



INITIATION AND DEVELOPMENT OF EYESPOT LESIONS
ON WHEAT BY PSEUDOCERCOSPORELLA HERPOTRICHOIDES

BY

SALLY HIGGINS

THESIS SUBMITTED TO THE UNIVERSITY
OF LONDON, FOR THE DEGREE OF PH.D.

PLANT PATHOLOGY DEPARTMENT
ROTHAMSTED EXPERIMENTAL
STATION,
HARPENDEN, HERTS AL5 2JQ

ABSTRACT

The aims of this project were to identify some of the factors affecting the initiation and development of eyespot (Pseudocercospora herpotrichoides (Fron) Deighton) lesions on wheat. Conidial germination and mycelial growth of P. herpotrichoides were studied both in vitro and in vivo, both in glasshouse and in field experiments.

Viable conidia were produced in abundance in the field on naturally infected straw from February to July 1982. In laboratory tests conidial production on naturally infected straw was optimal at 5°C and decreased with increasing temperature up to 25°C. Seedlings exposed amongst infected straw during rain collected conidia on all aerial parts, but P. herpotrichoides only colonised the coleoptile.

On seedlings, the severity of eyespot lesions increased with increasing inoculum dose (number of conidia) applied and for different isolates was correlated with their radial growth rates on potato dextrose agar at 20°C. On mature plants no such relationship was found between pathogenicity and growth in vitro; nor was disease severity related to that obtained on seedlings. Benzimidazole-tolerant isolates were as pathogenic to seedlings and mature plants as benzimidazole-sensitive isolates.

On agar P. herpotrichoides grew optimally at water potentials of -5 to -15 bars. Although heavy watering enhanced lesion development both in controlled environment room and in field

experiments, it was not clear whether this related to an effect of water potential.

The number of seedling leaf sheaths penetrated by P. herpotrichoides increased with increasing accumulated temperature ($T_o=6^{\circ}\text{C}$) but at higher temperature more leaf sheaths were produced and more were desiccated and sloughed off. Therefore the number of live leaf sheaths penetrated decreased with increasing accumulated temperature.

In field experiments development of existing lesions was enhanced by heavy rain when plants were in 'boot' (Growth stage 45); droughting during this period reduced severity of infection at harvest.

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CHAPTER 1

INTRODUCTION

Eyespot, caused by Pseudocercospora herpotrichoides Fron (Deighton) is a potentially serious problem of winter sown wheat and barley but attempts to predict the occurrence of severe epidemics have not been entirely successful. In the UK the first symptoms of eyespot disease are seen as indistinct, brown lesions on the leaf sheaths near to soil level, often affecting a high proportion of a crop. In some years these lesions develop slowly and have very little effect on yield. However, in other years lesion development is extensive and crops may lodge, resulting in yield losses of up to 40%. The factors determining whether lesions will become severe are not understood. Also it is not clear why lesions occur almost exclusively at the base of the plant. The aims of this study were:-

1. To examine the initial stages of infection in order to find the reason for the position of the lesions.
2. To identify some of the factors affecting the development of severe lesions.

1.1 Eyespot: the disease and the fungus

Pseudocercospora herpotrichoides Fron (Deighton), the causal fungus of eyespot on cereals, has a widespread distribution and occurs in many temperate countries including most of Europe and parts of North America, Africa and Australasia (Glynne & Salt, 1958). The most extensive and damaging lesions develop on winter sown cereals, which are particularly susceptible. Widespread

severe lesions decrease crop yields considerably, whereas widespread mild infections do not greatly affect yield (Jørgensen, 1964; Scott & Hollins, 1974). The factors influencing the development of relatively superficial lesions into extensive and damaging lesions are not understood.

P. herpotrichoides was first recorded as Cercospora herpotrichoides in 1911 by Fron but he regarded C. herpotrichoides as the conidial stage of Leptosphaeria herpotrichoides. Much of the uncertainty about the identity of C. herpotrichoides arose because isolates remained sterile in vitro. The mycelial phase was designated fungus x, whereas the conidial stage was named C. herpotrichoides. Later Foëx & Rosella (1933) showed that there was a close relationship between fungus x and C. herpotrichoides, since the conidia of the latter gave rise to the mycelial stage of fungus x. Sprague (1931) obtained similar results, so both investigators concluded that C. herpotrichoides and fungus x were the same fungus. By applying Koch's postulates, Sprague also established that C. herpotrichoides was the cause of eyespot on wheat. The method of liberation of conidia in the genus Cercospora is quite distinct. Pressure from within the conidiophore builds up, forcibly ejecting the conidia leaving a distinct scar. Further examination of the eyespot fungus by Deighton (1973) led to its reclassification into the genus Pseudocercospora in which the conidiophore scars are indistinct.

P. herpotrichoides belongs to the order Moniliales of the Deuteromycetes and reproduces by asexual conidia or by hyphal branches. In culture the vegetative mycelium is yellow to dark

brown and the conidiophores are simple or slightly branched, arising from macrohyphae. The conidia are colourless, acicular, straight or slightly curved, smooth and 3-7 septate, $26.5 - 47 \times 1-2 \mu\text{m}$ (Deighton, 1973).

Conidia usually germinate from terminal cells, but occasionally from other cells. Vegetative growth on Potato Dextrose Agar (PDA) is optimal at $20-25^{\circ}\text{C}$. Lower temperatures are required for sporulation (Glynne, 1953); incubation at 10°C under near ultra-violet light also aids sporulation in culture (Leach, 1967).

Eyespot epidemics follow a distinctive annual cycle (Hollins & Scott, 1980) consisting of four phases which overlap considerably; sporulation on infected stubble from the previous season, dispersal of conidia by rainsplash, infection of the new growing crop and disease development within infected plants.

1.2 Sporulation on infected stubble

Glynne (1953) reported that the fungus did not seem to produce spores within lesions on mature living plants. However, sporulation was abundant on infected pieces of straw lying on the soil after ploughing, especially during wet weather from September to April. Frost seemed to increase sporulation and dry weather to decrease it. Severe lesions produced more spores than small superficial lesions and continued to do so for several weeks.

Many conidia can be obtained by incubating naturally infected straws at temperatures between 5 and 20°C (Jørgensen, 1964), but two other techniques have proved useful for producing large numbers of conidia for experiments. Pang Chang & Tyler (1964) did this by

growing the fungus on distilled water agar (DWA) at 10°C under near ultra-violet light. Reinecke & Fokkema (1979) however preferred to use artificially infected straws in sterile soil, stored at 4°C, which could then be transferred to PDA plates at various temperatures.

Dispersal of P. herpotrichoides conidia was first investigated by Glynne (1953) who found that conidia could be collected on greased slides 5cm away from infected straw over which a fine mist was sprayed. None were caught when dry air was passed over the straws. When drops of water were allowed to fall from 12ft onto straws infected with sporulating P. herpotrichoides, the splash droplets produced contained conidia. In the field conidia are normally dispersed by rainsplash (Rowe & Powelson, 1973; Fitt & Bainbridge, 1983b) from infected straw debris to neighbouring young winter seedlings in which they initiate eyespot lesions. However, some infection may be initiated through direct mycelial contact. Most conidia are carried in the larger ballistic splash droplets for short distances (Fitt & Bainbridge, 1983b). However, some small droplets which become airborne may be carried considerable distances by thermal air currents, so helping to distribute the pathogen to more distant crops.

1.3 Germination and infection

Infection can take place soon after seedling emergence and in severe attacks young plants are killed outright (Gair, Jenkins & Lester, 1978). There is some conflict in the literature about the optimum temperature at which spore germination and initial growth take place. Defosse (1966) states that germination is

quickest at 23°C. However, others report that maximum mycelial growth occurs between 9 and 21°C (Deacon, 1973; Schrödter & Fehrmann, 1971).

Several wheat cultivars show some resistance to infection by the pathogen. This resistance is established early in the infection process at the time of colonisation of the coleoptile. Ultra-structural studies of wheat coleoptiles infected with P. herpotrichoides (Fehrmann & Mendgen, 1976) indicate that the fungus enters the host cell wall by a thin penetration peg aided by lytic enzymes. Staining with zinc chloride and potassium iodide reveals a penetration halo showing the action of these enzymes (Defosse, 1976). Host resistance may be shown at this stage by the deposition of an osmophilic compound on the cell wall at the site of penetration. The production of lamellae arranged across the cell may prevent further spread of the fungus.

Typically the symptoms of infection are seen in the spring as brown smudgy lesions on one side of the outer leaf sheaths just above soil level. As the fungus grows further towards the stem, the lesions become more typically "eye-shaped", with a "pupil" consisting of black dots of mycelium. The central area of the lesion is composed of dead cells thought to be killed by the action of a toxin which may be produced by the host as an immune response (Akai, 1951).

Isolates of P. herpotrichoides differ in the extent to which they infect cereals. There are R-type isolates which are equally pathogenic to rye and wheat, and with a feathery edge when grown in culture, W-type, which are pathogenic to wheat and barley, but

only slightly to rye, fast growing in culture with a smooth edge to the colony (Lange-de la Camp, 1966 ; Scott, Hollins & Muir, 1975) and C-types which are especially pathogenic to couch grass (Cunningham, 1981). Couch grass could be important in the survival of the pathogen from one cereal crop to the next during crop rotation, providing an "alternative" host in the ley headlands surrounding fields. Despite these differences between isolates there is no evidence to suggest that there is any physiological specialisation by P. herpotrichoides isolates (Scott, Hollins & Muir, 1975).

1.4 Lesion development and disease severity

Generally eyespot development is slow, commencing with the penetration of successive leaf sheaths until the culm is reached. Between each leaf sheath stroma or pseudoparenchyma is formed before penetration continues (Ward, 1976). Straw infected at an early stage may become weakened and twisted. This weakening of the straw base may, in some instances, cause lodging and a thinning of the crop. Eyespot induces straws to bend sharply at their weakest point and they fall over in many directions amongst the upright ones. This is known as straggling of the crop and is distinguished from weather lodging when all the straws tend to fall in one direction (Fig. 1). Severely infected straws yield about half the weight of grain produced by healthy ones, and straggling often makes it difficult or impossible to harvest a crop. The fungus spreads much faster in the parenchyma in the lumen region than it does nearer the outer surface of the culm. During moist weather in late season (June to July) the lumen of a large

Fig. 1. Photograph to show straggling of a winter wheat crop on the Rothamsted Farm



percentage of diseased culms becomes filled with wefts of smoke-grey mycelium. The stem is weakened and buckles easily at the point of infection.

Heald (1922), in the USA, investigated the relationship between planting date and severity of eyespot lesions. He found that lodging caused by the pathogen was most severe in wheat planted early ; no stems survived from wheat planted in August. Only mild lesions developed on wheat planted in October, but the yields were reduced. Similarly, Sprague (1937), also working in the USA, found that there was appreciably less infection in late-planted wheat and lesions were less severe. However, the higher yields obtained make it preferable for the UK farmers to sow their crops early in the season (Glynne, 1954).

Row spacing, which affects plant density, influences the amount of infection; this being greatest within densely planted crops (Foëx & Rosella, 1933). Such relationships may depend on the weather, and more especially rainfall (Sprague & Fellows, 1934). There was no appreciable difference in severity of lesions between crops with rows from 8 to 14 inches apart after seasons with high rainfall. However, after seasons with low rainfall wider spacing reduced eyespot, probably by improving aeration.

Lodging caused by P. herpotrichoides tends to be greatest in dense crops (Salt, 1953). In wheat sown at low rates with less than 30 heads per foot, there was no lodging and only 8 percent infection (Moore, 1949). The increase in available light to these plants promoted better plant development and strong root systems, preventing lodging. Wheat sown with more than 30 heads per foot

had 43 percent infected culms and 28 percent lodging. In the UK, Glynne (1950) noted that at low seed rates plants formed extra tillers which protected the central primary shoots from infection. Increased air movement at the base of plants may facilitate drying and possibly impede penetration by the fungus. During spring droughts, infected outer leaves are sloughed off more readily in thin than in dense stands. Thin stands also tend to be composed of sturdier plants and have a higher grain yield per unit of dry straw weight (Salt, 1953).

Eyespot development seems to be associated with the average amount of available soil moisture. For example Sprague (1937) found that disease severity was greatest at lower altitudes in fields into which soil water had drained from surrounding slopes. Eyespot lodging is also worse under moist soil conditions (Glynne, 1939). However, information on the effects of rainfall throughout the growing season on eyespot development is lacking and has been investigated in this study.

The fungus seems to be especially well adapted to survive under normal crop rotation as used in the long-term experiments at Rothamsted. Over a period of 30 years the proportion of wheat straws from these crops infected with eyespot ranged from 2 to 99%, including 1-81% severely infected. The pathogen survived well in the 5 years between wheat crops, partly on spring-sown barley and autumn-sown rye. The first wheat after ploughed up grass normally had very low%infection (Glynne, 1963).

When nitrogen is applied to crops it stimulates plant growth and can increase yield, but it also increases the likelihood of

lodging. Eyespot, when severe, can decrease the crop response to nitrogen by causing lodging (Glynne & Slope, 1959). Nitrogen applied between November and April is effective in reducing the severity of eyespot because new tillers are produced early enough to delay penetration of the main straws (Salt, 1953). Oort (1936) observed that phosphorous deficiencies favoured the development of eyespot. Potassium application did not enhance eyespot development (Gardineau & Guyot, 1925). The incorporation of cellulose-dominant organic matter, free of plant pathogens, into the soil may intensify soil microbial activity leading to a suppression of P.herpotrichoides (Blair & Morrison, 1949).

1.5 Disease Prediction And Control

Scott & Hollins (1978) attempted to produce a scheme for the prediction of eyespot severity and yield loss, based on spring disease incidence. However, incidence in the spring was not a reliable indicator of the subsequent rate of disease development, or of its effect of yield. Crops with less than 10% of shoots infected were not sufficiently affected to justify fungicide application. No prediction scheme, whether based on disease incidence in the spring (Polley & Clarkson, 1978) or weather conditions for infection (Fehrmann & Schrödter, 1972) has proved entirely successful. The summer weather in Britain is very unpredictable and our knowledge of the effects of environmental conditions on disease development is very limited. Temperature, for example, is known to have an important effect on the rate of disease development. The growth of the fungus through successive leaf sheaths increased with increase in ambient temperatures over a range of 6 - 18° C

(Ponchet, 1959; Scott, 1971). However, the variation in disease development from year to year suggests that other climatic factors may also be important (Scott & Hollins, 1978).

Currently control of eyespot involves a combination of cultivar resistance, fungicides and crop rotation but each of these methods has its limitations. Resistance to eyespot was first discovered in the cultivar Cappelle - Desprez and has proved to be one of the most important characteristics of this cultivar, contributing to its performance and its use as a parent of many newer cultivars (Ingle, 1983). Although the resistance of Cappelle - Desprez is incorporated into most cultivars grown in the UK, they are all attacked by P. herpotrichoides under conditions favouring the pathogen (Cook & King, 1979). Cereal breeders are trying to introduce more resistance into winter wheat cultivars from a wild relative of wheat (Aegilops ventricosa L.) in the French line VPM1. It is hoped that use of this resistance may minimise the need for fungicidal control. However, cultivars with this resistance are not yet commercially available.

The increase in cereal production over the past decade has been accompanied by an increase in the use of benzimidazole fungicides for control of eyespot. During the summer of 1981 the Agricultural Development Advisory Service (ADAS) received reports from farmers that MBC fungicides were failing to control eyespot. Subsequent tests showed that isolates of P. herpotrichoides from these sites were tolerant to MBC-generating fungicides. Griffin & Yarham (1983) surveyed field sites throughout the UK and found isolates of P. herpotrichoides showing benzimidazole

tolerance in 23 of them. Exactly how these isolates have arisen is not clear. Alternative strategies or new fungicides are therefore desirable for the future control of eyespot. The pathogenicity of these MBC-tolerant strains of P. herpotrichoides is investigated in this study.

Although crop rotation, or a 2 year break from cereals, will give some control of eyespot, the British agricultural structure is such that most arable land is devoted to more intensive cropping. The financial rewards from this method of farming are high enough to justify the method, despite losses due to pathogens. Even with rotations the eyespot fungus will remain viable for several years, if buried in soil on straw debris which has been ploughed in (Cox & Cock, 1962).

1.6 Scope of this thesis

Chapter 3 examines the initial stages of infection and eyespot lesion formation on young seedlings and investigates why lesions are normally found at the stem base near ground level. This section also includes a study of the way in which spores are distributed on plant surfaces and of the susceptibility of different plant tissues. Chapter 4 is concerned with the growth of isolates in vitro and the pathogenicity of MBC-tolerant isolates is compared to that of MBC-sensitive isolates. Chapter 5 provides information on the development of lesions throughout each season of a 3 year field survey. These data are accompanied by constantly monitored weather information. Chapter 6 is a final discussion of the results. The appendix contains tables of detailed results which are presented in summarised form in the body of the thesis.

CHAPTER 2

GENERAL METHODS AND MATERIALS

2.1 Fungal isolates

The isolates used in this study can be placed in four main categories:-

a) Seven isolates with a known history, obtained from T. W. Hollins (PBI, Cambridge). They were:-

<u>Isolate Code</u>	<u>Year isolated</u>	<u>Host</u>	<u>Location</u>
C79/241	1979	W. wheat cv. Huntsman	Cambridge
C80/255	1980	W. barley cv. unknown	Cambridge
C80/257	1980	Spring barley cv. unknown	Cambridge
C81/264	1981	W. wheat cv. Armada	Cambridge
C81/266	1981	W. barley cv. Maris Otter	Cambridge
C81/279	1981	W. barley cv. Gerbel	Norfolk
C81/284	1981	W. barley cv. unknown	Gloucestershire

b) Four isolates, obtained from T. Murray (Washington State University), which originated from regions of the USA with low annual rainfall (indicated in brackets):-

PH 79-1 (49cm), PH 80-2 (42cm), PH 80-4 (46cm), PH 81-2 (50cm).

c) Two isolates (W 2 and E 3), supplied by D. Yarham, ADAS, Cambridge and known to be ben zimidazole-tolerant.

d) One isolate (R-1) from winter wheat (cv. Armada) on the Rothamsted Farm in 1980.

Straw pieces with suspected eyespot lesions were surface sterilised in 10% sodium hypochlorite and then plated out onto PDA containing 100µg actidione per ml of media. The resulting mycelium was subcultured onto PDA and DWA plates and incubated at 20°C and at 10°C under ^{'Phillips' black} ultra-violet light respectively. The isolates were identified by the appearance of the grey characteristic mycelium and needle-like spores.

All isolates were used in studies of comparative pathogenicity and growth and, except W 2 and E 3, were compared for their ability to grow at different water potentials.

2.2 Maintenance of cultures

Mycelial cultures of each isolate were maintained under glycerol on potato dextrose agar (PDA) slopes in Bijou bottles and stored at 2°C. When required, sub-cultures were made from these stock cultures onto PDA plates, which were then incubated at 20°C. Sporulation was induced by growing isolates on plates of distilled water agar (DWA) under near ultra-violet light at 10°C for 5-6 weeks. Spore suspensions were prepared in sterile distilled water by dislodging spores with a sterile glass rod. After 6 weeks each plate produced enough spores to give 10ml of a suspension containing at least 10^3 spores/ml.

2.3 Preparation of wheat seedlings

Winter wheat cv. Armada was used in all experiments. It is susceptible to eyespot, being rated 4 in the scale of resistance (1-9) of the National Institute of Agricultural Botany (ANON, 1984).

It has been widely used by farmers because it produces grain with good milling and breadmaking qualities.

Seeds were soaked over-night and pre-germinated at 15-20°C for 2 days, or until the radicle and coleoptile were about 2mm long. These young seedlings were then transferred to pots containing Eff compost (a sand/loam mixture) which were kept either in a greenhouse at c. 15°C or in controlled environment cabinets or rooms. Seedlings were inoculated with P. herpotrichoides at the second leaf stage. Several methods were used.

- 1) A spore suspension was applied with a soft brush to the upper surface of the coleoptile (Bateman & Taylor, 1976). The stem was then enclosed in a polythene collar to maintain high humidity.
- 2) The coleoptile was inoculated with a spore suspension absorbed onto foam mounted on clips and the plants were placed under plastic covers. This method was devised at ICI Plant Protection Ltd, Jealott's Hill Research Station.
- 3) Sterile filter paper discs (5mm diameter) were shaken in a spore suspension. Discs were then placed, using forceps, between the coleoptile and the first leaf sheath and held in place with parafilm (fig.2). The number of conidia on discs was estimated by determining the weight of the disc before and after immersion in suspensions of known concentration and calculating the quantity of liquid absorbed. Ten discs were also stained with cotton-blue in lactophenol and the number of conidia present counted.

Method (3) was used in most experiments because it was relatively easy to use and produced uniform infection. Method (2)

Fig. 2. Photograph to show inoculation of seedlings using the paper disc method



was retained for inoculation of specific tissues on plants.

2.4 Assessments of disease severity

Lesion severity on the green leaf sheaths of wheat seedlings and the stems of mature plants was assessed using a modified version of the scale devised by Scott & Hollins (1974) for use with mature wheat:

- 0. Uninfected.
- 1. Slight. One lesion, or more, occupying in total less than half the stem circumference or outer green leaf sheath.
- 2. Moderate. One lesion, or more, occupying in total at least half the stem circumference or outer green leaf sheath.
- 3. Severe. One lesion, or more, occupying in total at least half the stem circumference and tissues softened so that lodging could occur.

CHAPTER 3

SPORULATION, SPORE GERMINATION AND DISPERSAL

3.1 Introduction

Most early work on the effects of temperature on the sporulation of P. herpotrichoides has been done using cultures on agar. For example Dickens (1964), working in the USA, found that the fungus sporulated abundantly on plates of cornmeal agar (CMA) that were placed outside and subjected to temperatures between -18° and 12°C . Glynne (1953) showed that conidia were produced from discs of mycelial culture on water agar, provided that they were incubated in the presence of water in an unheated greenhouse where temperatures fluctuated between -3° and 13°C . Jørgensen (1964) examined conidial production on straw from the field but this was not a quantitative study. Schrödter & Fehrmann (1971) made observations on sporulation in the field and concluded that maximum conidial production was between 3° and 4°C . Rowe & Powelson (1973) looked at the effects of periods of unfavourable temperature on sporulation. They found that alternating temperatures within a range of $8-12^{\circ}\text{C}$ gave moderate sporulation but this was prevented or reduced when temperatures were allowed to reach 0°C or below for more than 14h. Temperatures of 20°C or above for more than 10h interspersed with more favourable temperatures prevented sporulation.

Eyespot lesions are normally found at the base of the stem near ground level, occasionally in the axil of the leaf blade and leaf sheath, but very rarely on the green leaf blade (Glynne, 1953).

This observation has led to the assumption that the stem base is where most of the conidia collect. However, the distribution of splash-dispersed spores on naturally infected seedlings has not been examined. Differences in structure between plant tissues may affect their susceptibility to infection by germinating conidia of P. herpotrichoides. The coleoptile is especially susceptible to infection (Bateman & Taylor, 1976). The upper and lower surfaces of wheat leaves and sheaths differ in their structure and physiology. The upper surface, for example, has a thicker layer of wax, fewer stomata and more hairs than the lower surface (Percival, 1921). These factors may play an important part in the retention of conidia by wheat seedlings and also affect their subsequent germination. The chemical composition of the waxes found on leaf cuticles may affect their wettability (Netting, 1973) and influence spore retention. Rapilly & Foucault (1976) found that spores adhere most firmly to smooth surfaces because the area of contact is greatest.

This section describes experiments which examined the seasonal changes in numbers of conidia on infected straw on a field site in relation to temperature and rainfall. The samples of naturally infected straw were incubated at a range of temperatures and the production of conidia was assessed. The coleoptile, which is especially susceptible to infection, contains far less chlorophyll than the leaf blade. Germination and infection by P. herpotrichoides conidia were examined on plants kept under reduced lighting (to decrease chlorophyll) and on plants kept under normal lighting. The distribution of conidia on naturally infected seedlings and their subsequent germination and initial

growth were also examined. Known concentrations of conidia were used to inoculate seedlings and the severity of lesions which developed was assessed.

3.2 Sporulation on wheat straw in the field and at different temperatures

Straw stubble naturally infected with P. herpotrichoides was spread over the surface of an area of fallow ground (c. 40m²) on the Rothamsted Farm. Stubble was collected from a wheat crop known to have a high incidence of eyespot after the 1981 harvest and was spread densely over the area the following November. At weekly intervals from February to July 1982 samples of 50 straws were taken from this site. The roots were removed from groups of ten straws, cut to about 5cm length, which were placed in each of 5 McCartney bottles. To each bottle, a 10ml aliquot of sterile distilled water was added and the bottles were placed on an automatic shaker for 1h. The resultant suspension was decanted and made up to 10ml where necessary. The numbers of P. herpotrichoides conidia in each suspension were estimated from counts made with a haemocytometer slide. The viability of conidia was also estimated, using a 'hanging drop' preparation. A drop of spore suspension was placed centrally on a cover-slip which was inverted over the cavity of a slide to which it was held by spots of Vaseline. These preparations were incubated for 24h at 20°C in petri-dishes on wet filter paper and then 50 conidia were examined for germination. The presence of a germ-tube was taken to indicate germination and viability.

The washed straws were incubated at 5, 10, 15, 20 or 25°C. After 3 or 5 weeks, newly formed conidia were washed from these straws, counted and their viability determined by the same method.

Results

In February 1982, when mean weekly temperatures were c. 6°C , numbers of conidia per straw were very high; on average about 8×10^6 conidia were obtained from a 5cm length of infected straw (Fig. 3). By June numbers of conidia had declined to 2×10^4 per straw and mean temperatures had risen to c. 15°C . A regression analysis indicated a negative correlation ($P < 0.01$) between numbers of conidia per straw and mean weekly temperatures (Fig. 4) through the period February to July. This is in agreement with data obtained for production of conidia on washed straws at a range of temperatures. Conidial production was greatest at 5° and 10°C ; higher temperatures did not favour spore production. Dispersal of conidia from straws by rain accounts for some of the fluctuations in numbers present on the straws. ^(p 149) The viability of the conidia was similar throughout the sampling period. A mean of 85% of the conidia had germinated after 24h at 20°C .

Fig. 5 shows the effect of temperature on subsequent conidial production by one representative sample from ten examined between February and July 1982. At 25°C numbers of conidia per straw were low but bacterial growth was enhanced. Spores of Penicillium, Aureobasidium, Fusarium and Cylindrocarpus species were commonly observed in suspensions washed from straws incubated at 20°C . At temperatures of 15°C and above, fewer conidia of P. herpotrichoides were present on straws incubated for 5 weeks than on straws incubated for 3 weeks. ^(due to the germination of spores) This decrease, associated with the longer incubation period, was accompanied by an increase in the numbers of spores of other fungi and of bacteria. Conidia of P. herpotrichoides produced during subsequent incubation at different temperatures had a mean percentage germination of $57 \pm 8\%$; lower than that of

Fig. 3 Spore numbers with temperature and rainfall

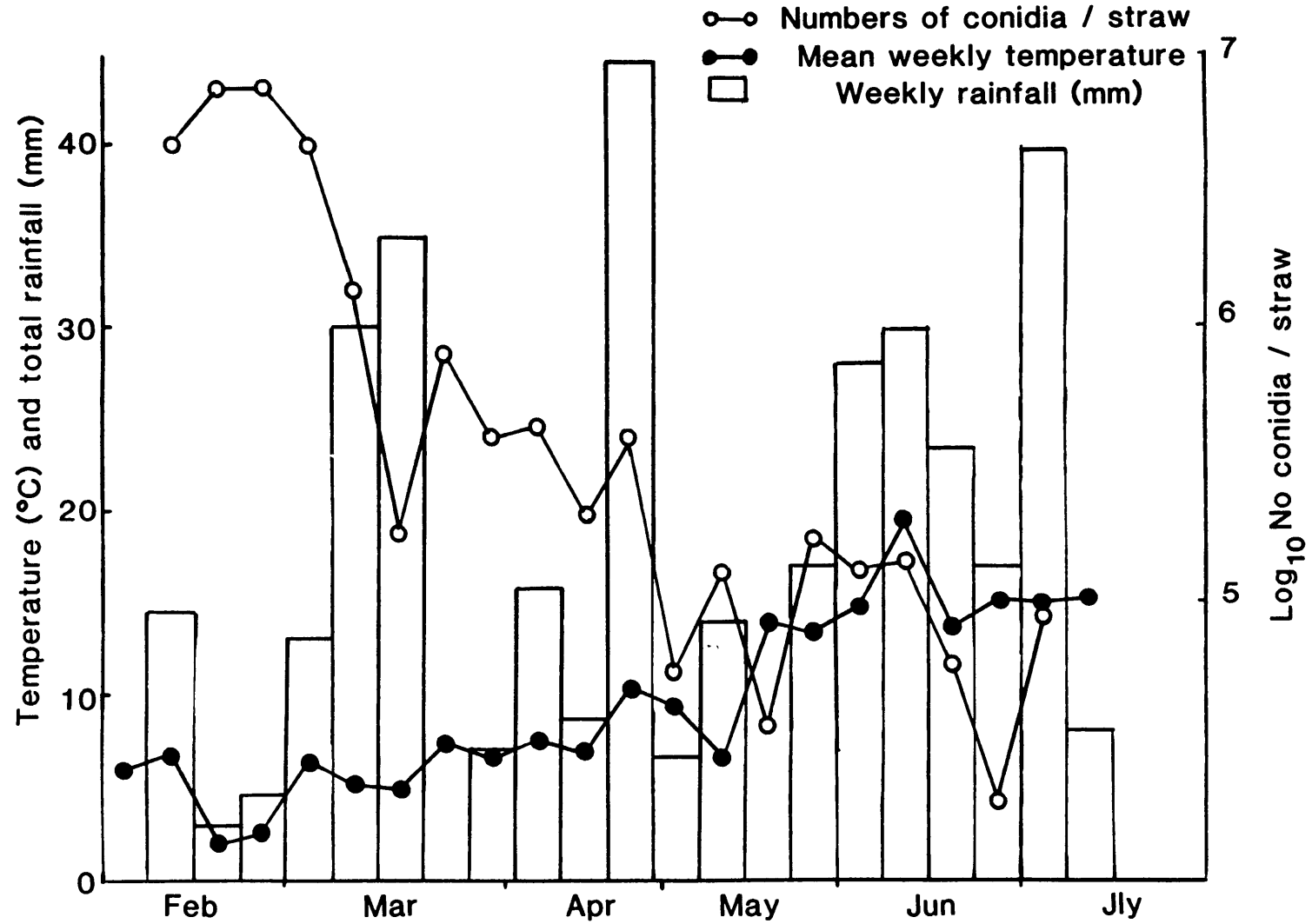


Fig.4 Regression of numbers of conidia produced on infected wheat straw on temperature

where $y = a + bx$
and Total sum of squares $S = \sum_{i=1}^N (y_i - \bar{y})^2$

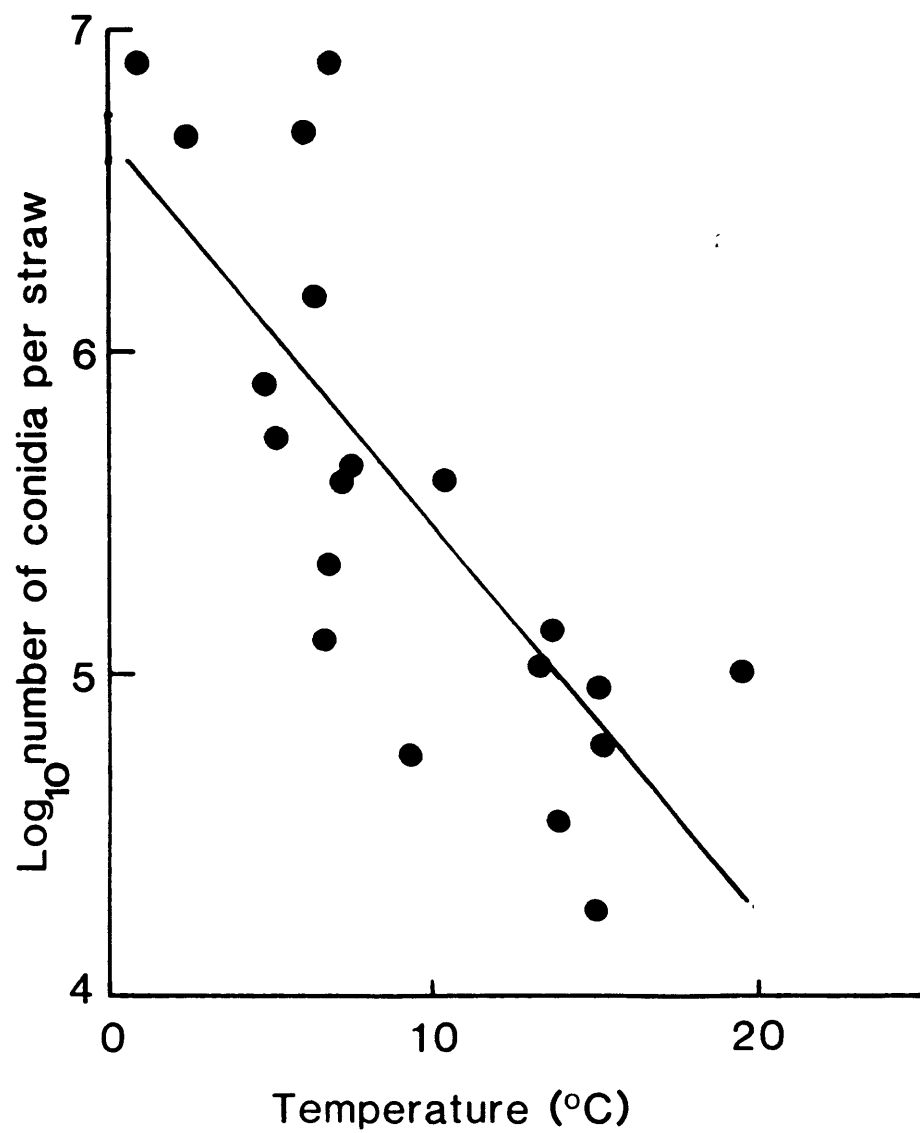
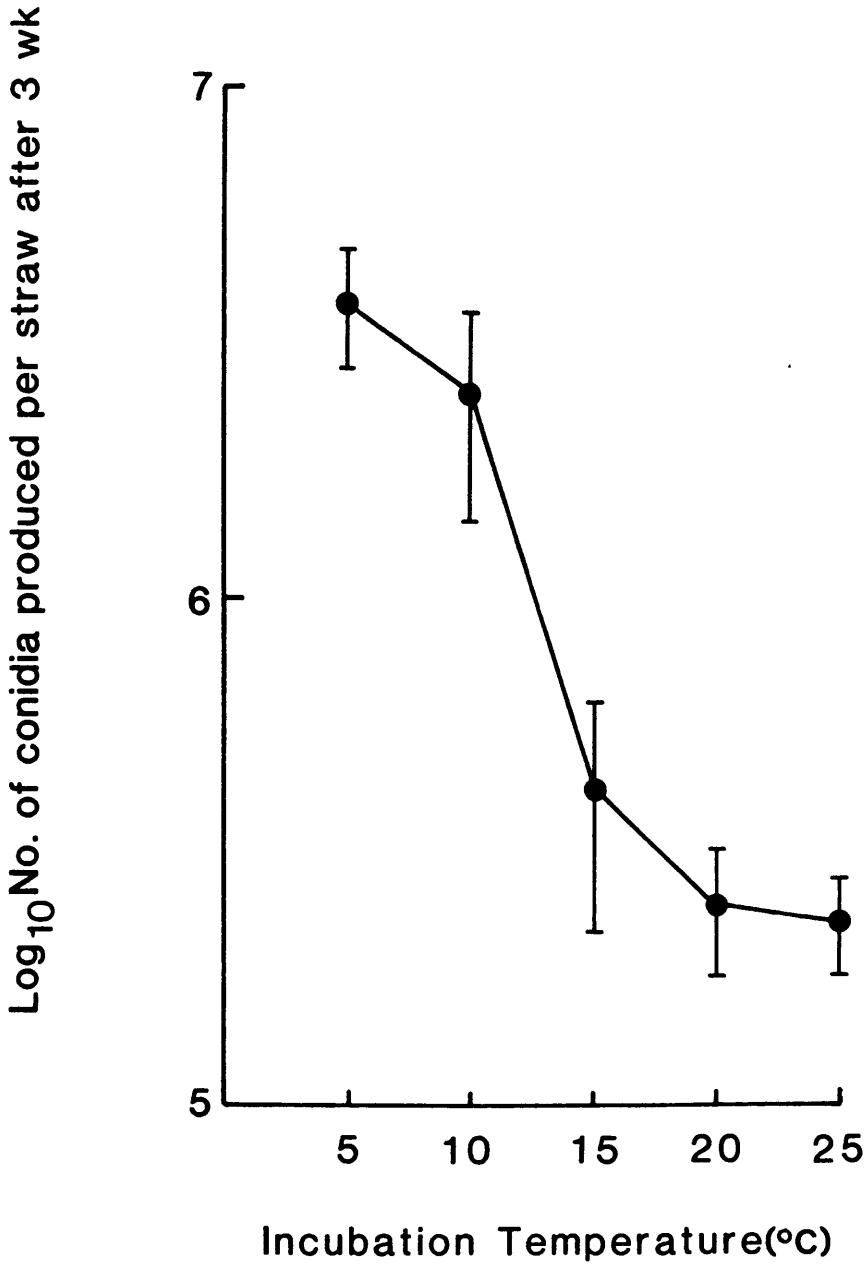


Fig 5. Sporulation of P.herpotrichoides on straw at different temperatures. Vertical bars indicated LSD ($P<0.01$). Data based on sample of straw from field in February 1982



conidia produced on straw in the field.

3.3 Distribution and retention of conidia on wheat seedlings

There were four experiments each with 10 pots (12cm diameter) of five seedlings grown to the third leaf stage. In two experiments (1 and 2) plants in each pot were sprayed with 10ml of a suspension containing 3×10^4 conidia/ml.

In a further two experiments (3 and 4) pots were sunk into the ground amongst infected straw on the field site (section 3.2). In experiments 1 and 2 half of the plants were subsequently exposed to rain (maximum of 2.5mm in 3.5h) and then all plants were dried off in a glasshouse. In experiment 3 plants were removed after rain (3.7mm in 4h) and received no further treatment. In experiment 4 all plants were removed from the site after rain (2.3mm in 3.4h) and half of them were exposed to a further period of rain (3.6mm in 4.5h). At intervals, the proximal and distal halves of the first, second and third leaves were cut from a sample of 25 plants. Three sections, 2cm long, of each tissue were selected at random and surface impressions prepared using collodion in amyl acetate (experiment 3) or double-sided sellotape (experiments 1, 2 and 4) (after Langvad, 1980). These impressions were stained with cotton-blue/lactophenol and examined to determine the number, orientation and percentage germination of conidia on each surface. Data were transformed ($\log_{10} (X+1)$) before analysis of variance.

Results

In the second 'natural' inoculation experiment (4) plants collected too few spores to make analysis of the results valid and it will not be considered further. Seedlings placed amongst naturally infected straw (experiment 3) and seedlings inoculated with an aerosol both collected conidia on the upper and lower surfaces of all leaf blades and leaf sheath bases. There was no significant difference in the number of conidia collected between the proximal and distal ends of the leaf blades. Table 1 shows the mean number of

Table 1. Retention of conidia on wheat seedlings

Mean total number of conidia/cm² *

<u>Leaf</u>	<u>Surface</u>	<u>Experiment 1</u>				<u>Experiment 2</u>				<u>Experiment 3</u>	
		<u>A</u>	<u>a</u>	<u>B</u>	<u>b</u>	<u>A</u>	<u>a</u>	<u>B</u>	<u>b</u>	<u>A</u>	<u>a</u>
First	adaxial	73	2.810	45	2.601	17	2.233	16	2.190	69	2.114
	abaxial	88	2.878	78	2.800	63	2.769	53	2.346	101	2.293
Second	adaxial	63	2.762	56	2.560	19	2.065	17	2.206	59	2.058
	abaxial	139	2.648	195	2.781	27	2.156	68	2.829	113	2.342
Third	adaxial	17	1.873	38	2.529	4	1.509	4	1.585	11	1.262
	abaxial	13	2.078	71	2.691	7	1.820	37	2.276	50	1.984
Stem base		-		-		-		-		15	1.424
SED between two			0.199				0.165				0.104
mean (log ₁₀ x+1)											
Df			48				48				24

*On plants sprayed with a suspension of conidia (Experiment 1 and 2) or exposed to natural inoculum (Experiment 3), after spraying or first rain (A) and after subsequent rain (B) (a and b are log₁₀(x+1) transformations of data).

conidia retained after inoculation and after subsequent rain. Subsequent rain did not significantly ($P < 0.05$) reduce the number of conidia present on the plant surfaces. Generally more conidia were collected on the lower (abaxial) than on the adaxial surface. There were significantly more spores lying over the anti-clinal plant cell walls after subsequent rain than after the initial inoculation (when results were analysed by a chi-square test) in two instances, one in each experiment (Table 2).

Table 3 shows numbers of conidia which had germinated within 24h, when the length of the spore was either above the anticlinal plant cell walls or in other orientations. In all three experiments the numbers of conidia above the anti-clinal walls which had germinated were significantly higher ($P < 0.001$) than the numbers in other orientations which germinated.

3.4 Susceptibility of plant tissue

Fifteen seedlings were planted in each of 12 pots (11cm diameter), in five rows of three seedlings, each with its radicle facing the same way. Coleoptiles then faced the same way, and so three plants could be inoculated together (Fig.6). At the first leaf stage, the coleoptile and proximal and distal ends of the leaf blade were inoculated using sponge clips impregnated with suspensions containing 4×10^6 conidia/ml.

All pots were covered with clear plastic tops to create high humidity. Additionally six pots were each covered with black polythene. All pots were then placed in a growth cabinet with alternating day and night temperatures of 15°C and 10°C for 12h periods. The cabinet was illuminated during the 12h day periods. Readings taken with a 'Lunasix 3' light meter inside the plastic tops indicated light intensities of 4,400 lux

Table 2. Orientation of conidia retained on wheat plant surfaces

Experiment 1

<u>Leaf</u>	<u>Surface</u>	<u>Sample 1:after spraying</u>		<u>Sample 2:after rain</u>	
		<u>Number of conidia over anticlinal cell walls</u>	<u>% of total number</u>	<u>Number of conidia over anticlinal cell walls</u>	<u>% of total number</u>
First	adaxial	127	34.6	76	33.3
	abaxial	144	32.5	126	32.0
Second	adaxial	113	35.6	108	38.0
	abaxial	234	33.4	327	33.5
Third	adaxial	10	11.6	52	27.2**
	abaxial	21	31.3	112	31.2

** refers to the level of significant difference by the chi-square test, ($P < 0.01$)
in the number of conidia lying over the anticlinal cell walls after rain.

Table 2. Orientation of conidia retained on wheat plant surfaces

Experiment 2

<u>Leaf</u>	<u>Surface</u>	<u>Sample 1:after spraying</u>		<u>Sample 2:after rain</u>	
		<u>Number of conidia over anticlinal cell walls</u>	<u>% of total number</u>	<u>Number of conidia over anticlinal cell walls</u>	<u>% of total number</u>
First	adaxial	33	37.0	40	48.7
	abaxial	118	37.2	123	45.8*
Second	adaxial	35	35.3	34	39.5
	abaxial	47	34.8	137	39.8
Third	adaxial	5	25.0	8	38.0
	abaxial	13	37.1	63	33.6

* refers to the level of significant difference by the chi-square test. ($P < 0.05$)
in the number of conidia lying over the anticlinal cell walls. after rain.

Table 3. Germination of conidia lying above anti-clinal cell walls and in other orientations

Experiment 1

	<u>Numbers of conidia *</u>		
	<u>Germinated</u>	<u>Not Germinated</u>	<u>Total</u>
Above anticlinal cell walls.	86	203	289
Other orientations	58	533	591
$\chi^2=56.4, P<0.001$			

Experiment 2

	<u>Number of conidia *</u>		
	<u>Germinated</u>	<u>Not Germinated</u>	<u>Total</u>
Above anticlinal cell walls.	40	93	133
Other orientations	12	173	185
$\chi^2=31.47, P<0.001$			

Experiment 3

	<u>Numbers of conidia *</u>		
	<u>Germinated</u>	<u>Not Germinated</u>	<u>Total</u>
Above anticlinal cell walls.	73	146	219
Other orientations	31	165	196
$\chi^2=16.9, P<0.001$			

* All plant surfaces combined.

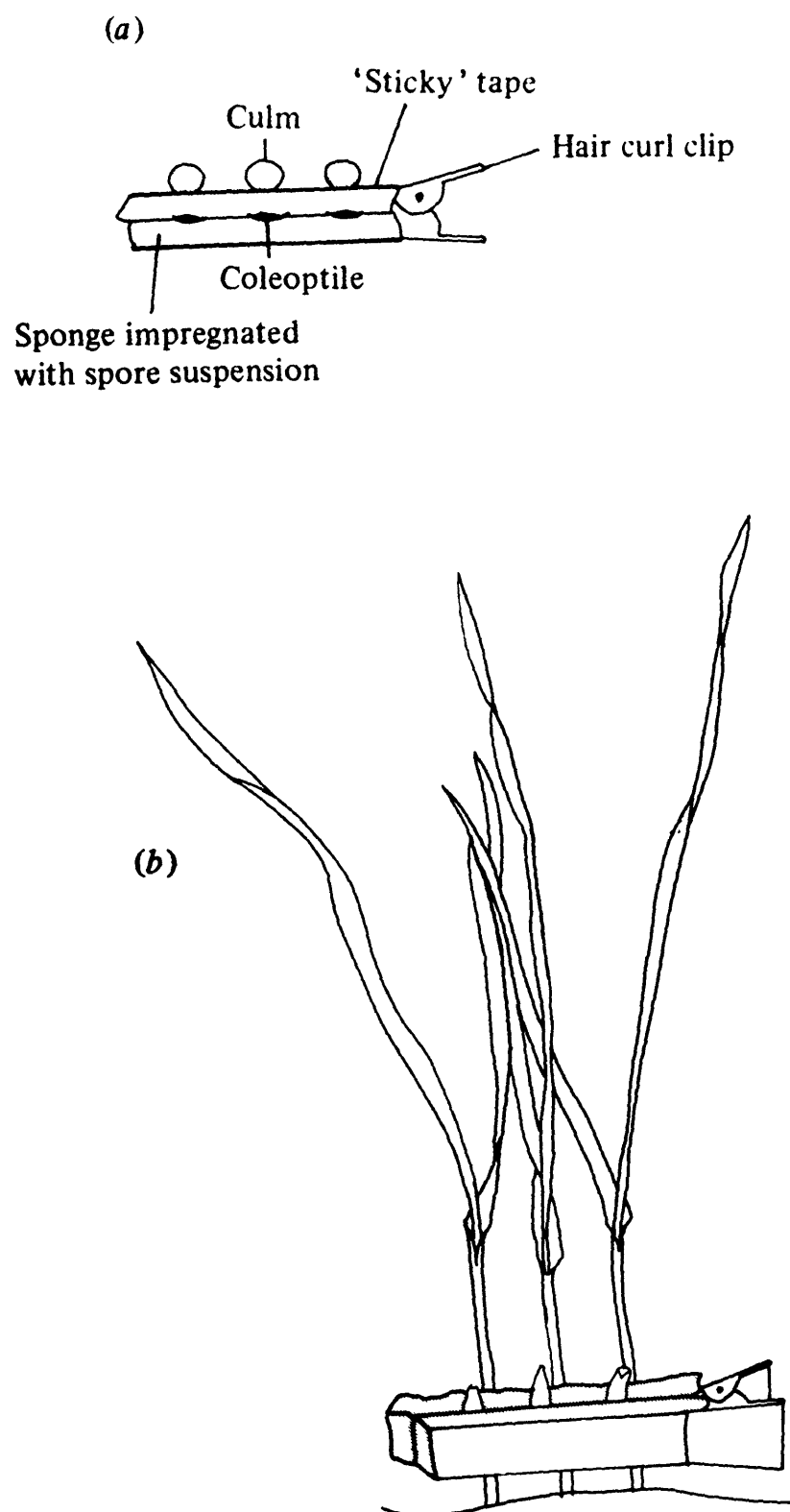


Fig. 6 Diagram of inoculation of wheat seedlings by *P. herpotrichoides*. a) T.S. b) Whole specimen.

and 525 lux beneath the clear cover and black polythene respectively. The sponge clips were removed after 16h and inoculated tissue was taken from three plants in each treatment at 16, 24, 40 and 56h after inoculation. Percentage germination was assessed from stained sellotape impressions. Leaf tissues were also mounted directly on slides stained with cotton-blue/lactophenol and examined for haloes indicating penetration.

Results

There was no evidence to suggest that reducing light intensity had any effect on germination of conidia on the different plant tissues. Conidia of P. herpotrichoides germinated much more quickly on the coleoptiles than on the proximal and distal ends of the leaf blades (Fig.7). A reduced analysis of variance test to compare the coleoptile with the leaf as a whole (proximal and distal end combined), at both light intensities, showed that there was significantly more ($P < 0.001$) growth on coleoptiles than on leaf blades (Table 4).

Occasionally hyphae were observed penetrating the cells of the leaf tips, but penetration haloes were far more common on the proximal ends of leaf blades and on the coleoptiles (Fig.8).

3.5 Lesion development in relation to the number of conidia in inoculum

Seedlings were grown to the first leaf stage in a cool glass-house ($10-15^{\circ}\text{C}$) in 32 pots (6cm diameter) each with five seedlings. These seedlings were then inoculated using filter paper discs soaked in sterile distilled water or in one of a range of suspensions containing different numbers of conidia (Table 5). Each disc was placed

Fig. 7 Germination of conidia on coleoptile (x) and on proximal (●) and distal (o) ends of leaf blade (light and dark treatments combined). Vertical bar indicates LSD ($P < 0.05$) for any pair of observations at any given time.

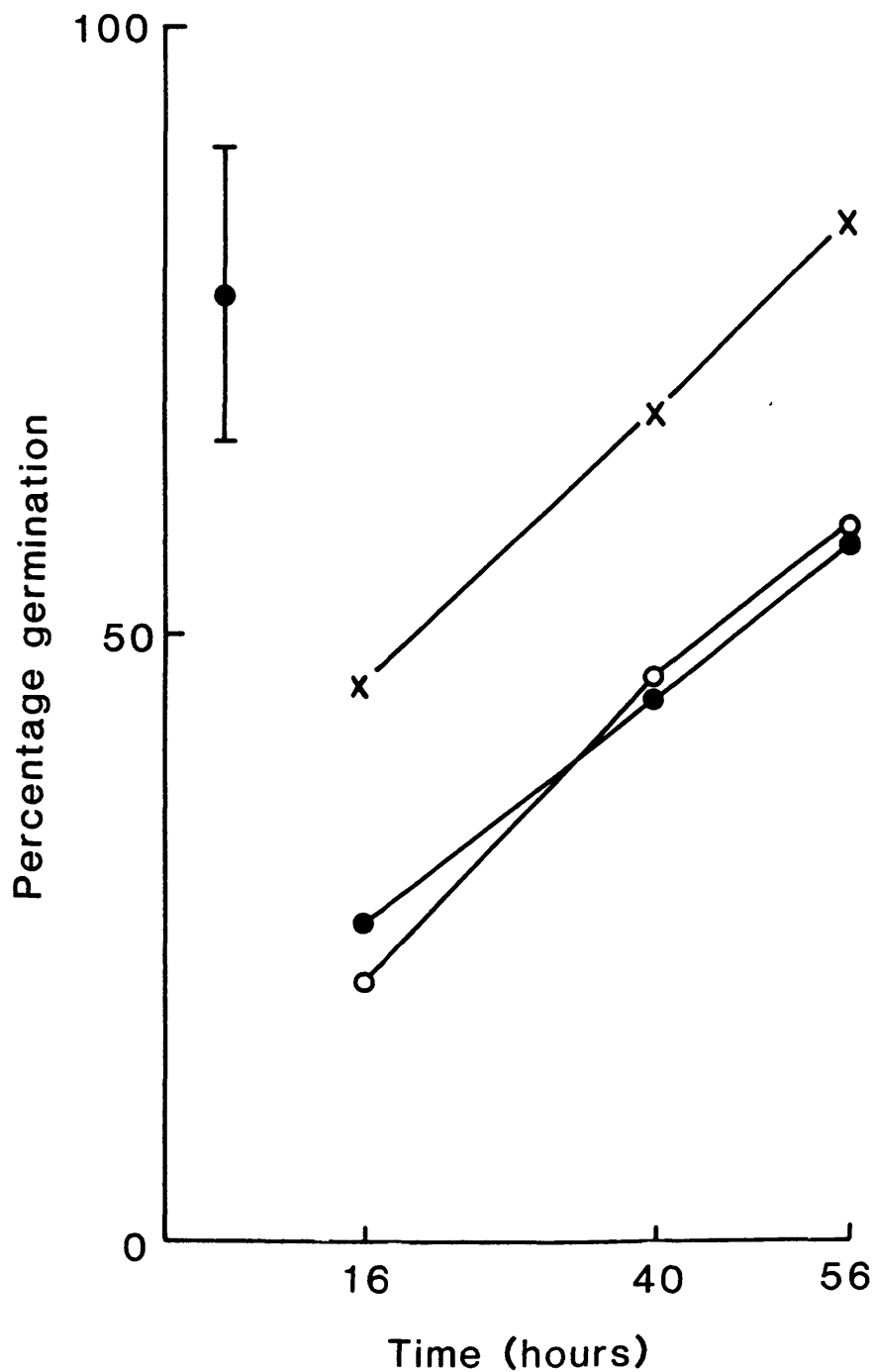


Table 4. Initial mycelial growth on different plant tissues under different light intensities

<u>Light intensity</u>	<u>Tissue</u>	<u>Mean percentage of conidia with hyphae longer than</u> <u>the length of the spore after:-</u>			
		16h	24h	40h	56h
525 lux.	Coleoptile	17.0	16.4	53.9	40.3
	Leaf (proximal end)	5.1	6.3	8.7	15.3
	Leaf (distal end)	0.8	2.3	13.2	20.9
4,400 lux.	Coleoptile	12.0	14.4	24.8	47.1
	Leaf (proximal end)	0.0	2.3	8.8	14.2
	Leaf (distal end)	2.3	3.0	8.6	20.8

Coleoptile v leaf (proximal and distal end), for both treatments ($P < 0.001$). The light intensity treatments - not significantly different.

Fig. 8. Photograph to show penetration haloes on the leaf blade caused by P. herpotrichoides hyphae

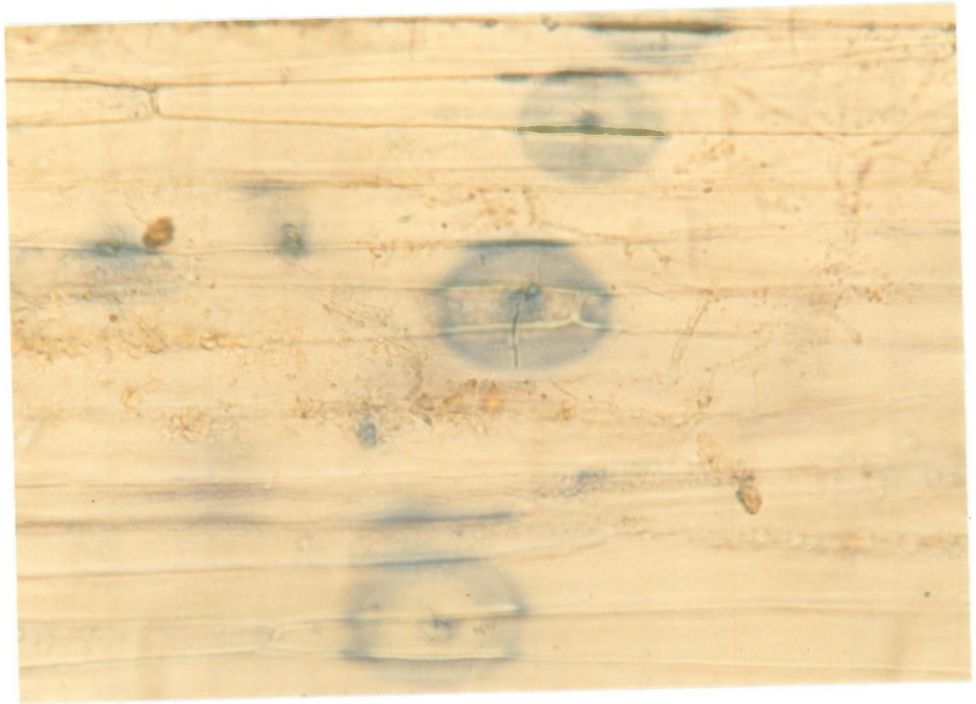


Table 5. Concentration of conidia used for inoculation

<u>Concentration of conidia/ml suspension</u>	<u>Calculated number of conidia per disc</u>
1	0.01
10	0.1
10^2	1.0
10^3	10
10^4	10^2
10^5	10^3
10^6	10^4

between the coleoptile and mainstem of each plant and held in place with parafilm (section 2.3). Each of the suspensions in the range 10^6 to 1 conidia/ml was inoculated onto ten replicate seedlings. Plants were kept in a glasshouse at c. 15°C and were sampled and scored for percentage infection and lesion severity (section 2.4) after 4 to 20 weeks. The number of leaf sheaths penetrated was also counted. Disease severity was calculated as:-

$$\left(\frac{\sum_{i=1}^3 n_i x_i}{3n_t} + \frac{\sum_{i=1}^9 n_i y_i}{9n_t} \right) \cdot 100$$

where x_i was lesion severity (0-3), y_i the number of leaf sheaths penetrated (1-9), n_i the number of mainstems with a given score and n_t the total number of mainstems in each sample.

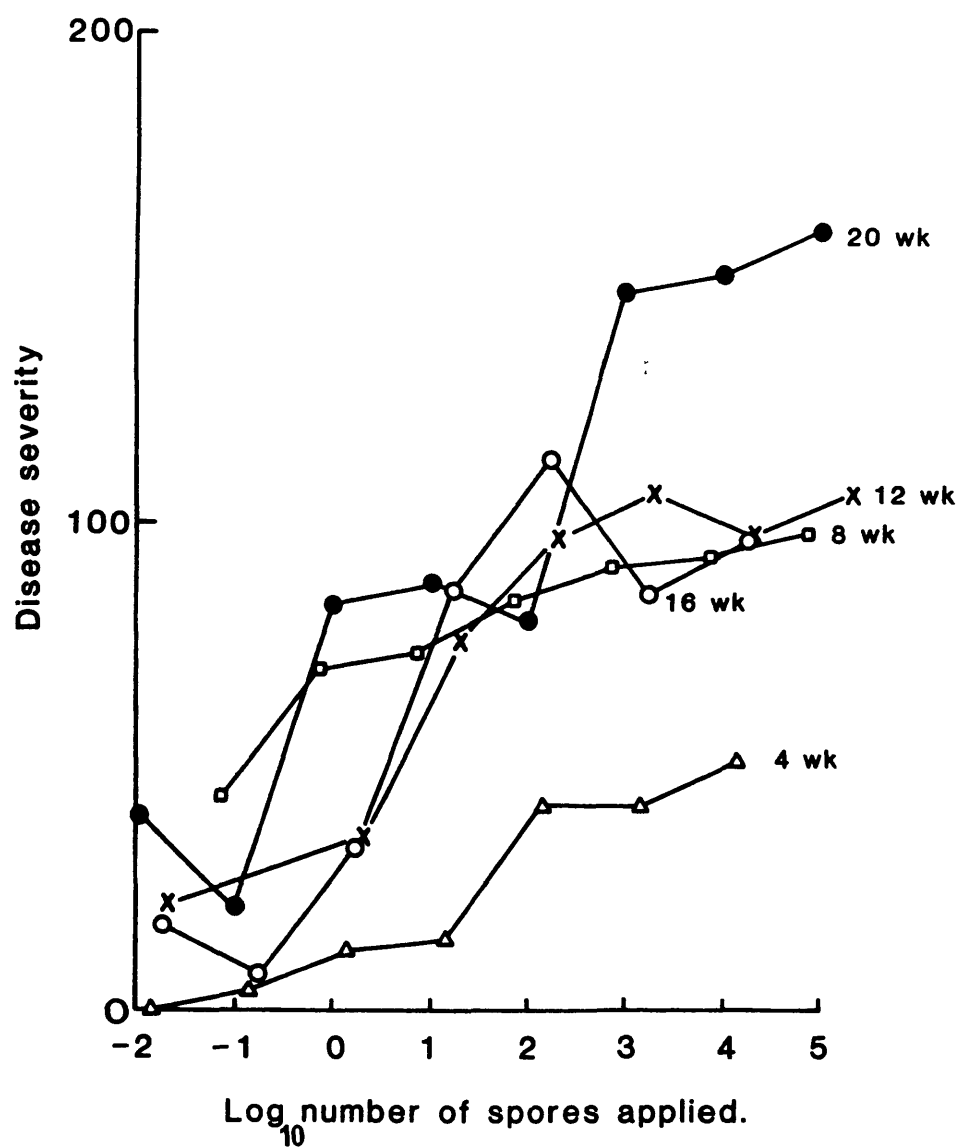
Results

The severity of infection increased as number of conidia applied to seedlings increased (Fig.9). This relationship held throughout the period of observation (4-20wk after inoculation). There was a rapid increase in disease severity ^{4 and 8wk and} between 16 and 20 wk when the mature plants reached 'booting' (growth stage 45, Zadoks et al., 1974).

3.6 Summary of results

Production of conidia in the field was favoured by temperatures of about 6°C . When P.herpotrichoides, growing on naturally infected straw stubble, was incubated at temperatures between 2°C and 25°C , maximum conidial production was at 5°C . At temperatures above 10°C , bacteria and fungal competitors were dominant. Conidia collected from the field were viable throughout the whole of the sampling period (February to July). Viability of conidia was less

Fig. 9 Lesion Severity with time and different numbers of conidia



when they were produced in the laboratory.

Wheat seedlings collected conidia on all plant surfaces and subsequent rain did not significantly reduce the number of conidia which remained 'attached'. Germination of conidia after 48h was greater for those which lay above the anti-clinal plant cell walls than for those which lay in other directions. Comparatively few conidia were collected at the base of the stem, and most were found on the lower surfaces of the leaves. Germination of conidia was much quicker on the coleoptile than on other plant tissues. Mycelial growth by P. herpotrichoides was only observed on the coleoptile even though penetration of the leaf blade was occasionally seen.

The induction of chlorosis, by reducing light intensity at which plants were grown, did not increase the susceptibility of wheat leaves to infection.

The severity of lesions formed on wheat seedlings, and the depth to which the fungus penetrated the leaf sheaths, was directly related to the initial numbers of conidia infecting the plant at the coleoptile. These differences in lesion severity were maintained throughout the growing period of the host plant upto 'booting stage' (growth stage 45).

CHAPTER 4

GROWTH AND PATHOGENICITY OF *P.HERPOTRICHOIDES* ISOLATES

4.1 Introduction

Several factors appear to influence the growth and pathogenicity of *P.herpotrichoides*. Isolates of the fungus vary in their ability to infect cereals. This was clearly demonstrated by Scott & Hollins (1977) in tests with wheat seedlings, though the differences were not consistent from one experiment to the next. They suggested that this inconsistency was caused by sub-culturing of the heterozygous mycelium or by changes in the environment between experiments. Although there is no evidence that isolates show physiological specialisation, Scott, Hollins & Muir (1975) demonstrated some isolate specialisation. The rye (r-type) isolates have been distinguished from the wheat (w-type) isolates by their slow growth rate and the feathery appearance of the colony edge when grown on cornmeal agar (Lange-de la Camp, 1966). How the growth rate in culture of the isolates is related to pathogenicity to wheat is not clear. Most work on the pathogenicity of *P.herpotrichoides* has been done with young seedlings (Scott & Hollins, 1977; Scott, Hollins & Muir, 1975) and not with mature plants on which the most damaging effects occur in terms of crop yields.

Variants of the fungus have arisen in response to the widespread use of benzimidazole fungicides. These have given good control of eyespot (Jordan & Tarr, 1980) and farmers have used them extensively over the last decade. It is estimated that in the UK 75% of the acreage planted with winter wheat and other cereals is now sprayed with MBC fungicides. However, failure to control eyespot on some

farms led to a survey of field sites throughout the UK by the Agricultural Development Advisory Service (Griffin & Yarham, 1983). They detected isolates of P.herpotrichoides with benzimidazole tolerance in 23 out of the 59 sampled. It is not clear how these MBC-tolerant isolates have arisen, or whether they are equally pathogenic in the absence of fungicides.

In the field the critical stage in the development of eyespot epidemics occurs when the plants are in "boot" (growth stage 45, Zadoks et al., 1974). In some years lesions which have been formed earlier remain restricted and do not cause undue damage; in other years lesions become severe and yield losses result. The role of temperature in determining which of these two situations prevails is not clear but temperature does affect infection markedly. Defosse (1967) found that the optimum temperature for infection of the first leaf sheath was at or below 15°C, while that for the progress to the second leaf sheath was near to 20°C. Increasing temperature over the range 6-18°C increased the penetration of the leaf sheaths by P.herpotrichoides (Scott, 1971). However, this was not a linear relationship and there appeared to be an initial "lag" phase in growth. This probably reflected the need for all environmental conditions to be suitable before infection could take place. Host water potential may also affect the growth and development of P.herpotrichoides. For example Bruehl & Manandhar (1972) suggested that the optimum temperature for growth of P.herpotrichoides in culture is progressively higher as the osmotic potential is lowered from near zero to about -20 bars. This suggests that the host water potential may affect growth and development of P.herpotrichoides in wheat.

The importance of water stress in the development of Fusarium foot rot of wheat in the Pacific Northwest of the USA was illustrated by Cook (1980). Plants on a south facing 30° slope developed severe Fusarium foot rot, whereas plants on an adjacent flat, low-lying area developed only mild infection or none at all. Plants growing on the slope were water-stressed, whilst those growing on the flat area into which water drained were not. Soil-borne pathogens are exposed mainly to matric water potentials between soil particles, but as a fungus enters the host, the osmotic potential of plant cells probably affects fungal growth. During stem elongation and ear production, the rate of vegetative growth is generally less (Barnell, 1936) and there is a decrease in the amount of soluble carbohydrates in the tissues (Barnell, 1938). These changes in the concentrations of sugars affect the osmotic water potential of the host. Spraying wheat plants with a sugar solution promoted growth of P.herpotrichoides on both susceptible and resistant winter wheat cultivars (Evans & Rawlinson, 1977). However, the herbicide "linuron" decreased the concentrations of both glucose and fructose in the stem base and also decreased the severity of eyespot.

This section examines the growth and pathogenicity of P.herpotrichoides isolates under different environmental conditions. The growth of benzimidazole-sensitive and benzimidazole-tolerant isolates was compared in vitro, in seedlings and in mature plants. The germination of conidia and growth of isolates were also tested at different water potentials. The isolates used in these experiments were obtained from either the dryland regions of the USA (section 2.1) or the wetter UK climate. Attempts were made to control the water potentials of plants grown in pots, in buckets and in lengths of nylon tubing by giving the plants different volumes of water. The

severity of lesions produced under these watering regimes was assessed. The effects of temperature on the production of leaf sheaths and on the fungal penetration of live leaf sheaths were also examined.

4.2 Isolate growth

Experiment 1

Mycelial plugs of all 14 isolates of P.herpotrichoides (section 2.1) were each inoculated onto four replicate PDA plates and incubated at 20°C. The diameters of the colonies were recorded over a period of 2 weeks to measure the radial growth rate of each isolate. Differences in growth characteristics between isolates were observed and it was decided to measure growth quantitatively by increase in dry weight. An agar plug taken from the edge of a colony of each isolate was placed in a McCartney bottle containing 20ml of freshly prepared potato dextrose broth (PDB). Three replicate bottles of each isolate were prepared. The bottles were incubated at c.20°C on a shaker for 2 weeks. The fungal mycelium which had grown was filtered off onto filter paper, placed in an oven at 80°C for c.12h and dry weights of each isolate were determined. This experiment was repeated once in McCartney bottles and then twice in conical flasks in which 75ml of PDB were used. Isolates were also grown on cellophane and the mycelial growth was scraped off onto filter paper and weighed after drying. However, this method was not very successful as the fungal growth was not easily separated from the cellophane.

Experiment 2

The cellulolysis rate (Garrett, 1983) of each isolate was measured. A wad of 10 Whatman No. 3 filter papers was added to each of 28 conical flasks together with 20ml of a nutrient solution.

This solution was composed of:-

NaNO ₃	5g	Ferric ammonium citrate	1mg
K ₂ HPO ₄	1g	Thiamin	0.1mg
MgSO ₄ ·7H ₂ O	0.5g	Biotin	0.01mg
Distilled water 1l.			

Flasks were autoclaved at 10^5 N/m^2 for 30 min. Then an agar disc (11mm diam) of PDA covered by an isolate of P. herpotrichoides was placed aseptically on the filter papers in each flask, with two replicates for each isolate. The flasks were incubated at 20°C for 7wk, when the loss in dry weight of the filter papers was calculated. The cellulolysis adequacy index was calculated using the formula (Garrett, 1966):-

$$\frac{\text{respiration rate of colony growing on cellulose}}{\text{unit extension of colony}} = \frac{\text{dry wt loss}}{\text{mm/24h}}$$

Results

Experiment 1

Isolates varied considerably in their rates of growth (Fig. 10) on PDA at 20°C, ranging from 3.3mm/day (isolate 255) to 0.6mm/day (isolate 279). There were also considerable differences in growth between the two benzimidazole-tolerant isolates, growth of isolate W-2 being much slower than that of isolate E-3. There was a significant difference in growth rate between the different isolates ($P < 0.05$, SED 0.7).

The mean dry weights of isolates when grown in PDB in McCartney bottles (a) and conical flasks (b) are given in Table 6. Although, within each experiment there were significant differences in growth between the isolates, there was no marked correlation between the

Fig.10 Mycelial growth rates of P.herpotrichoides isolates on PDA at 20°C

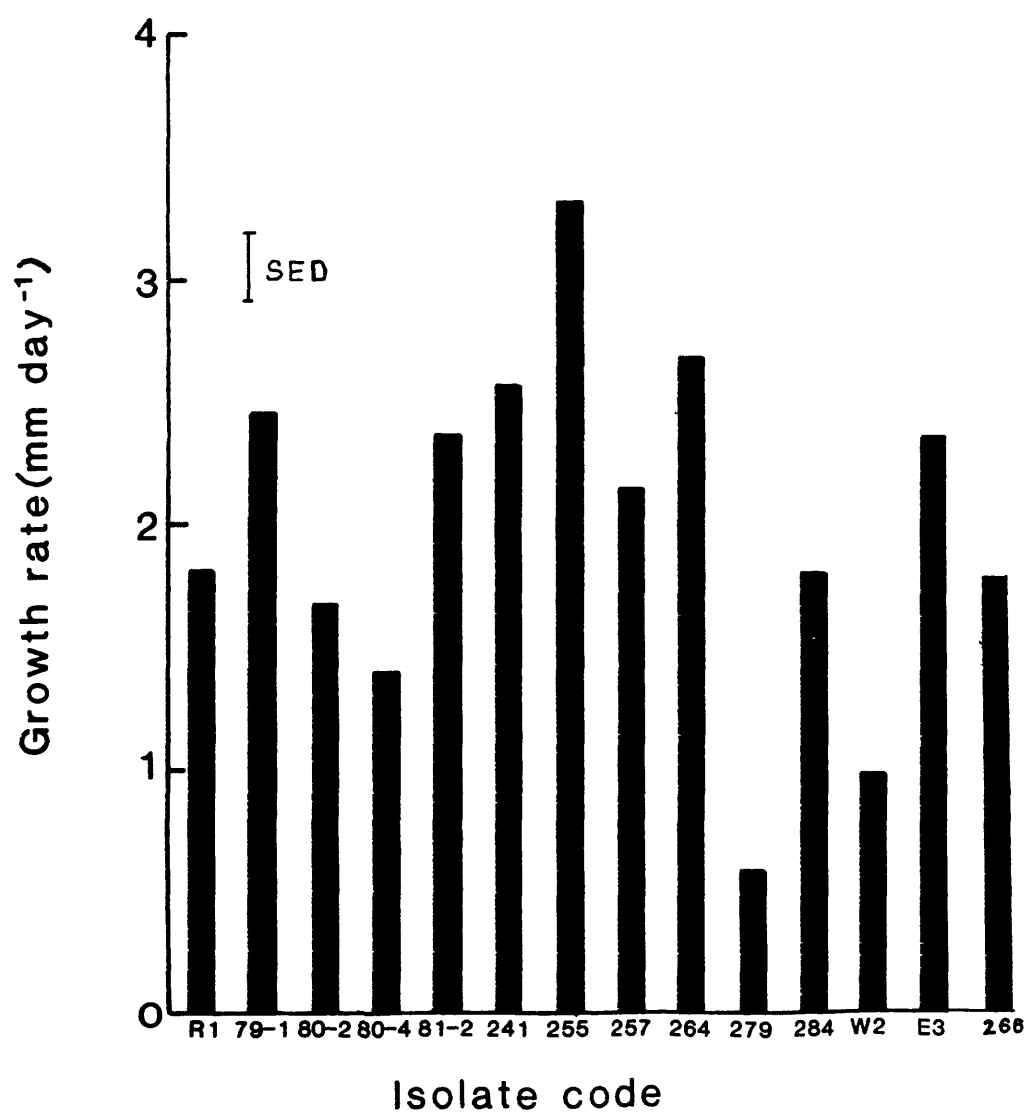


Table 6. Increase in dry weight of P. herpotrichoides isolates
after 2 weeks growth in PDB and their cellulolysis
adequacy index

Isolate	Mean increase in weight (g)		Cellulolysis Adequacy Index
	Experiment (a) (McCartney bottles)	Experiment (b) (Conical Flasks)	
R-1	0.080	0.955	0.014
79-1	0.081	0.573	0.0330
80-2	0.070	0.621	0.042
80-4	0.077	0.651	0.0467
81-2	0.096	1.061	0.0297
241	0.082	0.659	0.0243
255	0.058	0.832	0.0037
257	0.059	0.937	0.0175
264	0.057	0.787	0.0533
266	0.085	0.938	0.0490
279	0.074	0.959	0.1077
284	0.068	1.012	0.0305
W-2	0.088	0.726	0.0676
E-3	0.088	0.744	0.0043
SED	0.013	0.144	0.014
DF	69	67	13

growth of isolates in McCartney bottles and growth in conical flasks. Thus isolate 279, which ranked third in conical flasks, ranked ninth in McCartney bottles. Similarly there appeared to be no relationship between growth of isolates on PDA at 20°C and growth in the liquid medium. The two benzimidazole-tolerant isolates (W-2, E-3) showed similar increases in dry weight which were within the extremes recorded for the benzimidazole-sensitive isolates.

Experiment 2

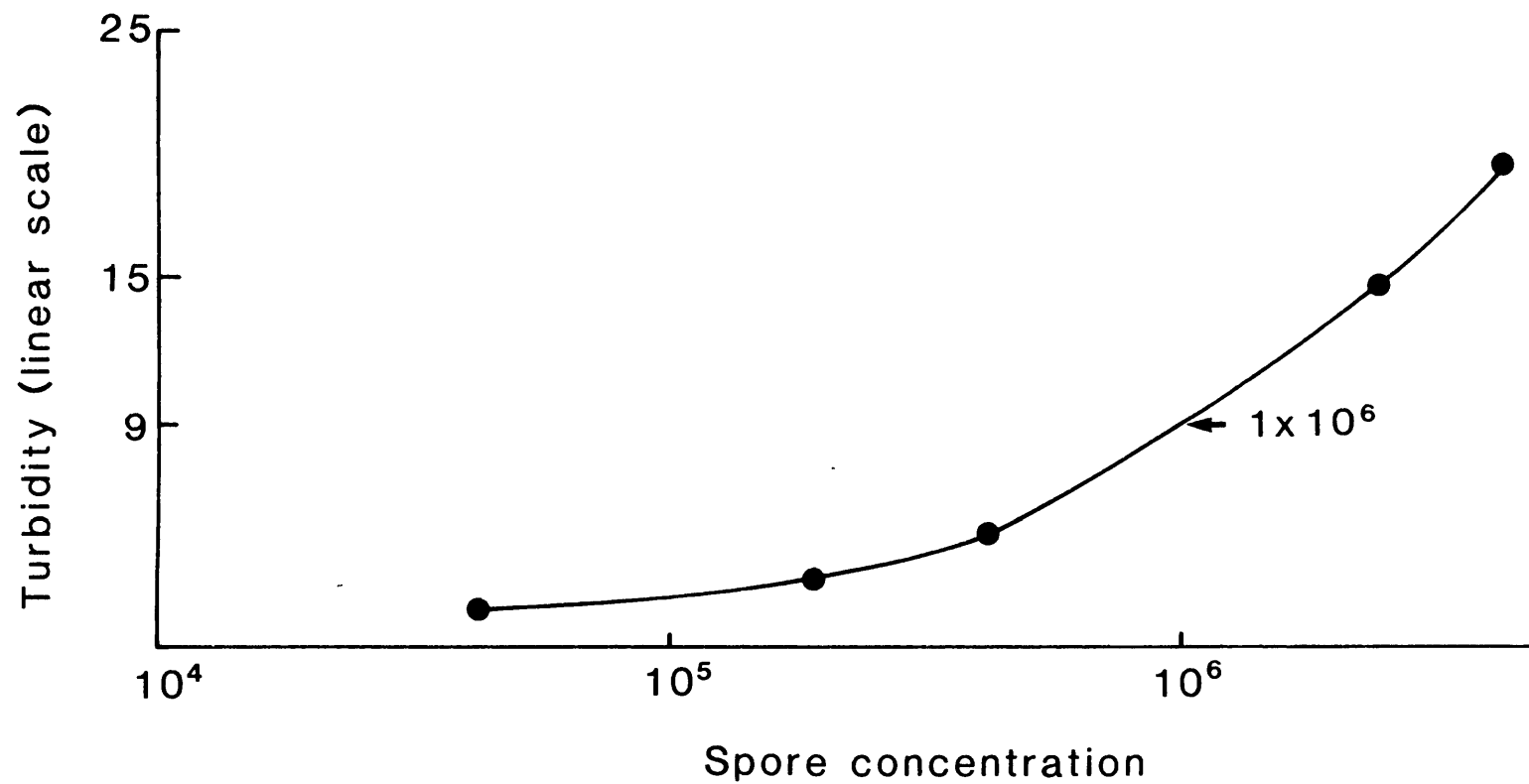
The Cellulolysis Adequacy Index (CAI) differed significantly between isolates. However, CAI was not correlated with an increase in dry weight when grown in PDB. The CAI of isolate W-2 was significantly greater than that of E-3 (both are benzimidazole-tolerant). This was probably a reflection of the difference in radial growth rate on PDA between the two isolates.

4.3 Pathogenicity of isolates

(1) To seedlings

To test the pathogenicity of isolates to wheat seedlings (cv. Armada) the filter paper disc method of inoculation was used (section 2.3). Spores were washed from DWA plates using sterile distilled water. Concentrations of spore suspensions were estimated using a haemocytometer slide and their corresponding turbidities were determined using a nephelometer (Eel Ltd, Hadleigh, Essex). From these results a standard curve was prepared (Fig. 11). Spore suspensions of all the isolates were adjusted to

Fig.11 Standard curve for concentration of P.herpotrichoides spores using the Nephelometer turbidity meter



the same concentration (10^6 /ml) by means of a turbidity reading on a nephelometer. Each suspension was then serially diluted by steps of 1/10 to 1 spore/ml.

Ten sterile filter paper discs (5mm diam) were placed in each spore suspension and each was used to inoculate a wheat seedling. The seedlings were grown in 12cm diam. pots in a sand/loam mixture with 5 plants per pot. After inoculation they were incubated at 10/15°C night/day temperatures for 4 weeks and then scored for disease severity (section 2.4) and depth of penetration of the fungus through the leaf sheaths. The disease severity scores were logit (i.e. $\log \frac{x}{1-x}$) transformed. A common regression of logit (disease severity) on spore concentration was then calculated for all of the isolates and each isolate was then compared for its deviance from this line.

Results

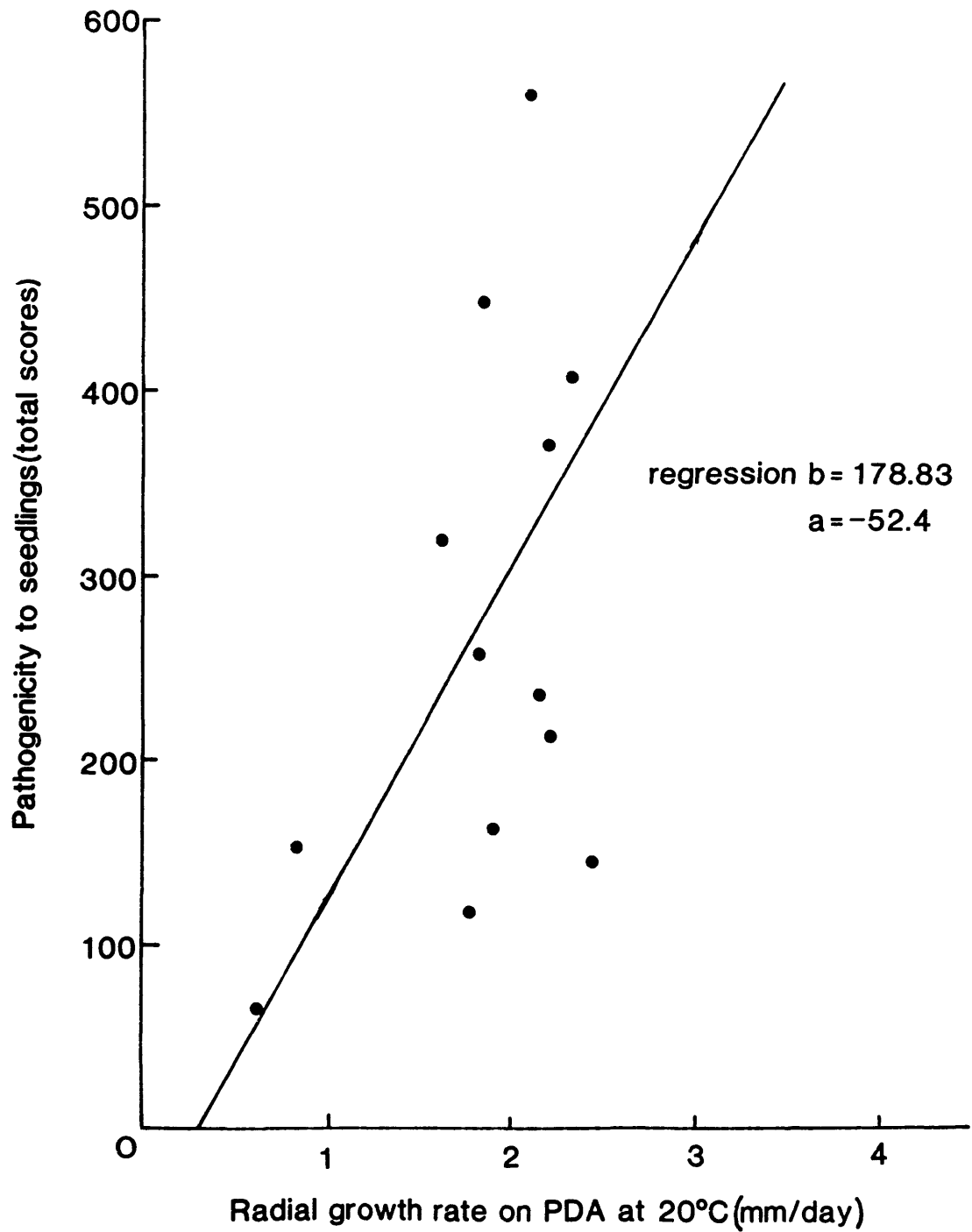
None of the control plants showed symptoms of eyespot lesion formation. All of the isolates tested produced more severe lesions as the number of conidia applied to the seedlings was increased, i.e. conidia appear to act together in the formation of lesions (Table 7). On the basis of the total pathogenicity score, isolate 255 was the most pathogenic and isolate 279 the least pathogenic. There was a significant correlation between the total pathogenicity score and the radial growth rate on PDA (Fig. 12, $P < 0.01$, 12 Df.). A correlation of $\log_{10}$ disease severity with $\log_{10}$ number of conidia applied was made for each isolate. All of the isolates gave a significant positive correlation (Fig. 13). The threshold value for infection, taken as the intercept of the x axis for each isolate, was found and this was also correlated to the rate of radial growth on PDA ($P < 0.05$). There was a significant

Table 7. Effect of conidial concentration on pathogenicity of *P.herpotrichoides* isolates
to wheat seedlings

Isolate	Pathogenicity score from inoculations with following concentrations of conidia/ml								Estimate of intercept on x axis for logit transformed data	
	<u>0</u>	<u>1</u>	<u>10</u>	<u>10²</u>	<u>10³</u>	<u>10⁴</u>	<u>10⁵</u>	<u>10⁶</u>	<u>Total</u>	
R-1	0	10.6	14.6	10.6	26.6	54.0	50.6	90.0	257.0	-4.028
79-1	0	0	14.6	20.0	44.6	98.6	114.6	114.6	407.0	-2.496
80-2	0	0	12.6	10.6	16.0	36.0	34.0	54.6	163.8	-3.973
80-4	0	0	2.0	18.0	35.3	59.3	89.3	114.6	318.5*	-2.698
81-2	0	5.5	5.5	10.6	16.0	66.6	57.3		236.0	-3.841
241	0	0	39.3	43.3	50.0	62.0	79.3	96.6	370.5	-1.695
255	0	58.0	64.0	99.3	100.6	120.6	115.3	128.6	561.0	1.641
257	0	21.3	37.3	78.0	104.6	102.6	110.6	116.6	370.5	-0.316
264	0	10.6	5.3	10.6	30.6	25.3	32.0	32.0	146.4	-6.625
266	0	12.6	21.3	32.6	70.6	88.0	112.6	110.6	448.3	-1.524
279	0	7.3	5.3	5.3	10.6	10.6	10.6	16.0	65.7	-10.015
284	0	5.5	16.0	10.6	21.3	21.3	26.6	26.6	318.5	-5.350
W-2	0	0	0	5.3	10.6	18.0	33.3	85.0	152.2	-4.947
E-3	0	0	0	10.6	22.0	52.6	54.0	74.0	213.2	-3.847

* estimated total using a mean value for 10⁶ conidia/ml

Fig.12 Regression analysis for pathogenicity to seedlings
and radial growth on PDA at 20°C



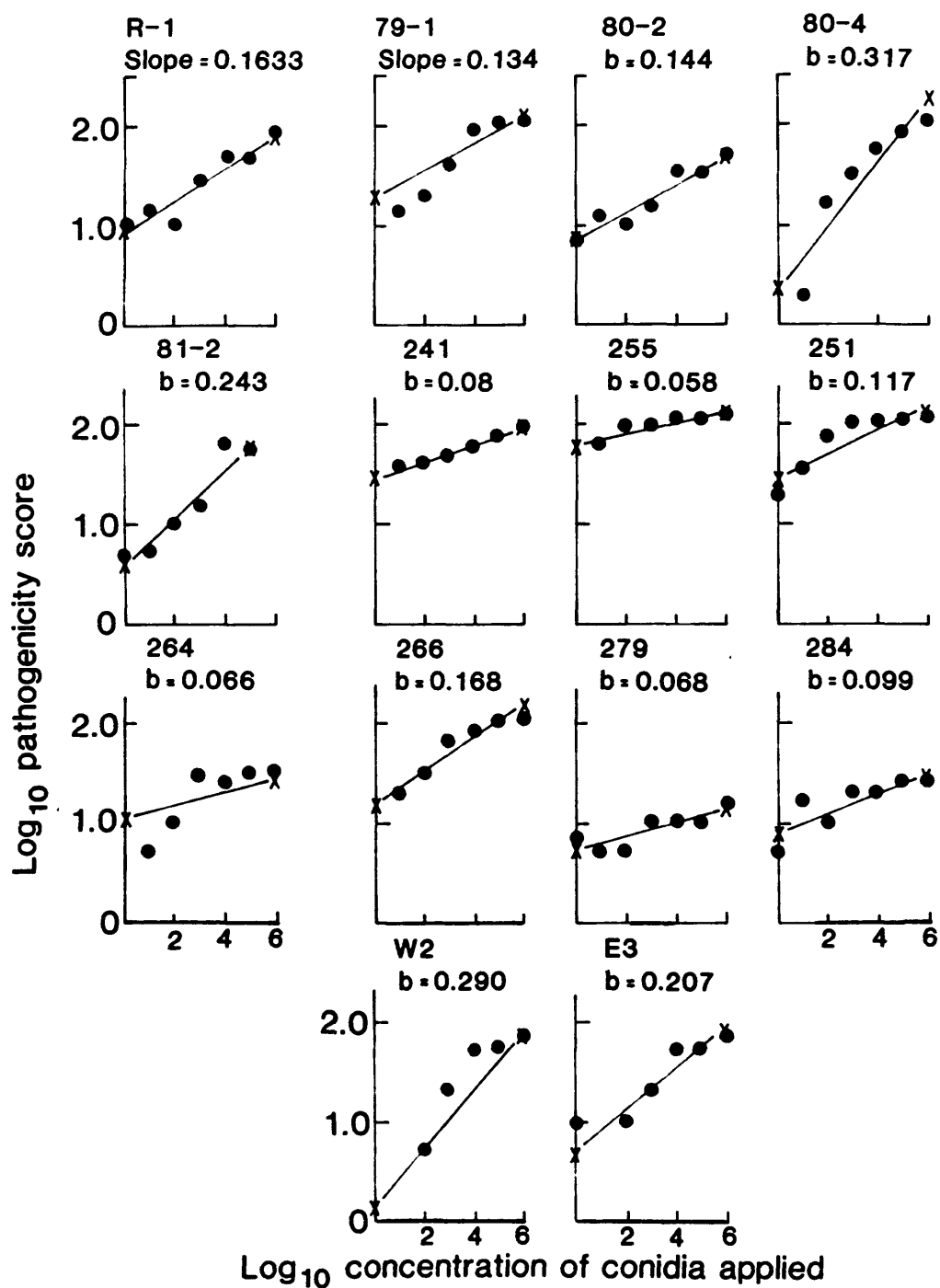


Fig.13 Regression analysis for each of the *P.herpotrichoides* isolates of pathogenicity to seedlings with concentration of conidia applied

difference between isolates in the logit transformed data with $\log_{10}$ concentration regression slopes ($P < 0.01$; $F(13,69)$), i.e. the rate of formation of severe lesions with the number of conidia applied. (fig 13)

There was a significant difference between isolates in the intercept values ($P < 0.01$; $F(13,69)$). (Table 7)

(2) To mature plants

Experiment 1 (1982/3)

Seeds of wheat (cv. Armada) were planted in twelve 2 gallon buckets containing a sand/loam mixture, and thinned to 30 plants per bucket at the first leaf stage of growth. Small packets of straw (c. 10g) were prepared in Sterilin autoclave bags and autoclaved at 10^5 N/m^2 for 30 min. Spore suspensions of each of 12 P.herpotrichoides isolates were prepared by washing spores from one-month-old cultures growing on DWA.

To each bag 5ml of a spore suspension (10^5 to 10^6 /ml) of one of the 12 isolates to be tested were added. The bags were incubated at 10°C under near ultra-violet light for 6wk to encourage colonisation of the straw and further sporulation. When the seedlings had reached the second leaf stage of growth, infected straw was spread over the soil surface in each of the 12 buckets. The buckets were left outside under netting throughout the season (January to July). In July when the plants had reached anthesis, samples of stems taken from each bucket (30 stems or all the stems present, whichever was the least) were scored for disease severity (section 2.4). All missing stems were scored 3 in the assumption that tillers had been killed outright by eyespot at an earlier stage of plant growth.

Experiment 2

Experiment 1 was repeated in 1983/84 to include two benzimidazole-tolerant isolates (E-3 and W-2). This second experiment included three replicate buckets for each isolate (i.e. a total of 42 buckets). These were arranged randomly under the netting.

Results

Experiment 1

After 2 months it was possible to see differences in growth between plants in different buckets and in several buckets some tillers had died as a result of severe eyespot infection. There were some significant differences in pathogenicity between isolates but generally these were not correlated with relative growth rates on PDA at 20°C. (section 4.2).

Experiment 2

Overall, there were again significant differences in pathogenicity between isolates (Table 8), and in general these were similar to those in Experiment 1. However, the correlation between the eyespot severity values in the two experiments just failed to reach the $P=0.05$ level of significance. There was no correlation between pathogenicity to mature plants and any measurements of isolate growth in culture nor was the pathogenicity of isolates to mature plants significantly correlated with pathogenicity to seedlings.

Table 8 Pathogenicity of *P.herpotrichoides* isolates to
mature wheat plants (cv. Armada).

<u>Disease severity score.</u>			
<u>Isolate</u>	<u>Experiment 1</u>	<u>Experiment 2</u>	<u>Mean of</u> <u>1 and 2</u>
R-1	42	60.3	51.1
79-1	61	43.6	52.3
80-2	58	46.0	52.0
80-4	66	49.3	57.7
81-2	84	71.0	77.5
241	56	43.3	49.7
255	59	63.6	61.3
257	48	52.3	50.1
264	52	36.6	44.3
266	57	46.3	51.7
279	30	45.3	37.7
284	45	44.6	44.8
W-2	-	37.0	37.0
E-3	-	57.0	57.0
SED	13.4	4.1	
Df	9	28	

4.4 Effect of water potential

(1) On isolate growth

The growth of isolates at different water potentials was compared. The water potential of PDA was controlled using osmotically adjusted glycerol water in place of distilled water in the preparation of the medium (Table 9) (Dallyn & Fox, 1980).

These values were calculated from the formula:-

$$100\text{ml of H}_2\text{O} \times \text{g glycerol} = \frac{92.1 \times \text{molality at } A_w \times 100}{1000}$$

given that the molecular weight of glycerol = 92.1

(Dube, Dodman & Flentje, 1971). The conversion of water activity (A_w) to water potential (ψ bars) is given by:

$$\psi = \frac{RT}{V} \times \log_{10} A_w + P$$

where: R = Ideal gas constant

V = Volume of 1 mole of water

T = Absolute temperature

P = Atmospheric pressure

The plates were poured when the agar was near to its setting point to minimise loss of water from the medium by evaporation. They were inoculated immediately after they had set.

Experiment 1

(a) The effect of water potential on the germination of conidia of the R-1 isolate of P.herpotrichoides was examined at 20°C.

Conidia were washed from DWA plates into 10ml of osmotically adjusted glycerol water (OAG). Approximately 3% of the conidia in suspension had germinated when washed from the DWA. From these suspensions 0.5ml aliquots were spread over the surface of PDA plates of the appropriate water potential. There were 3 replicate plates with water potentials of -150, -100, -69, -41 and -27 bars

Table 9. Quantities of glycerol used in the preparation of PDA at different water potentials.

Water potential (bars)	Glycerol (g) in 100ml water	Water activity
-5	1.84	0.9960
-10	4.14	0.9926
-15	5.53	0.9890
-20	7.36	0.9862
-27	9.21	0.9801
-41	14.73	0.9700
-69	24.86	0.9502
-100	36.84	0.9285
-150	55.26	0.8947

respectively. Plates with the same water potential were sealed in a polythene bag. After 24h at 20°C, a slice of agar (22 x 50mm) was removed from each plate and germination of about 1000 conidia was assessed, germination being taken as the first appearance of a germ tube.

(b) The effect of the water potential on mycelial growth rate was also examined. PDA plates were prepared using OAG at the same water potentials as in (a). These were inoculated with 6mm diam discs taken from the edge of a mycelial culture of the R-1 isolates.

The plates were then placed on small plastic platforms over OAG water of the corresponding water potential in sandwich boxes at 20°C. Each box was sealed inside a polythene bag to enable the plates to be viewed and scored without altering the humidity. The rate of growth was recorded as the increase in colony diameter.

Experiment 2

The effect of water potential on the mycelial growth rate of the R-1 isolate was also examined at three temperatures. Small petri-dishes (5cm diam) were prepared with PDA at water potentials of -5, -10, -15, -20 and -69 bars. Each plate was inoculated with three plugs of a thin layer of agar covered with mycelial growth. This was to prevent the introduction of excess amounts of water. Plates of the corresponding water potential were placed flat in polythene bags (3 per bag) and sealed using "lassotape". Each bag contained two replicate plates per treatment and one uninoculated plate to stabilise the relative humidity within the bag. The plates were incubated at 15, 20 or 25°C and the diameter of each colony was measured every 3 days for 15 days.

Experiment 3

Twelve plates each of isolates 255, 264, R-1, 79-1, 80-4 and 81-2 were prepared at water potentials of -5, -10, -15, -20 and -69 bars respectively. Each plate was inoculated with a plug of agar taken from a colony growing on a thin layer of PDA. Six plates of each isolates were then incubated at either 12 or 20°C. The diameter of each colony was measured after 7, 14 and 21 days of growth.

In all experiments the water potential of the agar medium was checked using a psychrometer (Protimeter Ltd, Marlow, Bucks). This measures the air dew point temperature. Dew formed on an electrically cooled, solid gold mirror is detected by optoelectronics and the temperature of the mirror (the dew point temperature) is continually monitored and stored in a memory. The instrument incorporates another transducer for measuring ambient temperature and the relative humidity can also be found. The water potentials of the media were estimated using conversion tables.

Results

Experiment 1

Germination of conidia was affected considerably by water potential (Table 10). At -41 bars about 30% fewer conidia germinated than at -27 bars. At -69 bars there was only 3% germination and conidia remained at the initial stages of germination (swelling and/or the production of a germ tube) even after a further 7 days incubation. Similarly, a reduction in water potential from -27 to -41 bars reduced mycelial growth by 74%. There was some growth at -69 bars but not at -100 or -150 bars.

Table 10. Effect of water potential on initial mycelial growth and
spore germination (isolate R-1)

Water potential (bars)	Mean % spore germination (after 24h)	SED	Mean colony diam(mm) after 25 days	SED
-150	2.6*	-	6.0**	-
-100	3.1*	-	6.0**	-
-69	3.1*	-	12.0	0.67
-41	63.2	0.89	13.8	2.48
-27	95.2	0.57	35.7	0.67

* Nil germination

** Nil growth

Experiment 2

Growth was more extensive at 20°C than at 15 or 25°C at all five water potentials tested including -69 bars (Table 11). Maximum growth at all three temperatures was at -10 bars water potential. After 15 days of incubation at 25°C the diameter of the colonies was on average 2mm less than at 15 and 20°C.

Experiment 3

Overall, water potential significantly affected growth of isolates (Table 12). Generally all the isolates grew best at water potentials of -5, -10 and -15 bars. Isolates 255 and 79-1 had significantly higher growth rates than the other four isolates at all water potentials and at both temperatures. There was significantly more growth at 20°C at the lower water potentials. There was no evidence to suggest that the radial growth of the dryland isolates from the USA (79-1, 81-2, and 80-4) was greater than that of the UK isolates at the lower water potentials (-20 and -69 bars).

Table 11. Growth of *P.herrpotrichoides* (isolate R-1) on osmotically adjusted PDA

Temperature (°C)	Water potential (bars)	Mean growth (mm) after:					days
		3	6	9	13	15	
15	-5	3.0	5.8	10.9	19.4	24.1	
	-10	3.5	7.1	14.5	25.6	30.8	
	-15	3.0	5.3	8.8	18.4	24.0	
	-20	3.0	6.2	10.8	19.8	24.3	
	-69	3.0	3.0	3.0	3.8	4.2	
	SED(20 Df)	0.13	0.22	0.66	0.62	0.52	
20	-5	3.2	6.8	13.2	21.7	25.3	
	-10	3.8	7.7	16.8	29.3	36.1	
	-15	3.0	6.8	14.2	25.8	31.1	
	-20	3.0	6.2	11.3	21.3	27.2	
	-69	3.0	3.2	4.1	5.7	6.6	
	SED(20 Df)	0.23	0.27	0.93	1.25	0.49	
25	-5	3.5	6.5	9.3	14.8	19.0	
	-10	3.6	7.1	10.7	16.5	22.6	
	-15	3.6	6.2	10.2	17.8	21.7	
	-20	3.5	6.8	10.7	16.3	21.0	
	-69	3.0	3.0	3.3	4.8	4.9	
	SED(20 Df)	0.49	0.29	2.93	0.87	0.57	

Table 12. Effect of temperature and media water potential on growth of *P. herpotrichoides*
(after 3 wk)

Incubation temperature	Water potential (bars)	Mean diameter of six replicate colonies (mm) of Isolate No.					
		255	80-4	264	79-1	R-1	81-2
12°C	- 5	42	28	26	36	40	31
	-10	40	33	26	34	41	27
	-15	37	37	23	30	38	26
	-20	34	32	22	32	32	26
	-69	11	10	13	13	13	10
20°C	- 5	50	47	55	55	34	44
	-10	50	39	53	53	50	41
	-15	52	43	52	52	51	38
	-20	42	39	52	52	42	40
	-69	13	13	19	19	16	13

Analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean square F(,300)	
Isolate	5	3872.1	774.4	132.1
Incubation temperature	1	11289.6	11289.6	1925.9
Water potential of media	4	39443.6	9860.9	1682.2
Incub. temp. x water pot.	4	1702.4	425.6	72.6
Incub. temp. x isolate	5	1636.8	327.3	55.8
Water pot. x isolate	20	2187.8	109.4	18.6
Incub. temp. x water pot. x isolate	20	1758.7	94.9	16.2

All the above F ratios are significant at $P < 0.001$

2) On lesion development in plants

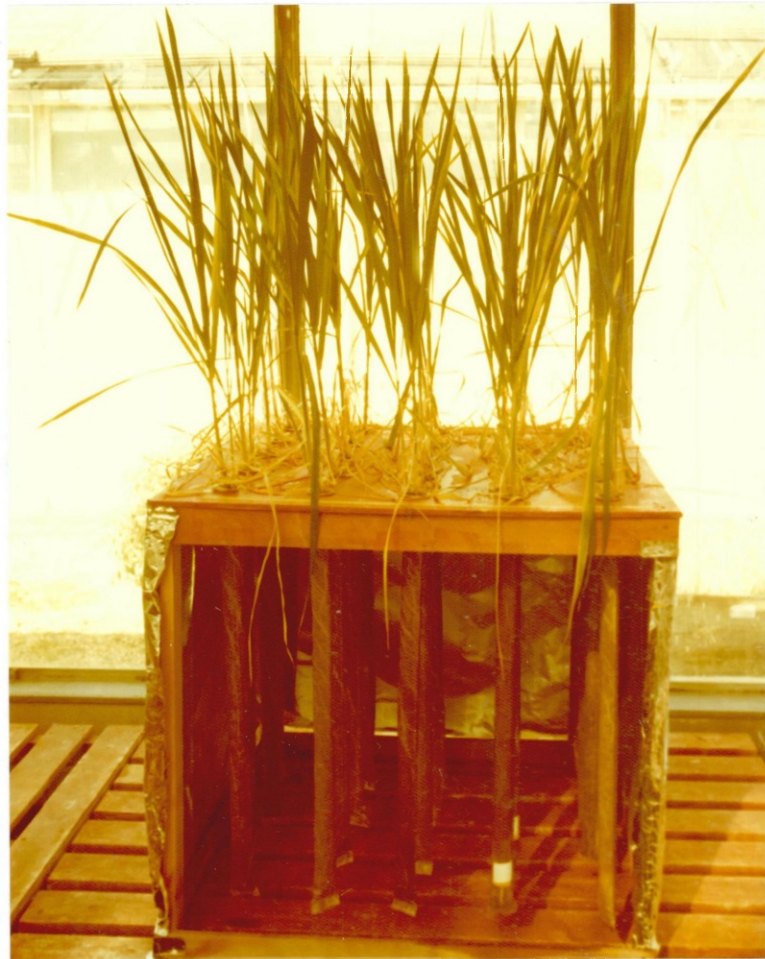
Preliminary experiments to create differences in water potential in pot-grown plants in a controlled environment were not entirely successful. The maximum difference in plant water potential created was only -2 bars, but lesion development was more extensive in plants which were heavily watered (-0.5 bars) ^{soil water potential} than those given other watering treatments. Plants adapt quickly to their environment when grown in pots, and young plants are not readily water stressed. Therefore in subsequent experiments plants were grown to maturity before watering regimes were introduced either a) in tubing or b) in buckets.

a) Tubing experiments

Experiment 1

Nylon "Layflat" tubing was cut into lengths of 50cm, heat-sealed at one end and filled with a sand/loam (17:3) mixture (Ayres, 1981). Pre-germinated wheat seeds (cv. Armada) were sown at a depth of 3cm. A brass curtain ring (3.0cm diameter) was placed over the open end and the tubing wrapped over it. Each tube was suspended from a wooden frame, the curtain ring acting as a support. Four wooden frames each with five rows of five tubes (Fig 14.) were placed in a glasshouse at c. 15°C. The ends of the tubes were perforated to allow for drainage. The frames were wrapped in aluminium foil to reduce the amount of light reaching the roots and to keep them cool. Seventy-five of the plants were inoculated with P.herpotrichoides (isolate R-1, 7×10^5 conidia/ml) using the filter paper disc technique, when they were at the first leaf stage of growth (section 2.3). The other 25 plants were

Fig. 14. Photograph showing the arrangement of the tubes in the experiment on the effects of water potential on lesion development.



uninoculated controls. All of the plants were watered to run off every day; once a week a 0.1% nitrogen, phosphorus and potassium solution was also applied to run off. After 6 weeks of growth, the plants had developed a yellow speckling of the leaves due to a lack of available nitrogen. Watering was then supplemented with 0.1% solution of NH_3NO_3 every 2 weeks and the plants regained their green appearance.

Different watering treatments were applied when the plants had reached the first node stage of growth (Growth stage 31). The inoculated rows of plants were allocated at random to treatments. The way in which the tubes were arranged in the frames meant that plants on the outer edges of the frames might be subjected to different lighting and relative humidities from plants in the middle of the frames. In order to identify these differences, if they were present, the outer edge rows were allocated two blocks of treatments and the inner rows then formed a further three blocks of treatments.

The watering treatments were as follows:-

- normal watering - watered every day to run off.
- droughted - watered to run off once every 7 days.
- heavily watered - the ends of the tubes were re-sealed using "Lassotape" and the plants watered until free water was visible throughout the column of soil daily.

Uninoculated plants watered every day to run off served as controls. These watering regimes were continued over a 5 week period, and the plants were then sampled. Each bag was split open and a 5cm length of root, cut from each plant, was placed in a hydraulic membrane^{press} (T & J Crump scientific instruments) and the water potential measured. Plants were scored for lesion severity (section 2.4) and the water potential of the stem was measured using a "pressure bomb" (designed and built at Rothamsted) (Fig 15). Pressure inside the sealed chamber was gradually increased until the first appearance of sap from the protruding cut stem; the pressure was then recorded and the values converted to water potential in bars ($-1 \text{ bar} = 14.5 \text{ lb/m}^2$).

Experiment 2

The experiment was repeated using two sets of frames. One set was placed in a controlled environment at $20 \pm 2^\circ\text{C}$ and the other in a glasshouse where the max/min temperature was recorded daily. None of the control plants in experiment 1 became infected and they were replaced by a further "dry" watering treatment - watered every other day to run off. The transformation logit (percentage lesions with scores greater than 1) was used before an analysis of variance.

Results

Experiment 1

Differences in plant water potential between treatments were achieved. Roots of droughted plants had a mean water potential of -13 bars whereas root water potentials of heavily watered and normally watered plants were about -3 bars (Table 13). Stem water

Fig. 15. Photograph of the pressure bomb used to measure water potential

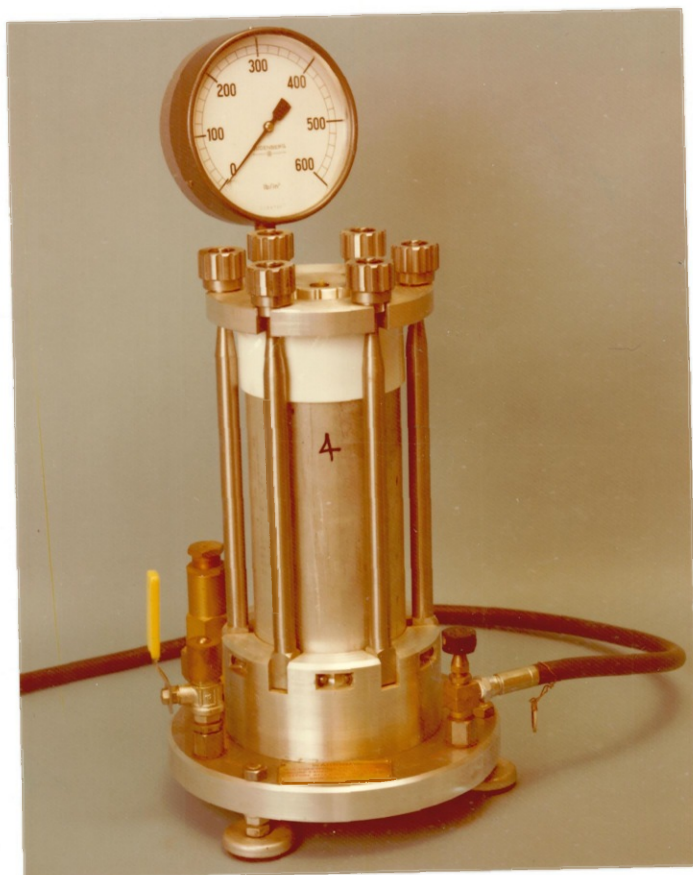


Table 13. Effect of watering regime on plant water potential
(mean values) and eyespot lesion severity

Experiment 1

<u>Treatment</u>	<u>Water potential (bars)</u>		<u>Severity score</u>	<u>Growth stage</u>
	<u>Root</u>	<u>Stem</u>		
Droughted	-13	-13	0.7	40
Normally watered	- 3	- 7	0.6	40
Heavily watered	- 3	- 6	1.2	42
Control	- 4	- 6	0.0	40
SED	1.2	1.5	0.1	1.4
DF	24	24	24	24

potentials tended to be lower in the stem than in the roots of normally or heavily watered plants. Disease severity scores were highest in the heavily watered plants ($P < 0.01$) but very similar in the normal and droughted plants, despite the difference in water potential.

Neither watering regime nor eyespot infection had any significant effect on plant growth^{stage}. Temperatures in the glasshouse went higher than normal and often reached 30°C during the watering period.

Experiment 2

The temperature in the glasshouse did not vary as much as in experiment 1, rarely reaching 20°C , and generally stayed within $\pm 2^{\circ}\text{C}$ of 15°C . There was a significant effect of watering treatment ($P < 0.01$) and the differences in plant water potential between treatments were much greater than in experiment 1. Lesion development was greater in the glasshouse at c. 15°C than in the constant environment at 20°C . Table 14 shows that mean disease severity was again greater at higher water potentials.

Table 14. Effects of watering regime and temperature on plant water potentials and eyespot lesion severity

<u>Temp</u>	<u>Treatment</u>	<u>Water potential (bars)</u>		<u>Severity score</u>
		<u>Root</u>	<u>Stem</u>	
20°C	Droughted	-18.6	-18.4	2.3
	Heavily watered	- 4.4	- 6.9	2.7
	Dry	- 7.0	- 8.3	2.1
	Normally watered	- 6.0	- 7.5	1.5
15°C	Droughted	-16.8	-15.9	2.0
	Heavily watered	- 3.5	- 5.8	2.8
	Dry	- 6.9	- 7.7	2.4
	Normally watered	- 6.8	- 8.0	2.1
	SED	1.1	0.8	0.2
	DF	12	12	12

At 20°C disease severity of the heavily watered plants was significantly greater ($P < 0.05$) than that of plants under other watering regimes. There was no significant differences in lesion severity between the droughted plants and those in the dry regime. Normally watered plants developed the least severe lesions. At 15°C the severity of lesions did not differ significantly between heavily watered plants and plants in the dry regime. However, lesion severity was significantly less on plants which were normally watered or droughted.

With heavily watered plants, temperature had no significant effect on disease severity but on plants watered normally and those in the dry regime lesion severity was greater at 15°C than at 20°C. By contrast, eyespot severity was greater on droughted plants at 20°C than at 15°C.

b) In buckets

Bundles of straw, c. 10g, were autoclaved in Sterilin autoclave bags for 30 min. at $10 \frac{5}{\text{N}}/\text{m}^2$ pressure. The straw was then inoculated with a spore suspension of one of three P.herpotrichoides isolates, washed from DWA plates using sterile distilled water. The isolates were chosen because they grew best at different water potentials, the optimum water potential being -10 to -15 bars for R-1, -5 to -20 bars for 79-1 and -5 bars for 264 (section 4.4).

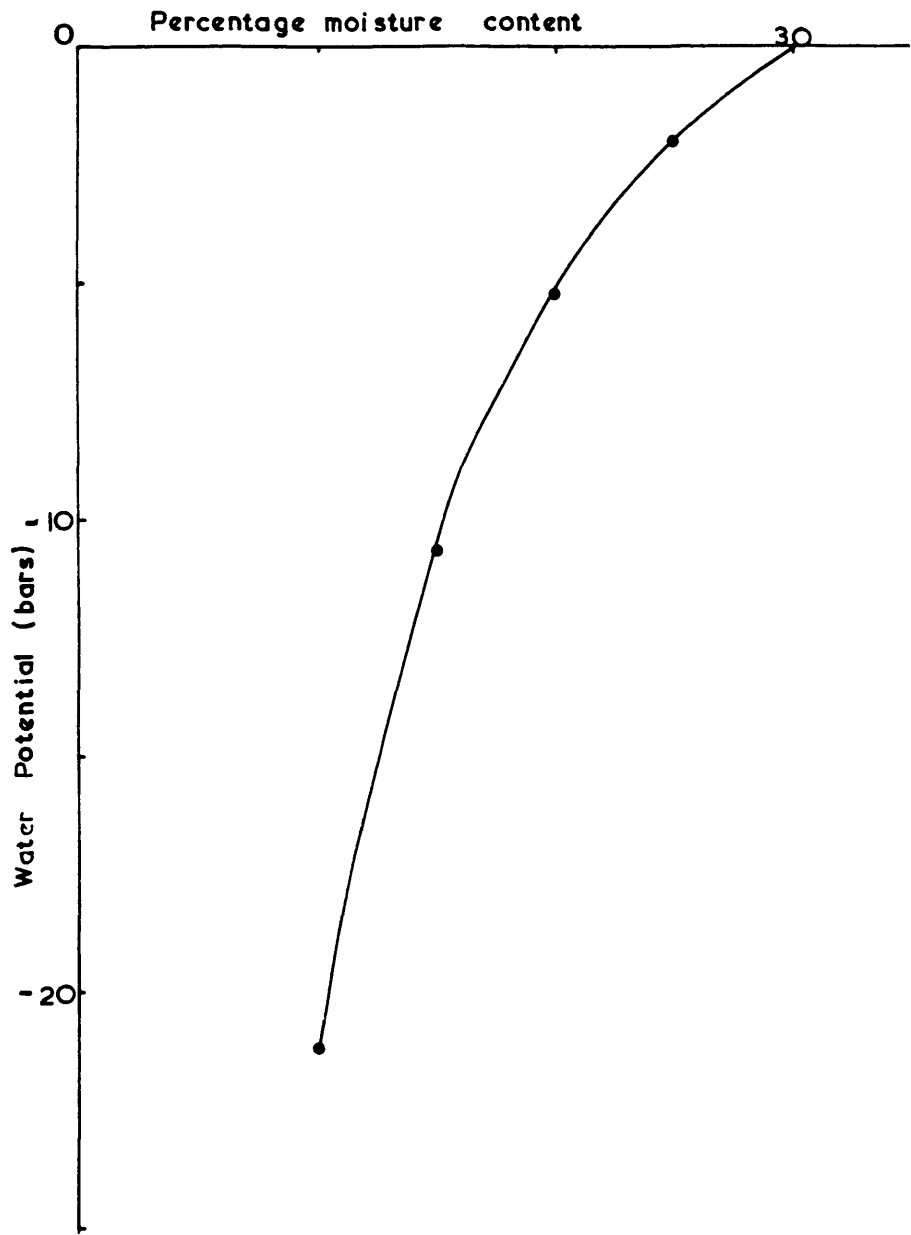
Eighteen 2 gallon buckets were filled with a sand/loam mixture (1:3) and sown with winter wheat seed (cv. Armada). Once the seedlings had emerged, the surface of the soil in the buckets was covered with the straw inoculum and plants were thinned to 30 per bucket (six buckets for each isolate) The buckets were left

outside under netting to grow under conditions comparable to those of the field. When plants reached the first node stage of growth (Zadoks et al., 1974, GS 31), two replicate buckets of each isolate were left unwatered, two were heavily watered every day and two were placed under a shelter and watered once every 7 days. The shelter was constructed using an "A"-shaped wooden frame covered with polythene.

It was hoped that the soil water potential within the buckets could be monitored continuously whilst buckets were receiving the different watering regimes. A psychrometer would have been the best apparatus to use, but unfortunately one was not available. It was not possible to standardise a solid block resistor for use in the buckets as this proved to be insensitive in the upper and lower ranges of water potential to which the plants were to be subjected. Therefore, a dew point meter (Protimeter Ltd, Marlow Bucks) was used to produce a standard curve to convert percentage soil moisture content to water potential (Fig. 16). During the period that the watering treatments were applied, soil was removed from the buckets at weekly intervals and the water potential measured, using the curve, from wet and dry weight measurements.

Watering treatments were continued for a 5 week period, after which the plants were sampled. Twenty-five stems or all of the stems present (whichever was least) were removed from each bucket. Each was scored for lesion severity (section 2.4) and plant growth stage; the water potential of each plant was measured using a membrane press. Missing plants were given a severity score of 3 on the assumption that they had been killed outright by the fungus.

Fig 16. Standard curve of water potential against percentage moisture content for soil from buckets



A similar experiment investigated the effects of watering regime alone on disease development. Six buckets were prepared as above with straw infected with the R-1 isolate of P.herpotrichoides. The buckets were divided into three groups and watered with the same watering regimes as in the previous experiment. After 5 weeks the plants were scored for lesion severity, water potential and plant growth stage.

Results

The following mean weekly water potentials ($\pm$ 1 bar) of soil removed from the buckets given the three watering treatments remained constant over the 5 week period of the treatments:

Normally watered	-2 bars
Heavily watered	0 bars
Droughted (place under shelter)	-4 bars.

Although the water potential of the droughted plants fell to -13 bars there was very little difference in water potential between normally watered and heavily watered plants (-6 to -10 bars). Isolate 79-1, from the dryland area of the Pacific Northwest of USA, showed no evidence of adaptation to growth on plants with low water potentials. Although the eyespot severity score for isolate 79-1 was greater, there was no significant difference in disease severity scores between isolates on the droughted plants (Table 15). There was no significant difference in the severity scores for isolates R-1 and 79-1 between watering regimes but lesions on normally and heavily watered plants inoculated with the isolate 264 were significantly less severe than those on droughted plants inoculated with isolate 79-1 (SED 11.6, 49 Df). All of the water potentials achieved in the plants were within the range of optimum growth of

Table 15. Effect of watering regime on plant water potential ($\bar{W}$) and eyespot severity (temperature range 6.3 to 25.9°C during watering)

	Isolate					
	R-1		79-1		264	
	<u>severity</u>	<u>$\bar{W}$</u>	<u>severity</u>	<u>$\bar{W}$</u>	<u>severity</u>	<u>$\bar{W}$</u>
Droughted	55.1	-13	65.3	-12	50.7	-13
Normally watered	54.7	- 9	56.6	-10	31.2	- 8
Heavily watered	56.1	- 7	53.3	- 8	33.3	- 6

water potential SED (98 DF) 2.5

the isolates (section 4.4; effect of water potential on mycelial growth) on agar. Temperatures were high during this period and differences in disease severity scores were not significant.

There was no significant difference between watering regimes in severity of lesions caused by isolate R-1. However, differences in water potential were not significant. Temperatures during the period of treatment were higher than average for the time of the year.

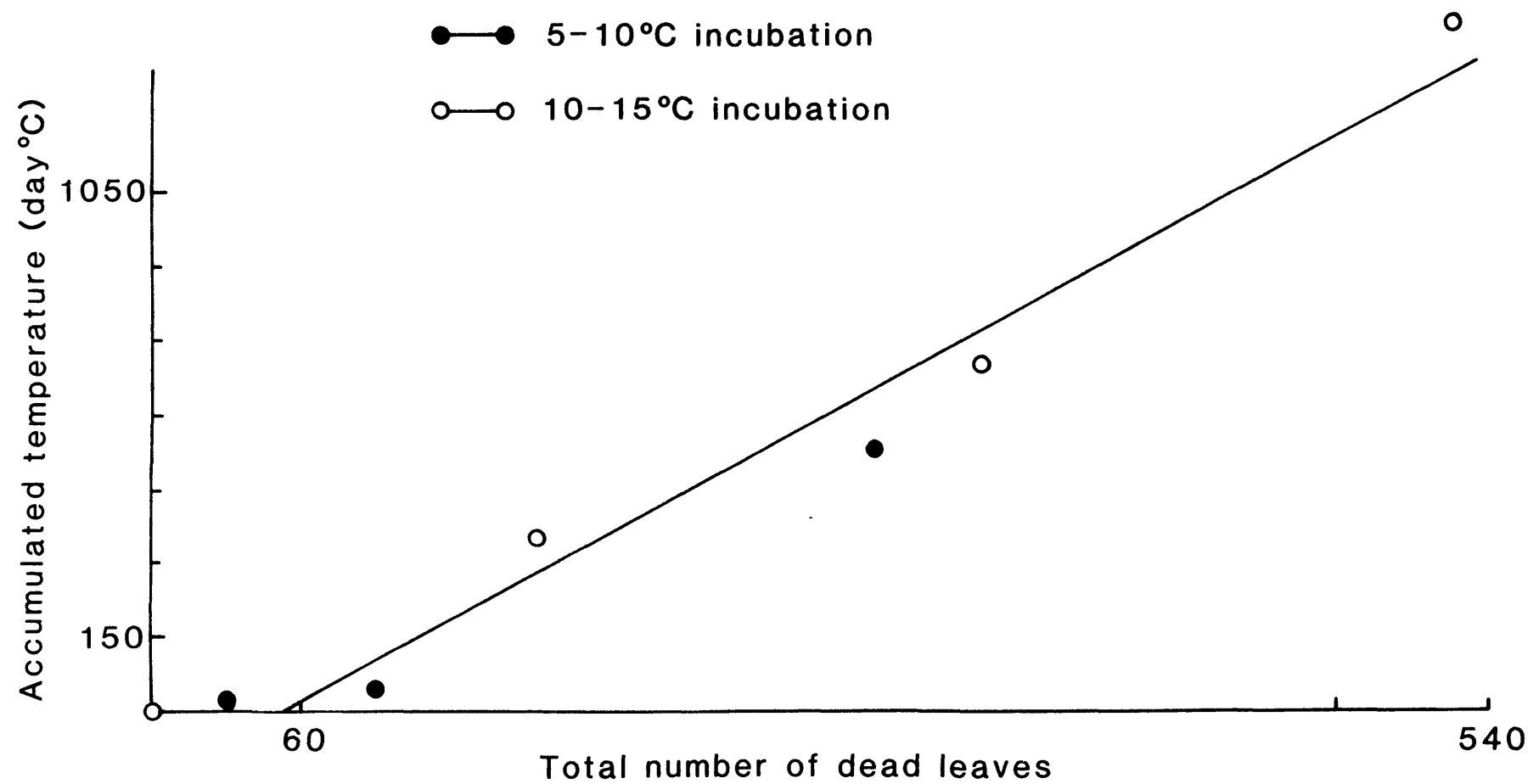
4.5 Temperature and fungal penetration

Pre-germinated wheat seeds were planted at five per pot (12cm diam) in soil-less compost. Ten pots were incubated at 5/10°C and ten at 10/15°C night/day temperatures. At the second leaf stage of growth plants were inoculated with a spore suspension (10^4 conidia/ml) of P.herpotrichoides using filter paper discs. This inoculation was repeated 4 weeks later and all the plants were sampled 16 weeks after the first inoculation. A daily record of maximum and minimum temperatures was kept and the accumulated temperature for each set of plants was calculated, using $T_0 = 6^\circ\text{C}$. The growth stage of each plant was noted, disease severity was assessed (section 2.4) and the number of leaf sheaths penetrated by the fungus (dead and live) and the total number of dead leaves recorded.

Results

There was a positive correlation between the number of dead leaves sloughed off the plants and the number of day-degrees accumulated above 6°C (Fig 17). However, there were ultimately fewer live leaf sheaths penetrated by the fungus when plants were incubated at 10/15°C than at 5/10°C. More leaf sheaths in total were penetrated at the higher temperatures but most of these were

Fig.17 Regression of total numbers of dead leaves produced with accumulated temperature (%variance accounted for 95.5 , SED =0.3)



dead by the assessment date (Table 16). There was a decrease in the number of live leaf sheaths penetrated with an increase in accumulated temperature (Fig 18). The more dead leaf sheaths that there were present on each plant, the fewer were the numbers of live leaf sheaths penetrated by the fungus (Fig. 19).

4.6 Summary of results

Significant differences were observed in the growth rates of isolates on PDA and in PDB. The pathogenicity of isolates to seedlings, but not to mature plants, was significantly correlated with radial growth on PDA. Neither growth in PDB nor the CAI of isolates was related to their pathogenicity to seedlings or mature plants. Benzimidazole-tolerant isolates were as pathogenic to seedlings and mature plants as benzimidazole-sensitive isolates.

There was some mycelial growth at -69 bars but conidia did not germinate at or below this water potential. The optimal temperature for growth at all water potentials examined was 20°C. Maximal growth at all temperatures was at water potentials in the range -5 to -15 bars. Isolates from the dryland region of the Northwest USA did not show any specialisation for growth at the lower water potentials.

In glasshouse/controlled environment experiments at 15°C and 20°C the most severe eyespot lesions developed in plants under a heavy watering regime, which had the greatest water potentials. The number of dead leaves produced by seedlings increased with the number of day-degrees accumulated, as did the number of leaf sheaths penetrated by the fungus. However, there was a decrease in the number of live leaf sheaths penetrated with increase in temperature.

Table 16. Effect of temperature on penetration of leaf sheaths
by P. herpotrichoides

Incubation time (wk)	Temp. of incubation (°C)	Accumulated temp.	Penetration score	No. live leaf sheaths penetrated	Total no. of dead leaves
4	10/15	350	43.8	67	156
	5/10	21	26.2	101	30
8	10/15	700	62.6	28	336
	5/10	42	44.4	131	91
16	10/15	1400	70.4	4	526
	5/10 *	533	55.2	31	293
SED (54 DF)			3.5	9.5	21.5

* These plants were grown in a glasshouse (temp. 10-28°C) for the first four weeks, when the 5/10°C controlled environment cabinet was not working

Fig 18 Regression of number of live sheaths penetrated with accumulated temperature

(% variance accounted for 74.7, SE=0.02)

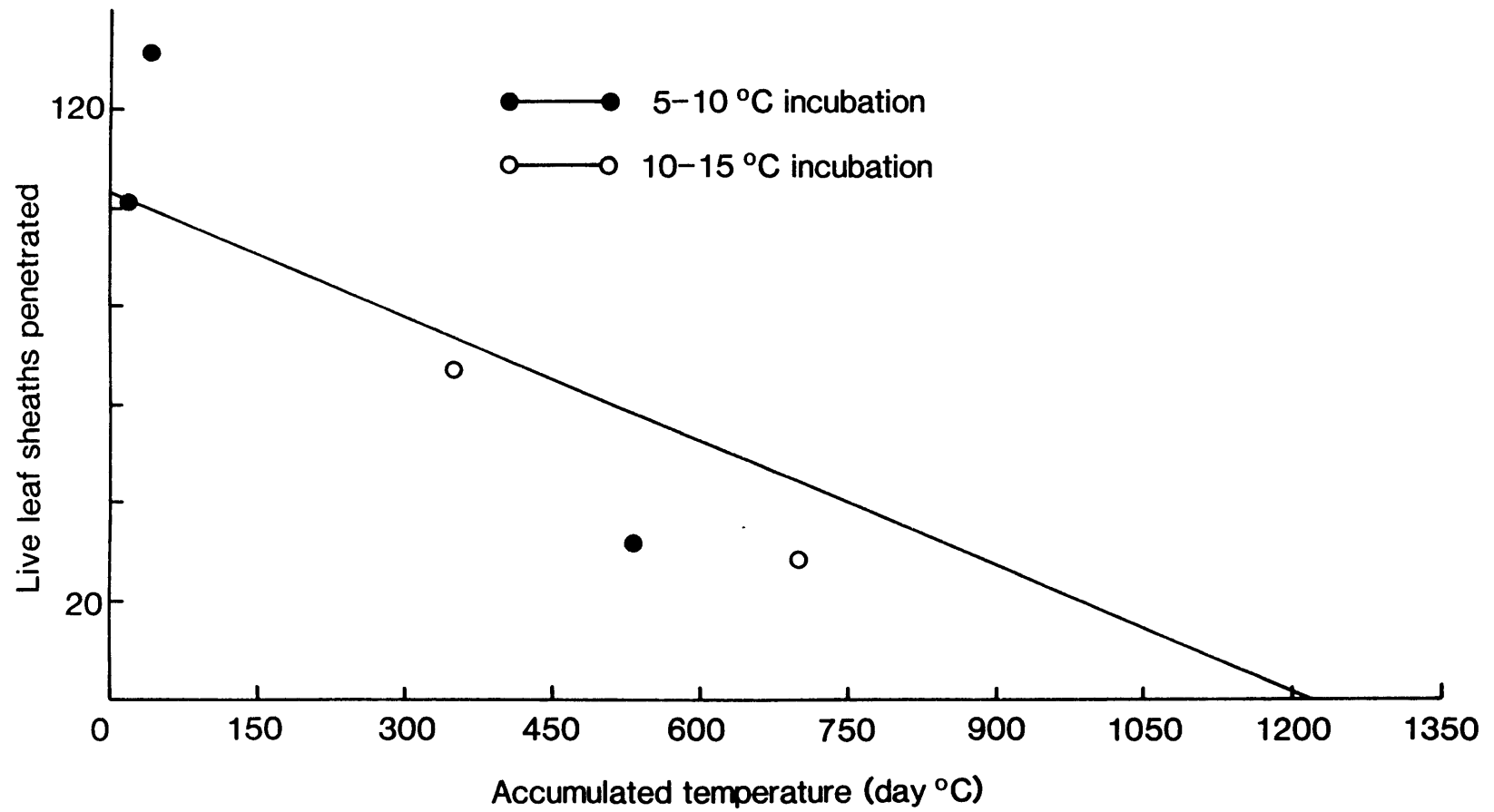
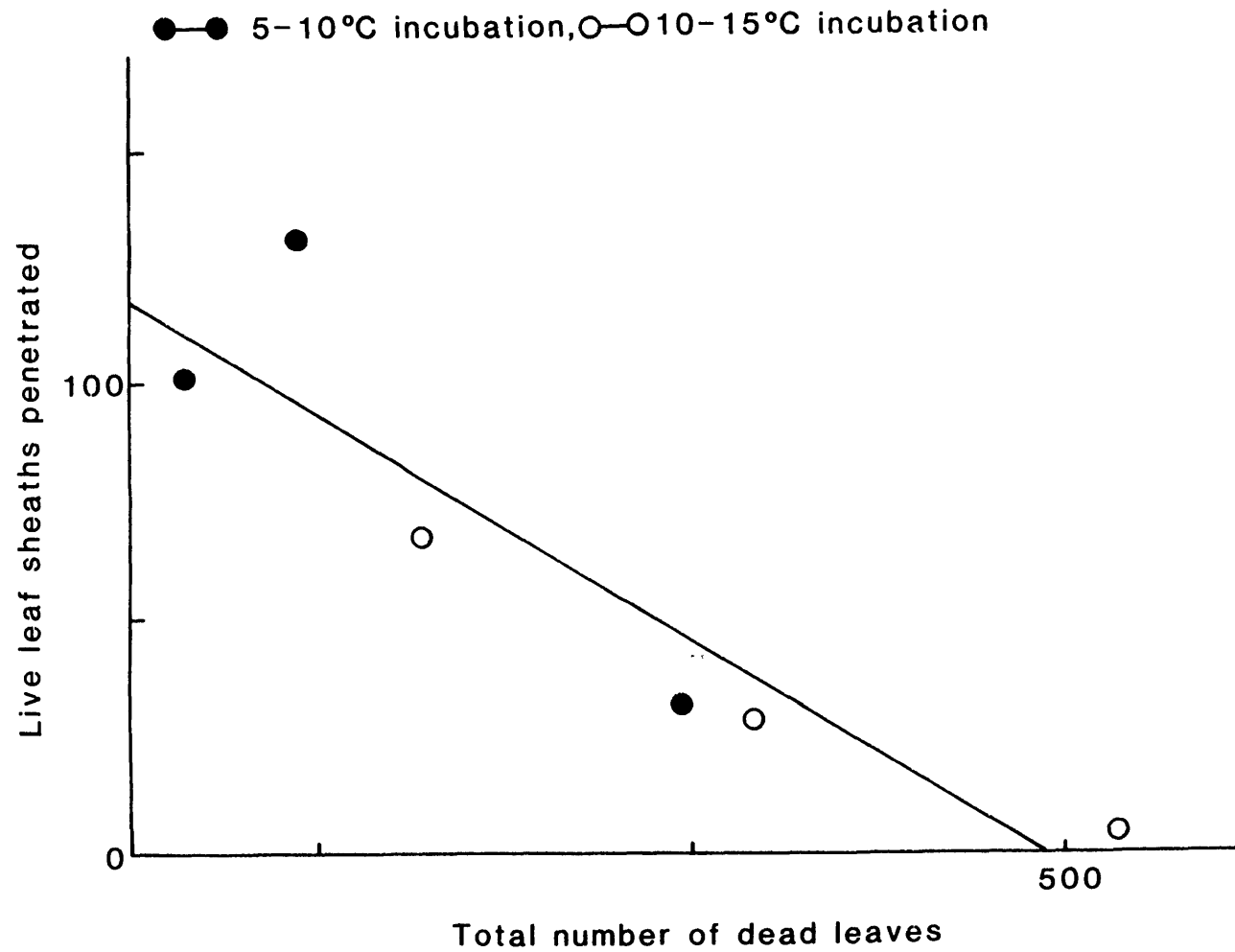


Fig.19 Regression of live leaf sheaths penetrated with total number of dead leaves present (%variance accounted for 78.7, SED = 0.05)



CHAPTER 5

DISEASE DEVELOPMENT IN THE FIELD

5.1 Introduction

Surveys by the Agricultural Development & Advisory Service (ADAS) in the UK show that eyespot is a relatively common disease of cereals. In 1983, 36% of all fields sampled had more than 10% moderate or severe eyespot and at harvest 10% of the fields were severely affected (ADAS disease report 1983, unpublished data). Eyespot (P. herpotrichoides) reduces wheat yield directly by altering the nutrition of the plant and indirectly by increasing the risk of lodging. Evidence for the direct effects of P. herpotrichoides was given by Scott & Hollins (1974), who showed that death of tillers in the spring, as a result of eyespot infection, reduced the final yields of individual wheat plants even when lodging was slight or absent. Severe eyespot lesions greatly reduced grain numbers per ear and 1000 grain weight of individual tillers. The former effect was, however, not present in the whole plots because of the compensatory growth of other tillers. Such host compensation is perhaps characteristic of a disease that spreads sufficiently slowly for neighbouring tillers to be infected to different extents. Scott & Hollins⁽¹⁹⁷⁴⁾ were unable to determine from their data whether compensation occurred within plants or between plants. Losses in grain yield per ear were mainly due to severe lesions, which caused a weakening of the stem and lodging, and not at all to slight lesions.

Currently there is no wheat cultivar which is completely resistant to infection by eyespot. Law et al. (1975) investigating three wheat cultivars (Chinese Spring, Cappelle-Desprez and Mara),

identified four chromosomes (1A, 2B, 5D and 7A) as being implicated in the control of eyespot resistance. Chromosome Cappelle-Desprez 7A gave increased resistance irrespective of the cultivar combination used. Anthocyanin pigmentation of the stem and coleoptile has been associated with eyespot resistance (Defosse & Vandam, 1969; Doussinault et al., 1974). Although the resistant cultivar Chinese Spring (Cappelle-Desprez 7A) was non-pigmented, chromosome 7 has been shown to carry genes for anthocyanin production (Law, 1966). It is likely that the association of pigmentation with resistance is due to the linkage between genes located on the group 7 chromosome.

Farmers now control eyespot by using fungicide sprays at growth stage 31 if more than 20% of tillers are infected in the spring. It is most likely that increased use of benzimidazole fungicides has caused the recent increase in the proportion of MBC-tolerant isolates of P. herpotrichoides and consequent control failures. There is thus a pressing need for an accurate scheme for eyespot prediction in order to limit the application of fungicide to high disease risk situations.

It has proved very difficult to predict yield loss due to eyespot. Polley & Clarkson (1978) showed that recommendations for spraying based on disease incidence in the spring could be very unreliable. They tested prediction curves for the disease produced in Germany by Schrödter & Fehrmann (1971) on two experimental sites in the UK. No yield response was obtained by spraying at one site, despite a high incidence of eyespot in the spring, whereas at the other site a yield increase of about 10% was obtained, presumably due to the control of the more severe eyespot lesions. These results indicate the importance of environmental factors in the development of eyespot infections between the spring and harvest. For example the crop at the second

site was much denser than the crop at the first site and may have provided a microclimate more conducive to eyespot development. Batts & Fiddian (1955) noted that when seed rate was increased by 40% in a field trial, only 12% more tillers were present at harvest because there was an increased incidence of disease under the dense canopy where moisture accumulated and the relative humidity at soil level was greater. Despite the difficulty in predicting yield loss from the amount of eyespot infection in the spring, Scott & Hollins (1978) observed that, in most instances, when more than 10% of the shoots were infected by eyespot in the spring, sufficient yield losses occurred to justify the cost of spraying with fungicides.

The dramatic changes in the climatic conditions from West to East across the Pacific North West area of America provided ideal conditions for studying effects of climate on the development of eyespot (Bruehl et al., 1968). The potential for eyespot development (climatic potential) was based on weather factors.

- 1) the length of time between 1 January and 30 July when mean daily temperatures averaged between 0 and 15°C;
- 2) the rainfall during these months when the temperature was in this range.

They concluded that where the climatic potential was high the risk of severe eyespot development was greater than in areas of low climatic potential. Where differences in eyespot development occurred between regions with similar climatic potential, they suggested that this was the result of water retention by the soil. Areas with deep soils retain moisture when the land is fallow and early sowing is possible. Early sowing, with considerable growth

during the autumn, was recognised as a pre-requisite for eyespot development in Washington state. The effects of climatic factors on eyespot development may operate through the internal changes in plants and there is some evidence that concentrations of carbohydrates and sugars influence disease development.

Spraying plants with sugar solutions increased the severity of eyespot lesions on both resistant and susceptible winter wheat cultivars (Evans & Rawlinson, 1977). However, Benada et al. (1981) investigated the amounts of sugar in leaf blades and leaf sheaths and found them to be higher in the blades where no infection takes place. They concluded that other factors must be involved and placed more importance on the physiological age of the tissue, actively growing young tissue being more resistant than physiologically older tissue. In the field the coleoptile is often the initial point of infection, due to its high susceptibility (Bateman & Taylor, 1976), but coleoptiles often die quite quickly. Benada (1981) measured all sugars present in the plants, whereas Evans & Rawlinson (1977) looked at the effects of glucose and fructose alone. Ward (1976) working with resistant and susceptible cultivars, found that during infection there was a greater increase in invertase resistant sugars in susceptible cultivars than in resistant cultivars. The effects of the changes in sugar content and water potential on development of P.harpotrichoides require further investigation. Since seasonal changes in the percentage of these sugars are known to occur, these too could presumably affect the course of eyespot development.

There is a continuous decrease in the percentage of sucrose and fructose present in winter wheat plants after March (Barnell, 1938).

There is also a significant rise in the amount of glucose between June and July and a decrease in amounts of soluble carbohydrates in tissues. This is probably because these energy reserves are being used up to provide for vigorous stem growth during this period. The water contents of plants during these periods of very high growth rate are generally reduced (Barnell, 1936). However, the way in which climatic conditions affect the water potential of the plants during this stage of growth have not been closely monitored.

The aims of this field experiment were to identify some of the factors affecting development of eyespot lesions in wheat crops by continuously monitoring the climatic conditions and regularly following the progress of eyespot development throughout three consecutive seasons.

5.2 The 1981/2 field experiment

On 1 October 1981 winter wheat (cv. Armada) was sown at two seed rates (100 and 300 kg/ha with a 17.5 cm row spacing) on a ploughed area (0.4 acres) on the Rothamsted Farm. The soil on this site is a flinty loam on clay with flints and is moderately well drained. Straws infected with P.herpotrichoides, taken from a naturally infected site, were spread over half the area sown at each seed rate. Nitrochalk fertiliser was applied to the plot on 20 April 1982 at the rate of 560 kg/ha.

Instruments for recording dry and wet bulb temperatures, rainfall and windspeed were placed in the uninoculated half of the crop in January 1982. Data from these instruments were recorded at 10 min intervals and stored on magnetic tape by a data logger. An electric moisture recorder was also placed in

the crop to measure the retention of moisture on plant leaves in plots sown at the two seed rates. Each of the six sensors of the leaf wetness recorder was attached to the oldest green leaf of a plant and was moved at weekly intervals to avoid excessive damage to the leaves. The electrical resistance across the leaves was measured and recorded on a chart at 20 min intervals.

In the early stages of growth, whole mounts of successive leaf sheath bases sampled were stained with cotton-blue in lactophenol and the depth of penetration noted. A sample of 10 plants was taken at random along a transect of each plot at weekly intervals until GS 17 (Zadoks et al., 1974) when the sample was increased to 25 plants. The percentage of plants infected with eyespot and severity of infection were assessed (section 2.4) and the height, growth stage and number of tillers infected were also noted. Assessments of disease severity and percentage infection were also made in the uninoculated half of the crop one week before harvest.

5.3 The 1982/3 field experiment

The site used for the 1981/82 experiment was sown with winter wheat (cv. Armada) by direct drilling on 2 November 1982. It had been hoped to plant earlier than this, but unfortunately the weather was too wet. The plot was again divided into halves with a high and low seed rate (300 and 100kg/ha with a 17.5cm row spacing). Since the whole of the previous crop had developed eyespot lesions by the 1982 harvest, no further straws were spread over the area. Meteorological instruments were again placed in the crop. To improve the method of sampling the site, the two areas were divided into 8 sub-plots marked with canes. Within each sub-plot six randomly placed 1m square quadrats were used as the sampling

areas. The total area of all the quadrats was one tenth of the whole site. One plant was sampled at random from each quadrat at weekly intervals from January to August and assessed for growth stage, eyespot severity and number of tillers infected. After the stem had formed, water potentials were monitored montaly by sampling three plants at random from each sub-plot (outside the quadrats). In the first four months of sampling, a hydraulic membrane press (T & J Crump Ltd., Essex) was used to measure the water potential of the oldest green leaf sheath from each plant sampled. However, once the crop had started to ripen, a pressure bomb (Fig.15) was used to measure the water potentials of the stems.

A dew point meter (Protimeter Ltd., Marlow, Bucks.) was used to calibrate soil water potential against percentage moisture content. Samples of soil were taken at weekly intervals from 5cm below the surface and the moisture contents determined from the wet and dry weights. The soil water potential could then be assessed from the calibration curve.

As in 1981/2 the crop environment was monitored with meteorological instruments connected to a data-logger. Additional measurements were obtained with three surface wetness and two humidity sensors. The surface wetness recorders were placed amongst the foliage of the crop. Water on the surface of the sensors was detected by differences in electrical resistance passing through them. One of the "Vaisala" humidity sensors was placed within the crop near ground level whilst the other was placed above the crop at a height of 2m.

Four weeks before harvest, the weather was warm and dry, with many of the plants showing drought symptoms. Ten rows of the plants in an area (in which there were no quadrats) on the outer edge of the high seed rate plot were divided into two sub-plots, each 1m x 3m. A sample of 30 plants was taken at random from each sub-plot and an assessment of eyespot severity was made. One of the sub-plots was watered daily with 15 gallons of water for 5 weeks throughout the period of dry weather during June and July. Samples of 30 plants were removed from each sub-plot at weekly intervals and disease severity was assessed.

In addition to the weekly samples taken from the Rothamsted field site, samples of naturally infected winter wheat were obtained from commercial farm sites via ADAS. These were taken (1) at growth stage 21 to 31 (Zadoks *et al.*, 1974) and (2) prior to harvest at about growth stage 79. Penetration of the leaf sheaths and eyespot severity were assessed on 25 tillers from each sample received. Penetration score was calculated as:

$$\frac{\sum_{i=1}^9 n_i y_i}{9n_t} \times 100$$

Where y_i was the number of leaf sheaths penetrated (1-9), n_i the number of mainstems with a given score and n_t the total number of mainstems in each sample. Details of planting dates and previous crops were noted where the information was available.

5.4 The 1983/4 field experiment

The field site used for the 1981/2 and 1982/3 experiments was sown with winter wheat (cv. Armada) by direct drilling on 9 September 1983. As the percentage of the crop affected in 1983 had been much

lower than in 1982, wheat straws, naturally infected by P.herpotrichoides, were taken from another site on the Rothamsted Farm and spread across the experimental site to increase the amount of inoculum. The plot was divided into areas with high and low seed rates (300 and 100kg/ha with a 17.5cm row spacing). The crop was treated with "Brittox" herbicide on 10 April 1984 at the rate of 1.1 l in 56 l of water/ha, with nitrochalk at 560kg/ha on 15 April 1984, and on 1 May with "Hytane" (another herbicide) at 2.5 l in 60 l of water/ha.

The area of the sub-plots was slightly less than in the previous experiments because paths, 0.5m wide, were made around each plot to reduce damage during sampling. The crop was sampled at weekly intervals and eyespot development and plant growth assessed. At weekly intervals the leaf primordia from 10 plants were dissected out to follow their apical development (Kirby & Appleyard, 1981). The dates of plant emergence, "double ridging", "anthesis" and when the caryopsis was hard were noted.

The rain gauge failed to operate correctly during December 1983 and after several attempts to repair it, in February 1984 it was decided to measure rainfall on a weekly basis using a 12cm diameter funnel over a conical flask sunk into the ground. The water potential of the soil and of the plants were initially measured at monthly intervals as in 1983 and then later in the season at weekly intervals. The plants for measurement were randomly selected from areas adjacent to the quadrats. Eyespot severity was recorded as a mean percentage score and calculated as:

$$\left(\frac{\sum_{i=1}^3 n_i x_i}{3n_t} \right) \times 100$$

where x_i was lesion severity (0-3), n_i the number of mainstems with a given score and n_t the total number of mainstems in each sample.

The relationship between eyespot development and previous weather was examined for all three seasons using a linear predictor model. In the model fitting, the expected proportion of eyespot severity is related to a linear combination or function of weather variables - temperature, accumulated temperature, accumulated and total rainfall and to each of these from one, two or three weeks previously. The different models were compared using percentage deviance accounted for, or the mean deviance for the residual. The best model has the largest percentage deviance accounted for or the smallest mean deviance. All model fitting was done on the Rothamsted computer using "Generalised linear interactive modeling" (Baker & Nelder, 1978).

5.5 Results

Eyespot severity and percentage infection differed between the three seasons. The results given below are examined for each of several environmental factors.

1) Rainfall

March 1982 was wet with 68mm of rain. Early spring incidence of eyespot was almost 100% (Fig. 20). At "booting" and anthesis (late May and early June) there was 110mm of rain in five weeks and the subsequent warm weather apparently favoured eyespot development since there followed a rapid increase in lesion severity. Before harvest 44% of the plants had severe lesions and almost 100% of the plants were infected. There was also an abundance of Fusarium spp., Rhizoctonia cerealis and several other pathogenic and saprophytic fungi.

In June 1983, when the plants were again in early "boot", there were by contrast very few rainy days and temperatures were often above 20°C (Fig. 20); this caused drought symptoms in the crop and apparently checked eyespot development. Reports of droughting were also received via ADAS from throughout England. In July there was 35mm of rain but this was not enough to cause a rapid increase in eyespot severity at the end of the season, such as occurred in 1982. Eyespot severity was significantly greater ($P < 0.05$) in the small watered area of the crop (Table 17).

Only 6.7mm of rain fell during April 1984 when the plants were at growth stage 31. Early May was also dry and eyespot severity remained low. Following heavy rain at the end of May

Fig.20 Rainfall (weekly total) and severity of eyespot (high seed rate, ●—●) on winter wheat grown on one site on the Rothamsted Farm for three consecutive seasons.[Break in the graph indicates change from scoring eyespot severity on leaf sheaths to the stem]

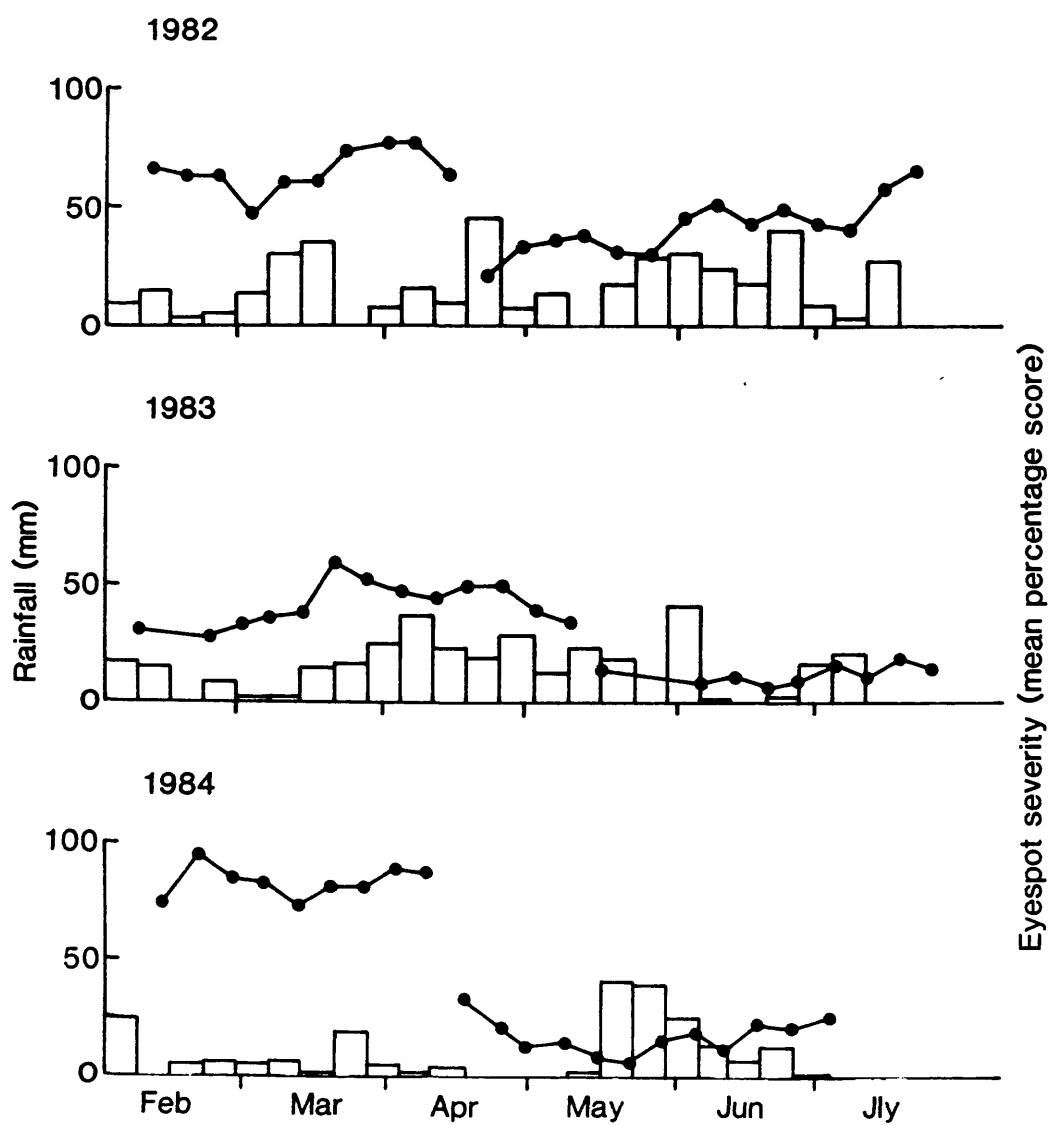


Table 17 Eyespot development in a small area of a winter wheat crop
on the Rothamsted farm (during 1983) with or without watering

<u>Date</u>	Eyespot severity scores*	
	<u>watered</u>	<u>not watered</u>
15 July	20.0	17.6
22 July	25.3	17.7
29 July	23.3	13.3
5 Aug	18.8	13.3
12 Aug	26.6	20.0
Total	114.0	81.9

SED ($\underline{P} < 0.05$, 8 Df) 3.1

* Mean percentage score for 30 plants

and moderate rainfall during June (total of 140mm) and steady increase in disease severity was observed.

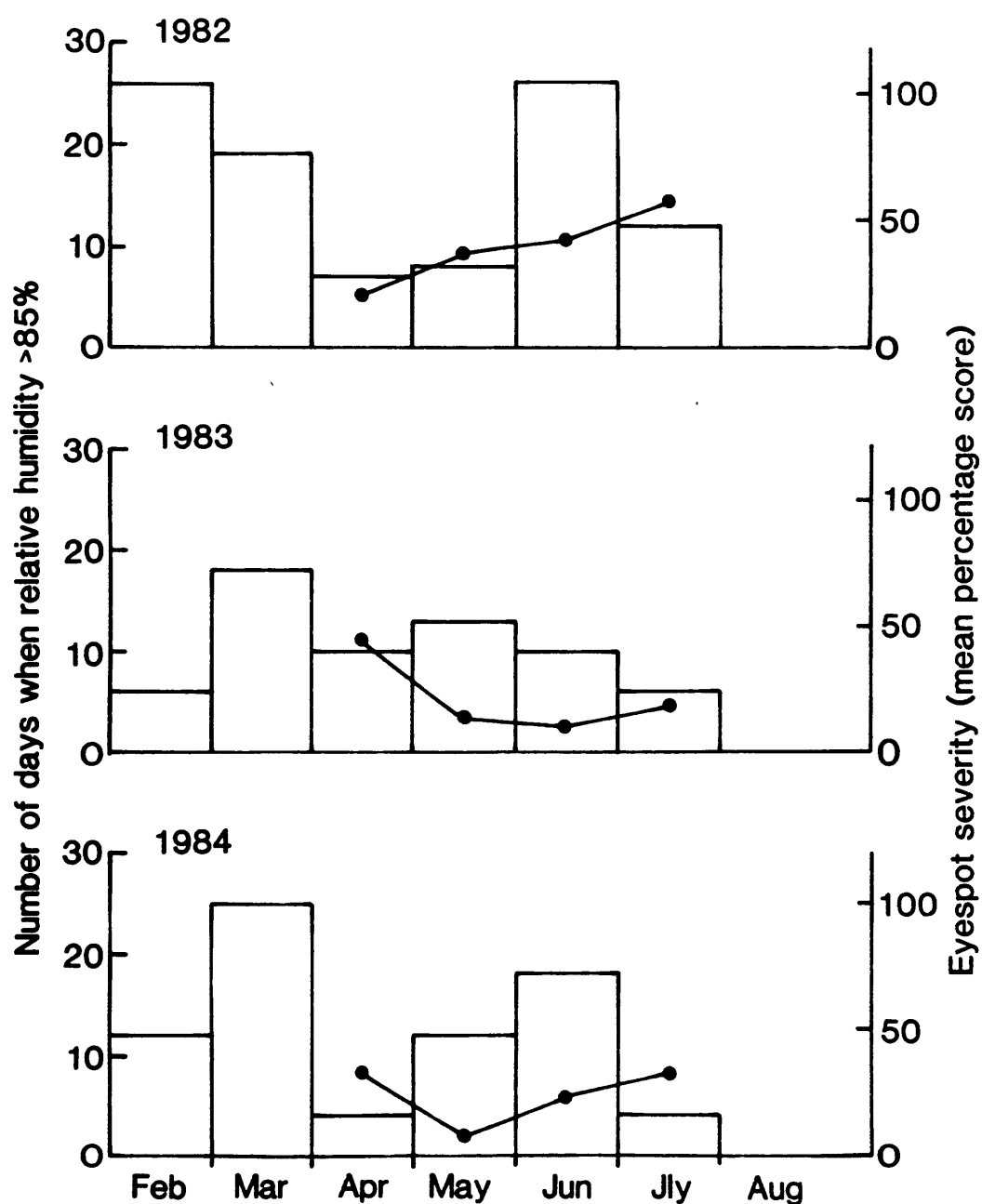
2) Relative humidity

Relative humidity was >85% on most days in February and March 1982, then in April and May averaged 70% (Fig. 21). A period of high relative humidity in June with 26 days when the relative humidity was greater than 85% was followed by rapid eyespot development. In the high seed rate area of the experiment leaf surfaces ~~remained~~ wet for longer because the humidity was c.9% greater in the denser crop. Eyespot severity was greater than in the low seed rate (thinner) crop.

There were only six days when the relative humidity was greater than 85% during February 1983 and this was during a period of snow cover. However, there were 18 such days during March. The late sowing of the crop delayed plant growth and the establishment of lesions was later than in 1982. In June and July the relative humidity averaged 79% and the temperature often reached 25°C and these factors appeared to have checked the development of established lesions.

Snow fell again during February 1984 and remained for about 2 weeks when relative humidity was c.30%. March 1984 was very humid and the establishment of infection and the development of eyespot lesions were more rapid than in 1983. During April, at the stem elongation stage, the relative humidity was low (average c.70%) and a large number of the outer leaf sheaths became desiccated and sloughed off. However, the relative humidity was generally 10% higher than this during May and June and established lesions became progressively more severe.

Fig 21 Number of days when the relative humidity was 85% (histogram) and severity of eyespot lesions (high seed rate, ●—●) on winter wheat (cv. Armada) grown on one site on the Rothamsted Farm for three consecutive seasons

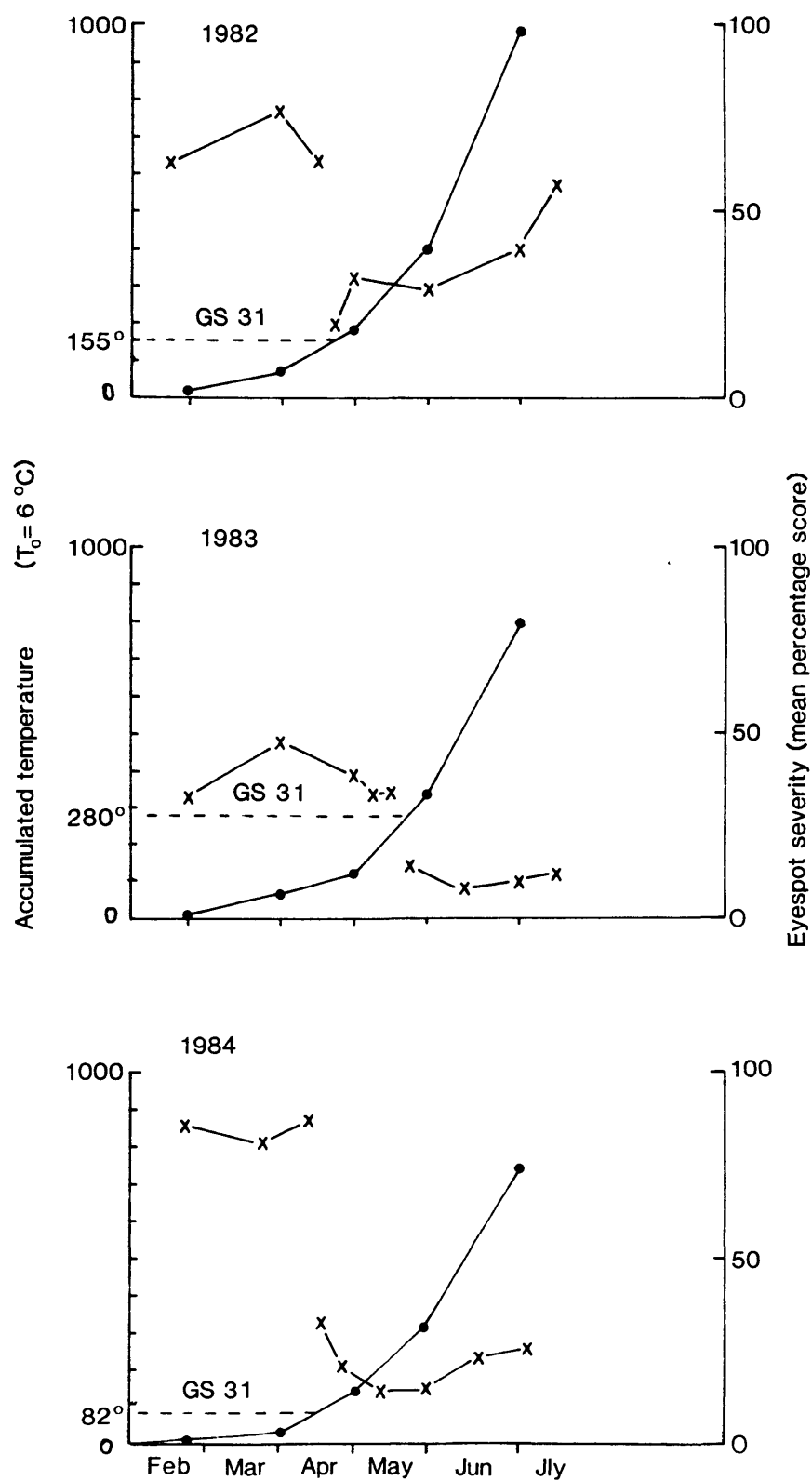


3) Temperature and planting date

The 1981/2 field experiment was sown in early October 1981 and by the spring nearly all the plants examined were infected. The 1983 crop was not sown until November 1982 and only 50% of the crop was infected by the spring of 1983. This difference in planting date not only caused a difference in the length of time the plants were exposed to inoculum, but also meant that plants at corresponding stages in growth were subjected to different temperatures. Stem elongation (growth stage 31) did not occur until mid-May 1983. The accumulated temperature (using $T_0 = 6^{\circ}\text{C}$) from January to this stage was 280 day-degrees (Fig. 22) and eyespot was not severe. Comparing these data with those for 1982 and 1984 at the same stage in crop growth, only 155 and 82 day-degrees respectively had accumulated by mid-April and eyespot severity was considerably greater than in 1983. Initially the eyespot severity score was 87% in 1984 but immediately after stem elongation a very dry spell with steadily increasing temperatures reduced the eyespot severity score to 14%. Lesion severity then slowly increased following rain during May and June.

The humid conditions prior to stem formation in April 1982 probably reduced the number of outer leaf sheaths which became desiccated and sloughed off. The gradual increase in temperature after this period ensured that lesion development was quite rapid. The temperatures during June and July 1982 oscillated around 15°C , which is optimal for growth of P. herpotrichoides in wheat plants (Defosse, 1967).

Fig 22 Accumulated temperature and eyespot severity (high seed rate, x—x) on winter wheat (cv. Armada) grown on one site on the Rothamsted Farm for three consecutive seasons, showing temperature accumulated (●—●) by plant growth stage 31 (Zadoks *et al*, 1974) [Break in the graph indicates change from scoring eyespot severity on leaf sheaths to the stem]



4) Water potential

The water potential of the soil just below the soil surface went down to -50 bars (Table 18) and that of the plants dropped to -15 bars in July 1983. Many of the plants wilted (Fig. 23). Eyespot incidence remained very low during this period and only reached 30% infection, with no severe lesions, prior to harvest. By contrast during 1984 the plant water potentials were generally much greater and there was a very gradual increase in eyespot severity from April until harvest in August.

5) 1983 ADAS samples

Information about the samples of four winter wheat cultivars received from ADAS is summarised in Table 19. Lesions which developed before harvest were more severe than those on Armada from the Rothamsted farm site. Generally the results show that the greater the depth of penetration of the leaf sheaths in the spring, the greater the disease severity at harvest. No information was available about the rates at which seed was planted on these sites.

6) Linear predictor model for eyespot development

Models were based on weekly eyespot severity scores from growth stage 31 (first node stage) to harvest. Weather variables were examined up to three weeks prior to each eyespot severity score recorded in order to identify any subsequent response in lesion development.

The best three-variable linear predictor model for all three consecutive seasons used mean temperature and accumulated temperature lagged by two weeks and rainfall.

Table 18 Water potentials of soil taken from 5cm below the surface
on the Rothamsted farm site, under winter wheat, for two consecutive
years

<u>1983</u>		<u>1984</u>	
Date	Soil water potential (bars)	Date	Soil water potential (bars)
10 Feb	-10.0		
22 Feb	- 4.0		
1 Mar	-11.4		
8 Mar	-12.7		
17 Mar	-13.2		
29 Mar	-14.5		
6 Apr	-10.6		
13 Apr	-13.2	16 Apr	-20.8
21 Apr	- 8.7		
26 Apr	-14.2	25 Apr	-28.5
3 May	-13.2		
10 May	-13.7		
17 May	-17.2		
10 Jun	-37.7	6 Jun	-14.1
17 Jun	-37.7	17 Jun	-21.2
23 Jun	-48.2		
27 Jun	-47.5	26 Jun	-34.7
30 Jun	-18.0	3 Jly	-50.0
12 Jly	-43.5	10 Jly	-50.0
14 Jly	-45.5		
21 Jly	-50.0		
28 Jly	-47.0		
5 Aug	-31.2		

Fig 23 Plant water potential and eyespot severity score (●—●), (high seed rate) on winter wheat (cv. Armada) grown on one site on the Rothamsted Farm for two consecutive seasons

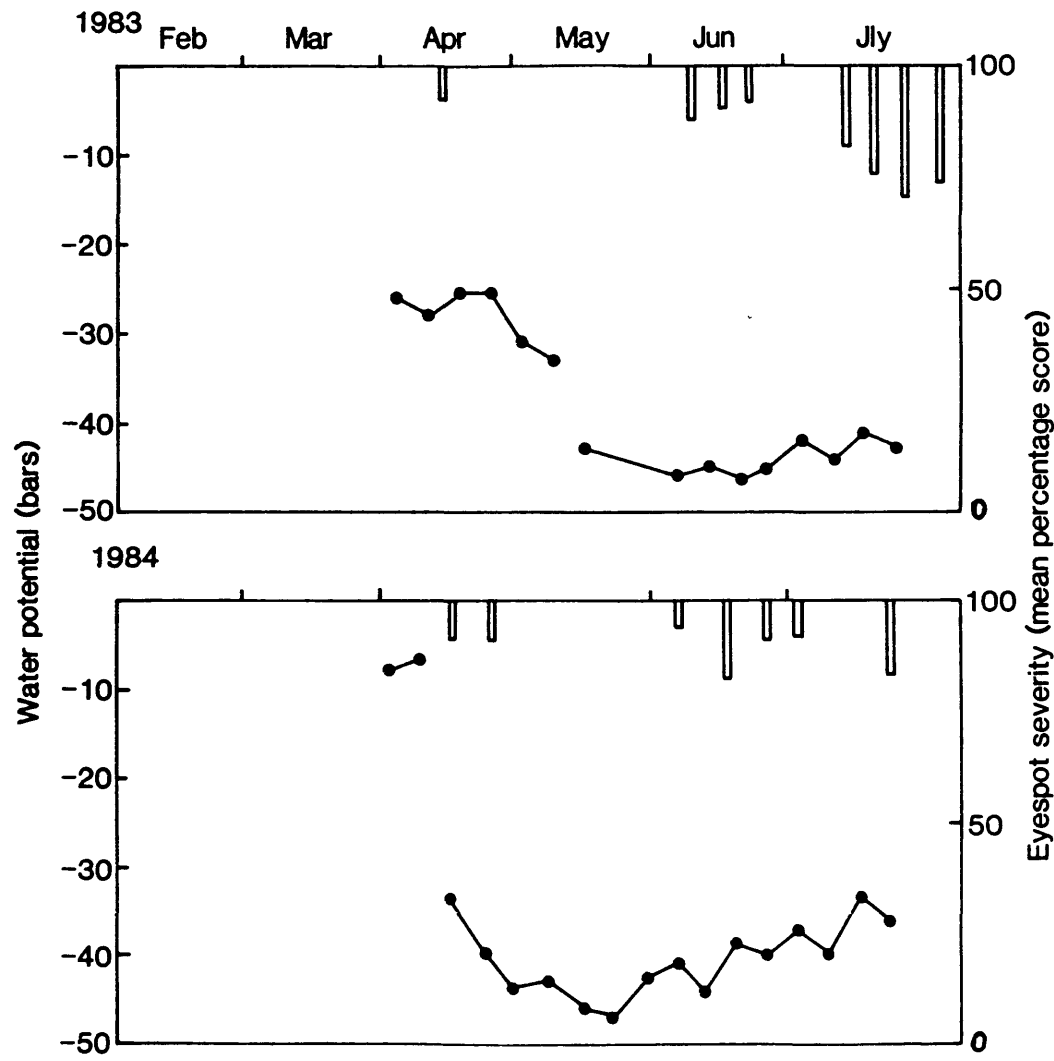


Table 19 Eyespot infection of winter wheat received from farm sites via ADAS in spring and
at harvest 1983

site	cultivar	planting date (1982)	<u>Spring</u>		<u>Harvest</u>	
			penetration score*	% infection	severity score*	% infection
Guiting Power	Avalon	18 Nov	21.3	48	33.3	60
Harnhill	Rapier	22 Nov	26.6	40	41.3	76
Ciren- cester	Norman	18 Sept	44.0	68	36.0	60
Swindon	Armada	18 Oct	46.0	80	60.0	95

* Mean percentage score for 25 plants.

week. However, this only accounted for 47.4% of the deviance.

The variables and the percentage deviance explained by the best model for each season are shown in Tables 20, 21 & 22.

Accumulated temperature appeared to be the most reliable variable, but because of the uniform way in which it increases every year, it is probably the least useful in a prediction model and other variables must also be considered.

5.6 Summary of results

The incidence of eyespot in a 3-year field experiment (1981/82, 1982/83 and 1983/84) was high in the spring of all three seasons. February and March 1982 were very wet and humid and lesions quickly became established, whereas during the same period in 1983 it was less humid and eyespot was not as severe. February 1984 was less humid than February 1982 but March was wet and lesions became established. Dry weather in April 1983 and late April/early May 1984 reduced eyespot incidence, as many of the leaf sheaths in which P.herpotrichoides was contained were sloughed off before the stem had formed.

There was heavy rainfall during June 1982, when the plants were in "boot" and plant growth was rapid; this was followed by increased eyespot development. When water was scarce at this growth stage, as in 1983, plants became water stressed and this, together with the high temperatures, limited lesion development. Watering a small area of the site when water was limiting increased the severity of lesions. June 1984 was warm with moderate rainfall and lesion development was moderate.

None of the prediction models produced satisfactorily explained the course of lesion development in all three seasons.

Table 20 Analysis of deviance for best fitting linear predictor models for

1981-82

<u>One-variable</u>	<u>Deviance</u>	<u>D.f.</u>	<u>Mean deviance</u>	<u>% Deviance explained</u>
Accum. temp.	53.45	1	53.45	
Residual	22.01	12	1.83	68.4
Total	75.46	13	5.80	
<u>Two-variable</u>				
Mean temp. lagged 1wk	56.34	2	28.17	
Accum. temp. lagged 3wk				
Residual	19.12	11	1.74	70.06
Total	75.46	13	5.80	
<u>Three-variable</u>				
Mean temp. lagged 1wk	58.65	3	19.55	
Accum. temp. lagged 3wk				
Total rain lagged 3wk				
Residual	16.81	10	1.68	71.04
Total	75.46	13	5.80	

Table 21 Analysis of deviance for best fitting linear predictor models for

1982-83

<u>One-variable</u>	<u>Deviance</u>	<u>D.f.</u>	<u>Mean deviance</u>	<u>% Deviance explained</u>
Accum. temp. lagged 3wk	3.07	1	3.07	
Residual	7.44	7	1.06	19.10
Total	10.51	8	1.31	
<u>Two-variable</u>				
Accum. temp. Accum. rain lagged 2wk	7.43	2	3.72	
Residual	3.07	6	0.513	60.94
Total	10.51	8	1.31	
<u>Three-variable</u>				
Accum. temp. lagged 3wk Accum. rain Mean temp. lagged 2wk	9.64	3	3.21	
Residual	0.87	5	0.175	86.70
Total	10.51	8	1.31	

Table 22 Analysis of deviance for best fitting linear predictor models for

1983-4

<u>One-variable</u>	<u>Deviance</u>	<u>D.f.</u>	<u>Mean deviance</u>	<u>% Deviance explained</u>
Accum. rain	17.45	1	17.45	
lagged 3wk				
Residual	45.12	13	3.47	22.34
Total	62.57	14	4.47	
<u>Two-variable</u>				
Mean temp.	29.26	2	14.63	
lagged 2wk				
Accum. rain				
lagged 3wk				
Residual	33.31	12	2.78	37.89
Total	62.31	14	4.47	
<u>Three-variable</u>				
Mean temp.	36.70	3	12.23	
lagged 2wk				
Accum. temp.				
lagged 2wk				
Total rain				
Residual	25.87	11	2.35	47.40
Total	62.57	14	4.47	

CHAPTER 6

DISCUSSION

6.1 Initial infection

The results presented in Section 3.2 suggest that in the UK, eyespot epidemics caused by P.herpotrichoides are not limited by lack of inoculum. Although the numbers of conidia on straws collected from the field showed a negative correlation with increasing mean weekly temperature (Fig. 4), they remained large during the early stages of the growing season. During February and March 1982 numbers of conidia were very large (8×10^6 /straw). Fluctuations in numbers reflected changes in the rate of spore production with temperature and in the rate of spore dispersal by rain (Rowe & Powelson, 1973). When pieces of infected stubble were incubated at temperatures from 5 to 25°C there was a decrease in numbers of conidia produced with increase in temperature and most conidia were produced on straw incubated at 5°C (Fig. 5). At 20°C and above other fungal competitors present on the straw (e.g. Penicillium and Fusarium spp.) were predominant. Macer (1961) reported sporulation of P.herpotrichoides on straw at 22.5°C but at this temperature other fungi such as Fusarium, Cochliobolus and Helminthosporium spp. were more competitive in colonising the straw. Straw from the Rothamsted farm incubated at 25°C produced an abundance of bacteria and few fungi. This effect of temperature on the sporulation of P.herpotrichoides on naturally infected straw in the presence of other fungal competitors may explain the decrease in numbers of conidia in the field later in the season, when temperatures often exceeded 20°C.

The high viability (c. 85%) of the conidia collected from the field during January to April suggests that low viability does not limit the numbers of plants which become infected and that other factors are involved. Temperatures in the range of 4 to 13°C are favourable for infection of wheat seedlings (Schrödter & Fehrmann, 1971), with an optimum of 9°C (Ponchet 1963). In the UK, temperature does not limit sporulation and infection early in the season since mean weekly temperatures rarely reach above 15°C.

It has often been assumed that the basal position of eyespot lesions on wheat straws is a result of conidia washing down the plant and collecting at this point. Evidence from this study on the distribution of conidia inoculated onto plant surfaces disproves this idea. Conidia which were either naturally dispersed or sprayed onto wheat seedlings were not readily removed by subsequent rain (section 3.3) and with time this would result in an accumulation of conidia over all the surfaces of the plant. This supports the view of Fitt & Bainbridge (1983a) that P.herpotrichoides conidia have adhesive properties. Most conidia were found on the abaxial surfaces of the leaf blades and especially on the lower leaves (Table 1). This may simply be due to the trajectory of most conidia onto this surface. However, the abaxial surfaces of leaves have very few hairs (Percival, 1921) and a smooth substrate allows maximum contact and firm adhesion by conidia (Rapilly & Foucault, 1976).

Conidia were observed to germinate on leaves, but penetration and colonisation of plant cells was only observed on coleoptiles (section 3.4). Conidia which were lying above anticlinal plant

cell walls had a significantly higher percentage germination than those which lay in other orientations (Table 3). Leakage of nutrients from plant cells, which often occurs at the anticlinal wall (Skoss, 1955), may have promoted germination of conidia. Water potential of the substrate was also shown to affect germination of conidia (section 4.4). Conidia were observed to swell visibly prior to germ tube production, probably due to the uptake of water. Therefore it is not surprising that low water potentials inhibited germination (Table 10). Under natural conditions the high water potentials necessary for germination are normally present since conidia are splash-dispersed (Fitt & Bainbridge, 1983b).

The coleoptiles of inoculated wheat seedlings were colonised by P.herpotrichoides much more quickly than the leaf blades (Fig. 7). Infection of the leaf sheaths, but not of the coleoptiles, is sometimes accompanied by localised thickening of the cell walls through which penetration hyphae pass (Bateman & Taylor, 1976). In the coleoptile, development of infection was associated with generalised necrosis. In leaf sheaths necrosis occurred in individual cells adjacent to areas where penetration was proceeding. The ease with which the coleoptile was colonised was probably due to its inherent susceptibility and was not because of a lack of chlorophyll. Induced chlorosis of leaves did not increase their susceptibility to infection and light intensity had no effect on germination and initial mycelial growth of P.herpotrichoides in vitro (Table 4). In contrast to these results, Reinecke & Fokkema (1981) found that infection of seedlings was most rapid in the dark. However, this was in the presence of Microdochium bolleyi (an antagonist to

P.herpotrichoides). They suggested that there could be a reduction in the production of fungitoxic compounds in the dark.

Lesions are normally observed at the stem base (Glynne, 1953) and it is probable that high susceptibility of the coleoptile in the initial stages of infection determines the position of the lesion. The coleoptile tissues rapidly senesce, but stem base lesions caused by P.herpotrichoides can be initiated even after the coleoptile has died. Hollins & Scott (1980) exposed plants to inoculum throughout the season (October to July); all developed lesions provided suitable incubation conditions were available, even after the coleoptile had disintegrated. Ageing of the stem base tissues seems to alter their susceptibility to infection so that, providing sufficient conidia inoculate the plants and the microclimate is suitable for infection, lesions will develop.

6.2 Lesion formation, isolate growth and pathogenicity

Lesion severity increased with an increase in numbers of conidia in the inoculum applied to the seedlings (Fig. 9). This suggests that in some way the conidia act together in the formation of lesions. Germinating conidia on the coleoptile surface were often observed (both in controlled temperature room and in field experiments) to fuse together to form a dense mycelial mat over the host surface. From these results (Table 7) it appears that only one spore might initiate a lesion. It is unlikely that this happens in the field, since most conidia are spread over short distances in splash droplets containing many spores (Fitt & Bainbridge, 1983b; Fitt & Nijman, 1983). Many young seedlings probably become infected by groups of spores. The numbers of conidia which initiate a lesion on plants could influence the course of lesion development.

The differences in lesion severity between seedlings with different numbers of conidia applied was still evident when the plants reached maturity.

Although lesion severity increased with increase in the number of conidia applied to the seedlings for all of the isolates tested, there were differences between isolates in pathogenicity to seedlings (and these were reflected in their total pathogenicity scores) with conidial numbers (Table 7). However, the ranking of isolates in their pathogenicity to seedlings was not correlated with the ranking in pathogenicity to mature plants (Table 8). Scott et al. (1975), in their experiments using small plots (0.7m^2), found little difference between isolates in pathogenicity to mature plants whereas Cunningham (1981), using troughs, did not find differences between isolates in the incidence of eyespot they caused. However, Bruehl et al. (1968), using large plots (36m^2), found no differences in final yield between plants infected with different P.herpotrichoides isolates.

It is not clear how the pathogenicity of isolates to seedlings relates, if at all, to the pathogenicity to mature plants. Scott et al. (1975) used the same four isolates in their seedling tests in the glasshouse as in the field, but the pathogenicity rankings were quite different. Scott & Hollins (1977) suggested that the differences between their pathogenicity tests with seedlings may have occurred because of changes in the cultivars, in the isolates, or in the environment. Both temperature (Lange-de la Camp, 1966) and duration of experiment (Macer, 1966) affect the results of such pathogenicity tests, possibly by acting on the host resistance genes. Plant resistance to P.herpotrichoides is possibly expressed

by different mechanisms depending on the age of the plant. The observations of Khan & Bouriquet (1984) on infected seedlings reveal that the inter and intracellular hyphae of P.herpotrichoides form dense segmented filaments in the sensitive cultivars. In resistant cultivars only short segments of hyphae were observed and these eventually degenerated and dilated. They concluded that there was a fungitoxic reaction to fungal penetration by the cultivar. Resistance may be altered by the environment, e.g. by heat shock, if genes controlling toxin production or cell lignification are temperature-controlled (Bell, 1981). Heat sensitivity may explain some of the differences between pathogenicity to mature plants and pathogenicity to seedlings, since in the field plants experience a gradual increase in temperature with the approach of summer. The total number of genes conferring host resistance and their method of operation is not understood (Law et al., 1975). Epidermal cell walls become thickened and lignified prior to contact with the fungus and pith parenchyma cell walls become lignified in diseased tissues. The strengthening of cell wall structure aids host resistance and becomes more apparent as the host approaches maturity (Murray & Bruehl, 1983).

Isolates W-2 and E-3, which were benzimidazole-tolerant, were as pathogenic to wheat seedlings and to mature plants as the benzimidazole-sensitive isolates (Tables 7 & 8). Brown et al. (1984) found that benzimidazole-tolerant isolates did not differ from susceptible isolates in pathogenicity to seedlings. This is of particular importance since Griffin & Varham (1983) reported that in none of their field trials where MBC fungicides were used was there total control of eyespot. Isolates of P.herpotrichoides showing MBC resistance were detected in 6 of the 15 winter wheat

fields examined. They reported two main growth types of MBC-tolerant isolates; the fast-growing even types and the slow-growing feathery types, of which the latter were predominant.

The growth rates of isolates on PDA incubated at 20°C were positively correlated with their pathogenicity to seedlings (Fig. 12). Of the two benzimidazole-tolerant isolates, W-2 was a slow-growing feathery type with a low pathogenicity to seedlings and E-3 was a smooth fast-growing type which was more pathogenic to the seedlings. In this case the relative pathogenicity shown by the two isolates was maintained on mature plants in the bucket experiments (section 4.3). The Cellulolysis Adequacy Index and all the other growth measurements for isolates were not correlated with their pathogenicity to seedlings or to mature plants.

The results suggest that the extent of fungal penetration before plant growth stage 31 (Zadoks et al, 1974), when the stem was formed, determines the initial severity at growth stage 31 and the percentage of plants infected at harvest. Temperatures of 10°C and above early in the year favoured fungal penetration of leaf sheaths, but decreased the numbers of live leaf sheaths penetrated as they increased both production of new leaf sheaths and the desiccation and death of leaf sheaths (Table 16). Increased leaf sheath production may delay penetration of the newly-formed stem and reduce the final severity of lesions. Defosse (1967) reported that the optimum temperature for infection of the first leaf sheath was at or below 15°C, while that for progress to the second leaf sheath was near 20°C. From field investigations, Schrödter & Fehrmann (1971) stated that the optimum temperature for infection of wheat seedlings by P.hierpotrichoides is approximately 9°C. Ponchet (1959; 1963) found

that the rate of growth of the pathogen in pure culture increased linearly with temperature over the range 0-20°C and suggested that the rate of progress of the disease in the host responded similarly. In artificially inoculated field experiments the rate of progress of the fungus through the wheat tissues was proportional to their accumulated temperature since inoculation (Ponchet, 1963). Scott (1971) also examined the effects of temperature on P.herpotrichoides in seedlings, using mycelial inoculum. He observed that the number of leaf sheaths penetrated increased with an increase in temperature over the range 6-18°C. This increase in penetration depth was a response to temperature by the fungus alone and not an undetected host response. Scott suggested that in the field warm weather during the winter and spring would increase the risk of existing infections becoming damaging. However, the findings of this study suggest that cooler temperatures in the early spring promote moderate plant growth and allow rapid fungal penetration through successive leaf sheaths to the stem (Fig. 18). This would then result in high disease incidence and increased lesion severity by early summer.

The temperature response observed by Scott⁽¹⁹⁷¹⁾ was not linear and in all cases he observed an initial lag phase prior to penetration. This lag phase may reflect both the need for all environmental conditions to be suitable, before penetration can take place and the formation of stroma or pseudoparenchyma by the fungus between and before penetration of leaf sheaths (Ward, 1976). Further investigation is required to discover whether high relative humidity at the higher temperatures (10-15°C) could reduce the production and desiccation of leaf sheaths and

stimulate fungal growth.

The most important period in the development of established lesions is between growth stage 31 and harvest. The period between growth stage 31 and "booting" (GS. 45) is a time of rapid cell growth and extension. It is during this period that plants are most susceptible to water stress which may cause a weakening of their defensive mechanisms. The relationship between plant water potential and lesion development is not clear. Water stress in plants caused by droughting or by excessive watering at suitable temperatures lowered plant resistance and/or increased fungal growth (Tables 13 & 14). Although differences in stem water potentials between the droughted plants and the normally watered plants were created in a second experiment (section 4.4), increased eyespot severity was not directly correlated to higher water potentials. Droughted plants ($\Psi = -18$ bars) at 20°C had a greater eyespot severity score than the normally watered plants ($\Psi = -6$ bars). Heavy watering at c. 15°C appeared to increase lesion severity (Table 14). Clearly a combination of factors is involved in the progress of lesions.

Sprague (1937) was the first to investigate the effects of soil moisture on lesion severity. Plants were grown in drainage pipes in an unheated glasshouse from January to May. Different watering regimes were applied from sowing; plants watered every other day, with 16% available moisture (near saturation), developed the most severe eyespot lesions. Generally lesion severity decreased with a decrease in the frequency of watering. No reference was made to the effect of watering regime on plant growth. Watering regimes of this type affect the relative humidity at the

stem base and more precise controls are needed to investigate these effects. The extent of water stress created by the droughting treatment in the tubing experiment (section 4.4) was illustrated by the unusual situation that the root water potentials were lower than those of the stems (Table 14). Water normally flows from regions of high water potential to regions of low water potential and stem water potentials were lower than root water potentials in all the plants from the other watering regimes.

Water potential does have a direct effect on the growth of P.herpotrichoides. The optimum range for mycelial growth was -5 to -15 bars (Table 12) and although growth occurred at -69 bars and below, it was minimal (Table 10). P.herpotrichoides is therefore well adapted to the host plant since in the UK plant water potentials rarely reach below -15 bars during growth (W. Day, personal communication). The ability of mycelia to survive the dry conditions presented by straw stubble left after harvest is reflected in the ability of P.herpotrichoides to grow at very low water potentials. The effects shown by Sprague (1937) probably relate more closely to the requirement for moisture for the germination of conidia and lesion establishment than to effects on lesion development.

Growth isolates from the temperate UK climate and of isolates from the dryland Pacific northwest of the USA at different water potentials was examined both in vitro and in vivo (Table 12). There was no evidence to suggest isolate specialisation and adaptation to dry conditions and isolates showed similar optimal water potentials (-5 to -15 bars) for growth. Although the dryland isolate 79-1 produced the most severe eyespot when inoculated onto droughted plants, it was not significantly different from the

UK, temperate isolates and was therefore not considered to be especially adapted to dry conditions. Similarly Bruehl & Manandhar (1972) reported a lack of evidence for the development of dryland ecotypes amongst the isolates they tested. They did not examine spore germination at different water potentials, but did observe mycelial growth at -100 bars. The increase in growth with increasing temperature at low water potentials (-28 bars) which they observed was not confirmed in this study, but this may have been because a different water potential was used (-25 bars).

6.3 Effects of weather factors on eyespot development in the field

When rainfall is high during late winter, early spring and when the crop is in "boot", the probability of severe eyespot is great (Fig. 20). During February and March 1982, the cool moist weather enhanced the sporulation of P.herpotrichoides on straws, and dispersal of conidia onto developing seedlings. The following June there was frequent and heavy rain which maintained a high relative humidity within the crop and the development of established lesions was extensive (Fig. 21). Sprague (1937) observed that the most severe attacks of eyespot occurred in areas having an average rainfall of c. 51cm per year. In regions of Washington State, USA, where annual precipitation was only 34 to 37cm the disease was slight except where moisture accumulated in the soil in localised areas. Glynne (1939) also observed that eyespot lodging was favoured by moist soil conditions. However, the observed differences in eyespot severity between areas with identical annual rainfall indicated the importance of other factors. The total rainfall for 1982

on the Rothamsted farm was 86cm, well above the limit for lesion development specified by Sprague.

The spring of 1983 was, as in the previous year, moist and warm and the numbers of conidia present on the straws were therefore large. However, the very dry period that followed, when the plants were in "boot", probably checked the development of lesions and at harvest only 30% of the crop was infected and there were no severe lesions (Fig. 20).

The spring of 1984 was drier than the previous two springs, and it was cooler than the spring of 1982, and the numbers of conidia which were dispersed from straw onto the plants were probably less and, although initial severity of eyespot was similar to that of 1982, leaf sheath penetration was probably less (Fig. 20). Temperatures in June and July 1984 were slightly below those of 1983 and rainfall, although quite low, was more frequent. Before harvest, almost 50% of the crop was infected by P.herpotrichoides, with about 2% severe infection, higher than in 1983.

Sprague (1937) observed that in Oregon, USA, although air temperature reached 15°C which is near optimal for P.herpotrichoides growth, at soil level where nearly all lesions occur temperatures often reached 40°C or more, which would certainly have limited fungal growth. In this study temperatures just above soil level often exceeded 30°C during June/July 1983. Sprague⁽¹⁹³⁷⁾ also noted that high soil moisture deficiencies were normally associated with these very high temperatures and were important in restricting eyespot development. This suggestion is verified by the 1983 results, since daily watering of a confined area of the field site increased the severity of lesions which developed (Table 17).

In the summer in the UK relative humidity is very closely related to rainfall; during the wet June of 1982 the relative humidity remained above 85% for almost two weeks and temperatures of around 15°C were ideal for growth of P.herpotrichoides (Defosse, 1967). All these factors combined probably contributed to the rapid increase in disease severity which followed. As the crop ripened, the difference in disease severity between the two stands increased, the severity being greater on plants in the high seed rate (300kg/ha) plot. More tillers are produced by plants sown at low seed rate than ~~by~~ plants sown at a high seed rate and these tillers protect central shoots from infection (Glynne, 1953).

High water potentials during "booting" stage of crop growth, generally promote lesion development, but the direct effects of water potential on eyespot development are not clear. During June and July 1983 soil and plant water potentials became very low and plants showed symptoms of droughting; the development of eyespot lesions was restricted. However, how much this was an effect of relative humidity, sowing date and earlier weather conditions in the same season is not known. 1983 samples from farm sites, did have a high percentage of severe lesions despite the dry summer. Information on the differences between soil water retentions at specific sites was not available, but the soils did appear quite different from one another and from that of the Rothamsted farm. June 1984 was wetter than June 1983 and soil and plant water potentials remained moderately high throughout the period of ear development and grain ripening; ~~thus~~ soil moisture was not limiting as in 1983. Lesion development was moderate and showed a slow steady increase towards harvest (Fig. 23).

Although it has been shown that high soil moisture content generally enhances eyespot development (Sprague, 1937; Glynne, 1939), temperature must also be considered. Dickens (1964) grew seedlings at different soil temperatures, gave them either high (near saturation) or low (droughted) moisture levels and examined the effects on the numbers of tillers which became infected. All tillers of plants at 6-13°C were severely infected and there was no observable difference between soil moisture treatments in disease severity. However, at 17-18°C, significantly more tillers became infected at the low than at the high soil moisture. He suggested that a relatively high carbohydrate content of the tillers caused by water deficiency may have provided more suitable nourishment for the fungus resulting in a greater number of infections. The carbohydrate content of different grasses is increased by decrease in temperature, possibly as a result of a water deficiency due to reduced water absorption at low temperatures (Brown, 1939).

Generally early planting of a crop exposes the crop to the inoculum for longer and increases the risk of severe eyespot. The late sowing of the 1983 crop (2 November 1982) resulted in its slower development compared with 1982 and 1984 crops (October and September planting dates). Consequently the crop was not exposed to the inoculum for the same length of time during the period of peak spore production. The stem elongation stage of plant growth was reached approximately four weeks later in 1982 and 1984 (Fig. 22). This delay in crop growth meant that more day-degrees ($T_0 = 6^{\circ}\text{C}$) had accumulated between February and stem elongation. Warm dry weather causes the drying out and sloughing off of the outer leaf sheaths and as this is where the fungus is initially contained, progress of

lesions can be restricted.

The first experiments relating date of planting to severity of eyespot lesions were carried out in the USA by Heald (1922). The least lodging occurred in wheat which was planted in October but yields were less than those obtained from September-sown wheat. It is because of these differences in yield that farmers have shown a preference for early-sown crops. Sprague (1937) also found appreciably less infection in late-sown than early-sown wheat; there were fewer infected culms and the lesions were less severe.

Several commercial wheat cultivars show some resistance to infection by P.herpotrichoides but this only reduces eyespot infection. Samples obtained from farm sites via ADAS included the cultivars Rapier and Norman (Table 18). Despite the similarity in planting date (within 4 days of each other), Rapier had a low penetration score and percentage infection in the spring, but its disease severity was greater than that of Norman by harvest. This may have been due to local differences in weather conditions, soil types etc. but Rapier is also slightly more susceptible to infection by P.herpotrichoides (Anon, 1984).

Generally observations made on these cultivars indicated that the greater the depth of penetration of the leaf sheaths in the spring, the greater was the final disease severity at harvest. Information was not available on the rates of seed planted and the influence of other differences in environmental factors should also be considered when comparing the severity of eyespot lesions on crops from different sites.

6.4 The linear predictor models

In all three consecutive years examined the "three-variable" models accounted for the highest percentage deviance or gave the lowest mean deviance. The models fitted the data for the harvest years 1982 and 1983 but this was because these two seasons showed extremes of eyespot severity. June 1982 was exceptionally wet, whereas June 1983 was very dry and warm; eyespot was very severe in 1982 but only affected the crop slightly in 1983. By contrast June 1984 was warm but with frequent moderate rain, and none of the variables examined gave a good basis for a predictive model.

The "three-variable" models for 1982 and 1983 explained 71 and 86% of the deviance but accumulated temperature lagged by 3wk was the only variable common to both models. Although temperature has been shown to be important in disease development (Ponchet, 1959; Scott, 1971), the variation in eyespot severity from year to year suggests that other climatic factors are important (Scott & Hollins, 1978). Rainfall accumulated and total rain for each week were both shown to be important by the three-variable models and soils with high moisture retention capacities favour eyespot lesion development (Sprague, 1937). Possibly rainfall maintains a high relative humidity at the stem base, delaying the desiccation of the outer cells of the stem tissue and their subsequent lignification. This may then enable extensive fungal growth and lesion development. No model could satisfactorily explain eyespot development for all three years and more data need to be recorded to obtain further combinations of weather factors and examine their

effects on eyespot development before an accurate prediction scheme can be produced. However, the models produced in this study are a useful indicator of the variables likely to be of most importance in such a model.

REFERENCES

- AKAI, S. (1951). On the anatomy of necrotic lesions on leaves and stems on plants affected by pathogenic fungi. Memoirs of the college of Agriculture, Kyoto University 61, 33.
- ANON. (1984). NIAB Recommended varieties of Cereals 1983. Farmers Leaflet No. 8.
- AYRES, P.G. (1981). Root growth and solute accumulation in pea in response to water stress and powdery mildew. Physiological Plant Pathology 19, 169-180.
- BAKER, R.J. and NELDER, J.A. (1978). The GLIM System Release 3. Oxford: Numerical Algorithms Group.
- BARNELL, H.R. (1936). Seasonal changes in the carbohydrates of the wheat plant. New Phytologist 35, 229-266.
- BARNELL, H.R. (1938). Distribution of carbohydrates between component parts of the wheat plant at various times. New Phytologist 37, 85-112.
- BATEMAN, G.S. and TAYLOR, G.S. (1976). Significance of the coleoptile in establishing seedling infection on wheat by Pseudocercospora herpotrichoides. Transactions of the British Mycological Society 67, 95-101.
- BATTS, C.C.V. and FIDDIAN, W.E.H. (1955). Effect of previous cropping on eyespot in four varieties of winter wheat. Plant Pathology 4, 25-28.

- BELL, Alois, A. (1981). Biochemical Mechanisms of Disease Resistance. Annual Review of Plant Physiology 32, 21-81.
- BENADA, J. (1981). Priciny zmen odolnosti vuci steblolamu (Cercospora herpotrichoides). Sbor. Uytiz-Ochr. Rostl. 17, 26-36.
- BENADA, J. and VANOVA, M. (1981). Moznosti Prognozy napadeni a signalizace osetreni psenice proti steblolamu (Cercospora herpotrichoides). Sbor. Uytiz-Ochr. Rostl. 17, 37-44.
- BLAIR, I.D. and MORRISON, L. (1949). Wheat diseases and insect pests. Department of Science and Industrial Research, Wellington, New Zealand. Information Series number 3. pp. 59. (John McIndoe Ltd. Dunedin).
- BROWN, E.M. (1939). Some effects of temperature on the growth and chemical composition of certain pasture grasses. University of Mo Agricultural Experimental Station Research Bulletin. 299. pp. 76.
- BROWN, M.C., TAYLOR, G.S. and EPTON, H.A.S. (1984). Carbendazim resistance in the eyespot pathogen Pseudocercospora herpotrichoides. Plant Pathology 33,
- BRUEHL, G.W. and MANANDHAR, J.B. (1972). Some water relations of Cercospora herpotrichoides. Plant Disease Reporter 56, 594-596.

BRUEHL, G.W., NELSON, W.L., KOEHLER, F. and VOGEL, O.A. (1968).

Experiments with Cercospora foot rot (straw breaker) disease of winter wheat. Washington Agricultural Experimental Station Bulletin 694, 1-14.

COOK, R.J. (1980). Fusarium root rot of wheat and its control in the Pacific Northwest. Plant Disease 64, 1061-1066.

COOK, R.J. and KING, J.E. (1979). Eyespot and sharp eyespot of wheat and barley. Ministry of Agriculture, Fisheries and Food. Advisory Leaflet 321, pp. 7. MAFF, Middlesex.

COX, J and COCK, L.J. (1962). Survival of Cercospora herpotrichoides on naturally infected straws of wheat and barley. Plant Pathology. 11(2), 65.

CUNNINGHAM, P.C. (1981). Occurrence, role and pathogenic traits of a distinct pathotype of Pseudocercospora herpotrichoides. Transactions of the British Mycological Society 76, 3-15.

DALLYN, H. and FOX, A. (1980). Spoilage of materials of reduced water activity by Xerophilic fungi. In Microbial growth and survival in extremes of environment (eds. G.W. Gould and J.E.L. Corry). Academic Press, London.

DEACON, J.W. (1973). Pseudoparenchyma produced by Pseudocercospora herpotrichoides. Transactions of the British Mycological Society 60, 537-545.

DEFOSSE, L. (1966). Les premiers stades de l'infection du Cercospora herpotrichoides Fron sur froment, orge, seigle et avoine. Bulletin des Recherches Agronomiques de Gembloux NS 1, 561-569.

DEFOSSE, L. (1967). Etude, en conditions experimentales, des facteurs qui régissant l'inoculation et l'infection de froment par Cercospora herpotrichoides Fron. Bulletin des recherches Agronomiques de Gembloux NS2, 38-51.

- DEFOSSE, L. (1976). Recherches histo¹chimiques sur la nature des réactions parietales formés en réponse à la pénétration de Cercospora herpotrichoides dans le coleoptile du blé. Parasitica 32, 147-157.
- DEFOSSE, L., and VANDAM, J. (1969). La sensibilité de genre Triticum au Cercospora herpotrichoides Fron. II (1) Sélection des espèces moins sensibles (2) Résultat d'une hybridation interspécifique T. aethiopicum + T. vulgare. Bulletin des Recherches Agronomiques de Gembloux NS 4, 411-419.
- DEIGHTON, F.C. (1973). Studies on Cercospora and allied genera. IV Cercospora sacc., Pseudocercospora gen. Nov. and Pseudocercosporidium gen. Nov. Mycological Papers, 133. Commonwealth Mycological Institute, Kew, Surrey, England.
- DICKENS, L.E. (1964). Eyespot footrot of winter wheat. Cornell University Agricultural Experimental Station Ithaca. Memoirs 390.
- DOUSSINAULT, G., KOLLER, J., TOUVIN, H. and DOSBA, F. (1974). Utilisation des géniteurs VPM¹ dans l'amélioration de l'état sanitaire du blé tendre. Annales de l'Amélioration des Plantes 24, 215-241.
- DUBÉ, A.J., DODMAN, R.L. and FLENTJE, N.T. (1971). The influence of water activity on the growth of Rhizoctonia solani. Australian Journal of Biological Science 24, 57-65.
- EVANS, M.E. and RAWLINSON, C.J. (1977). Stem sugars : A possible factor affecting the resistance of wheat to Pseudocercospora herpotrichoides. Phytopathologische Zeitschrift 89, 37-43.
- FEHRMANN, M. and MENDGEN, K. (1976). Ultrastructure in wheat coleoptiles after infection with Pseudocercospora Herpotrichoides. Phytopathologische Zeitschrift 83, 267-280.
- FEHRMANN, M. and SCHRÖDTER, H. (1972). Ökologische Untersuchungen zur Epidemiologie von Cercospora herpotrichoides IV. Erarbeitung eines praxisnahen Verfahrens zur Bekämpfung der

Halmbruckkrankheit des Weizens mit systemischen fungiziden.

Phytopathologische Zeitschrift 74, 161-174.

FITT, B.D.L. and BAINBRIDGE, A. (1983a). Recovery of Pseudocercospora herpotrichoides spores from rain-splash samples. Phytopathologische Zeitschrift 106, 117-182.

FITT, B.D.L. and BAINBRIDGE, A. (1983b). Dispersal of Pseudocercospora herpotrichoides spores from infected wheat straw. Phytopathologische Zeitschrift 106, 214-225.

FITT, B.D.L. and NIJMAN, D.J. (1983). Quantitative studies on the dispersal of Pseudocercospora herpotrichoides spores from infected wheat straw by simulated rain. Netherlands Journal of Plant Pathology 89, 198-203.

FOEX, E., and ROSELLA, E. (1933). Quelques observations sur le piétin des céréales. Revue de pathologie, végétale et d'entomologie agricole de France 20, 172-187.

GAIR, R., JENKINS, J.E.E. and LESTER, E. (1978). Cereal pests and diseases 2nd Edition Farming Press, Ipswich.

GARDINEAU, Mlle. et GUYOT, L. (1925). De quelques facteurs qui influencent le développement de la maladie du piétin du blé. Revue de Pathologie Végétale et d'Entomologie Agricole de France 12, 317-344.

GARRETT, S.D. (1966). Cellulose-decomposing ability of some foot rot fungi in relation to their saprophytic survival. Transactions of the British Mycological Society 49, 57-68.

- GARRETT, S.D. (1983). Weight losses of unsterilised filter-paper caused by colonies of cereal root rot fungi. Transactions of the British Mycological Society 81, 421-423.
- GLYNNE, M.D. (1939). Eyespot lodging of wheat caused by Cercospora herpotrichoides Fron. Agricultural Progress 16, 70-74.
- GLYNNE, M.D. (1950). Close cereal cropping. Effect of cultural treatments of wheat on eyespot, take-all and weeds. Agriculture 56, 510-514.
- GLYNNE, M.D. (1953). Production of spores by Cercospora herpotrichoides. Transactions of the British Mycological Society 36, 46-51.
- GLYNNE, M.D. (1954). Soil-borne diseases of cereals. Royal Agricultural Society, England, Journal 115, 41-46.
- GLYNNE, M.D. (1963). Eyespot (Cercospora herpotrichoides) and other factors influencing yield of wheat in the six course rotation experiment at Rothamsted (1930-60). Annals of Applied Biology 51, 189-214.
- GLYNNE, M.D., and SALT, G. (1958). Eyespot of wheat and barley. Rothamsted Experimental Station Report for 1957, 213-241.
- GLYNNE, M.D., and SLOPE, D.B. (1959). Effects of previous wheat crops, seed rate and nitrogen on eyespot, take-all, weeds and yields of two varieties of winter wheat. Annals of Applied Biology 47, 187-199.
- GRIFFIN, M. and YARHAM, D. (1983). Fungicide resistance - MBC resistance in the eyespot fungus. Agrospray FBC Ltd. Technical Information Issue No. 6.
- HEALD, F.D. (1922). Footrot of wheat. Washington Agricultural Station Bulletin 175, 36.

- HOLLINS, R.W. and SCOTT, P.R. (1980). Epidemiology of eyespot (Pseudocercospora herpotrichoides) on winter wheat, with particular reference to the period of infection. Annals of Applied Biology 95, 19-29.
- INGLE, E. (1983). In Wheat a guide to varieties from the Plant Breeding Institute Seed Development Organisation Ltd. Cambridge.
- JORDAN, V.W.L. and TARR, H.S. (1981). Timing of fungicide for eyespot control. In Strategies for control of Cereal Disease. (Ed. J.F. Jenkyn and R.T. Plumb), Blackwell, Oxford.
- JØRGENSEN, J. (1964). Some observations on the effect of temperature on sporulation of Cercospora herpotrichoides Fr. on straw. Acta Agriculturae Scandinavica 14, 12-20.
- KHAN, A.R. and BOURIQUET, R. (1984). Réactions histologiques de blés résistants ou non à l'infection par Pseudocercospora herpotrichoides. Phytopathologische Zeitschrift 109, 309-317.
- KIRBY, E.J.M. and APPLEYARD, M. (1981). Cereal Development Guide. (Ed. A. Barron). Cereal Unit, PBI, Cambridge.
- LANGE-DE LA CAMP, M. (1966). Die Wirkungsweise von Cercospora herpotrichoides Fr. Erreger der Halmbruchkrankheit des Getreides I. Phytopathologische Zeitschrift 55, 34-66.
- LANGVAD, F. (1980). A simple and rapid method for qualitative and quantitative study of fungal flora of leaves. Canadian Journal of Microbiology 26, 666-670.
- LAW, C.N. (1966). The location of genetic factors affecting a quantitative character in wheat. Genetics 53, 487-498.
- LAW, C.N.; SCOTT, P.R.; WOORLAND, A.J. and HOLLINS, T.W. (1975). The inheritance of resistance to eyespot (Cercospora herpotrichoides) in wheat. Genetical Research, Cambridge 25, 73-79.

- LEACH, C.M. (1967). Pseudocercospora herpotrichoides : storage and mass production of conidia. Canadian Journal of Botany 45, 1999-2016.
- MACER, R.C.F. (1961). Saprophytic colonisation of wheat straw by Cercospora herpotrichoides Fron. and other fungi. Annals of Applied Biology 49, 152-164.
- MACER, R.C.F. (1966). Resistance to eyespot disease (Cercospora herpotrichoides Fron) determined by a seedling test in some forms of Triticum, Aegilops, Secale and Hordeum. Journal of Agricultural Science, Cambridge 67, 389-396.
- MOORE, H.I. (1949). The problem of lodging. Journal for the Ministry of Agriculture 56, 314-316.
- MURRAY, T.D. and BRUEHL, G.W. (1983). Role of the hypodermis and secondary cell wall thickening in basal stem internodes in resistance to strawbreaker foot rot in winter wheat. Phytopathology 73, 261-267.
- NETTING, A.G. (1973). The physio-chemical basis of leaf wettability in wheat. Planta 114, 289-309.
- OORT, A.J.P. (1936). De oogulkkenziekte van de granen veroorzaakt door Cercospora herpotrichoides Fron. Tijdschrift over plantenziekten 42, 179-210.
- PANG CHANG, E.W., and TYLER, L.J. (1964). Sporulation by C. herpotrichoides on artificial media. Phytopathology 54, 729-736.
- PERCIVAL, J. (1921). The Wheat Plant. A monograph, pp. 463. London, Duckworth Co. Ltd.
- POLLEY, R.W. and CLARKSON, J.D.S. (1978). Forecasting cereal diseases epidemics. In Plant Disease Epidemiology (Ed. P.R. Scott and A. Bainbridge). 141-150, Blackwell, Oxford.
- PONCHET, J. (1959). La maladie du piétin-verse des céréales: Cercospora herpotrichoides Fron. Importance agronomique, biologie, épiphytologie. Annales des Épiphyties 20, 45-98.

- PONCHET, J. (1963). Influence de la temperature sur l'évolution des symptomes du Cercospora herpotrichoides Fron sur blé. Congrès de Biometeorologie, Pau 1963 (Cyclostyled report).
- RAPILLY, F. and FOUCAULT, B. (1976). Premières études sur la retention de spores fongiques par des epidermes foliaires. Annales de Phytopathologie 8, 31-40.
- REINECKE, P. and FOKKEMA, N.J. (1979). Pseudocercospora herpotrichoides storage and mass production of conidia. Transactions of the British Mycological Society 72, 329-331.
- REINECKE, P. and FOKKEMA, N.J. (1981). An evaluation of methods of screening fungi from the haulm base of cereals for antagonism to Pseudocercospora herpotrichoides in wheat. Transactions of the British Mycological Society 77, 343-350.
- ROWE, R.C. and POWELSON, R.L. (1973). Epidemiology of Cercospora foot rot of wheat: Disease spread. Phytopathology 63, 984-988.
- SALT, G.A. (1953). A field experiment on wheat infected with eyespot. Annals of Applied Biology 40, 224-225.
- SCHRÖDTER, H. and FEHRMANN, H. (1971). Ökologische untersuchungen zer Epidemiologie von Cercospora herpotrichoides. Phytopathologische Zeitschrift 71, 97-112.
- SCOTT, P.R. (1971). The effect of temperature on eyespot Cercospora herpotrichoides in wheat seedlings. Annals of Applied Biology 68, 169-175.
- SCOTT, P.R. and HOLLINS, T.W. (1974). Effect of eyespot on the yield of winter wheat. Annals of Applied Biology 78, 269-279.
- SCOTT, P.R. and HOLLINS, T.W. (1977). Interaction between cultivars of wheat and isolates of Cercospora herpotrichoides. Transactions of the British Mycological Society 69, 397-403.

- SCOTT, P.R. and HOLLINS, T.W. (1978). Prediction of yield loss due to eyespot of winter wheat. Plant Pathology 27, 125-131.
- SCOTT, P.R; HOLLINS, T.W. and PATRICIA MUIR. (1975). Pathogenicity of Cercospora herpotrichoides to wheat, barley, oats and rye. Transactions of the British Mycological Society 65, 529-538.
- SKOSS, J.D. (1955). Structure and composition of the plant cuticle in relation to Environmental Factors and Permeability. Botanical Gazette 117, 55-72.
- SPRAGUE, R.A. (1931). Cercospora herpotrichoides Fron. the cause of Columbian basin footrot of winter wheat. Science 24, 51-53.
- SPRAGUE, R.A. (1937). Influence of climatological factors in the development of Cercospora footrot of winter wheat. United States Department of Agriculture Circular 451, 1-40.
- SPRAGUE, R.A. and FELLOWS, H. (1934). Cercospora footrot of winter cereals. Technical Bulletin of the United States Department of Agriculture 428, 1-24.
- WARD, M.R. (1976). The biology of eyespot disease on winter wheat. Ph.D. thesis, Hull University.
- YARHAM, D.J. and NORTON, J. (1981). Effects of cultivation methods on disease. In Strategies for the Control of Cereal Disease. (Ed. J.F. Jenkyn and R.T. Plumb), Blackwell, Oxford.
- ZADOKS, J.C; CHANG, T.T. and KONZAK, C.F. (1974). A decimal code for the growth stages of cereals. Weed Research 14, 415-421.

ACKNOWLEDGEMENTS

I am very grateful to Dr. B. D. L. Fitt and Dr. B. E. J. Wheeler for their guidance and encouragement during this investigation.

I gratefully acknowledge the excellent technical assistance of my husband for the photography, Mr R. White and Mr R. Payne for analysis of results and statistical advice, Mrs L. Castle for the diagrams, Mr H. Jones and his staff for assistance with the field experiments, Miss N. Ross for the typing.

I also thank the Perry Foundation and the Frank Horne Trust for their financial support.

APPENDIX

Numbers of *P. herpotrichoides* spores on straw collected from
the field site on the Rothamsted Farm with mean weekly
temperature for 1982

<u>Week Ending</u>	<u>Weekly Temperature(°C)</u>	<u>Number of Conidia/ml</u>
9-2	6.0	4.8×10^6
16-2	6.8	8.1×10^6
23-2	1.9	8.1×10^6
2-3	2.4	4.7×10^6
9-3	6.3	1.5×10^6
16-3	5.2	5.5×10^5
23-3	4.8	8.1×10^5
30-3	7.2	4.0×10^5
13-4	7.5	4.5×10^5
20-4	6.8	2.2×10^5
29-4	10.4	4.1×10^5
4-5	9.3	5.7×10^4
11-5	6.7	1.3×10^5
18-5	13.9	3.6×10^4
25-5	13.3	1.7×10^5
8-6	19.6	1.3×10^5
16-6	13.7	1.4×10^5
22-6	15.2	6.1×10^4
29-6	15.0	1.9×10^4
6-7	15.3	9.2×10^4

Sporulation of *P. herpotrichoides* on straw at different temperatures

Sample	Number of conidia/ml				
	Temperature of incubation (°C)				
	5	10	15	20	25
1	1.0×10^6	3.4×10^5	2.3×10^5	9.3×10^4	3.1×10^4
2	2.8×10^6	1.1×10^6	7.1×10^5	1.5×10^5	2.6×10^4
3	7.0×10^5	4.9×10^5	4.8×10^4	3.8×10^4	7.0×10^3
4	1.8×10^6	1.5×10^5	1.7×10^5	5.3×10^4	6.0×10^3
5	1.9×10^5	4.8×10^4	1.7×10^4	7.0×10^3	4.0×10^3
6	1.5×10^5	7.6×10^4	7.8×10^4	7.0×10^4	1.5×10^4
7	3.8×10^6	2.5×10^6	4.2×10^5	2.5×10^5	2.3×10^5
8	4.1×10^6	5.4×10^5	3.7×10^5	1.1×10^5	8.8×10^4
9	1.1×10^6	2.0×10^5	1.8×10^5	7.9×10^4	3.8×10^4
10	4.4×10^6	1.6×10^6	2.2×10^5	9.8×10^4	6.5×10^4

Germination of conidia on the coleoptile and leaf blade
in the light and in the dark with time

	Mean percentage germination		
	<u>after time (h)</u>		
<u>Light treatment</u>	<u>16</u>	<u>40</u>	<u>56</u>
Coleoptile	44.0	61.2	83.2
Leaf (distal end)	25.5	49.7	52.2
Leaf (proximal end)	21.2	48.0	66.1
<u>Dark treatment</u>			
Coleoptile	32.8	66.9	84.2
Leaf (distal end)	21.6	49.0	61.7
Leaf (proximal end)	18.9	37.2	65.6

Standardisation of suspensions of *P. herpotrichoides*
conidia with their turbidity

Number of conidia <u>counted/ml</u>	<u>Turbidity</u>
4.2×10^4	1.5
4.2×10^5	4.5
4.2×10^6	19.5

Standardisation of soil percentage moisture content
for soil from the buckets and water potential

<u>Percentage moisture content of soil</u>	<u>Water potential (bars)</u>
10	-21.2
15	-10.6
20	- 5.3
25	- 2.1
30	0.0
35	0.0

Fungal penetration through leaf sheaths with temperature

Incubation time (wk)	Temperature of incubation (°C)	Temperature accumulated at sampling (T ₀ = 6°C)	Penetration score	Total no. of live sheaths penet- rated	Total no. of dead leaves
4	10 - 15	350	43.8	67	156
	5 - 10	21	26.2	101	30
8	10 - 15	700	62.6	28	336
	5 - 10	42	44.4	131	91
16	10 - 15	1400	70.4	4	526
	5 - 10	533	55.2	31	293

DISEASE SEVERITY ASSESSMENTS 1982

DATE	GROWTH STAGE	DISEASE HIGH S.R*	SEVERITY LOW S.R*	% INFECTION	
				HIGH S.R*	LOW S.R*
11-2	13.6	66.6		100	
18-2	13.9	63.3		100	
25-2	14.8	63.3		100	
4-3	14.8	46.6		100	
11-3	15-2	60.0		100	
18-3	15-2	60.0		90	
24-3	14.9	73.3		100	
2-4	16.3	76.6		100	
8-4	15.7	76.6		90	
15-4	17.1	63.3		90	
22-4	15.9	20.0	25.3	52	60
29-4	20.1	32.0	24.0	64	64
6-5	25.7	34.6	26.6	80	60
13-5	31.8	37.3	34.6	76	72
20-5	32.8	30.6	29.3	68	80
27-5	36.6	29.3	29.3	72	72
3-6	52.6	44.0	28.0	80	68
10-6	64.8	50.6	41.3	88	84
17-6	64.9	42.6	28.0	80	64
24-6	72.8	48.0	34.6	80	76
1-7	75.1	42.6	45.3	92	76
8-7	79.5	40.0	33.3	76	72
15-7	83.8	57.3	48.0	88	84
22-7	89.2	64.0	45.3	96	88
29-7	91.8	70.6	45.3	100	88
5-8	92.5	78.6	65.3	100	100

*SR = Seed Rate

DISEASE SEVERITY ASSESSMENTS 1983

DATE	GROWTH STAGE	PENETRATION		DISEASE SEVERITY		% INFECTION	
		HIGH S.R*	LOW S.R*	HIGH S.R*	LOW S.R*	HIGH S.R*	LOW S.R*
5-1	11.2					12.0	12.0
18-1	12.3	2.7	2.7	13.1	14.5	22.9	28.0
25-1	12.7	7.6	5.5	21.5	22.9	41.6	35.4
1-2	14.3	6.2	5.0	23.6	20.1	33.3	31.2
8-2	15.1	8.3	-	31.9	-	45.8	
23-2	15.1	7.9	7.6	28.4	28.4	37.5	39.5
2-3	16.8	20.1	9.7	33.3	34.7	39.5	45.8
8-3	18.0	21.5	15.6	36.8	42.1	45.8	50.0
15-3	18.4	16.6	14.2	38.8	29.8	43.7	31.2
22-3	19.7	23.6	25.0	59.7	38.1	62.5	72.9
29-3	19.7	18.6	22.0	52.7	53.4	56.2	54.2
6-4	19.7	21.5	15.6	47.9	40.2	47.9	41.7
13-4	20.8	18.4	19.0	44.4	48.6	47.9	52.0
20-4	20.8	21.5	21.5	49.4	56.1	50.0	47.9
26-4	20.8	24.3	16.6	49.3	31.2	50.0	33.3
3-5	29.2	21.8	19.7	38.8	37.5	39.5	37.3
10-5	30.3	15.6	18.4	34.0	34.0	37.5	35.4
17-5	34.1	25.0	12.1	13.8	6.2	41.6	18.7
7-6	41.7			8.3	2.0	20.8	6.2
14-6	47.1			10.4	7.6	29.1	22.9
21-6	58.3			6.9	3.4	20.8	10.4
27-6	62.5			9.7	5.0	20.8	16.6
5-7	68.6			16.3	8.3	27.0	16.6
12-7	73.3			11.8	8.3	27.0	22.9
19-7	77.8			18.0	13.1	43.7	33.3
26-7	80.8			14.5	14.5	35.4	31.2
3-8	87.0			13.8	11.1	35.4	29.1
9-8	90.6			11.8	8.3	31.2	22.9

* SR = Seed Rate (100 or 300kg/ha)

DISEASE SEVERITY ASSESSMENTS 1984

DATE	GROWTH STAGE	PENETRATION		EYESPOT SEVERITY		% INFECTION	
		HIGH SR*	LOW SR*	HIGH SR*	LOW SR*	HIGH SR*	LOW SR*
9-1	18.3	30.8	37.1	71.5	67.6	79.0	70.8
16-1	19.2	40.9	49.3	76.3	82.6	85.4	72.9
27-1	19.2	43.4	45.1	70.8	84.0	72.9	85.4
30-1	19.0	45.8	46.8	83.3	83.3	85.4	83.3
13-2	19.0	53.4	45.1	74.3	77.0	75.0	77.0
21-2	19.4	62.1	50.6	95.1	80.5	97.9	83.3
28-2	30.0	50.7	44.4	85.4	86.1	85.4	89.6
6-3	30.0	41.3	41.3	83.3	80.5	83.3	85.4
13-3	30.0	43.0	43.7	72.9	70.8	72.9	70.8
20-3	30.0	57.9	55.9	81.2	81.2	81.2	81.2
27-3	30.0	57.2	58.3	81.2	79.1	81.2	79.1
2-4	30.0	60.4	60.4	89.5	89.5	89.5	89.5
9-4	30.0	65.9	65.2	86.8	85.4	89.5	85.0
16-4	30.9	58.6	63.5	33.3	28.5	77.0	70.8
24-4	31.7	56.2	54.8	20.8	20.1	52.0	54.1
30-4	38.5			13.1	12.5	35.4	35.4
8-5	40.4			14.5	16.6	29.1	29.1
16-5	40.4			8.3	16.6	20.8	35.4
22-5	41.3			6.2	13.8	14.5	27.0
30-5	41.4			15.2	20.8	35.4	41.8
5-6	56.4			18.7	9.0	43.7	20.8
12-6	65.3			11.8	29.8	29.1	62.5
19-6	69.0			22.9	22.9	56.2	56.2
26-6	72.4			20.8	22.2	43.7	50.0
3-7	77.5			25.7	26.3	54.2	60.4
10-7	79.4			20.1	25.7	43.7	60.4
17-7	86.3			33.3	40.3	62.5	79.2
24-7	91.9			27.7	25.0	58.3	58.3

* SR = Seed Rate (100 or 300 kg/ha)

WEATHER DATA 1981-2

DATE	TEMPERATURE (°C)					HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET BULB <i>DEPRESSION</i>	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS %
27-1	3.9	0.9	6.1	0.4	0.0	94	0.0			
28-1	8.3	-2.0	1.7	0.7	0.5	88	0.7			
29-1	10.9	2.8	3.8	0.7	2.2	88	0.0			
30-1	9.4	7.5	8.1	0.6	4.3	91	0.0			
31-1	10.4	6.8	8.8	1.2	6.7	85	0.0	0.7	5.7	84.5
1-2	7.5	6.0	7.0	0.7	7.4	91	0.0			
2-2	7.7	5.0	6.8	0.5	8.0	93	0.0			
3-2	6.2	-0.4	4.8	0.9	8.0	86	0.0			
4-2	9.8	0.2	2.8	0.4	9.0	92	0.0			
5-2	11.1	1.2	4.5	0.4	10.3	94	2.4			
6-2	10.7	6.7	8.3	0.5	12.9	93	0.6			
7-2	7.4	3.0	7.4	0.1	13.2	97	6.7	9.7	6.0	78.9
8-2	9.2	0.4	4.8	-	14.0	96	0.1			
9-2	12.2	1.5	6.3	0.9	16.0	88	0.0			
10-2	10.4	4.1	8.5	0.7	17.7	91	0.0			
11-2	9.9	4.7	6.7	0.7	19.3	89	3.9			
12-2	9.9	1.1	7.2	0.4	20.3	95	9.8			
13-2	10.1	4.8	6.1	0.5	22.0	96	0.1			
14-2	9.1	-0.7	8.4	0.7	22.0	91	0.6	14.5	6.8	78.5

WEATHER DATA 1981-2

DATE	TEMPERATURE (°C)					HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T _o =6°C)				TEMP (°C)	LEAF WETNESS (%)
15-2	4.4	1.1	2.7	-	22.0	91	0.0			
16-2	2.4	1.6	2.0	-	22.0	80	0.3			
17-2	2.6	0.5	1.5	-	22.0	81	2.5			
18-2	4.6	0.0	2.3	-	22.0	100	0.1			
19-2	3.5	2.2	2.9	-	22.0	98	0.0			
20-2	1.4	-1.4	0.0	-	22.0	74	0.0			
21-2	5.7	-1.0	2.3	-	22.0	80	0.0	2.9	1.9	78.8
22-2	5.4	-3.5	0.9	-	22.0	81	0.0			
23-2	3.6	-3.6	0.0	-	22.0	89	0.0			
24-2	1.4	-4.7	1.6	-	22.0	90	0.1			
25-2	3.9	-2.9	0.5	-	22.0	100	0.3			
26-2	5.7	1.2	3.4	-	22.0	81	1.3			
27-2	9.5	2.4	5.9	-	22.1	97	0.3			
28-2	11.0	4.3	7.6	-	25.0	100	2.6	4.6	2.4	76.8
1-3	9.1	6.9	9.2	0.2	33.0	97	4.3			
2-3	9.9	2.2	7.9	0.8	33.0	90	4.1			
3-3	9.5	6.0	6.2	1.1	34.7	84	0.7			
4-3	8.5	3.1	7.5	1.3	35.0	83	1.6			
5-3	10.1	-1.0	4.5	0.9	36.4	87	0.1			
6-3	8.1	0.5	3.5	0.4	36.9	94	2.2			
7-3	7.8	3.0	5.4	-	37.3	65	0.0	13.0	6.3	54.5

WEATHER DATA 1981-2

DATE	MAX	MIN	TEMPERATURE (°C)			HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
			DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS (%)
8-3	8.4	-3.2	2.6	-	37.9	71	1.1			
9-3	9.4	0.0	4.7	-	38.8	79	16.0			
10-3	11.4	1.1	6.2	-	40.3	100	2.3			
11-3	8.3	1.2	4.7	-	40.8	69	9.5			
12-3	7.6	3.1	5.4	-	41.2	90	0.0			
13-3	9.2	0.8	5.0	-	42.0	68	0.0			
14-3	10.5	1.7	7.6	1.2	43.2	84	1.1	30.0	5.2	40.8
15-3	7.5	6.9	5.9	0.5	50.4	93	19.5			
16-3	7.4	0.4	3.4	0.6	50.8	90	2.9			
17-3	8.4	0.2	5.8	1.1	51.4	85	5.5			
18-3	8.8	2.0	5.4	-	52.1	84	2.0			
19-3	7.1	0.5	3.8	-	52.3	96	3.7			
20-3	6.5	3.2	4.8	-	52.4	86	0.6			
21-3	7.4	1.5	4.5	0.6	52.8	91	0.6	34.8	4.8	82.0
22-3	11.7	3.6	7.6	1.6	55.0	89	0.0			
23-3	11.3	0.5	6.9	1.9	56.4	75	0.0			
24-3	12.5	-0.3	6.1	-	58.2	84	0.0			
25-3	12.5	-1.2	7.2	-	61.1	75	0.0			
26-3	16.2	-0.4	7.9	-	64.6	78	0.0			
27-3	16.9	-0.7	8.1	-	68.4	69	0.0			
28-3	12.0	1.4	6.7	-	70.3	97	0.0	0.0	7.2	58.6

WEATHER DATA 1981-2

DATE	TEMPERATURE (°C)					HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS(%)
29-3	7.5	3.2	4.9	0.7	70.6	88	2.0			
30-3	7.5	2.1	4.7	0.8	71.0	87	0.4			
31-3	9.0	2.0	5.1	1.6	71.7	77	3.0			
1-4	11.5	1.9	6.1	1.1	73.5	85	1.7			
2-4	10.9	3.0	6.7	0.5	75.2	92	0.0			
3-4	14.9	1.5	8.2	-	78.5	86	0.0			
4-4	16.1	3.8	9.9	-	83.0	76	0.0	7.1	6.5	52.6
5-4	17.6	5.4	11.5	-	83.6	64	3.9			
6-4	10.7	8.4	9.5	-	98.2	90	7.9			
7-4	14.6	7.7	11.1	-	109.3	94	4.0			
8-4	7.5	2.5	5.0	-	109.7	81	0.0			
9-4	8.0	-0.9	3.6	-	110.2	96	0.0			
10-4	10.5	4.3	7.4	-	112.0	81	0.0			
11-4	7.0	1.4	4.2	-	112.3	70	0.0	15.8	7.5	44.5
12-4	7.9	0.9	4.3	-	112.7	69	0.0			
13-4	8.7	1.1	3.8	-	113.4	74	0.0			
14-4	13.2	0.8	7.0	-	115.7	57	0.0			
15-4	15.1	0.1	7.5	-	118.7	85	0.0			
16-4	15.1	2.9	9.0	-	122.5	69	0.0			
17-4	12.8	3.2	8.0	-	125.2	69	0.0			
18-4	12.0	3.5	7.7	-	127.6	67	8.6	8.6	6.8	28.4

WEATHER DATA 1981-2

DATE	TEMPERATURE (°C)					HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T _O =6°C)				TEMP (°C)	LEAF WETNESS (%)
19-4	13.2	1.3	7.2	-	130.0	72	9.7			
20-4	15.1	-0.5	11.5	2.9	132.9	67	9.3			
21-4	15.4	7.1	11.2	2.8	144.1	69	10.0			
22-4	15.9	5.2	10.6	1.9	148.9	77	4.9			
23-4	12.9	6.9	11.1	3.7	158.8	59	9.9			
24-4	12.4	5.6	9.7	1.9	161.9	77	0.8			
25-4	18.6	7.1	11.5	2.1	174.8	76	0.0	44.6	10.4	29.6
26-4	13.5	5.3	9.2	1.6	178.3	81	0.0			
27-4	18.1	5.3	10.7	2.3	184.2	74	0.0			
28-4	18.4	4.7	11.4	2.9	190.1	68	0.0			
29-4	12.8	5.0	8.2	1.6	193.2	80	3.5			
30-4	14.2	3.7	8.8	1.4	193.2	83	0.6			
1-5	11.4	2.8	8.4	2.4	198.6	70	0.5			
2-5	12.1	2.7	7.9	2.8	200.8	65	2.0	6.6	9.3	46.6
3-5	12.4	4.2	6.6	1.3	203.8	82	1.0			
4-5	10.8	0.2	5.5	-	204.8	68	0.1			
5-5	10.6	-2.5	4.0	-	205.9	64	12.7			
6-5	7.9	1.7	4.8	-	206.4	84	0.1			
7-5	10.3	0.8	8.4	1.8	207.5	78	0.0			
8-5	12.4	1.3	7.9	1.9	209.5	76	0.0			
9-5	17.4	1.5	10.0	2.9	214.1	66	0.0	6.7	6.7	50.8

WEATHER DATA 1981-2

DATE	MAX	MIN	TEMPERATURE (°C)			HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
			DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS (%)
10-5	17.2	5.9	10.2	2.9	219.6	66	0.0			
11-5	19.6	1.7	11.2	3.9	225.4	57	0.0			
12-5	21.3	6.5	13.5	3.8	239.3	60	0.0			
13-5	24.1	7.2	14.2	4.6	254.9	55	0.0			
14-5	24.3	5.6	13.8	4.3	264.0	57	0.0			
15-5	26.8	7.7	17.0	4.1	281.2	63	0.0			
16-5	21.3	7.4	16.8	3.4	295.5	69	0.0	0.0	13.9	26.1
17-5	17.6	7.6	11.6	1.1	308.1	86	1.8			
18-5	18.3	5.3	13.5	3.1	315.1	68	0.0			
19-5	16.7	5.8	13.4	2.3	319.4	76	1.7			
20-5	14.3	0.6	16.6	0.6	333.3	94	0.0			
21-5	17.9	11.6	15.4	1.3	348.0	87	3.4			
22-5	16.6	8.0	13.4	0.5	360.3	95	4.0			
23-5	14.4	8.6	11.6	0.5	371.8	95	6.2	17.1	13.3	48.5
24-5	16.3	7.6	15.4	1.6	383.8	83	0.0			
25-5	18.3	12.0	15.1	1.9	398.9	81	0.0			
26-5	22.0	7.1	16.2	1.8	413.5	82	27.6			
27-5	15.0	10.3	11.9	0.1	426.1	86	0.4			
28-5	18.1	6.5	13.9	1.4	438.4	84	0.0			
29-5	22.0	6.0	15.8	2.1	452.5	80	0.0			
30-5	23.0	8.5	15.3	2.1	468.2	79	0.0	28.0	14.8	42.7

WEATHER DATA 1981-2

DATE			TEMPERATURE (°C)			HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS (%)
31-5	25.9	9.9	17.7	1.5	486.1	86	0.2			
1-6	28.8	15.5	20.8	2.9	508.2	75	0.0			
2-6	28.8	12.5	17.9	1.3	528.9	88	8.7			
3-6	24.8	13.5	19.3	0.9	548.0	91	2.5			
4-6	28.0	14.6	21.0	0.9	569.4	92	9.8			
5-6	27.4	14.3	20.5	0.8	590.2	93	7.5			
6-6	27.3	16.7	20.2	0.7	612.2	94	1.1	29.8	19.6	61.6
7-6	25.6	15.3	19.4	1.1	632.6	90	0.0			
8-6	26.0	15.7	19.3	1.3	653.5	89	0.0			
9-6	24.7	13.7	18.3	1.5	672.7	86	0.0			
10-6	21.4	12.0	16.4	1.8	689.4	83	3.8			
11-6	20.8	13.2	16.0	1.2	706.4	88	4.8			
12-6	18.3	11.2	13.6	0.3	721.1	96	14.8			
13-6	15.0	9.8	12.3	1.1	733.5	88	0.0	23.4	13.7	35.8
14-6	20.1	10.1	16.5	2.3	748.6	77	0.0			
15-6	18.5	9.1	13.5	1.2	762.5	87	0.6			
16-6	21.5	10.8	15.8	1.4	778.6	86	0.0			
17-6	22.8	11.4	16.1	1.6	795.7	84	6.8			
18-6	18.6	11.7	14.0	0.5	810.8	95	2.9			
19-6	17.6	11.4	14.2	0.2	825.3	98	6.7			
20-6	24.1	9.3	16.2	1.4	842.0	86	0.0	17.0	15.2	84.5

WEATHER DATA 1981-2

DATE	TEMPERATURE (°C)					HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T ₀ = 6°C)				TEMP (°C)	LEAF WETNESS (%)
21-6	17.2	12.6	14.6	0.3	856.9	97	0.5			
22-6	18.5	12.2	15.0	0.0	872.3	99	5.8			
23-6	17.9	11.5	14.6	0.1	887.0	99	3.6			
24-6	21.5	13.2	16.2	0.6	904.3	93	0.6			
25-6	16.6	12.5	14.7	0.2	918.9	97	13.1			
26-6	17.3	11.6	14.2	0.1	933.3	100	14.3			
27-6	21.3	11.4	15.6	1.2	949.7	89	1.7	39.6	15.0	76.6
28-6	18.8	12.1	15.1	0.9	965.1	91	2.9			
29-6	21.7	11.0	15.4	1.7	981.5	83	0.0			
30-6	21.6	9.1	15.4	1.5	997.2	84	2.1			
1-7	17.3	12.4	14.7	0.3	1021.1	98	0.5			
2-7	22.2	13.0	16.8	1.2	1029.7	89	0.6			
3-7	22.7	11.2	15.7	1.6	1046.6	84	1.9			
4-7	17.3	8.8	13.5	0.9	1059.7	90	0.0	8.0	15.2	31.7

WEATHER DATA 1983

DATE	TEMPERATURE (°C)					HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T _o =6°C)				TEMP (°C)	LEAF WETNESS (%)
31-1	7.4	-2.6	6.8	0.0	10.9	100	15.4			
1-2	7.4	-2.6	2.7	0.0	10.9	100	0.7			
2-2	7.4	-2.6	3.1	0.0	10.9	100	0.1			
3-2	3.9	0.6	1.9	0.5	10.9	92	0.0			
4-2	7.8	-6.0	3.4	0.1	11.3	98	6.5			
5-2	4.6	-3.4	3.1	1.0	11.3	84	5.8			
6-2	3.8	2.1	3.4	0.4	11.3	94	2.6			
7-2	2.7	0.3	0.6	0.2	11.3		1.2	16.9	3.1	33.0
8-2	2.0	-0.8	-0.1	-0.2	11.3		0.3			
9-2	0.0	-2.5	-1.7	-0.2	11.3		0.0			
10-2	0.4	-4.5	-1.9	0.0	11.3	100	13.3			
11-2	1.4	-2.2	0.2	-0.2	11.3		1.3			
12-2	1.6	-1.6	0.2	-0.4	11.3		0.0			
13-2	0.5	-3.1	-1.6	-0.5	11.3		0.0			
14-2	1.1	-4.4	-2.0	-0.5	11.3	100	0.0	14.9	-0.9	40.0

WEATHER DATA 1983

DATE	TEMPERATURE (°C)					HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS (%)
15-2	0.8	-2.5	-0.4	-1.1	11.3		0.0			
16-2	0.1	-1.6	-1.2	-1.0	11.3		0.0			
17-2	-2.6	-2.6	-2.6	0.0	11.3		0.0			
18-2	7.4	-2.6	-0.6	0.0	11.3		0.0			
19-2	-2.6	-2.6	-2.6	0.0	11.3		0.0			
20-2	-2.6	-2.6	-2.6	0.0	11.3		0.0			
21-2	2.4	-2.9	-0.1	1.0	11.3		0.0	0.0	-1.4	44.3
22-2	2.1	-3.8	-1.1	0.4	11.3		0.0			
23-2	3.6	0.2	1.5	1.0	11.3	80	0.0			
24-2	4.3	2.2	3.7	0.0	11.3	100	0.4			
25-2	6.2	3.8	4.5	0.0	11.3	100	2.9			
26-2	10.9	3.9	7.2	0.7	13.1	91	1.2			
27-2	7.8	2.1	5.2	0.8	13.6	88	6.3			
28-2	6.7	0.2	4.2	1.5	13.8	78	0.2	11.0	4.2	

WEATHER DATA 1983

DATE	MAX	MIN	TEMPERATURE (°C)			HUMID- ITY %	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
			DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS (%)
1-3	7.1	2.6	4.2	0.6	13.9	91	0.5			
2-3	5.9	4.3	5.1	0.4	13.9	94	0.5			
3-3	9.6	1.2	4.6	0.7	15.0	90	0.0			
4-3	6.3	-0.1	2.3	0.5	15.0	92	1.1			
5-3	9.5	4.6	7.0	1.2	16.0	84	0.0			
6-3	10.2	1.9	6.4	1.1	17.4	85	0.0			
7-3	10.1	-0.5	4.4	0.7	18.5	90	0.0	2.1	4.8	
8-3	12.6	2.1	6.6	0.8	20.1	89	0.0			
9-3	9.1	0.1	3.7	0.6	20.7	91	0.0			
10-3	13.9	1.8	10.1	1.0	23.2	88	0.0			
11-3	10.5	4.0	6.4	0.7	24.2	90	0.0			
12-3	11.0	4.5	7.1	1.0	26.0	87	0.0			
13-3	13.3	7.7	9.5	0.9	29.1	89	1.6			
14-3	10.7	-0.7	4.6	0.6	37.9	91	0.6	2.2	6.8	

WEATHER DATA 1983

DATE	TEMPERATURE (°C)					HUMID- ITY %	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS(%)
15-3	10.8	-0.4	4.6	1.1	39.1	84	0.0			
16-3	11.4	6.8	8.8	0.3	40.2	96	1.4			
17-3	15.2	9.0	10.8	0.7	44.4	92	0.3			
18-3	12.8	9.2	10.7	0.7	55.0	92	1.9			
19-3	11.9	4.1	7.8	0.8	65.6	90	0.1			
20-3	10.7	2.2	5.6	0.6	66.3	91	4.7			
21-3	8.4	1.6	3.6	1.4	66.9	79	6.7	15.1	7.4	29.9
22-3	9.0	2.4	5.1	1.6	67.4	77	2.8			
23-3	9.9	3.4	6.4	0.7	68.3	90	5.6			
24-3	5.2	-0.4	2.0	1.0	68.3	84	0.7			
25-3	6.9	0.6	2.9	0.6	68.6	91	3.7			
26-3	7.0	3.4	4.7	0.0	68.6	100	3.3			
27-3	7.7	-0.6	2.8	0.0	69.0	100	0.4			
28-3	8.0	0.4	4.0	0.0	69.3	100	0.0	16.5	3.3	27.7

WEATHER DATA 1983

DATE	MAX	MIN	TEMPERATURE (°C)			HUMID- ITY (%)	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
			DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS (%)
29-3	9.0	2.6	5.8	0.0	69.9	100	3.1			
30-3	8.7	1.8	5.2	0.0	70.6	100	1.2			
31-3	9.7	1.3	3.1	0.0	71.4	100	4.1			
1-4	6.3	2.2	3.9	0.0	71.4	100	6.2			
2-4	7.4	-1.3	2.7	0.0	71.7	100	1.8			
3-4	7.9	0.3	3.5	0.0	72.1	100	7.8			
4-4	9.1	0.0	4.0	0.0	72.9	100	0.6	24.8	4.0	
5-4	8.4	0.2	5.0	0.8	73.5	88	8.7			
6-4	10.6	1.8	4.6	0.0	74.2	100	0.8			
7-4	10.3	0.2	5.6	1.3	75.2	82	1.9			
8-4	10.6	3.6	6.9	2.2	76.7	71	0.0			
9-4	13.1	1.4	6.4	0.0	78.1	100	6.5			
10-4	12.6	5.1	7.8	0.0	81.5	100	11.5			
11-4	6.7	2.2	4.9	0.0	81.8	100	6.8	36.4	5.8	
12-4	7.7	-0.3	3.6	0.0	82.9	100	0.0			
13-4	10.6	-3.0	5.9	0.0	85.1	100	0.0			
14-4	10.7	2.4	6.7	0.0	89.5	100	0.0			
15-4	18.0	2.4	9.9	0.0	94.3	100	0.0			
16-4	18.7	1.9	10.8	0.0	102.1	100	0.2			
17-4	9.6	4.9	7.3	0.0	102.1	100	11.2			
18-4	4.7	1.4	3.2	0.0	102.1	100	10.7	22.1	6.7	

WEATHER DATA 1983

DATE	TEMPERATURE (°C)					HUMID- ITY %	WEEKLY TOTAL	MEAN WEEKLY		
	MAX	MIN	DRY BULB	WET	ACCUM. (T _O = 6°C)			TEMP (°C)	LEAF WETNESS (%)	
19-4	6.5	2.8	4.1	0.0	103.0	100	0.2	19.2	7.4	
20-4	9.2	-1.0	4.6	0.0	105.5	100	5.6			
21-4	13.9	3.5	7.6	0.0	108.6	100	0.3			
22-4	13.4	3.8	8.5	0.7	118.5	91	2.5			
23-4	14.0	5.2	9.2	1.1	118.5	86	1.5			
24-4	14.6	1.1	8.7	1.8	121.5	78	9.1			
25-4	13.5	6.4	9.5	1.4	131.6	83	0.0			
26-4	15.0	4.4	12.1	2.4	135.7	74	5.4			
27-4	14.7	7.3	10.1	1.1	145.9	87	0.0	28.4	10.0	85.6
28-4	15.7	7.0	10.7	1.7	156.2	80	0.0			
29-4	14.8	3.9	9.5	1.4	160.0	83	8.3			
30-4	15.8	5.3	10.6	1.6	163.7	81	10.1			
1-5	12.6	6.7	9.5	0.3	173.4	96	4.5			
2-5	9.5	6.3	7.7	0.8	175.1	90	0.1			
3-5	11.0	6.2	7.9	1.3	183.7	83	0.2			
4-5	13.3	4.0	10.5	2.1	186.8	76	0.0			
5-5	16.7	6.9	12.1	1.7	198.6	81	3.7	12.1	10.6	72.6
6-5	16.9	10.6	13.4	0.8	212.3	91	0.4			
7-5	16.2	9.6	11.6	1.0	224.7	89	2.3			
8-5	14.6	7.9	10.8	0.6	236.4	93	3.6			
9-5	13.9	3.6	8.4	1.3	239.5	84	1.9			

WEATHER DATA 1983

DATE			TEMPERATURE (°C)			HUMID- ITY %	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS(%)
10-5					241.7	90	2.8			
11-5	13.2	6.5	10.6	1.9	244.2	78	5.2			
12-5	12.6	6.2	9.0	0.9	253.6	89	5.4			
13-5	15.0	6.8	10.0	1.2	264.3	85	1.2			
14-5	17.3	5.9	11.3	0.0	269.0	80	0.0			
15-5	15.1	5.1	9.0	0.7	272.8	91	5.9			
16-5	16.6	4.6	14.4	2.8	276.6	72	3.1	23.6	10.4	93.8
17-5	13.4	7.7	12.0	3.0	287.1	68	2.9			
18-5	15.7	6.6	10.3	0.4	297.7	95	6.5			
19-5	15.1	6.6	10.3	0.6	307.7	93	0.6			
20-5	15.9	4.9	10.2	0.7	310.9	92	7.1			
21-5	16.7	6.3	10.1	0.5	314.6	94	1.2			
22-5	15.6	5.6	10.6	1.1	318.5	87	0.0			
23-5	17.4	5.6	11.5	1.2	322.4	86	0.0	18.3	10.7	56.9
24-5	17.0	5.4	11.3	2.8	334.3	69	0.0			
25-5	12.6	6.0	9.0	2.1	339.6	74	0.0			
26-5	13.1	4.3	9.5	2.5	348.9	70	0.0			
27-5	16.5	7.1	11.6	1.4	351.0	84	7.5			
28-5	9.5	7.0	8.2	0.2	359.1	97	2.8			
29-5	15.0	5.9	10.2	0.5	368.9	94	0.2			
30-5	18.6	6.0	12.1	0.8	374.0	91	0.0	10.5	10.2	52.7

WEATHER DATA 1983

DATE			TEMPERATURE (°C)			HUMID- ITY	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET	ACCUM. (T _O =6 C)				TEMP (°C)	LEAF WETNESS (%)
31-5	21.6	8.6	15.0	1.0	338.3	90	24.1			
1-6	21.6	11.6	16.1	0.7	404.5	93	0.2			
2-6	18.4	10.6	12.9	0.5	417.9	95	4.7			
3-6	18.6	10.3	14.6	0.5	428.7	95	1.4			
4-6	20.0	11.9	16.3	2.7	444.6	74	0.2			
5-6	22.5	12.2	16.0	1.1	459.9	89	0.0			
6-6	22.5	9.2	15.4	0.9	473.1	91	0.0	30.6	15.1	
7-6	24.0	10.7	17.1	1.1	489.6	89	0.0			
8-6	23.3	15.1	19.4	2.3	508.8		0.0			
9-6	18.9	10.6	16.4	2.7	523.5	74	0.0			
10-6	18.9	8.1	16.1	3.5	537.0	67	0.0			
11-6	18.4	11.4	13.9	1.0	551.4	90	0.5			
12-6	21.4	8.0	14.3	1.9	565.8	81	0.0			
13-6	19.9	6.3	12.7	1.8	571.3	81	0.4	0.9	15.7	25.2
14-6	19.0	10.2	14.4	2.6	584.3	74	0.2			
15-6	15.5	6.4	12.5	2.8	595.7	70	0.0			
16-6	16.0	4.7	12.4	2.4	606.0	74	0.0			
17-6	18.5	12.5	15.6	1.1	619.1	89	0.0			
18-6	23.6	9.3	14.5	1.4	633.1	86	0.0			
19-6	25.8	7.2	15.4	2.5	646.7	76	0.0			
20-6	25.9	9.5	16.7	1.7	663.0	84	0.0	0.2	14.5	19.9

WEATHER DATA 1983

DATE	TEMPERATURE (°C)		HUMID-		WEEKLY TOTAL	MEAN WEEKLY TEMP (°C)	WEEKLY LEAF WETNESS (%)
	MAX	MIN	DRY BULB	WET BULB	ACCUM. (T _o =6°C) %	RAIN (mm)	
21-6	25.4	10.6	16.8	1.5	678.6	86	0.0
22-6	20.2	9.1	10.9	0.1	693.2	99	2.1
23-6	19.6	10.6	15.9	0.7	708.3	93	0.0
24-6	15.3	12.1	15.3	0.2	721.0	98	0.0
25-6	25.8	11.4	16.6	1.5	736.5	86	0.0
26-6	23.7	10.1	16.4	1.7	751.9	84	0.0
27-6	14.8	10.3	12.7	1.6	764.0	83	0.0
						2.1	14.9
							49.1
28-6	18.8	5.7	12.3	0.5	776.7	94	0.0
29-6	20.2	12.6	15.9	1.4	793.1	86	16.3
30-6	18.5	11.8	15.0	1.3	807.1	87	0.0
1-7	20.5	7.1	13.7	1.6	819.8	83	0.1
2-7	24.9	12.5	17.5	1.9	837.1	83	0.0
3-7	24.1	7.6	18.5	4.0	852.9	65	0.0
4-7	25.7	12.0	21.4	5.3	871.7	58	0.0
						16.4	16.3
							40.9
5-7	26.1	11.8	22.4	5.5	890.6	58	0.0
6-7	24.9	14.6	17.9	1.3	910.3	88	0.4
7-7	23.7	13.1	18.1	0.3	928.7	97	20.6
8-7	25.5	14.9	22.6	1.9	948.9	84	0.0
9-7	25.4	13.6	20.8	1.3	968.4	89	0.0
10-7	24.2	13.9	20.0	1.4	987.4	88	0.0
11-7	28.5	13.6	21.9	2.1	1008.4	83	0.0
						21.0	20.5

WEATHER DATA 1983

DATE	MAX	MIN	TEMPERATURE (°C)			HUMID- ITY %	RAIN (mm)	WEEKLY TOTAL	MEAN WEEKLY	
			DRY BULB	WET	ACCUM. (T ₀ =6°C)				TEMP (°C)	LEAF WETNESS (%)
12-7	27.2	14.5	21.9	2.6	1029.2	79	0.0			
13-7	28.1	13.6	22.3	2.6	1050.0	79	0.0			
14-7	29.5	13.6	23.1	2.9	1071.5	77	0.0			
15-7	30.7	16.0	24.4	3.8	1094.8	71	0.0			
16-7	35.8	15.6	24.4	3.8	1117.6	71	0.0			
17-7	35.9	16.6	23.6	3.2	1139.6	75	0.0			
18-7	30.4	17.5	22.3	3.2	1160.5	74	0.0	0.0	23.1	
19-7	29.0	15.1	20.6	2.7	1179.4	77	0.0			
20-7	23.5	14.1	18.4	2.6	1195.8	77	0.0			
21-7	30.8	10.0	18.7	4.4	1210.9	62	0.0			
22-7	32.0	12.2	20.2	4.1	1229.8	66	2.8			
23-7	24.5	15.7	19.0	0.8	1248.7	94	1.0			
24-7	24.1	14.2	19.0	1.3	1266.3	88	0.6			
25-7	29.2	16.4	20.5	2.0	1285.7	83	0.0	4.4	19.4	
26-7	30.7	14.6	21.4	2.4	1306.0	80	0.0			
27-7	30.1	14.6	21.4	2.4	1326.0	79	0.0			
28-7	30.9	15.4	21.5	4.4	1345.1	65	0.0			
29-7	35.0	14.1	22.6	4.7	1367.2	64	0.0			
30-7	35.8	15.6	23.6	4.3	1389.2	67	0.0			
31-7	31.0	14.9	20.9	1.7	1404.9	85	9.6			
1-8	20.9	10.9	15.7	1.8	1435.7	82	0.0	9.6	24.9	

WEATHER DATA 1984

DATE	MAX	MIN	TEMPERATURE (°C)			HUMID- ITY %	RAIN (mm)	COLLECTED WEEKLY TOTAL	MEAN WEEKLY	
			DRY BULB	WET BULB	ACCUM. (T ₀ = 6°C) <i>DEPRESSION</i>				TEMP (°C)	LEAF WETNESS(h)
31-1	6.0	0.1	1.3	0.5	1.6	92	0.3	20.1	2.8	18.1
1-2	8.1	0.2	1.5	0.3	2.1	95	4.1			
2-2	9.1	1.1	4.9	1.4	2.9	80	1.7			
3-2	11.7	4.0	9.1	0.3	5.2	96	4.1			
4-2	10.6	2.0	5.4	0.0	6.5	100	2.2			
5-2	9.0	1.6	2.7	1.7	7.3	73	1.5			
6-2	9.6	2.2	9.0	1.5	8.2	82	4.9			
7-2	7.6	0.7	3.1	1.0	8.6	84	7.1	25.6	5.1	
8-2	6.5	2.6	5.4	1.4	8.7	20	0.0			
9-2	6.8	1.5	2.4	1.0	8.9	84	0.0			
10-2	8.0	-0.6	2.0	0.6	9.4	90	0.0			
11-2	7.5	1.7	6.5	0.4	9.8	95	0.0			
12-2	6.4	3.7	4.2	0.4	9.9	94	0.0			
13-2	5.2	-4.2	-0.9	-0.5	9.9	100	0.0			
14-2	6.8	-3.1	1.4	1.0	10.1	73	0.0	0.0	3.0	7.6

WEATHER DATA 1984

DATE			TEMPERATURE (°C)			HUMID- ITY %	RAIN (mm)	COLLECTED WEEKLY TOTAL	MEAN WEEKLY TEMP	LEAF WETNESS (h)
	MAX	MIN	DRY BULB	WET	ACCUM. (T _o =6°C)					
15-2	0.6	-3.9	-2.1	0.0	10.1	89	0.0			
16-2	2.7	-1.9	-0.1	0.2	10.1	93	0.0			
17-2	6.8	-2.2	0.9	0.5	10.3	90	0.0			
18-2	6.3	-3.0	0.8	0.8	10.4	76	0.0			
19-2	2.8	-4.2	-1.4	0.8	10.4	83	0.0			
20-2	4.6	-3.9	0.0	0.8	10.4	84	4.1			
21-2	7.6	-2.6	4.9	0.9	10.8	87	5.8	5.7	0.4	18.4
22-2	4.6	3.6	4.6	0.2	10.8	97	5.6			
23-2	3.1	1.0	2.5	0.1	10.8	98	0.0			
24-2	3.5	0.5	1.1	0.6	10.8	100	0.0			
25-2	2.8	0.1	1.2	1.0	10.8	100	0.6			
26-2	2.6	-0.5	0.8	0.2	10.8	100	1.7			
27-2	3.2	0.5	2.6	0.0	10.8	100	1.9			
28-2	2.9	1.9	2.4	0.1	10.8	98	1.1	6.2	2.1	
29-2	7.1	1.1	1.8	0.2	11.0	97	0.0			
1-3	11.2	1.6	7.1	0.8	12.6	89	1.4			
2-3	4.5	2.6	3.4	1.7	12.6	74	0.0			
3-3	6.4	0.9	2.9	1.7	12.6	74	0.1			
4-3	7.0	-1.0	2.6	0.7	12.9	89	1.7			
5-3	8.8	2.5	6.7	0.0	13.6	100	0.0			
6-3	12.1	4.6	6.7	0.1	16.3	99	0.0	5.7	4.4	

WEATHER DATA 1984

DATE			TEMPERATURE (°C)			HUMID- ITY %	RAIN (mm)	COLLECTED WEEKLY TOTAL	MEAN WEEKLY TEMP	LEAF WETNESS(h)
	MAX	MIN	DRY BULB	WET	ACCUM. (T ₀ =6°C)					
7-3	9.5	1.5	5.9	0.8	17.1	89	0.0			
8-3	5.5	0.0	2.5	0.9	17.1	86	0.0			
9-3	6.0	1.0	2.7	0.8	17.1	87	0.0			
10-3	6.6	1.2	3.8	0.7	17.3	89	4.9			
11-3	10.6	3.5	5.4	0.4	19.0	94	6.0			
12-3	7.7	0.9	4.3	0.4	19.4	94	0.0			
13-8	2.2	0.1	2.3	0.5	19.4	92	0.8	6.4	3.8	17.4
14-3	8.2	0.1	3.6	1.0	19.9	85	0.0			
15-3	4.2	0.1	3.0	0.5	19.9	92	0.0			
16-3	4.2	0.1	1.3	1.3	19.9		0.0			
17-3	4.5	0.1	2.4	1.5	19.9	76	0.0			
18-3	4.5	0.9	2.0	0.6	19.9	90	0.0			
19-3	5.9	-0.4	1.9	1.2	19.9	80	0.0			
20-3	9.9	-0.8	4.9	1.6	20.9	77	0.0	1.8	2.7	12.1
21-3	9.9	0.6	3.3	0.6	21.9	91	0.2			
22-3	11.1	-0.6	3.8	0.6	23.1	91	0.1			
23-3	11.5	-0.4	6.7	1.9	24.5	75	23.6			
24-3	6.3	0.1	5.2	0.1	24.6	99	7.9			
25-3	6.8	2.1	5.6	0.9	24.8	87	10.8			
26-3	7.5	4.7	6.0	0.5	25.2	93	0.2			
27-3	9.8	1.3	5.2	0.2	26.2	97	3.6	19.8	5.1	16.3

WEATHER DATA 1984

DATE	TEMPERATURE (°C)						HUMID- ITY	RAIN (mm)	COLLECTED WEEKLY TOTAL	MEAN WEEKLY TEMP	LEAF WETNESS(h)
	MAX	MIN	DRY BULB	WET	ACCUM. (T _O =6°C)						
28-3	7.1	1.3	4.1	0.0	26.4		100	0.0			
29-3	10.4	-0.2	4.8	0.7	27.5		90	0.0			
30-3	12.7	-1.7	3.8	0.6	29.2		91	1.9			
31-3	6.5	1.3	3.6	0.7	29.3		89	0.0			
1-4	6.5	-0.9	2.9	1.7	29.3		71	0.0			
2-4	9.1	-1.9	4.7	2.3	30.1		67	0.0			
3-4	9.5	-4.2	2.4	1.5	31.0		76	0.0	4.5	3.7	12.3
4-4	11.8	-2.7	2.9	1.6	32.4		75	0.0			
5-4	7.6	-3.1	2.6	1.0	32.8		84	1.2			
6-4	5.3	2.9	4.0	0.2	32.8		97	1.3			
7-4	8.6	3.7	5.7	0.6	33.5		91	0.0			
8-4	9.0	3.6	5.8	0.6	34.2		92	0.0			
9-4	9.7	3.9	5.4	1.0	35.1		86	0.4			
10-4	8.7	3.8	5.7	1.8	35.8		75	0.6	2.5	4.6	12.6
11-4	10.5	3.9	8.7	1.3	37.5		84	2.8			
12-4	11.5	0.4	6.0	2.2	38.9		70	0.0			
13-4	14.0	-0.2	8.5	2.8	41.3		66	0.0			
14-4	16.6	-0.9	4.7	3.5	44.9		59	0.0			
15-4	10.7	0.9	7.9	1.5	46.1		81	0.0			
16-4	10.5	1.1	7.0	2.3	47.2		70	0.2			
17-4	15.0	-1.8	6.4	2.0	49.7		73	0.0	4.2	7.7	13.1

WEATHER DATA 1984

DATE	TEMPERATURE (°C)		HUMID-		ACCUM. (T _O =6°C)	ITY %	RAIN (mm)	COLLECTED WEEKLY TOTAL	MEAN WEEKLY TEMP	LEAF WETNESS(h)
	MAX	MIN	DRY BULB	WET						
18-4	17.0	2.2	9.5	2.5	54.3	70	0.0			
19-4	19.7	5.7	11.4	3.2	61.1	65	0.0			
20-4	20.0	3.7	11.1	2.4	67.5	73	0.0			
21-4	23.4	1.9	12.5	2.7	75.2	71	0.0			
22-4	18.4	7.5	12.2	2.1	22.1	77	0.0			
23-4	18.9	3.6	10.5	3.1	94.0	65	0.0			
24-4	20.9	2.3	10.8	3.0	100.5	66	0.0	0.0	11.1	7.1
25-4	21.5	4.2	12.6	3.7	107.8	61	0.0			
26-4	21.8	4.3	12.3	3.7	115.2	61	0.0			
27-4	19.9	4.4	10.8	3.2	121.8	64	0.0			
28-4	18.7	1.8	10.1	3.3	127.1	62	0.0			
29-4	15.2	2.6	8.3	2.7	130.8	67	0.0			
30-4	15.2	2.5	12.1	5.5	134.6	43	0.0			
1-5	16.4	3.6	10.0	1.5	140.8	82	0.0	0.0	10.8	5.9
2-5	15.5	4.7	7.8	0.9	145.2	88	0.0			
3-5	11.2	5.2	8.0	1.4	147.6	82	0.0			
4-5	15.0	4.4	11.2	1.6	151.7	82	0.0			
5-5	11.7	5.6	9.4	1.8	154.5	78	0.0			
6-5	11.7	6.2	7.4	0.8	163.4	90	0.0			
7-5	10.4	3.0	7.1	3.1	164.9	60	0.0			
8-5	10.1	2.4	6.9	2.5	166.0	67	0.0	0.0	8.2	

WEATHER DATA 1984

DATE			TEMPERATURE (°C)		ACCUM. (T ₀ =6°C)	HUMID- ITY %	RAIN	COLLECTED WEEKLY TOTAL	MEAN WEEKLY TEMP	LEAF WETNESS(h)
	MAX	MIN	DRY BULB	WET						
9-5	19.3	-1.2	9.0	2.7	170.9	67	0.1			
10-5	14.5	5.4	9.2	1.4	175.0	83	0.4			
11-5	13.4	1.9	7.5	2.7	177.6	66	0.0			
12-5	13.5	1.9	8.9	3.5	180.4	58	0.0			
13-5	18.0	2.0	9.5	4.1	185.4	54	0.0			
14-5	17.6	3.7	10.6	3.1	190.6	65	0.0			
15-5	14.1	4.2	10.1	1.5	194.2	82	0.9	2.0	9.2	10.3
16-5	19.0	7.9	11.6	1.4	207.6	84	2.4			
17-5	11.6	8.2	9.3	0.1	217.6	99	1.8			
18-5	13.6	7.9	10.3	1.0	228.3	88	0.0			
19-5	19.5	2.0	10.6	1.8	234.0	79	0.0			
20-5	11.7	3.5	7.5	0.6	236.3	92	6.9			
21-5	14.3	5.1	8.9	0.6	240.2	93	22.8			
22-5	12.6	7.6	9.9	0.1	250.3	99	3.7	41.0	9.7	16.6
23-5	16.4	5.6	12.6	2.7	255.4	71	0.4			
24-5	20.5	8.2	15.8	3.1	269.7	70	5.3			
25-5	11.6	7.7	10.1	0.8	279.4	90	6.3			
26-5	8.9	7.7	8.6	0.0	287.7	100	22.6			
27-5	9.0	3.9	5.2	0.1	288.6	99	4.2			
28-5	9.4	5.1	7.9	0.4	290.1	95	0.7			
29-5	14.6	6.0	7.7	1.2	300.4	84	0.0	29.7	9.7	

WEATHER DATA 1984

DATE	TEMPERATURE (°C)		HUMID-		ACCUM. (T ₀ =6°C)	ITY %	RAIN	COLLECTED WEEKLY TOTAL	MEAN WEEKLY	
	MAX	MIN	DRY BULB	WET					TEMP	LEAF WETNESS(h)
30-5	18.7	4.6	14.6	2.8	306.7	72	0.0			
31-5	18.0	5.6	16.6	3.2	312.6	70	5.8			
1-6	12.8	6.7	9.7		322.2	95	6.3			
2-6	16.1	6.0	11.5		333.4	68	8.5			
3-6	15.5	9.1	12.3		345.7	84	0.0			
4-6	16.0	4.4	10.2		350.3	71	4.7			
5-6	15.4	9.7	12.8	1.0	362.8	89	0.0	25.3	12.5	5.3
6-6	20.9	5.0	13.1	1.4	370.0	85	13.2			
7-6	21.1	11.4	15.0	0.6	386.2	94	0.0			
8-6	23.1	9.4	14.7	1.3	402.5	87	0.0			
9-6	24.5	7.6	15.1	1.5	418.5	85	0.0			
10-6	27.4	8.5	16.9	1.6	436.5	85	0.0			
11-6	17.3	11.0	13.7	0.5	450.6	95	0.0			
12-6	22.1	11.3	15.5	1.4	467.3	86	0.0	13.0	14.8	11.2

WEATHER DATA 1984

DATE			TEMPERATURE (°C)			HUMID- ITY %	RAIN* (mm)	COLLECTED WEEKLY TOTAL	MEAN TEMP	WEEKLY LEAF WETNESS (h)
	MAX	MIN	DRY BULB	WET	ACCUM. (T _O =6 °C)					
13-6	20.4	13.4	16.2	1.4	484.2	86	0.0			
14-6	24.7	10.3	16.9	1.6	501.7	85	0.0			
15-6	27.4	7.0	16.6	1.9	518.9	82	0.0			
16-6	23.8	9.6	17.2	1.2	535.6	89	0.0			
17-6	28.2	11.0	17.6	0.7	555.2	93	5.7			
18-6	27.7	11.4	18.4	1.0	574.8	91	0.0			
19-6	25.0	11.5	16.3		584.4	99	0.0	7.2	17.0	8.9
20-6	25.6	14.6	16.8		596.9	91	~~4.4~~			
21-6	19.3	12.7	17.1		608.0	64	0.0			
22-6	19.8	8.4	16.6		608.6	80	~~2.0~~			
23-6	17.5	6.3			620.4	49	~~7.3~~			
24-6	19.3	7.6			632.3	61	0.0			
25-6	20.4	11.9			645.7	88	0.0			
26-6	27.0	12.6	21.6	3.2	665.5	74	0.0	13.5	18.0	1.3

WEATHER DATA 1984

DATE	TEMPERATURE (°C)					HUMID- ITY %	RAIN* (mm)	COLLECTED WEEKLY TOTAL	MEAN TEMP	WEEKLY LEAF WETNESS (h)
	MAX	MIN	DRY BULB	WET	ACCUM. (T _O =6°C)					
27-6	25.1	10.1	16.6	2.1	683.1	80	0.0			
28-6	18.6	9.5	12.7	1.2	697.2	87	0.4			
29-6	20.5	6.3	13.5	2.1	710.6	78	0.4			
30-6	23.2	7.3	14.2	2.3	726.3	76	0.0			
1-7	26.3	8.2	17.0	2.5	743.5	77	0.1			
2-7	20.4	8.6	13.1	1.8	758.0	81	0.1			
3-7	25.3	7.2	15.2	2.7	774.1	73	0.0	0.7	14.6	6.9
4-7	26.9	6.8	15.9	8.6	791.1	75	0.0			
5-7	31.4	7.6	29.5	3.7	810.6	75	0.0			
6-7	33.7	12.2	21.1	3.9	833.6	68	0.0			
7-7	36.8	11.6	21.4	4.3	857.8	65	0.0			
8-7	38.6	11.8	23.0	4.8	883.0	63	0.0			
9-7	29.5	13.1	19.0	1.8	904.3	84	0.6			
10-7	31.6	13.6	24.0	5.5	925.6	59	0.0	0.5	21.9	8.5

WEATHER DATA 1984

DATE	MAX	MIN	TEMPERATURE (°C)			HUMID- ITY %	RAIN* (mm)	COLLECTED WEEKLY TOTAL	MEAN TEMP	WEEKLY LEAF WETNESS (h)
			DRY	WET	ACCUM. (T _O =6°C)					
			BULB							
11-7	29.9	11.2	18.1	2.5	946.1	77	0.1			
12-7	23.9	12.2	16.7	1.3	964.1	87	0.5			
13-7	25.3	12.2	15.3	2.3	982.8	77	3.0			
14-7	20.6	13.5	16.6	0.3	999.8	97	4.7			
15-7	21.2	9.1	18.7	0.7	1014.9	94	0.5			
16-7	23.6	8.8	16.2	1.9	1031.1	82	0.0			
17-7	27.4	8.0	19.0	2.8	1048.4	75	0.1	11.5	17.2	11.3
18-7	26.0	13.6	18.9	1.8	1068.6	84	0.1			
19-7	25.6	12.9	14.5	1.8	1087.8	83	0.1			
20-7	23.4	12.0	15.5	1.4	1105.5	86	0.1			
21-7	30.2	9.1	19.0	2.7	1125.1	76	0.0			
22-7	31.8	12.8	19.5	2.6	1147.4	77	1.5			
23-7	28.4	11.1	17.3	1.8	1167.1	83	0.0			
24-7	28.8	11.3	18.2	3.3	1187.1	70	0.0	1.5	17.8	8.5

* recorded by Rothamsted meteorological station

WEATHER DATA 1984

DATE	TEMPERATURE (°C)					HUMID- ITY %	RAIN* (mm)	COLLECTED WEEKLY TOTAL	MEAN TEMP	WEEKLY LEAF WETNESS (h)
	MAX	MIN	DRY BULB	WET	ACCUM (T _O = 6 °C)					
25-7	25.6	10.1	16.5	2.4	1204.9	77	0.0			
26-7	26.0	10.0	17.5	2.3	1222.9	79	0.0			
27-7	32.6	10.0	20.4	4.5	1244.2	63	0.0			
28-7	35.0	14.1	22.2	5.1	1268.7	60	0.0			
29-7	36.6	9.4	22.6	5.3	1291.7	59	0.0			
30-7	35.5	15.9	20.8	4.1	1317.4	66	0.0	0.0	20.0	2.9

* recorded by Rothamsted meteorological station

Addendum

The linear predictor model compares the observed disease severity (y) for each of the independent weather variables ($x_1, x_2 \dots x_i$) with an expected value of y [$E(y)$]. The three basic components of the generalised linear model are:

1) The error structure

As the disease severity was recorded as a percentage, y is assumed to have a binomial distribution with variance

$$V_y = y(1-y)$$

2) The linear predictor (X)

where $X = b_0 + b_1x_1 + b_2x_2$,

b_0 is the intercept on the x -axis and b_1, b_2 etc. are the estimated coefficients.

3) The link function

The expected value of y is related to the linear predictor by a link function

$$X = f(\mu)$$

In the case of binomial data we chose the logit link function which is

$$X = \log_e \frac{\mu}{100 - \mu}$$

where μ is expressed as a percentage.

The inverse of this function gives the expected value of y as follows

$$\begin{aligned} E(y) &= f(X) \\ &= \frac{100 e^{(b_0 + b_1x_1 + b_2x_2)}}{1 + e^{(b_0 + b_1x_1 + b_2x_2)}} \\ &= \frac{100}{1 + e^{-(b_0 + b_1x_1 + b_2x_2)}} \end{aligned}$$

FACTORS AFFECTING SPORE GERMINATION AND INITIAL GROWTH OF *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* ON WHEAT

By SALLY HIGGINS

Plant Pathology Department, Rothamsted Experimental Station, Harpenden, Herts, AL5 2 JQ

Wheat coleoptiles inoculated with *Pseudocercospora herpotrichoides* spores were much more susceptible to colonization and infection than first leaf laminae. It is unlikely that susceptibility was due to a lack of chlorophyll, since fungal colonization of plants grown at low light intensity to induce chlorosis was similar to that of plants grown under normal lighting. Similar numbers of spores were found on first, second and third leaves of seedling plants of wheat exposed to natural inoculum from crop debris on a field site. Spore germination on agar was insensitive to light, but very sensitive to changes in water potential; at -27 bars 95 % of the spores germinated but at potentials below -41 bars only 63 % germinated. Germination on agar was not affected by changes in spore concentration over a range of 1.5×10^3 to 5.2×10^6 spores ml^{-1} .

Lesions of eyespot, caused by *Pseudocercospora herpotrichoides* (Fron) Deighton, usually occur near the soil level on wheat culms. Occasionally lesions have been reported to form elsewhere on the stem, but very rarely on the green leaf blade (Glynne, 1953). It is not known why they normally develop at the base of wheat plants; both host and environmental factors could be involved. Factors which might be important include host resistance (which varies between cultivars and between plant tissues) and the relative humidity, temperature and light intensity at the plant surface.

Cappelle-Desprez and many of the currently grown wheat cultivars show resistance to eyespot but the genetic basis for this resistance is not understood (Law, Scott, Worland & Hollins, 1975). Following their work on seedling infection, Bateman & Taylor (1976) showed that the coleoptile was more susceptible to infection than the leaf sheath, but cultivar resistance was not expressed in the early stages of plant growth. Relative humidity and temperature have a direct influence on both spore germination and fungal growth; for example Defosse (1966) found that although 20°C was the optimum temperature for spore germination, host cell penetration and infection occurred at 10° . These observations were supported by Schrodter & Fehrmann (1971), who went on to show that for high disease incidence to occur, favourable temperature/humidity conditions must be continuous initially over a 15 h period. Bruehl & Manandhar (1972) studied the effects of water activity and temperature on mycelial growth. They tested several isolates of *P. herpotrichoides* on potato dextrose agar (PDA) and obtained growth at water potentials of -4 to -10

bars between 1 and 25° . At higher temperatures growth was favoured by a drier environment; at 29° maximum hyphal extension was favoured by water potentials of -8 to -22 bars. Cook & Papendick (1972) in the Pacific North West of the U.S.A. state that wheat leaf water potentials are about -10 to -20 bars in cool moist weather. The water potential of wheat-stem tissue is somewhere between that of roots in moist soil and leaves. However, the relevance of these results to disease development *in vivo* under U.K. conditions has yet to be ascertained.

This paper describes a series of investigations which set out to examine some of the factors affecting spore germination and initial mycelial growth of *P. herpotrichoides* both *in vitro* and on wheat plants.

MATERIALS AND METHODS

Effects of light

Spores of a field isolate of *P. herpotrichoides* were washed from one-month-old cultures on distilled water agar (DWA) plates with 15 ml of sterile distilled water. Aliquots of this suspension (0.5 ml) were inoculated on to PDA plates and spread with a sterile glass spreader. Plates were incubated at 12° in continuous darkness, continuous light or alternating periods of 6 h darkness and 6 h light. Spore germination and initial mycelial growth were assessed after 18 and 42 h of incubation. Ten spores were counted on each of five replicate plates (using an eye-piece graticule marked in squares) at a magnification of $\times 100$. The extent of initial mycelial growth was measured by recording the number of graticule squares (each square $118 \mu\text{m}$) traversed by the hyphae from each spore and then

Table 1 Derivation of index of initial mycelial growth of *Pseudocercospora herpotrichoides*

Number of 118 μ m graticule squares traversed	1-2	3-4	5-7	8-9	10-11	> 11
Growth index value	1	2	3	4	5	6

converting to an index value (Table 1). The sum of the index values for 50 spores counted constituted the growth index for the treatment

Effects of water activity

To examine the effect of water activity on spore germination, spore suspensions in osmotically adjusted glycerol (OAG) water Dallyn & Fox (1981), were prepared from 6-week old cultures. From these suspensions, 0.5 ml aliquots were spread over the surface of PDA plates of the appropriate water potential prepared using OAG water in place of distilled water. There were three replicates of plates with water activities (A_w) of 0.90, 0.93, 0.95, 0.97 and 0.98. They were incubated at 20° in sealed polyethylene bags. After 24 h the percentage of spores which had germinated at each water activity was recorded, approximately 1000 spores were counted on each plate.

The effect of water activity on mycelial growth was also examined. PDA plates were prepared as before using OAG water. These were inoculated with 6 mm diam agar discs taken from the edge of a mycelial culture of *P. herpotrichoides*. They were then placed on small plastic platforms over OAG water of the corresponding water activity in sandwich boxes at 20°. Each box was sealed inside a polyethylene bag to enable the plates to be viewed and scored without altering the humidity. The rate of growth was recorded by measuring the increase in colony diameter.

Effects of spore concentration

Spore suspensions were prepared by collecting, in 15 ml of sterile distilled water, spores from DWA plates of two-month-old cultures of *P. herpotrichoides*. Following haemocytometer counts the suspensions were diluted to give spore concentrations between 5.2×10^6 and 1.5×10^3 spores ml⁻¹. Aliquots (0.25 ml) of each concentration were inoculated on to five replicate DWA plates, incubated at 20° and examined after 24, 30, 46 and 50 h. Ten spores at random on each plate were assessed for germination and initial mycelial growth (Table 1). Germination was taken as being a swelling of the spore and the production of one or more germ tubes, and initial mycelial growth as

the extension of the germ-tube to a length greater than that of the spore itself.

Spore distribution on wheat plants surfaces

Wheat seeds (cv. Armada) were grown on moistened filter paper in germination trays for 2 days at 20°. They were then planted, 5 per pot in 10 pots, 10 cm diam, containing 'Eff' compost, and incubated at about 10° until the second leaf stage. The pots were then transferred to a field site of straw debris infected with sporulating *P. herpotrichoides* and sunk into the ground. The pots were removed after the first heavy rainfall. Five pots were placed in a cold frame outside until a second rainfall the following day. The plants were allowed to dry before examination.

Six randomly selected leaves and bases were cut into 2 cm lengths from the first, second and third leaves respectively. An imprint of each leaf piece was made using double-sided clear adhesive tape, mounted on a glass slide and stained with cotton blue in lactophenol for observation. The number of spores on each section was counted and their position on the plant noted. The data were transformed using $\log_{(10)} + 1$ before analysis of variance.

Effects of growing plants under different light intensities on spore germination and initial mycelial growth on plant tissues

Wheat seeds (cv. Armada) were grown on moistened filter paper in germination trays for 2 days at 25°. They were then planted, 15 per pot in 12 pots, 11 cm diam, containing 'Eff' compost (a peat/sand mixture). Seedlings were planted in threes, each with its radicle facing the same way. Coleoptiles then faced the same way so that three could be inoculated at a time (see Fig. 1). All the pots were covered with clear plastic tops to create humid conditions and placed in the light. Six pots were then covered with black polyethylene to reduce the light intensity from 4400 to 525 lx. The seedlings were grown in a growth cabinet with alternating day/night temperatures of 15° and 10° for 12 hourly periods.

At the first leaf stage, the coleoptile, the proximal end and the distal end of the leaf blade were

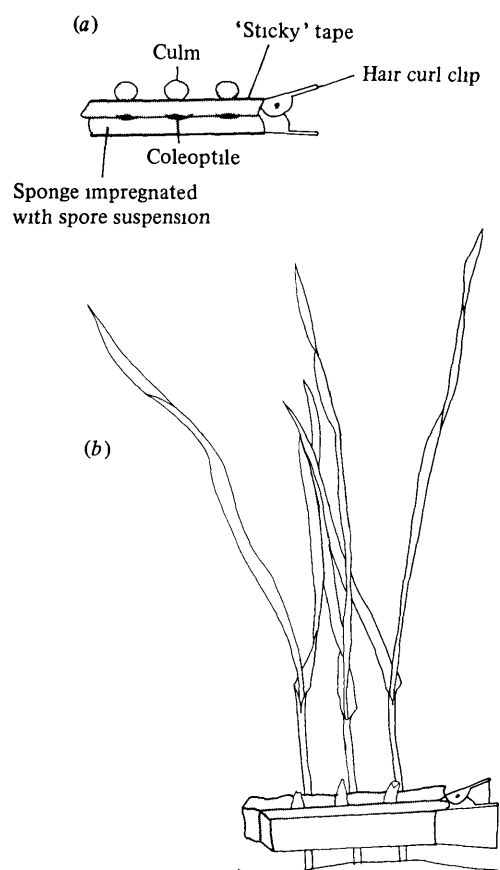


Fig 1. Diagrammatic representation of inoculation of wheat seedlings with spores of *Pseudocercospora herpotrichoides* (a) Transverse section; (b) whole specimen

inoculated. Hair curl clips, with strips of sponge taped to each half, were saturated with a spore suspension of *P. herpotrichoides* (4×10^6 spores ml^{-1}) and then attached to 3 plants in a pot (M. A. Kemp, pers. comm.). After 16 h, the clips were removed and the inoculated areas examined. A transfer of each inoculated area was made by pressing it onto the surface of double-sided sticky tape attached to a microscope slide (Langvad, 1980). The slides were stained with cotton blue in lactophenol and germination and initial mycelial growth assessed microscopically (Table 1).

Inoculated tissue was removed from 3 plants of each treatment 16, 24, 40 and 56 h after inoculation, stained similarly and examined for penetration haloes. Further experiments investigated the growth of mycelium after 1, 2 and 3 weeks of incubation

RESULTS

Effect of light

Germination was unaffected by light-period duration. Over 95% of the spores germinated and produced mycelial growth after 18 h in all treatments and after 42 h there were no differences in mycelial growth.

Effect of water activity

Spore germination was optimal at A_w 0.98 but at 0.97 the percentage germination was approximately 30% less (Table 2). At A_w 0.95 only 3% of the spores were showing the first signs of germination (i.e. swelling and production of germ tubes). After a further 7 days incubation they had not developed beyond this stage.

After 25 days growth, mycelial extension was greatest on DWA with A_w 0.98. A reduction in A_w from 0.98 to 0.97 (equal to change in water potential from -27 to -41 bars) reduced mycelial growth by 74%. *P. herpotrichoides* grew at A_w 0.95 (-69 bars), but not at 0.93 and 0.90 (-100 and -150 bars).

Effects of spore concentration

At the higher concentrations the spores became clumped together, making growth assessment very difficult. For example, at 5.2×10^6 spores ml^{-1} , the growth indices were approximately 30% below the maximum. Linear regression of growth on spore concentration was not significant.

Spore distribution on wheat plant surfaces

Generally there were significantly more spores on the abaxial surface of the leaves (Table 3, $P < 0.05$). The second leaves (at an angle of $\approx 45^\circ$) collected most spores after the first rainfall but the first leaf (parallel to the ground) retained most spores after subsequent rain. Spores were retained over the whole surface of the plant after the subsequent fall of rain but there were fewer spores at the base of the plant than on the leaves. There was no significant difference in spore numbers between plants that were exposed during the first rain and those also exposed to subsequent rain $P > 0.05$.

Effects of growing plants under different light intensities on spore germination and initial mycelial growth on plant tissues

In all the experiments the coleoptile was significantly more susceptible to fungal colonization than the

Table 2. *Effect of water potential on spore germination and initial mycelial growth of Pseudocercospora herpotrichoides*

Water activity (A_w)	Water potential (bars)	Mean percentage spore germination after 24 h	Standard error	Mean colony diam initially 60 after 25 days (mm)	Standard error
0.90	-150	3	0.04	6	—
0.93	-100	3	0.08	6	—
0.95	-69	3	0.28	12	0.67
0.97	-41	63	0.89	14	2.48
0.98	-27	95	0.57	36	0.67

Table 3. *Spore distribution and retention on wheat plant surfaces*

Leaf	Angle to ground	Surface	Total number of spores present after	
			First rain	Subsequent rain
First	360°	Abaxial	34	49
		Adaxial	40	21
Second	45°	Abaxial	66	32
		Adaxial	24	30
Third	90°	Abaxial	31	26
		Adaxial	20	24
Base of stem	90°	—	27	21
		Total	242	203

proximal and distal ends of the leaf blade ($P < 0.001$; Fig. 2). As there was no significant difference between the light and dark treatments the results for both are combined in Fig. 2.

After 56 h the coleoptile showed the first visible signs of infection, i.e. tissue browning. Spores germinated at a similar rate on both ends of the leaf blade, but this was delayed by about 20 h compared with germination on the coleoptile. Mycelial growth was extensive on the coleoptile, whereas after 56 h it was minimal on the leaf blade (Table 4).

DISCUSSION

The coleoptile of wheat seedlings was colonized by *P. herpotrichoides* much more quickly than the leaf blade. Although differences in the micro-environment at the surface of the tissues may occur, the restriction of eyespot lesions to the base of the culm of wheat plants is probably due to differences in inherent susceptibility to infection between tissues. This is supported by the fact that when young seedlings were exposed to a natural inoculum, spores were detected on all of the wheat plant surfaces examined and none escaped inoculation.

The high susceptibility of the coleoptile does not appear to be due to the lack of chlorophyll. Substantially decreased light intensity and hence

decreased levels of chlorophyll in the leaves, did not alter their susceptibility nor did light intensity have any effect on germination and initial mycelial growth *in vitro*. Reinecke & Fokkema (1981) however, found that in the presence of *Microdochium bolleyi* (an antagonist to *P. herpotrichoides*) infection was more rapid in the dark. They suggested that there could be a reduction in the production of fungitoxic compounds in the dark.

The initial restriction of colonization to the coleoptile is probably due to its rapid senescence by comparison with other plant tissues. Ageing of these tissues alters their susceptibility to infection as shown by Hollins & Scott (1980). In their experiments, plants exposed to inoculum throughout the season from October to July all developed lesions provided suitable incubation conditions were available, even after the coleoptile had disintegrated.

Spore germination and initial mycelial growth on agar were very sensitive to small changes in water activity. The spores swell visibly prior to germ-tube production probably due to the uptake of water. It is not surprising therefore that low water potentials (i.e. reductions in amounts of available water) inhibited germination and mycelial growth.

Most previous workers (e.g. Bruehl & Manandhar (1972), Cook & Papendick (1972)) have investigated the effects of water activity on mycelial growth

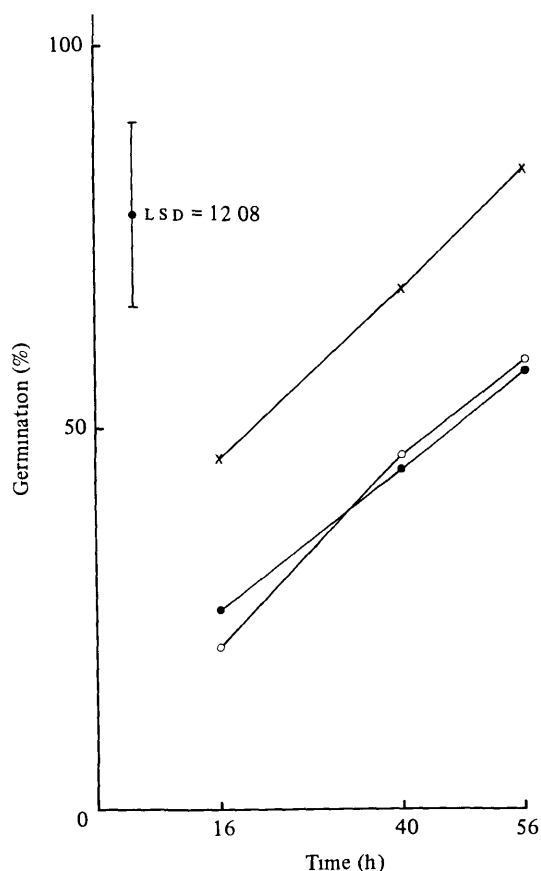


Fig. 2 Spore germination (light and dark treatments combined). $\times$ — $\times$, Coleoptile, $\bullet$ — $\bullet$, proximal end of leaf blade, $\circ$ — $\circ$, distal end of leaf blade. Vertical bar is for $P < 0.05$ for any pairs of observations at any given time

alone; the results reported here suggest that spore germination requires a higher water potential than mycelial growth, possibly due to a relatively low nutrient level in the spores (Cook & Duniway, 1981) and water could be very important in the initial stages of infection and colonization of the host.

The spores are surrounded by a mucilagenous layer which may be removed by dilution during splash dispersal. This layer may contain chemicals which inhibit spore germination. Glynne (1953) observed that spores produced on young plants did not germinate in the surrounding water until the suspension was diluted. However, on DWA there was no relationship between spore concentrations used and germination at 20° .

The experiments suggest that for germination and infection to proceed both host and environmental factors are important. These factors are inter-related since changes in the environment can create small changes in the micro-climate of the host. There are also small differences in the host micro-environment between individual plants (e.g. nutrient status and water activity) which may have important effects on fungal infection. Varietal resistance mechanisms (Law *et al.*, 1975) and general plant resistance to infection are still not understood and require further investigation.

The author wishes to thank Dr B. D. L. Fitt for his valuable advice during the study and also in the preparation of this manuscript, and the Perry Foundation for its research grant.

REFERENCES

- BATEMAN, G. L. & TAYLOR, G. S. (1976). Seedling infection of two wheat cultivars by *Pseudocercospora herpotrichoides*. *Transactions of the British Mycological Society* **67**, 95–101.

Table 4. Initial mycelial growth on different plant tissues under different light intensities

Tissue	Mean percentage of spores with hyphae longer than the spore after			
	16 h	24 h	40 h	56 h
525 lux				
Coleoptile	17.0	16.4	53.9	40.3
Leaf (proximal end)	5.1	6.3	8.7	15.3
Leaf (distal end)	0.8	2.3	13.2	20.9
4400 lux				
Coleoptile	12.0	14.4	24.8	47.1
Leaf (proximal end)	0.0	2.3	8.8	14.2
Leaf (distal end)	2.3	3.0	8.6	20.8

SED = 8.56

Coleoptile v. leaf (proximal and distal end) difference significant ($P < 0.001$) Dark v. light treatment – not significant

- BRUEHL, G. W. & MANNANDHAR, J. B. (1972) Some water relations of *Cercospora herpotrichoides*. *Plant Disease Reporter* **56**, 594-596.
- COOK, R. J. & DUNIWAY, J. M. (1981) Water relations in the life-cycles of soilborne plant pathogens. In *Water Potential Relations in Soil Microbiology*. Soil Science Society of America Special Publication **9**, 119-140. Madison, Wisconsin, U.S.A.
- COOK, R. J. & PAPENDICK, R. I. (1972) Influence of water potential of soils and plants on root diseases. *Annual Review of Phytopathology* **10**, 349-368.
- DALLYN, H. & FOX, A. (1981) Spoilage of materials of reduced water activity by xerophilic fungi. In *Microbial Growth and Survival in Extremes of Environments* (ed. G. W. Gould & J. E. L. Carry), pp. 129-239. London: Academic Press.
- DEFOSSE, L. (1966). Les premiers stades de l'infection du *Cercospora herpotrichoides* Fr. sur froment, orge, seigle et avoine. *Bulletin des Recherches Agronomiques de Gembloux* **NS 1** (4), 561-569.
- GLYNNE, M. D. (1953) Production of spores by *Cercospora herpotrichoides*. *Transactions of the British Mycological Society* **36**, 46-52.
- HOLLINS, T. W. & SCOTT, P. R. (1980) Epidemiology of eyespot (*Pseudocercospora herpotrichoides*) on winter wheat, with particular reference to the period of infection. *Annals of Applied Biology* **95**, 19-29.
- LANGVAD, F. (1980) A simple and rapid method for qualitative and quantitative study of the fungal flora of leaves. *Canadian Journal of Microbiology* **26**, 666-670.
- LAW, C. N., SCOTT, P. R., WORLAND, A. J. & HOLLINS, T. W. (1975) The inheritance of resistance of eyespot (*Cercospora herpotrichoides*) in wheat. *Genetical Research, Cambridge* **25**, 73-79.
- REINECKE, P. & FOKKEMA, N. J. (1981) An evaluation of methods of screening fungi from the haulm base of cereals for antagonism to *Pseudocercospora herpotrichoides* in wheat. *Transactions of the British Mycological Society* **77**, 343-550.
- SCHRODTER, H. & FEHRMANN, H. (1971) Ökologische untersuchungen zur Epidemiologie von *Cercospora herpotrichoides* II. Die Abhängigkeit des Infektionserfolges von einzelnen meteorologischen Faktoren. *Phytopathologische Zeitschrift* **71**, 97-112.

(Received for publication 24 January 1983)