periods. Their relevance is shown by the loss of vigour in culture and, more importantly, the loss of capacity to sporulate and of virulence, exhibited in the many isolates obtained from Long Ashton Research Station which had been stored as agar cultures under mineral oil for long periods. Presumably these cultures had been much more vigorous soon after isolation and before long storage.

Similar changes in cultures have been reported by Piening (1963) and Boyd (1968). Khan and Boyd (1969) however reported that conidia could be stored at 5°C in the lyophilized state for six months with no loss of virulence.

A simple and effective alternative method was developed in which segments of leaves of susceptible barley were inoculated, incubated to allow extensive colonization and abundant sporulation, and then kept in the dried state at 2°C for six months. After this period there were no or only slight decreases in spore germination, vigour of growth or in virulence compared with 10 day old spores. Time did not allow development of this method but it is likely that spores

could be kept much longer at 2°C with no serious loss of important characters, and that the period of storage could be increased greatly by storing at much lower temperatures.

The main points from the research described in this thesis are, therefore: the loss of vigour and of virulence on prolonged storage of agar cultures under mineral oil; the variation in virulence of isolates and on susceptibility of barley cultivars with winter cultivars somewhat more susceptible than spring cultivars; the very striking effects of light quality and times of exposure on sporulation; the close correspondence in assessments of responses to inoculation of barley and a wide range of other members of the Gramineae in tests using segments of detached leaves and leaves of young seedlings in controlled conditions and in the field; the wide range of responses to an isolate virulent on cultivated barley including highly resistant responses comprising darker green cells, or chlorotic cells; the substantial growth and sporulation of the pathogen on Hordeum spp. other than cultivated barley and on plants usually considered non-host such as three species of Triticum, and Lolium perenne and with significant growth and sporulation on a range of other cereals and grasses; the significant sporulation on moribund tissue in which responses earlier had been highly resistant with very restricted growth of the pathogen; the decrease in susceptibility from first to second to third leaves of seedlings which was shown to depend on age

of leaf; the similar germination and growth on leaves of host and non-host plants but a decline in numbers of appressoria and penetrations from them with increasing resistance; the induction of moderate increases in susceptibility or resistance, systemic and local, by pre-inoculation (but not simultaneous inoculation) of, respectively, a virulent and less virulent isolate; the free production of pseudothecia on infected tissues of host and non-host plants; the maintenance of virulence after passage through non-host plants; the retention of viability of spores, vigour of growth from spores and of virulence of spores produced on segments of leaves and kept at 2°C for six months.

REFERENCES

AL-ALI, B. (1978). Contribution à L'étude de l'*Helminthosporium teres* Sacc. Parasite de l'orge. *Thèse de Doctorat de Troisième cycle, No. 33.. E.N.S.A.T., Toulouse, France*.

AL-ALI, B., ALBERTINI, L., BARRAULT, G. (1979). Etude de la sensibilité de variétés d'orge originaires de France et du Proche-Orient à l'*Helminthosporium teres* Sacc. *Bulletin de la Société d'histoire naturelle de Toulouse. France*. *115*, 11-12.

BARNETT, H.L., and LILLY, V.G. (1950). Influence of nutritional and environmental factors upon asexual reproduction of *Choanephora cucurbitarum* in culture. *Phytopathology* *40*, 80-89.

BERDUGO, J. (1968). Morphologie, Biologie et symptomologie de *Pyrenophora teres*. These de Docteur ingenieur, No. 190. Universite Paul Sabatier de Toulouse, France.

BRAVERMAN, S.W. (1960). The *Helminthosporium gramineum* complex and related species on cereals and forage grasses. *Phytopathology* *50*, 688-691.

BUTLER, E.J. and JONES, S.G. (1955). Plant Pathology, MacMillan, London, 441-445.

CADDEL, J.L., and WILCOXSON, R.D. (1975). Sources of resistance to net blotch of barley in Morocco. Plant Disease Reporter 59, 491-494.

CLIFFORD, B.C., and JONES, D. (1980). Net blotch of barley. U.K. Cereal Pathogen Virulence Survey. Annual Report, 71-77.

CLIFFORD, B.C., BUONO, R.D. and JONES, E.R.L. (1984). Net blotch of barley. U.K. Cereal Pathogen Virulence Survey. Annual Report, 64-69.

C.M.I. Map No. 364, ed.2, (1968). Distribution Maps of plant diseases. Map N: 364, ed. 2, Issued 1. vii.

CROWELL, I.H. (1941). Canadian Plant Disease Survey 41, No. 5, 299-300. Cited in Piening, L.J. (1961).

DICKSON, J.G. (1956). Diseases of field crops. MacGraw-Hill, New York and London.

DRECHSLER, C. (1923). Some graminicolous species of Helminthosporium. Journal of Agricultural Research 24, 241-256.

ELLIS, M.B. (1971). Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

ELLISTON, J.E., KUĆ, J. and WILLIAMS, E.B. (1971). Induced resistance to bean anthracnose at a distance from the site of the inducing interaction. Phytopathology. 61, 1110-1112.

ERIKSSON, J. (1914). Maladies cryptogamiques des plantes agricoles et leur traitement, ITCF, Paris, France.

EVANS, S.G. (1969). Observations on the development of leaf blotch and net blotch of barley from barley debris. Plant Pathology, 18, 116-118.

GRAY, G.G. (1966). Genetic systems in the net blotch diseases complex of barley. Ph.D. Thesis, North Dakota State University, USA.

HARGREAVES, J.A. (1982). The nature of the resistance of oat leaves to infection by Pyrenophora teres. Physiological Plant Pathology 20, 165-171.

HESSAYON, D.G. (1982). The Cereal disease expert. Pbi Publications. Britannica House, Waltham Cross, Herts, England.

IBRAHIM, A.N., EL-FAHL, A.M. (1977). Susceptibility of barley plants of different ages to net blotch. Ontogenetic predisposition. Agricultural Research Review. 55, 13-15.

JAMES, W.C. (1967). Proceeding of the 4th British Insecticide, Fungicide Conference. 1, 111-114.

JONES, D.G. and CLIFFORD, B.C. (1978). Cereal diseases, their pathology and control. Pervian Press. BASF, United Kingdom.

JORDAN, V.W.L. (1981). Aetiology of barley net blotch caused by Pyrenophora teres and some effects on yield. Plant Pathology 30, 77-87.

JORDAN, V.W.L., BEST, G.R. (1981). Evaluation of fungicide treatments for control of barley net blotch caused by Pyrenophora teres. Proceeding 1981 British Crop Protection Conference - Pests and Diseases, 249-258.

JORDAN, V.W.L. (1982). Barley net blotch. Long Ashton Research Station, leaflet 6, 1-6.

JORDAN, V.W.L., and ROYLE, D.J. (1983). Barley net blotch: Cultural and environmental effects. Long Ashton

Research Station, Crop Protection Division leaflet 4, 1-4.

JORDAN, V.W.L., ALLEN., and ELIZABETH, C. (1984). Barley net blotch: influence of straw disposal and cultivation methods on inoculum potential, and on incidence and severity of autumn disease. Plant Pathology, 33, 547-559.

KEELING, B.L., BANTTARI, E.E. (1965). A comparison of the development of Pyrenophora teres on resistant and susceptible varieties of barley. Phytopathology, Annual Abstracts: Aps 55, 1063.

KENNETH, R. (1962). On the taxonomy, morphology and geographic origin of Pyrenophora teres Drechsler, and species. Bulletin of the Research Council of Israel 11D, 55-82.

KENNETH, R., ATZMON, Y., KHAIR, J., KOLTIN, Y.I., WAHL, I. (1967). Problems of breeding barley resistant to net blotch in Israel. The first Israel Congress of Plant Pathology. 46-47.

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 BOYD, W.J.R. (1968). Gramineae species harbouring Drechslera teres in Western Australia. Australian Journal of Science 30, 373.

KHAN, T.N., and BOYD, W.J.R. (1968). Long term preservation of Drechslera teres by freeze drying. Phytopathology 58, 1448-1449.

KHAN, T.N. (1973). Host specialization by Western Australian Isolates causing net blotch symptoms on Hordeum. Transaction of the British Mycological Society 61, 215-220.

KILPATRICK, R.A. (1966). Induced sporulation of fungi on filter paper. Plant Disease Reporter 59(2), 789-790.

KING, J.E. (1972). Surveys of foliar diseases of spring barley in England and Wales 1967-70. Plant Pathology 21, 23-35.

KING, J.E. (1977). Surveys of foliar diseases of spring barley in England and Wales 1972-75. Plant Pathology 26, 21-29.

KING, J.E. (1982). Survey 1980/81 of diseases of winter barley in England and Wales. MAFF, ADAS, Plant Pathologist's Technical Conference, 1-7.

KUĆ, J. (1982). Plant immunization-mechanisms and practical implications. In 'Active Defense Mechanisms in Plant'. Ed. by R.K.S. Wood, pp. 157-178. Plenum Press. New York and London.

LANGERON, M., VANBREUSEGHEM, R. (1952). Précis de Mycology. Masson et Cie et. Paris, P.400.

LEACH, C.M. (1961). The sporulation of Helminthosporium oryzae as affected by exposure to near-ultraviolet radiation and dark periods. Canadian Journal of Botany 39, 705-715.

LEACH, C.M. (1967). Interaction of near-ultraviolet light and temperature on sporulation of the fungi Alternaria, Cercosporella, Fusarium, Helminthosporium, and Stemphylium. Canadian Journal of Botany 45, 1999-2016.

LITLEFIELD, L.F. (1969). Flax rust resistance induced by prior inoculation with an avirulent race of Melampsora lini. Phytopathology 59, 1323-1328.

LOCKE, T. (1981). Net blotch of barley. MAFF, Agriculture Science Service, Crop Pests and Diseases, 46-49.

LUKENS, R.J. (1960). Conidial production from filter paper cultures of Helminthosporium vagans and Alternaria solani. Phytopathology 50, 867-868.

MACHACEK, J.E., WALLACE, H.A.H. (1942). Net blotch of barley. Review of Plant Pathology 52, No. 5, 69-290. Cited in Shipton, W.A. (1973).

MATHRE, D.E. (1982). Compendium of barley diseases. American Phytopathological Society. Minnesota, U.S.A.

MATKIN, D.A. and CHANDLER, P.A. (1957). The U.C-type soil mixes. In: The UC-system for producing healthy container-grown plants, Ed. by Baker, K.F. 68-85. Univ. Calif. Agric. Exp. Station.

McDONALD, W.C. (1967). Variability and the Inheritance of morphological mutants in Pyrenophora teres. Phytopathology 57, 747-755.

METCALFE, D.R., CHIKO, J.W., MARTENS, J.W. and TEKAUZ, A. (1977). Reaction of barleys from the Middle-East to Canadian pathogens. Canadian Journal of Plant Science 57, 995-999

MAFF (1984). Winter barley
MAFF (Publications), booklet 2063.
Lion House, Willowburn Estate,
Alnwick, Northumberland, NE66 2PF

MILLER, P.M. (1955). V-8 juice agar as a general-purpose medium for fungi and bacteria. Phytopathology 45, 461-462.

MOORE, W.C., SMITH, A., PETHYBRIDGE, G.H. (1934). Bulletin of the Ministry of Agriculture and Fisheries 79, V1 + 117 pp.

MOORE, W.C. (1943). Bulletin of the Ministry of Agriculture and Fisheries 126, 100pp.

ONESIROSAN, P.T., and BANTTARI, E.E. (1969). The effect of light and temperature upon sporulation of Helminthosporium teres in culture. Phytopathology 59, 906-909.

OUCHI, S., OKU, H., HIBINO, C. and AKIYAMA, I. (1974). Induction of accessibility and resistance in leaves of barley by some races of Erysiphe graminis. Phytopathologische Zeitschrift 79, 24-34.

OUCHI, S. and OKU, H. (1981). Susceptibility as a process induced by pathogens. In: 'Plant disease control, resistance and susceptibility'. Ed. by R.C. Staples and G.H. Toenniessen, 33-44. John Wiley

and Sons, New York.

OUCHI, S., and OKU, H. (1982). Physiological basis of susceptibility induced by pathogens. In: Plant infection: the physiological and biochemical basis, Ed. by Asada, Y.; Bushnell, W.R.; Ouch, S. and Vance, C.P., 117-136. Japan Scientific Societies press, Tokyo and Springer-Verlag Berlin.

PIENING, L.J. (1961). The occurrence of Pyrenophora teres on barley straw in Alberta. Canadian. Plant. District Survey. 41, No. 5, 299-300.

PIENING, L.J. (1963). Physiological and nutritional studies of a conidial strain of Drechslera teres (Sacc.) Shoemaker and a mycelial variant. Canadian Journal of Microbiology 9, 479-490.

PIENING, L.J. (1968). Development of barley net blotch from infested straw and seed. Canadian Journal of Plant Science 48, 623-625.

PON, D.S. (1949). Physiological specialization and variation in Helminthosporium teres. Phytopathology 39, 18 (abst.).

PUTERILL, K.M. (1954). Some graminicolous species of

Helminthosporium and *Curvularia* occurring in South Africa. *Bothalia* 6, 347-378.

RAPILLY, F., and PONCHET, J. (1962). The use of explants of Gramineae for the study of the genus *Helminthosporium*. *C.R. Acad. Agric. Fr.* 48, 390-392.

RINTELEN, J. (1969). Studies on yield losses due to net blotch on barley (Pathogen: *Pyrenophora teres*). *Z. pflkrankh. pflpath pflschutz* 76 No. 3, 147-152.

RAVN, F.K. (1900). Nogle *Helminthosporium*-arten og de of dem frem. Kaldte sygdomme hos byg og havre. *Bot. Tids.* 23, 101-322.

SELIM, A.K.A., OMAR, A.M., DESOUKI, S.M., and MOHAMED, L.R.M. (1973). Inheritance of mature plant reaction to net blotch disease of barley (II). *Agricultural Research Review* 51, No. 6, 99-105.

SHEODHAN, S. (1962). Biotic Factors Affecting barley net blotch (*Helminthosporium teres*). Epidemiology. *Indian Phytopathology* 15, 195-202.

SHEODHAN, S. (1963). Effect of the age of the host and age of the pathogen on the incidence of the net blotch

of barley (Helminthosporium teres Sacc.). Indian Phytopathology 16, 133-137.

SHEODHAN, S. (1963). Effect of moisture on the incidence of net blotch of barley (Helminthosporium teres Sacc.). Indian Phytopathology 16, 128-132.

SHEODHAN, S. (1963). Effect of temperature on secondary infection and development of net blotch of barley. (Helminthosporium teres Sacc.). Indian Phytopathology 16, 94-97.

SHIPTON, W.A., KHAN, T.N., and BOYD, W.J.R. (1973). Net blotch of barley. Review of Plant Pathology 52, 269-290.

SHOEMAKER, R.A. (1962). Drechslera ITO. Canadian Journal of Botany 40, 829-830.

SMEDEGARD-PETERSEN, V. (1971). Pyrenophora teres. f. maculata f. nov. and Pyrenophora teres f. teres on barley in Denmark. Royal veterinary and Agricultural University Yearbook - 124-144.

SMEDEGARD-PETERSEN, V. (1972). The perithecial and pycnidial stages of Pyrenophora teres and P. graminea in Denmark. Friesia. 10, 61-85.

SMEDEGARD-PETERSEN, V. (1974). Reduction in yield and grain size of barley due to attack by the net blotch fungus Pyrenophora teres. Yearbook of the Royal Veterinary and Agricultural University, Copenhagen, 108-117.

SMEDEGARD-PETERSEN, V. (1976). Pathogenesis and genetics of net-spot blotch and leaf stripe of barley caused by Pyrenophora teres and P. graminea. Copenhagen, Denmark, DSR Forlag.

SMEDEGARD-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, N.J.G., and PUTTERILL, K.M. (1932). Pycnidia produced by Helminthosporium parasites of cereals and wild grasses. Review of Applied Mycology 12, (1933), 162-163.

SPRAGUE, R. (1950). Diseases of Cereals and Grasses in North America. The Ronald Press Company - New York.

SPRAGUE, R. (1955). Check list of the fungi of the Glacier

Bay National Monument, Alaska. Cited in Review of Applied Mycology 35.

SRINIVASAN, M.C., CHIDAMBARAM, P., MATHUR, S.B., and NEERGAARD, P. (1971). A simple method for Inducing sporulation in seed-borne fungi. Transaction of the British Mycological Society 56(1), 31-34.

TALVIA, P. (1966). The occurrence of plant diseases in 1965. Maatalous ja Koetoiminta. 20.

THOMAS, M.R. (1984). A simple method for producing suspensions of Pyrenophora teres Spores. Bulletin of the British Mycological Society 18, 60-61.

TSUCHYA, K., and KIRATA, K. (1973). Growth of various powdery mildew fungi on barley leaves infected preliminarily with the barley powdery mildew fungus. Annals of Phytopathological Society of Japan. 39, 396-403.

VAN CAESEELE, L., and GRUMBLES, J. (1979). Ultrastructure of the interaction between Pyrenophora teres and a susceptible barley host. Canadian Journal of Botany 57, 40-47.

VARGAS, J.M., and WILCOXSON, R.D. (1967). The effect of

temperature and light on sporulation of Helminthosporium dictyoides on artificial media. Phytopathology 75, 834 (Abstr.).

WEBSTER, J. (1951). Graminicolous Pyrenomycetes II. The occurrence of the perfect stage of Helminthosporium teres in Britain. Transaction of the British Mycological Society 34, 309-317.

WESTERN, J.H. (1971). Diseases of crop plants, MacMillan Press Ltd., London. 205-225.

WILSON, K.I., ALBELDAWI, A.S., and SHAMSALDIN, S. (1981). Plant Protection Research Station, Abou Ghraib, Baghdad. F.A.O. - Plant Protection Bulletin 29, 78.

WINTER, W.E. (1977). Presence of Drechslera teres Sacc. Shoem on fodder rye seeds. Boletin de la Sociedad Argentina de Botanica 18, 65-78.