NECROTIC SPOTTING ON BARLEY LEAVES ASSOCIATED WITH *ERYSIPHE GRAMINIS* INFECTION AND OTHER FACTORS

by

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DEDICATION

I would like to dedicate this thesis to my parents, May and John.

DECLARATION

This is to declare this thesis has been carried out by myself and has not been accepted for any previous application for a degree. All information and assistance obtained from other sources has been acknowledged in the appropriate places.

Karene Gardner Sutherland
June 1989

Part of the work described in this thesis has been published in a summarised form.

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ABSTRACT

Although brown lesions on barley leaves are usually attributable to a readily defined parasitic or non-parasitic factor, the causes of certain forms of necrotic spotting are sometimes more difficult to explain. A classification is given of the types of symptoms that may present diagnostic problems and the symptom types are associated with particular cultivars. In some cases necrotic responses to powdery mildew infection were related to cultivar, but, for other cultivars, relatively severe symptoms of brown spotting occurred independently of mildew. The results of experimental work indicated that where nutrient deficiencies gave rise to browning, the symptoms produced were generally characteristic of the mineral element involved rather than the cultivar, but that cultivar-specific symptoms were associated with herbicide injury or high temperature stress. Saprophytic organisms or deposits of pollen on leaves could not be implicated in the browning process. Observations from *in vitro* culture work with barley plantlets grown aseptically from embryos suggested that certain cultivars might be predisposed to accumulate high phenolic concentrations in response to stress, and this might be indicative of vulnerability to cultivar-related browning in the fields. Histological examinations showed differences between brown lesions due to mildew infection and those due to other, cultivar-related, factors. From a review of studies on brown spotting symptoms in barley leaves, it is suggested that genetic factors which render cultivars prone to visible necrotic responses to stress stimuli represent a potential source of yield reduction.

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ABBREVIATIONS

ANON	anonymous
B	browning
b	regression coefficient
cm	centimetre
°C	degrees Celsius
df	degrees of freedom
F-	untreated with fungicide
F+	treated with fungicide
g	grammes
Gs	growth stage
kg/ha	kilogrammes per hectare
l/ha	litres per hectare
M	mildew
M-	uninoculated
M+	inoculated with mildew
m	metre
ml	millilitre
mm^2	square millimetre
ms	mean square
NL1	first year of National List trial
NL2	second year of National List trial
p	probability
r	correlation coefficient
RLT	recommended list trial
SB	spring barley
SED	standard error of the difference
sig	significance
TS	transverse section
UV	ultra-violet
VR	variance ratio
WB	winter barley
%	percentage
<	less than
±	plus or minus

SECTION 1

GENERAL INTRODUCTION

Necrotic spots or brown lesions are a common type of symptom on barley leaves from field-grown crops, small plot field trials or glasshouse-grown plants. In some cases the necrosis can be readily attributed to colonisation of tissues of a susceptible host by a pathogenic organism and can be prevented by chemical control; in other cases a clearly identified abiotic cause is indicated. There are, however, further instances where the cause is less obvious. The necrosis could be an expression of a host resistance or non-host response to infection or of other biological interactions. Alternatively, it may have no identifiable biotic cause, being regarded as ''physiological'', a description which does not adequately explain the cause of such browning. The necrotic-type symptoms which currently present the most frequent diagnostic problems are associated with certain barley cultivars, in particular some of the more recent which have been bred from the German cultivar Trumpf and its parent, Diamant. A distinctive 'target-spot' symptom has also been observed in the cultivar Midas [(Proctor x Wong) x Mildew resistant A x Golden Promise]. In addition to presenting diagnostic difficulties, and problems with disease scoring and varietal assessment in trials work, these browning symptoms can also be associated with yield loss. It is possible that the brown spotting is often non-parasitic in origin, but large brown spots which may develop with Carnival (Trumpf x Maris Bulbeck) have been reported to be controlled by fungicide (Lockhart, 1981) and infection with *Erysiphe graminis* has been implicated or associated with some forms of brown lesions.

The present study was aimed at investigating factors responsible for cultivar-related symptoms in the form of brown areas of tissue in barley leaves and to examine the basis for any relationship with infection by *E. graminis*.

The appearance of brown areas of tissue in plants is generally, but not always, associated with necrosis of cells. In the present work the term browning or brown lesion is taken to refer to a visual symptom which may be derived from one or other

of a number of physiological or biochemical responses. Roberts and Boothroyd (1972) characterised necrosis as involving the degeneration of protoplasts followed by death of cells, tissues, organs or whole plants. Symptoms expressed before necrosis of protoplasts include yellowing, wilting and hydrolysis. In those expressed after death, tissues usually turn brown as spots, flecking, irregular blotches, stripes, streaks, marginal scorching or extreme blight of organs. The normal life-cycle of a plant involves cell division and elongation, tissue differentiation, organogenesis, and eventual senescence leading to tissue death. This ordered sequence may be disrupted at any point in the plant's development, resulting in premature senescence, abnormal growth or the various forms of browning described above. Factors which may be involved in this disruption include abiotic factors, such as mechanical injury and adverse environmental conditions, and biotic factors, including insects, animals, necrotrophic and biotrophic pathogens (Gahan, 1981; Figure 1.1). In this section a summarised classification of the various factors associated with the death of tissues is presented.

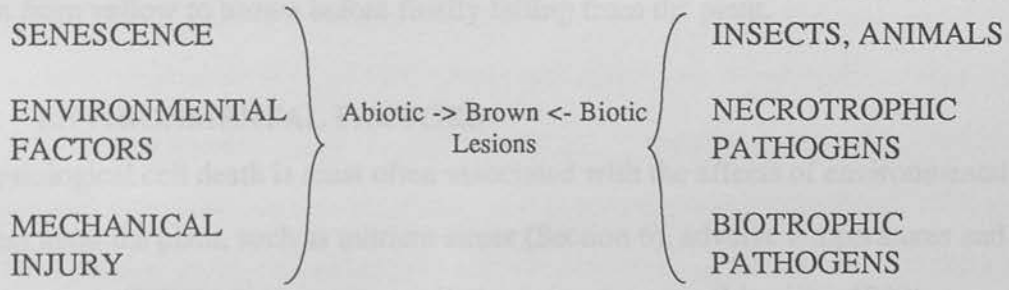


FIGURE 1.1: Schematic representation of the main factors involved in the death of plant tissue or the expression of brown symptoms.

1.1 SENESCENCE

Senescence, the complex process of ageing that eventually leads to death, is an integral part of the life cycle of a growing plant. In annuals and biennials this may take the form of death of the whole plant once fruiting has occurred (Ray, 1972). It may, alternatively, involve the colouring of deciduous leaves from green to gold or

rust in the autumn or dieback of spring bulbs in early summer (Leopold, 1980). Leopold categorised senescence into four groups: total plant death, top senescence, deciduous senescence and progressive senescence. The last grouping, also known as sequential senescence, is shown by cereal crops, in which each leaf emerges, expands to maturity then dies (Briggs, 1978). Once the ears of cereal plants have emerged from the leaf sheath and the grains begin to fill, all leaves tend towards senescence: senescence is not simply an uncontrolled breakdown of a plant, leaf or organ, but a series of events which occur in a controlled and ordered manner leading to loss of function and eventual breakdown (Bidwell, 1979). The process is first recognised by a loss of chlorophyll, the leaves turning from green to yellow, leading to a reduction in photosynthesis followed by a breakdown of chloroplasts, mitochondria, proteins and nucleic acids (Sexton and Woolhouse, 1984). Starch disappears early in the process, appearing as reducing sugars (Thimann, 1980). Breakdown products such as amino acids, sugars and nutrients within the dying leaves are retranslocated to younger, actively growing leaves. In the latter stages of senescence, leaves gradually turn from yellow to brown before finally falling from the plant.

1.2 ENVIRONMENTAL FACTORS

Physiological cell death is most often associated with the effects of environmental stress upon the plant, such as nutrient stress (Section 6), adverse temperatures and water stress (Section 8), excessive radiation or toxic gases (Manners, 1982). Macmillan (1918) observed a brown spotting disease on the pods of beans which developed prior to harvest. In shaded areas symptoms did not occur and it was concluded the spotting was the result of excess radiation from the sun. Toxic air pollutants and smog, the result of fumes from chemical plants, car exhaust fumes and smoke from city houses, can produce disorders in plants, associated with a build up of ozone, peroxyacetyl nitrate (PAN), fluorine, hydrogen sulphide, sulphur dioxide and ethylene (Middleton, 1961). Such air pollutants can remove the protective

epicuticular waxes from around the stomata of conifer needles, predisposing them to attack by pathogens or further adverse environmental conditions (Huttunen and Soikkeli, 1984).

Ozone is particularly injurious, causing chlorotic lesions on cereal leaves at concentrations as low as 0.25 ppm (Hill, Pack, Treshow, Downs and Transtrum, 1961). The mesophyll cells of cereals are sensitive to ozone, with chloroplast disruption and loss of chlorophyll leading to chlorosis. Necrotic bleaching may also occur as a result of plasmolysis of cells, inhibition of mitochondrial activity and disruption of semi-permeable membranes (Hill *et al.*, 1961; Rich, 1964). A condition known as weather fleck on tobacco, in which small brown to black spots appear on leaves, can be reproduced under controlled conditions by exposing plants to ozone (Walker, 1961). Similarly, ozone-treated apples produce brown sunken areas around the lenticels and grapes showed brown spots on leaves (Rich, 1964). The necrotic spotting in these examples is due to dead tissue rather than a brown pigmentation of the tissues (Hill *et al.*, 1961).

Sulphur dioxide and fluorine can cause the death of epidermal cells in some plants if allowed to build up to toxic levels (Mansfield and Freer-Smith, 1984). If the sulphur dioxide is converted to hydrogen sulphate by the plant, chlorosis of the leaves develops (Darley and Middleton, 1966). If it is converted to hydrogen sulphite then necrosis results. Sulphur dioxide may also cause premature flower and leaf drop in ornamentals (Metcalfe, 1941). Fluorine gas results in leaf necrosis of gladiolus and leaf marginal necrosis of Italian prune (Metcalfe, 1941). Ethylene is important in promoting chlorosis and eventual premature senescence (Bidwell, 1979).

Smog, a mixture of smoke and fog, leads to the symptom known as silver leaf in herbaceous crops, where PAN causes a distention of guard cells leading to stretching, rupture and eventual collapse of epidermal cells (Darley and Middleton, 1966). The mesophyll cells dehydrate and shrink, the spaces filling up with air so

leading to the silvering. If leaves are exposed to short periods of smog only, then chlorosis occurs (Middleton, 1961).

1.3 MECHANICAL DAMAGE

Mechanical damage, although caused by such factors as wind, rain, insect and animals, has many attributes associated with responses to infection by many pathogens (Stakman and Harrar, 1957) and will therefore be considered in the context of pathological invasion.

By far the most economically important and widely studied form of cell death is that due to invasion or attempted invasion by a pathogen, whether it be fungal, bacterial or viral. Fungal pathogens comprise the largest group and further discussion is confined to this category. Based on the nature of their nutritional relationship with their hosts, fungal pathogens can be divided into necrotrophs and biotrophs.

1.4 NECROTROPHIC PATHOGENS

Necrotrophic organisms are so called because they utilise and develop on dead tissue which they have killed. They include leaf spot (*Septoria* spp) and anthracnose (*Colletotrichum* spp) pathogens which give rise to small lesions, but where multiple infection points lead to extensive necrosis, and such as *Pythium* spp which form massive spreading lesions, blight, soft and dry rots (Agrios, 1969). Death of tissues or cells is often brought about by the introduction of substances from the invading pathogen onto or into the host. Enzymes such as cutinase, cellulase, hemicellulase and pectinase (Kollatukudy and Crawford, 1987) are released by numerous pathogens, both necrotrophs and biotrophs, in order to effect entry of plants, breach cell walls, spread within plant tissues and gain access to protoplasts. In addition, phosphatidases and proteases might affect cell membranes and be involved in the necrosis of cells (Tarr, 1972). In reference to cell wall-degrading enzymes, Cooper (1984) has indicated that degradation of primary cell walls is often rapid and

extensive during infection by facultative parasites which cause necrosis: on the other hand, successful infection by obligate parasites requiring living protoplasts must involve minimal changes to host walls.

Enzymatic digestion may predispose cells to attack by a second group of pathogen-produced substances, the toxins, which are typical of many of the necrotic leaf diseases such as the leaf spots and blights caused by *Alternaria* spp, net-blotch of barley (*Pyrenophora teres*) and blight of oats (*Helminthosporium victoriae* = *Drechslera victoriae*) (Wood, 1967). Some toxins, the host-specific toxins, are responsible for the actual symptoms of the disease of a particular species or cultivar, acting as primary determinants of disease, whereas others, the host non-specific toxins, may be viewed as secondary determinants (Goodman, Kiťaly and Wood, 1986). Toxins can produce either chlorosis or necrosis depending on their site of action. *Alternaria alternata* f. sp *kikuchiana* toxin (AK toxin) causes a release of electrolytic substances from the cells of Japanese Pear through damage and increased leakage of the plasma membrane (Nishimura and Kohmoto, 1983). Similar effects were found with other *Alternaria* toxins (ACT-A, AF and AM), the wilt toxins lycomarasmin and fusaric acid (*Fusarium oxysporum* f. *lycopersici*) and victorin (*H. victoriae*) (Husain and Kelman, 1959; Goodman, Kiřály and Zaitlin, 1967; Kohmoto, Otani and Nishimura, 1987). In the case of *Alternaria* AK toxin, after membrane damage there is *de novo* synthesis of mRNA and proteins which are involved in necrosis (Kohmoto *et al.*, 1987). However, the increased permeability of the semi-permeable membrane alone would eventually lead to loss of cellular function.

Chlorosis is brought about by several different means. Phaseolotoxin (*Pseudomonas syringae* pv *phaseolicola*) on beans inhibits the functioning of ornithine carbamoyltransferase (Nishimura and Kohmoto, 1983). Tabtoxin (*P. syringae* pv *tabaci*) inhibits glutamine synthetase, leading to a build up of ammonia

(derived from photorespiration) to toxic levels, uncoupling of photophosphorylation and chlorosis (Gilchrist, 1983). This wildfire toxin of tobacco reduces chlorophyll content through competitive inhibition of the amino acid methionine (Goodman *et al.*, 1967). These examples indicate that an important role of toxins is their interference in amino acid biosynthesis. A more direct effect on photosynthesis is brought about by tentoxin (*Alternaria alternata* f. *tenuis*) which inhibits photophosphorylation and uncouples electron transport of photosynthesis (Gilchrist, 1983). Although chlorosis does not result in immediate death of cells, lack of assimilation will eventually lead to organ or plant death through premature senescence (see Section 1.1). The breakdown products of senescence can then be used by the pathogen.

1.5 BIOTROPHIC ORGANISMS

Obligate biotrophic organisms, such as the rusts (*Puccinia* spp) and powdery mildews (*Erysiphe* spp) are wholly dependent on living cells of the host plant for nutrients (Yarwood, 1957) and characteristically cause little acute damage to the host with successful infections. Active resistance to infection by such pathogens is often associated with a hypersensitivity response where cells die rapidly at the immediate site of infection restricting the pathogen to a few cells (Müller, 1959; Farkas and Király, 1962). The hypersensitive reaction involves changes in several complex biochemical pathways including respiration and photosynthesis. Since the changes in these pathways are similar in most host-biotrophic organism relationships, they will be discussed in relation to barley/*E. graminis* in Sections 4 and 5.

This section has outlined some of the factors which are known to cause cell, tissue or organ death of plants. The following sections deal with some of these factors in more detail and consider their possible involvement in brown lesion development in barley leaves of certain cultivars. Firstly, however, an attempt is made to present a classification of the symptoms of brown lesions on barley leaves and cultivar relationships.

SECTION 2

CLASSIFICATION OF BROWN LESION SYMPTOMS OF BARLEY LEAVES

2.1 INTRODUCTION

The occurrence of necrotic lesions on the surface of leaves, stems and fruits of crop plants has already been described in general terms along with an account of possible causes (Section 1). The present section is concerned more specifically with barley leaves and with symptoms they may show which have no easily defined cause but which are cultivar-related. Reports on barley field trials have considered that 'necrotic' or brown lesions on the leaves of certain plants may often be the response of resistant barley cultivars to infection by *Erysiphe graminis* f. sp *hordei* Marchal (Yarham, 1982). However, precise evidence of the causal nature of these cultivar-related symptoms has been limited. In an attempt to obtain a better understanding of this problem it was decided that, in the first instance, a descriptive classification of the symptoms involved would be helpful. A survey was therefore carried out of the more distinctive symptoms shown on the leaves of barley plants grown in the field or glasshouse, in relation to cultivar and other factors.

2.2 MATERIALS AND METHODS

Leaf samples were taken from National List / Recommended List winter and spring barley trials in 1984-1986, small plot field experiments and glasshouse experiments, comprising 10 representative leaves per plot. Leaves were pressed and dried between sheets of blotting paper for a few weeks then stored in 'Kristal' transparent bags (254 x 44 mm) for future reference, when descriptions of symptoms were recorded, complemented by photographs.

2.3 RESULTS

From the observations, six main categories of browning symptoms were identified and these are indicated in Table 2.1, along with the cultivars which often expressed symptoms typical of each category. Symptoms will be described as a brown lesion, where lesion is defined as an area of disordered tissue.

TABLE 2.1: Main brown lesion symptoms identified on leaves of barley cultivars.

Category	Cultivar	Parents	Descriptions
1	Javelin	Athos[b] x Trumpf	Large brown 'thumb-print' lesions; mid-dark brown background; 1-2 mm dark brown spots within *(Plate 2.1)*
2	Tasman	Trumpf[a] x Maris Bulbeck	Lesion 0.5-1.5 cm diam; slight browning of background; dense dark brown speckling within lesion *(Plate 2.2)*
	Acclaim	Had 46813/68[b] x Triumph	
3	Midas	(Proctor x Wong)[a] x Mildew Resistant 'A' x Golden Promise	'Target-Spot' - alternate rings of brown and green tissue *(Plate 2.3)*
	Carnival	Trumpf[a] x Maris Bulbeck	
4	Triumph	Diamant[a] x St 1402964/6	Distinct dark brown or chocolate lesions, 2-5 mm diam; many per leaf *(Plate 2.4)*
	Heriot	Triumf[d] x HB855/467/8	
	CWB117/5/9/5	Igri[b] x HJ51/15/3	
5	Vista	PF52213[b] x Claret	Distinct dark brown lesions; 1-2 mm diam; many per leaf *(Plate 2.5)*
	Marinka	(Alpha x SVP 67.4)[b] x Malta	
6	Golden Promise	❊ ray mutant[a] of Maythorpe	'Senescent' browning *(Plate 2.6)*
	Corgi	Triumph[c] x 15530 Co	
	Gerbel	(Ager x Jumbo)[a] x FDE 244-95	
	Maris Otter	Pioneer[a] x Proctor	

[a]Anon, 1983a
[b]Anon, 1984a
[c]Baum, Bailey and Thompson, 1985
[d]Ellis, 1986
(breeding pedigrees are shown in Appendices 2.1-2.2)

Category 1 (Plate 2.1) refers to symptoms found on the leaves of the spring barley cultivar Javelin or cultivars with Javelin in the breeding background. Typically, these were brown 'thumb-print' lesions, 1 or 2 cm in length, consisting of a brown background within which were small (1-2 mm) dark brown spots or speckling. Usually, a large area of green tissue was still visible but in more extreme cases the lesions extended over the whole leaf surface, leaving no green tissue visible. In less severe cases, the lesions were smaller and paler in colour (lower leaf, Plate 2.1). It was noted, whilst carrying out a glasshouse experiment, that when lesions appeared they began as small pale brown areas which progressively enlarged. Javelin had the cultivars Trumpf and Athos as parents.

Category 2 includes the cultivars Tasman and Acclaim, both of which showed similar reactions (Plate 2.2). Lesions were 1 cm or more in diameter and, like Javelin, had a brown background containing small dark brown spots within. Unlike Javelin, the lesions in this category were mainly circular but at the leaf edge could extend for a few centimetres, thus taking on the appearance of Javelin-like lesions. The lesions of this category never extended over the whole leaf surface. Tasman, like Javelin, had the cultivar Trumpf as one of its parents and Acclaim contained part of the Triumph genotype.

Category 3 contains the cultivar Midas which often showed target-spot lesions, each comprising a central brown spot surrounded by circles of brown tissue at various intervals (Plate 2.3). Some lesions from Category 2 had a similar appearance, but those on Midas did not have a dark background colouring. Most of the leaf remained green. Midas was bred mainly from mildew susceptible cultivars but has a major resistance gene. The cultivar Carnival can also be placed into this category, Carnival having Trumpf as a parent.

Category 4 consists of those cultivars which showed dark or chocolate brown lesions, 2 to 5 mm in diameter (Plate 2.4). Most often there were numerous lesions

PLATE 2.1: Thumb-print lesions on the leaves of spring barley cultivar Javelin.

PLATE 2.2: Circular lesions on the leaves of spring barley cultivars Acclaim and Tasman.

PLATE 2.3: Target-spot lesions on the leaves of spring barley cultivar Midas.

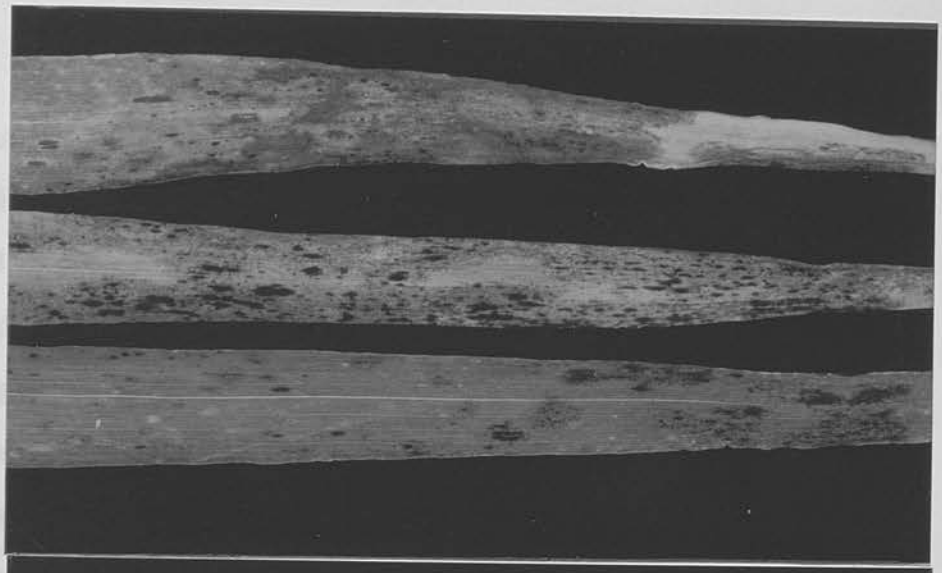


Plate 2.1

Plate 2.2

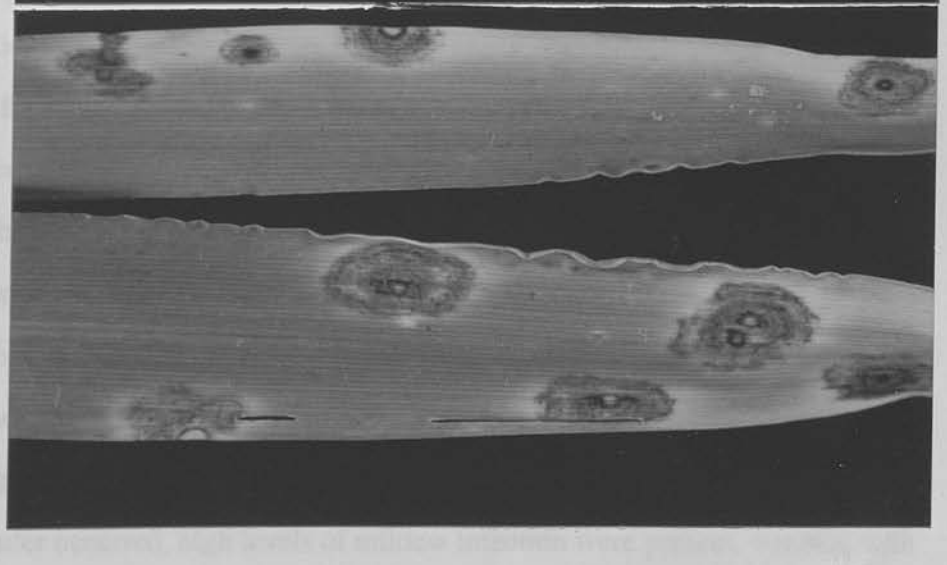


Plate 2.3

per leaf, but some leaves showed only a few lesions. This category included the spring cultivars Triumph and Heriot and the winter cultivars CWB 117/5/9/5 and Podium. Both spring cultivars had Trumpf as a parent or part of their genetic background.

Category 5 is similar to Category 4 but the lesions were smaller with diameters of 1 or 2 mm and included cultivars Vista and Marinka (Plate 2.5).

Category 6 includes all cultivars showing rust brown or dark brown lesions covering much of the leaf, with associated chlorosis. This browning was distinct from the all-over browning shown by Javelin and was associated with advanced stages of successful infection with *E. graminis* f. sp *hordei*. This has been called 'senescent browning' and was exhibited by cultivars such as Golden Promise, Corgi, Gerbel and Maris Otter (Plate 2.6).

Table 2.1 summarises the more characteristic brown lesion symptoms produced respectively on the leaves of particular barley cultivars. However, many of these symptoms were produced only under certain conditions. Browning on leaves of Javelin was similar at all National List trial sites in 1984, the only year this cultivar was in trials, with only the severity of symptoms differing. However, small plot field experiments in 1986 and outdoor and glasshouse pot experiments in 1984/1986 showed little of such symptoms, with only one or two plants per experiment showing the typical thumb-print lesions and most plants showing a general spotting of the leaves, as exemplified by Triumph leaves illustrated in Plate 2.7. Acclaim and Tasman, which on the whole tended to show distinct lesions (Plate 2.2), could also show the more general spotting which was associated with limited growth of mildew, symptoms being similar to those of Golden Promise (Category 6) but with a more intense brown colour (Plate 2.7). Midas, also, may in some cases show target-spots (Plate 2.3) but at other times may show browning similar to that of Category 6. When the latter occurred, high levels of mildew infection were present, whereas with

target-spots there were only traces of mycelial growth in the centre of each lesion. Triumph and Heriot, when grown under field conditions with no fungicide applied, tended to show symptoms similar to Category 6 (Plate 2.7). However, Heriot when grown in the glasshouse, consistently developed large chocolate brown spots (Plate 2.4) irrespective of whether mildew was present or absent through control by fungicides. In fungicide-treated field trial plots a small percentage of Heriot plants actually produced symptoms similar to those of Tasman and Acclaim (Plate 2.2).

Golden Promise and Corgi illustrated typical senescent browning associated with advanced stages of mildew infection. When a fungicide was applied the levels of browning on these cultivars were negligible (see Section 3).

Leaf position appeared to affect symptoms shown in certain cultivars. In general, brown symptoms were most frequent towards the base of the plant, this being especially true for cultivars with much mildew infection, such as Golden Promise and Corgi. However, some cultivars exhibited few lesions on the lower leaves: this was true for cultivars such as Javelin and Acclaim. For example, on Javelin it was mainly the uppermost leaves which showed the typical thumb-print lesion; on plants grown in the glasshouse leaves were observed not to develop lesions until flag leaves were fully emerged. Such Javelin lesions began as small spots which gradually enlarged to give the background colour within which smaller, darker spots appeared. From 36 mature Javelin plants, only two or three actually produced small lesions in this case and no extensive thumb-print lesions ever developed.

The winter barley cultivar Marinka was easily identified in the field because only the flag and second top leaf developed distinct spots (Plate 2.5). Lower leaves exhibited senescent browning to varying degrees. The lower leaves of the spring barley cultivar Tasman also showed senescent browning on occasions but mostly lesions were in Category 2 (Plate 2.2).

PLATE 2.4: Brown spots on the leaves of spring barley cultivar Heriot grown under glasshouse conditions.

PLATE 2.5: Small brown lesions on the leaves of spring barley cultivar Vista.

PLATE 2.6: Senescent browning on the leaves of spring barley cultivar Golden Promise, with old mildew mycelium present on the leaf.

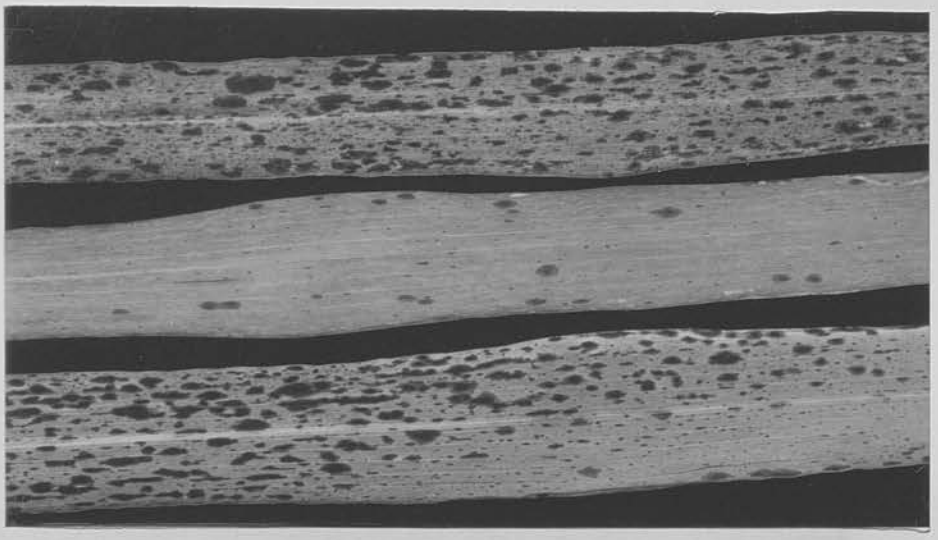


Plate 2.4

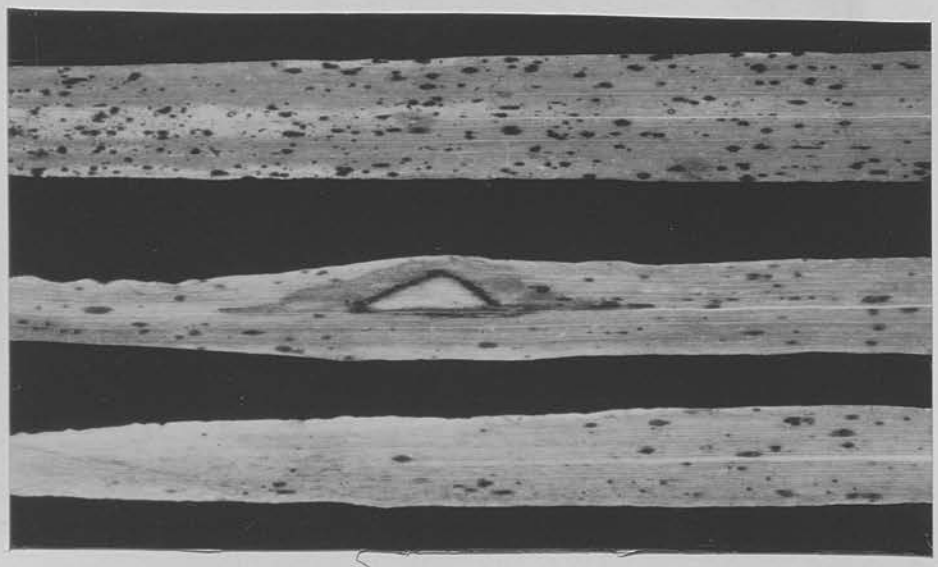


Plate 2.5

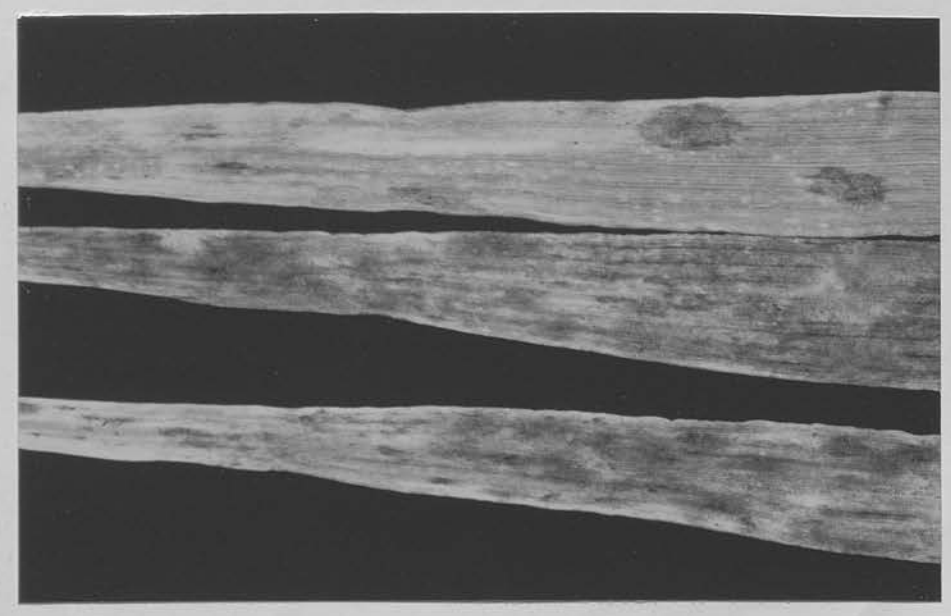


Plate 2.6

PLATE 2.7: Browning on leaves of spring barley cultivar Triumph given no fungicide treatment.

2.4 DISCUSSION

From general observations of barley leaves from a range of cultivars, brown lesion symptoms could be identified which were associated with particular cultivars but their expression was also dependent upon the conditions under which the cultivars had been grown. This was seen, for example, in the case of cultivars such as Javelin and Heriot, both of which showed different symptoms under field conditions compared with those in the glasshouse. Benada (1965) reported that mildew-infected barley plants showed chlorosis or brown spotting if grown in field conditions, but if grown under artificial illumination in the glasshouse never developed browning. Benada associated such browning, arising from mildew infection, with the pH of the host tissues: if below pH 7 chlorosis developed but if above pH 7 then brown patches ensued. The lack of browning under glasshouse conditions was attributed to the low pH in the plant tissues. This explanation does not hold for the observations on Javelin and Heriot, since brown patches were shown in glasshouse conditions, even when artificially illuminated, as well as in field conditions. However, other cultivars such as Midas and Triumph showed little or no brown spotting in the glasshouse. Benada (1969) noted that brown patches did not appear until after ear emergence. Plants in field trial conditions in this present study were not observed until after ear emergence, therefore it is not possible to say when symptoms appeared. However, glasshouse studies showed browning occurred well before ear emergence with Heriot but coincided with ear emergence on Javelin.

In considering the general genetic background of cultivars listed in Table 2.1, the cultivar Diamant featured in the ancestry of certain cultivars showing distinctive browning symptoms, i.e. Acclaim, Carnival, Heriot, Javelin, Tasman and Triumph (Appendix 2.1). It is most likely, if the browning is linked with a genetic factor, that the genes conditioning browning originated in Diamant, with the variation in expression depending on other modifying genetic factors and on the effect of the environment.

Golden Promise, Midas and Vista and the winter barley cultivars Gerbel and Maris Otter all have Binder x Gull as common ancestors (Appendix 2.2). However, these cultivars appeared several generations back in the respective breeding backgrounds, which are very complex, and it is unlikely that the browning response is wholly controlled by the genomes derived from such ancestral parents. Moreover, Gerbel, Golden Promise and Maris Otter showed senescent type browning (Plate 2.6) whereas Vista produced small lesions (Plate 2.5) and Midas varying responses (Plates 2.3 and 2.7). Corgi (Plate 2.6) displayed senescent browning but was bred from Triumph (Appendix 2.2) which could show distinctive spotting.

CWB 117/5/9/5 and Marinka were the result of two selections from a common breeding programme (Appendix 2.2), namely Malta and 'another'. The symptoms shown by CWB 117/5/9/5 and Marinka were both in the form of distinct spotting with only the size and distribution of the spots differing. The differences could again be due to other components of the genotypes present or the effect of the environment.

Although a common ancestor was recognised for many of the cultivars named in this section, the form of brown symptoms could vary with no clearly defined genetical relationships being identified. The problem is further complicated by plants showing brown lesions in response to a range of factors which do not interact obviously with the host genotype. In the following sections some of the factors which may be involved with cultivar-related symptoms are investigated further.

SECTION 3

FIELD SURVEY OF THE INCIDENCE OF BROWN LESIONS ON BARLEY LEAVES AND THEIR ASSOCIATION WITH *ERYSIPHE GRAMINIS* F. SP *HORDEI* INFECTION

3.1 INTRODUCTION

Numerous reports have been published on the development of brown lesions on the leaves of barley plants grown under controlled environmental conditions (Benada, 1965, 1969; Masri and Ellingboe, 1966a; McCoy and Ellingboe, 1966), but no surveys have been carried out into the distribution and the extent of this type of symptom. Channon, Mawson and Boyd (1984) noted that brown lesions occurred in field plots infected with mildew but took it no further. Some of the causes of such lesions have already been discussed generally (Section 1). Environmental factors cannot be fully controlled under field conditions but those of nutrient supply or level of pathogen infection can be determined by adding nutrients to soil or fungicides to the crop. With respect to pathogen attack, powdery mildew (*E. graminis* f. sp *hordei*) may be recognised as the most important barley leaf pathogen in Britain today (Yarham, 1982). Infection of barley leaves with powdery mildew is often associated with brown spots resulting from a hypersensitive response to invasion (Wood, 1967) or from premature senescence (Jenkyn and Bainbridge, 1978).

In recent years extensive browning has appeared on the leaves of many barley cultivars grown in National/Recommended List trials in the south-east of Scotland. This present survey aimed to investigate the extent of browning on various barley cultivars and its association with mildew infection and other factors.

3.2 MATERIALS AND METHODS

Surveys were carried out over the three years 1984 to 1986 on National/ Recommended List trials at various sites in south-east Scotland (Figure 3.1). Small plot experiments using specific cultivars were also set up at Bush Estate, several miles south of Edinburgh. Details of the various trial sites are summarised in Table 3.1. All trials were set up in a split-plot design. In the large plot trials, fungicide treatment represented the main plot with cultivar the sub-plots. In the 1985 small plot trial cultivar formed the main plot with fungicide the sub-plot. In the 1986 small plot

FIGURE 3.1: Location of survey sites (o) in South-east Scotland.

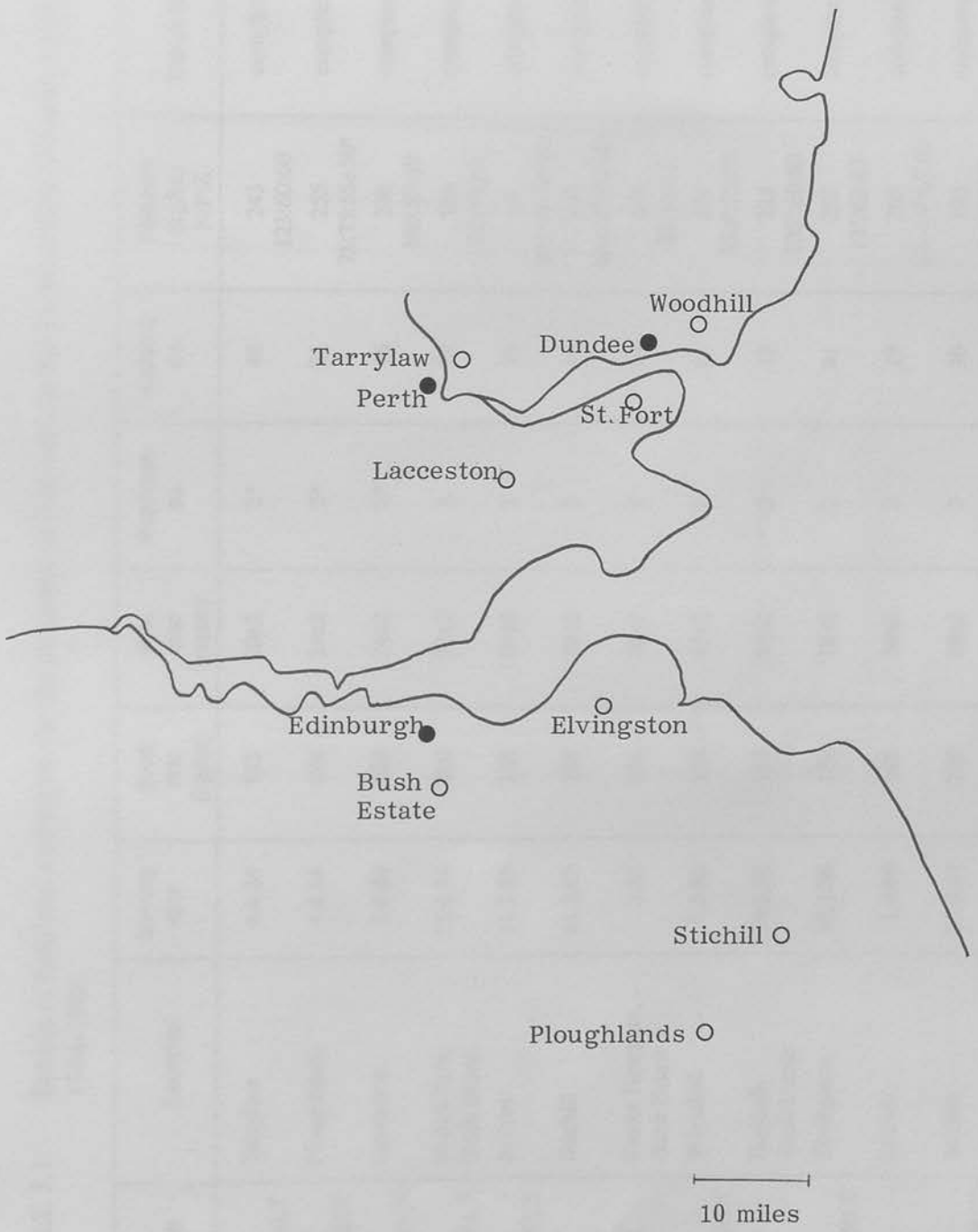


TABLE 3.1: Details of field trials surveyed for the incidence of leaf mildew and brown lesions in barley cultivars over the period 1984-1986.

Crop	Location	Sowing date	Seed rate (kg/ha)	Plot size (metre)	Replicate no.	Cultivar no.	Fertiliser (kg/ha) N:P:K	Fungicide	Scoring G.S	Scoring Date
S.B NL2/RLT	Tarrylaw	6.4.84	188	20x2	2*	46	243 123:60:60	complex[1]	68 83	4.7.84** 26.7.84
S.B NL2/RLT	Ploughlands	4.4.84	188	24x2	2*	21	255 75:75:55+50[a]	complex[2]	58 83	26.6.84** 24.7.84
S.B NL2/RLT	Lacceston	5.4.84	188	24x2	2*	46	278 160:59:59	complex[1]	87 87	27.7.84** 5.8.84
S.B NL2/RLT	March Park, Bush Estate	13.4.84	200	32x2	2	21	500 22:10:10	complex[2]	63 83	7.7.84 18.7.84
S.B NL2/RLT	St Fort	11.3.85	188	20x2	2	19	64 ratio not known	complex[1]	71 83	4.7.85*** 23.7.85
S.B RLT	Stichill	14.3.85	188	20x2	2	19	310 90+40[a]:90:90	complex[2]	71 83	9.7.85*** 26.7.85
S.B RLT	Easter Howgate, Bush Estate	3.85	180	20x2	2	19	500 22:11:11	complex[2]	71 83	10.7.85*** 1.8.85
S.B NL2/RLT	Woodhill	7.5.86	188	18x2	2	41	280 130[a]:75:75	complex[2]	85	22.7.86
S.B	Boghall, Bush Estate	19.3.86	188	18x2	3	19	225 105[a]:60:60	complex[1]	87	25.7.86
S.B NL2/RLT	Elvingston	26.3.86	188	18x2	2	41	251 125:63:63	complex[1]	87	30.7.86
S.B	Stichill	1.4.86	188	18x2	2	19	265 65+45[a]:65:90	complex[1]	87	23.7.86
W.B NL2/RLT	St Fort	21.9.84	200	20x2	2	30	190 30[a]:80:80	complex[3]	73	18.6.85

Table 3.1 (continued)

Crop	Location	Sowing date	Seed rate (kg/ha)	Plot size (metre)	Replicate no.	Cultivar no.	Fertiliser (kg/ha) N:P:K	Fungicide	Scoring G.S	Scoring Date
W.B NL2/RLT	Stichill	19.9.84	200	20x2	2	29	380 180[a]:100:100	complex[2]	83	27.6.85
W.B NL2/RLT	Woodhill	11.10.85	200	18x2	2	31	210 30[a]:90:90	complex[2]	85	3.7.86
W.B NL2	Stichill	2-4.10.85	200	18x2	2	31	220 30[a]:80:80	complex[2]	85	1.7.86
W.B NL1	St Fort	21.9.84	200	20x2	2	28	190 30[a]:80:80	-	72	19.6.85
W.B NL1	Stichill	19.9.84	200	20x2	2	29	380 180[a]:100:100	-	84	2.7.85
S.B	Bush House, plots	18.4.85	180	4x2	4	6	120 22:11:11	Milgo E 1.5 l/ha 12/7/85		various
S.B	Bush House, plots	13.5.86	hand drilled	1.5x5 rows	4	5	applied but details not known	Milgo E G.S 55-59	85	25.7.86

Key:

S.B spring barley
W.B winter barley
NL1 National List first year of trial
NL2 National List second year of trial
RLT Recommended List trial
kg/ha kilogrammes per hectare
N:P:K nitrogen:phosphorus:potassium
G.S growth stage (Zadocks, Chang and Konzak, 1974)

* only 1 replicate for browning on plots treated with fungicide
** first date mildew, second date browning
*** scored on two dates - mean of scoring dates used in results
a top dressing

1 fenpropimorph (1.0 l/ha) G.S 31
propiconazole + tridemorph (1.0 l/ha) G.S 45
2 complex fungicide spray programme but details not known
3 propiconazole (0.5 l/ha) late Oct/early Nov
fenpropimorph (1.0 l/ha) before spring barley emerges
carbendazim+prochloraz (1.5 l/ha) + Corbel (1.0 l/ha) G.S 31
propiconazole (0.5 l/ha) G.S 49
+ some cultivars fuberidazole+triadimenol seed dressing

trial inoculum density represented the main plot and cultivar the sub-plot. The layout of the two small plot field experiments are shown in Appendices 3.1 and 3.2. Plots were scored visually for percentage leaf area covered in mildew and browning on the top four leaves after the flag leaf had emerged (small plot experiments excepted).

In 1984 levels of mildew were recorded from data obtained from the Crop Production Advisory and Development Unit (CPAD) at Bush Estate. Where levels of mildew on the susceptible cultivars Golden Promise and Gerbel (Wolfe, Slater and Minchin, 1981) were above 10% (Leadbeater and McHale, 1987), results were analysed using the general statistics package Genstat 4.04. If levels were below 10% no statistical analyses were carried out, since most of these levels were between 0 and 2% and no further information would be gained from analysis (A. Hunter, pers. comm.). Statistical analyses were carried out on the percentage data and on angular transformations of the data (angular transformation = 180/PI x ARCSIN ($\sqrt{x}$/100) where x is a percentage) (Alvey, Galway and Lane, 1982). Except where indicated results are shown as angles.

The results are considered under four headings, as follows:

3.3.1 Incidence of browning symptoms in NL/RL field trials with spring barley cultivars in relation to year, site, general mildew incidence, cultivar and fungicide treatment.

3.3.2 Incidence of browning symptoms in second year (NL2) field trials on winter barley cultivars in relation to year, site, general mildew incidence, cultivar and fungicide treatment.

3.3.3 Incidence of browning symptoms in first year (NL1) field trials on winter barley cultivars in relation to site, general mildew incidence and cultivar.

3.3.4 Small plot field experiments to observe the incidence of mildew and browning on the leaves of selected spring barley cultivars treated or untreated with fungicide.

3.3 RESULTS

3.3.1 Incidence of browning symptoms in NL/RL field trials with spring barley cultivars in relation to year, site, general mildew incidence, cultivar and fungicide treatment

(a) Year and site effects

Levels of mildew and browning in relation to year, site and fungicide treatment are shown in Table 3.2. Mildew was generally more frequent in 1984 than in 1985 and was present in only slight amounts in 1986. From the data available, mildew was effectively controlled by fungicide treatment. The incidence of browning was greater in 1984 and 1985 compared with that in 1986: fungicide treatment reduced the average amounts in all three years but the extent of reduction differed between years.

TABLE 3.2: Mean percentage leaf area (angular transformation) affected by mildew (M) and browning (B) on spring barley cultivars untreated (-) or treated (+) with fungicide, grown at eight trial sites in the south-east of Scotland during 1984 to 1986.

	1984				1985				1986			
	M		B		M		B		M		B	
Trial site	-[a]	+	-	+	-	+	-	+	-	+	-	+
Ploughlands	7	-	9	5	-	-	-	-	-	-	-	-
Bush	11	-	13	<1	9	<1	10	6	4	<1	7	4
Lacceston	10	-	9	5[b]	-	-	-	-	-	-	-	-
Tarrylaw	9	-	16	4[b]	-	-	-	-	-	-	-	-
Stichill	-	-	-	-	3	<1	12	8	2	<1	5	3
St Fort	-	-	-	-	4	<1	11	8	-	-	-	-
Elvingston	-	-	-	-	-	-	-	-	<1	<1	5	4
Woodhill	-	-	-	-	-	-	-	-	<1	<1	6	3

a - data obtained from CPAD; b - 1 replicate only

In all three years the levels of mildew and browning varied between sites but not necessarily in the same way. Thus, mildew was generally more severe at Bush but only in 1986 was the extent of browning greatest at this site. The reduction in browning achieved with fungicide was broadly similar at different sites within any one year, except in 1984 when the reduction in browning was greater at the Bush site than at others.

(b) Cultivar and fungicide treatment effects

(1) Sites showing a high incidence of mildew on untreated susceptible cultivars

The mean levels of mildew and browning on cultivars common to sites showing high levels of mildew are shown in Table 3.3 and Figure 3.2 (Appendix 3.3). No data were considered for mildew from fungicide treated plots as the disease incidence was negligible for all cultivars. The extent of browning was on average also substantially reduced by fungicide treatments but not completely eradicated.

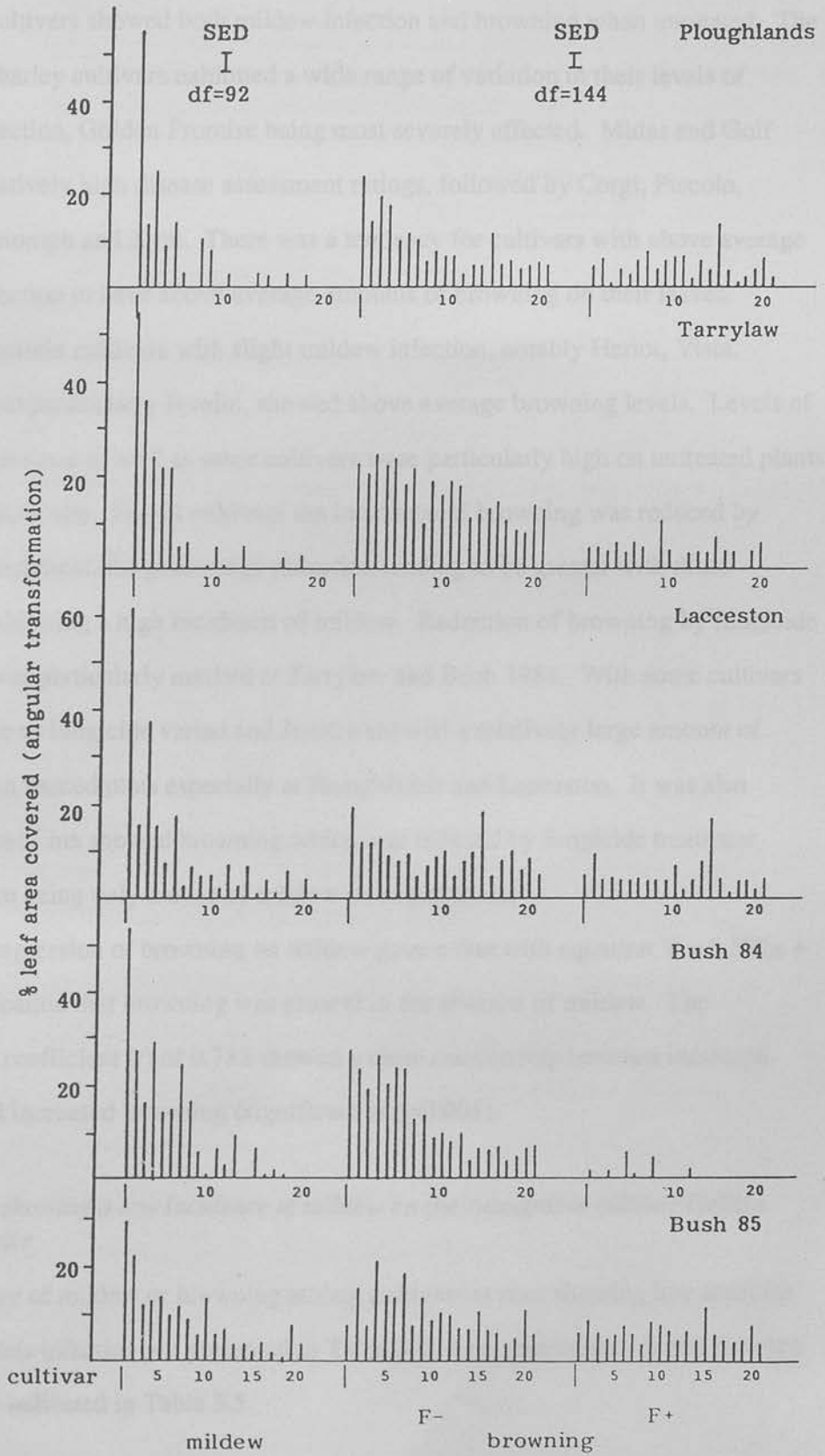
TABLE 3.3: Percentage leaf area (angular transformation) showing mildew and brown lesions for spring barley cultivars untreated (-) or treated (+) with fungicide and grown at sites showing a high incidence of *E. graminis* infection on Golden Promise (averaged for five sites).

Number	Cultivar	Mildew		Browning	
		-	+	-	+
1	Golden Promise	51	*	21	4
2	Midas	24	*	17	6
3	Golf	23	*	17	3
4	Corgi	18	*	21	4
5	Piccolo	15	*	16	4
6	Tasman	10	*	16	6
7	Triumph	9	*	17	5
8	Kym	9	*	8	2
9	Heriot	5	*	12	7
10	Vista	5	*	10	5
11	Acclaim	4	*	11	4
12	Natasha	4	*	9	3
13	Nairn	4	*	8	5
14	Celt	3	*	7	4
15	Javelin	2	*	13	10
16	Tweed	2	*	8	4
17	Themis	2	*	7	2
18	Klaxon	2	*	6	4
19	Delta	2	*	5	2
20	Doublet	1	*	9	5
21	Lina	<1	*	7	2
SED ±		1.8	between fungicide		1.5
			within fungicide		1.4
(df)		(92)			(144)

* not recorded but negligible in all cases.

FIGURE 3.2: Percentage leaf area (angular transformation) showing mildew and brown lesions for spring barley cultivars untreated (F-) or treated (F+) with fungicides and grown at five sites showing a high incidence of *E. graminis* infection (cultivars ordered as in Table 3.3).

(see overleaf)


SED
I
df=92
SED
I
df=144
Ploughlands
Tarrylaw
Lacceston
Bush 84
Bush 85
% leaf area covered (angular transformation)
cultivar
mildew
F-
F+
browning

All cultivars showed both mildew infection and browning when untreated. The individual barley cultivars exhibited a wide range of variation in their levels of mildew infection, Golden Promise being most severely affected. Midas and Golf showed relatively high disease assessment ratings, followed by Corgi, Piccolo, Tasman, Triumph and Kym. There was a tendency for cultivars with above average mildew infection to have above average amounts of browning on their leaves. However, certain cultivars with slight mildew infection, notably Heriot, Vista, Acclaim, and particularly Javelin, showed above average browning levels. Levels of browning on these as well as other cultivars were particularly high on untreated plants at the Tarrylaw site. For all cultivars the incidence of browning was reduced by fungicide treatment, the percentage reduction tending to be greater with those cultivars exhibiting a high incidence of mildew. Reduction of browning by fungicide treatment was particularly marked at Tarrylaw and Bush 1984. With some cultivars the response to fungicide varied and Javelin showed a relatively large amount of browning on treated plots especially at Ploughlands and Lacceston. It was also observed that Lina showed browning which was reduced by fungicide treatment despite there being only a trace of mildew on this cultivar.

The regression of browning on mildew gave a line with equation $Y = 0.322x + 8.7$, an indication that browning was present in the absence of mildew. The correlation coefficient (r) of 0.788 showed a close relationship between increased mildew and increased browning (significant at $p=0.001$).

(2) Sites showing a low incidence of mildew on the susceptible cultivar Golden Promise

The presence of mildew or browning among cultivars at sites showing low amounts of *E. graminis* infection are presented in Table 3.4. The approximate levels for each cultivar are indicated in Table 3.5.

TABLE 3.4: Numbers of plots of spring barley cultivars showing presence or absence of mildew pustules and brown lesions on their leaves, for plots untreated (F-) or treated (F+) with fungicide, grown at sites with less than 10% mildew infection on Golden Promise.

Site	Mildew*	Browning*			
		F-		F+	
		+	-	+	-
St Fort	+	17	0	1	0
1985	-	2	0	18	0
Stichill	+	13	0	1	0
1985	-	6	0	18	0
Woodhill	+	1	0	1	0
1986	-	28	0	23	5
Elvingston	+	8	0	2	0
1986	-	21	0	27	0
Stichill	+	9	0	0	0
1986	-	10	0	18	1
Bush	+	14	0	0	0
1986	-	5	0	19	0
Total	+	62	0	5	0
	-	72	0	123	6

* mildew or browning present (+) or absent (-)

From the total assessments it can be seen that of cultivar plots untreated with fungicide, 62 (46%) gave both mildew and browning on leaves, whereas 72 (54%) showed browning in the absence of mildew. No plot was completely free of browning over the total assessments of untreated plots. If treated with fungicides, 123 plots assessed (92%) produced browning in the absence of mildew, with five (4%) still showing the presence of mildew along with browning. However, in six cases neither mildew nor browning occurred. Thus, although treatment with fungicide eradicated mildew on most plots, it rarely eliminated browning.

TABLE 3.5: Indices of levels of mildew and brown lesions on leaves of spring barley cultivars, untreated (F-) or fungicide treated (F+), at sites with less than 10% mildew infection on Golden Promise (percentage leaf area covered; angular transformation).

Cultivar (No. of sites tested in brackets)		Mildew		Browning	
		F-	F+	F-	F+
Golden Promise	(6)	5-10	0	10-15	5-10
Cameo	(2)	5-10	0	5-10	5-10
Golf	(6)	5-10	0	5-10	1-5
Vista	(6)	1-5	<1	5-10	5-10
Acclaim	(2)	1-5	0	15-20	10-15
Corniche	(6)	1-5	0	10-15	5-10
Triumph	(6)	1-5	0	10-15	5-10
Heriot	(6)	1-5	0	5-10	5-10
Corgi	(6)	1-5	0	5-10	5-10
Celt	(6)	1-5	0	5-10	1-5
Natasha	(6)	1-5	0	5-10	1-5
Tweed	(6)	1-5	0	5-10	1-5
Flute	(6)	1-5	0	5-10	1-5
Klaxon	(6)	1-5	0	5-10	1-5
Kym	(6)	1-5	0	5-10	1-5
Doublet	(6)	<1	0	5-10	5-10
Blenheim	(4)	<1	0	5-10	5-10
Regatta	(6)	<1	0	5-10	5-10
Clansman	(2)	0	1-5	5-10	1-5
Goldpiece	(2)	0	0	10-15	10-15
Camargue	(6)	0	0	5-10	5-10
Shadow	(2)	0	0	5-10	5-10
Spirit	(2)	0	0	5-10	5-10
Sherpa	(6)	0	0	5-10	1-5
Formula	(2)	0	0	5-10	1-5
Fox	(2)	0	0	5-10	1-5
Prisma	(2)	0	0	5-10	1-5
Oasis	(2)	0	0	5-10	1-5
Ayr	(4)	0	0	1-5	1-5
Joline	(2)	0	0	1-5	1-5
Kingpin	(2)	0	0	1-5	0

The numbers of cultivars developing symptoms differed at each site. At St Fort and Stichill, 1985, and Bush, 1986, most of the cultivars on untreated plots were infected with mildew; at Stichill, 1986, numbers of cultivars with or without mildew were similar while at Woodhill, 1986, and Elvingston, more cultivars were uninfected. The lowest frequency of mildew on untreated plots occurred at Woodhill, but five of the 28 cultivars which showed no mildew but the presence of browning, when untreated, gave no browning when fungicide was applied.

Table 3.5 shows the responses of individual cultivars: thirteen out of the thirty-one cultivars did not show mildew symptoms in the absence of fungicide but all except fungicide-treated Kingpin showed browning symptoms. Of all the cultivars assessed, Acclaim and Goldpiece tended to show most browning. The regression of browning on mildew for fungicide untreated plots gave a line with the equation $Y = 0.577x + 6.7$. Again high levels of browning were present in the absence of mildew. The correlation coefficient $r = 0.423$ ($p=0.05$) indicated a relationship between mildew and browning but this relationship was poor. There was, however, a trend towards reduced browning with fungicide application.

3.3.2 Incidence of browning symptoms in second year (NL2) field trials on winter barley cultivars in relation to year, site, general mildew incidence, cultivar and fungicide treatment

Winter barley cultivars in the second year of trials were observed in 1985 and 1986. The differences between the two years and the various sites are shown in Table 3.6. Mildew occurred much more frequently in 1985 compared with 1986, while browning tended to be only slightly greater in the earlier year. Differences in mildew between sites in 1985 were significant (Appendix 3.4), whereas those in 1986 were slight. Fungicide treatment substantially reduced mildew but reduced browning only slightly.

TABLE 3.6: Mean percentage (angular transformation) leaf area affected by mildew and browning on NL2 winter barley cultivars untreated (F-) or treated (F+) with fungicide in relation to year and site.

Site	Year							
	1985				1986			
	Mildew		Browning		Mildew		Browning	
	F-	F+	F-	F+	F-	F+	F-	F+
St Fort	18	3	16	9	-	-	-	-
Stichill	11	1	12	8	<1	0	11	7
Woodhill	-	-	-	-	2	0	10	6

Since levels of mildew were much higher in 1985 compared with those in 1986, the two sets of data were treated separately.

Analysis of results for 1985 (Appendix 3.4) indicated that, for cultivars common to the two sites, significant differences in infection levels were evident. Fungicide reduced mildew on all cultivars but there was a significant interaction between fungicide and cultivar, with different degrees of response to fungicide for the different cultivars (Table 3.7). With respect to browning, the average incidence was only very slightly reduced by fungicide: there was a significant interaction between fungicide treatment and cultivar (Appendix 3.4) with only certain cultivars showing a sigificant reduction with fungicide treatment. It was observed that CWB 117/77/9/7 and CWB 117/5/9/5 produced very clear spotting symptoms on leaves (Table 2.1) which, although reduced by fungicide treatment, were still produced extensively. Cultivars Magie and Marinka also exhibited distinctive spotting of the leaves which was only slightly reduced by fungicide: in the case of Marinka symptoms were confined to the upper two leaves.

TABLE 3.7: Percentage leaf area (angular transformation) showing mildew and brown lesions for NL2 winter barley cultivars untreated (F-) or treated (F+) with fungicide and grown at sites showing a high incidence of *E. graminis* infection on Gerbel (averaged for two sites).

Cultivar	Mildew		Browning	
	F-	F+	F-	F+
Gerbel*	30	4	20	8
Gerbel	29	4	18	8
Curlew	26	4	11	5
Maris Otter	21	4	9	6
Maris Otter*	20	6	15	7
Podium	20	3	18	12
CWB 117/77/9/7	19	4	21	13
CWB 40/1/8/6	18	2	17	10
Kaskade*	18	2	18	10
Pirate*	17	2	16	7
Nevada*	16	2	12	8
MMG 7802/14	14	3	12	7
Magie*	14	2	18	9
Panda*	14	1	16	7
Serenade	14	1	9	4
Igri*	13	2	12	8
Panda	12	3	15	6
Igri	12	2	13	8
UN 480	12	1	14	8
Marinka*	12	0	16	12
CWB 22/6/13	11	2	13	9
Torrent	11	1	18	12
CWB 117/5/9/5	11	0	16	12
Halcyon*	9	2	8	5
Vixen	8	3	15	10
NFC 113/1/80	8	1	14	9
Mallard	7	2	8	7
Opera	5	1	14	8
Pipkin	3	0	11	5
SED± between fungicide		2.6		2.2
within fungicide		2.4		1.7
(df)		(112)		(112)

* seed treated with fuberidazole + triadimenol

The regression of browning on mildew ($Y = 0.220X + 11.1$) again indicated the presence of browning in the absence of mildew with the correlation ($r = 0.416$), being in this case poor ($p=0.05$).

A summary of results for 1986 is presented in Table 3.8.

TABLE 3.8: Numbers of NL2 winter barley cultivars showing presence or absence of mildew pustules and brown lesions on their leaves, for plots untreated (F-) or treated (F+) with fungicide, grown at sites with less than 10% mildew infection on Gerbel.

Site	Mildew	Browning			
		F-		F+	
		+	-	+	-
Woodhill	+	14	0	0	0
	-	17	0	31	0
Stichill	+	3	0	0	0
	-	28	0	30	1
Total	+	17	0	0	0
	-	45	0	61	1

+ presence of mildew or browning
- absence of mildew or browning

Of the untreated plots grown at the two sites, almost 75% showed browning in the absence of mildew, the remainder showing mildew and browning together. In all fungicide treated plots mildew was not recorded and all but one showed some browning of leaves. The two sites showed differences in mildew incidence: at Woodhill approximately 45% of cultivars, when untreated with fungicide, showed mildew but at Stichill only 10% of the cultivars when untreated had any signs of mildew infection.

Full details of individual cultivar responses are shown in Table 3.9. Eleven cultivars had mildew infection between 1 and 5 (per cent, angular transformation), with three showing less than 1 and the rest nil. Browning was seen on all cultivars, untreated or fungicide treated, particularly Plaisant untreated with fungicide and the general level of browning tended to be reduced by fungicide treatment.

Regression of browning on mildew for untreated plots gave a line with equation $Y = -1.410X + 3.6$. The correlation coefficient $r = -0.251$ ($p>0.05$) indicated the absence of a relationship between mildew and browning.

TABLE 3.9: Indices of levels of mildew and brown lesions on leaves of NL2 winter barley cultivars, untreated (F-) or fungicide treated (F+), at sites with less than 10% mildew infection on Gerbel (percentage leaf area covered; angular transformation).

Cultivar	Mildew		Browning	
	F-	F+	F-	F+
Gerbel	1-5	0	10-15	5-10
Kaskade	1-5	0	10-15	5-10
Igri	1-5	0	10-15	5-10
Magie	1-5	0	5-10	5-10
Sec 9911GH	1-5	0	5-10	1-5
FR 207/1	1-5	0	5-10	5-10
Marinka	1-5	0	5-10	5-10
Maris Otter	1-5	0	5-10	1-5
Sec 8747GH	1-5	0	5-10	5-10
Halcyon	1-5	0	5-10	1-5
CWB 240/331/4	1-5	0	5-10	5-10
Pirate	<1	0	10-15	5-10
CWB 240/187/5	<1	0	10-15	5-10
BR 481 B42	<1	0	5-10	1-5
Plaisant	0	0	15-20	5-10
Torrent	0	0	10-15	5-10
NFC 69/2/80	0	0	10-15	5-10
MMG 8050/1200	0	0	10-15	5-10
MMG 7809/79	0	0	10-15	5-10
Opera	0	0	10-15	5-10
Nevada	0	0	10-15	5-10
CWB 193/52/1	0	0	10-15	5-10
MMG 7802/4/6	0	0	10-15	5-10
ACK 75/456/12	0	0	10-15	5-10
G4114	0	0	10-15	1-5
NFC 97/6/80	0	0	5-10	5-10
Vixen	0	0	5-10	5-10
Fallon	0	0	5-10	5-10
Cebeco 7927	0	0	5-10	5-10
Panda	0	0	5-10	1-5
Pipkin	0	0	1-5	<1

3.3.3 Incidence of browning symptoms in first year (NL1) field trials on winter barley cultivars in relation to site, general mildew incidence and cultivar

Levels of mildew and browning on NL1 cultivar trials where no fungicides were used, were scored only in 1985. The results are summarised in Tables 3.10 and 3.11 and analysis of the data presented in Appendix 3.5. Mildew and browning were prevalent at both sites, with a somewhat higher browning to mildew ratio at Stichill. Mildew infection was most severe on Jeff and Gerbel which also showed relatively large amounts of browning. Certain cultivars, however, showed extensive browning with low rates of mildew infection, notably MMG 8050/1200. The regression of browning on mildew gave a line with equation $Y = 0.290X + 12.0$, showing browning in the absence of mildew, but the correlation coefficient of 0.458 ($p=0.001$) indicated a relationship between an increase in mildew and an increase in browning.

TABLE 3.10: Mean percentage (angular transformation) leaf area affected by mildew and browning on NL1 winter barley cultivars grown at two sites.

	Mildew	Browning
St Fort	14	16
Stichill	9	14

3.3.4 Small plot field experiments to observe the incidence of mildew and browning on the leaves of selected spring barley cultivars treated or untreated with fungicide

Most cultivars in two small plot studies were already identified from National List trials as being prone to exhibit browning of leaves.

For the 1985 study, the results of assessments carried out at intervals from about 6 to 15 weeks after sowing for percentage leaf area covered in mildew and browning are illustrated in Figure 3.3. On all cultivars the general trend was for both mildew and browning to increase with time after sowing. Cultivars Golden Promise, Koru and Midas all had mildew levels higher than browning levels, whereas for

TABLE 3.11: Percentage leaf area (angular transformation) showing mildew and brown lesions for NL1 winter barley cultivars, grown in 1985.

Cultivar	Mildew	Browning
Jeff	31	22
Gerbel	22	22
Sec 991GH	18	17
CWB 269/223/2	19	18
Maris Otter	17	14
SES 65/81	16	16
4617 GH	16	14
Igri	13	15
Sec 8747GH	13	19
NFC 27/1/82	12	12
ACK 75/456/12	11	16
BR 521	11	17
Firlbeck 4500	11	12
RG 5458	10	12
CWB 240/331/4	6	14
LP 282/70	8	12
Panda	8	17
MMG 7802/416	8	18
CWB 240/187/5	9	13
CEBECO 7927	7	14
NFC 69/2/80	6	14
MMG 8050/1200	5	21
G4114	6	14
BR 481 B42	6	12
MMG 7809/79	6	11
MMG 8053/1200	6	9
SED ±	2.81	1.73
(df)	(54)	(54)

cultivars Triumph, Carnival and Tasman the opposite was true. Although mildew did not appear until about 6 weeks after sowing, browning of leaves was already evident at this time, especially for cultivars more prone to show symptoms.

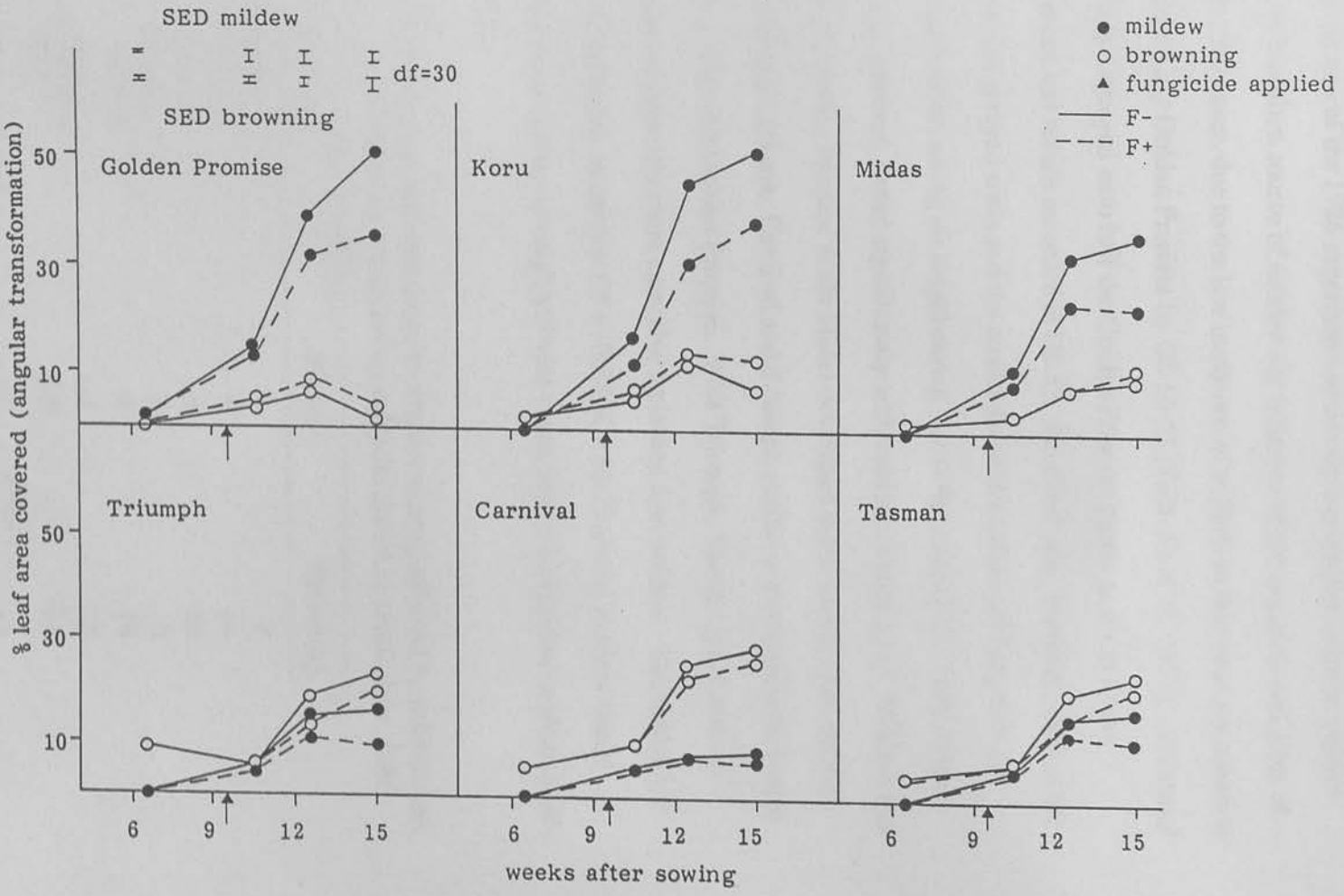
Analyses of the results (Appendix 3.6) indicated significant main effects of fungicide and cultivar on mildew with an interaction between fungicide and cultivar at about 13 weeks. Only cultivar significantly affected browning but there was a significant interaction between cultivar and fungicide at 13 and 15 weeks. The data for individual cultivars at 12.5 weeks are shown in Table 3.12.

TABLE 3.12: Percentage leaf area (angular transformation) affected by mildew and browning of six spring barley cultivars, untreated (F-) or treated (F+) with ethirimol, 12.5 weeks after sowing.

	Mildew		Browning	
	F-	F+	F-	F+
Koru	46	31	12	15
Golden Promise	39	31	6	8
Midas	33	24	8	9
Tasman	17	13	21	16
Triumph	16	11	19	13
Carnival	8	8	26	23
SED between cultivar	2.7		1.7	
± within cultivar	2.7		1.9	
(df)	(30)		(30)	

Funigicide treatment showed its greatest effect on mildew incidence with mildew susceptible cultivars Koru, Golden Promise and Midas, but reduced browning only on cultivars showing a lower incidence of mildew, the reduction being significant only with Tasman and Triumph. The general pattern was towards high mildew/low browning or low mildew/high browning with b = -0.407 for untreated and b = -0.328 for ethirimol treated cultivars in the regression of browning on mildew and a negative correlation (r = -0.764 and -0.576 for F- and F+ respectively) between

FIGURE 3.3: Percentage leaf area covered in mildew and browning of spring barley cultivars untreated (F-) or treated (F+) with ethirimol (angular transformation).



the two factors, both showing a relationship between increased mildew and reduced browning (p=0.001).

It was the aim of the 1986 experiment to use the susceptible cultivar Golden Promise as an inoculum source of mildew for infection of the experimental plots of test cultivars. However, due to the low incidence of mildew in this year, no infection had appeared on any Golden Promise by GS 50-55 (Zadocks *et al.*, 1974). Ethirimol was nevertheless sprayed onto half the Golden Promise plants to act as a non-inoculum source and results recorded at GS 85. No effect was, however, seen of the treatments on the sprayed areas and the results showed no effect of fungicide on levels of mildew or browning on neighbouring plots (Appendix 3.7): both mildew and browning, however, varied significantly with cultivar (Table 3.13). Mildew was most severe on Golden Promise while Midas developed significantly more mildew than the remaining cultivars. Carnival and Triumph exhibited the highest incidence of browning along with Golden Promise. With Triumph, Tasman and Carnival browning was substantially more extensive on leaves than mildew. The calculated regression of browning on mildew ($Y = -0.879X + 16.7$) tended to show that if mildew was low browning was high and vice versa, with a correlation coefficient of -0.383 (p=0.05).

TABLE 3.13: Percentage leaf area (angular transformation) affected by mildew and browning of six spring barley cultivars grown in small plots, 1986.

Cultivar	Mildew	Browning
Midas	9	8
Triumph	5	16
Tasman	5	10
Javelin	4	6
Carnival	4	18
SED ±	1.4	1.2
(df)	(24)	(24)
Golden Promise	14	14
SED ±	3.1	4.4
(df)	(3)	(3)

It should be noted that cultivar Javelin showed 'thumb-print' lesions (Plate 2.1) on one or two plants per plot only. The rest developed a more general spotting, as did all other cultivars.

3.4 DISCUSSION

Surveys of winter and spring barley field trials in south-east Scotland failed to show a clearly defined or consistent relationship between the extent of mildew infection and of brown lesions on the leaves of barley plants. In some cases a positive correlation between the two factors was found and in other cases a negative one. There was a trend towards a closer correlation between mildew and browning at moderate mildew levels compared with that at low mildew levels which was possibly related to mildew induced senescence (Table 3.14).

TABLE 3.14: Correlation of browning on mildew associated with moderate or low mildew levels on untreated barley plots (averaged over several sites).

Crop	Mildew level	Correlation coefficient	Probability
SB	moderate	0.788	0.001
	low	0.423	0.05
WB NL2	moderate	0.416	0.05
	low	-0.251	0.05
WB NL1	moderate	0.458	0.001

moderate - angular transformation 9-15
low - angular transformation <9

However, when the reductions in browning due to fungicide application were compared at high, moderate and low mildew sites there were no marked trends (Table 3.15), and the reduction in browning was variable for different mildew levels, suggesting that factors as well as mildew were involved.

TABLE 3.15: The effect of fungicide treatment on overall levels of browning at trial sites according to mildew levels found on untreated plots.

Mildew levels	Site	Crop	% Reduction in levels of browning
High	St Fort	WB	43
Moderate	Bush 84	SB	92*
	Tarrlaw	SB	75*
	Lacceston	SB	44*
	Bush 85	SB	40
	Stichill 85	WB	33
Low	Ploughlands	SB	44*
	Stichill 85	SB	33
	St Fort	SB	75
	Bush 86	SB	43
	Stichill 86	SB	40
	Elvingston	SB	20
	Woodhill	SB	50
	Stichill 86	WB	36
	Woodhill	WB	40

High - angular transformation >15
Moderate - angular transformation 9-15
Low - angular transformation <9

*note that mildew was scored on different date to browning at these sites.

The behaviour of individual cultivars with respect to their expression of mildew and browning, moreover, was not always the same. However, some broad groupings of cultivars can be made. For spring and winter barley cultivars three distinct groups may be postulated (Table 3.16), those with high, moderate or low levels of mildew infection. Within each group cultivars could be sub-divided according to levels of browning on leaves.

TABLE 3.16: Grouping of spring and winter barley cultivars according to level of mildew and browning found on leaves.

Levels of mildew	Levels of browning	Cultivars			
		Spring		Winter	
High	High	Golden Promise	F	Gerbel	F
		Midas	F	Podium	F
		Golf	F	Jeff	*
		Corgi	F	CWB 117/77/9/7	F
				CWB 269/223/2	*
				SEC 991GH	*
				SES 65/81	*
	Moderate			Curlew	F
				Maris Otter	F
				4617GH	*
	Low			Kaskade	F
Moderate	High	Piccolo	F	Pirate	F
		Triumph	F	Serenade	F
				Panda	F
				CWB 40/1/86	*
				SEC 8747GH	*
				ACK 75/456/12	*
				BR 521	*
	Moderate	Tasman	F	Igri	F
				Nevada	
				Magie	F
				Firlbeck 4500	*
				CWB 117/5/9/5	
				MMG 7802/14	F
				NFC 27/1/82	*
				RG 5458	*
	Low	Kym	F		
	High			CWB 193/52/1	*
				MMG 7802/416	*
				MMG 8050/1200	*

Table 3.16 (continued)

Levels of mildew	Levels of browning	Cultivars			
		Spring		Winter	
Low	Moderate	Vista	F	Torrent	F
		Heriot	F	Halcyon	
		Natasha	F	Vixen	F
		Acclaim	F	Mallard	
		Javelin		Opera	F
				UN 480	F
				CWB 240/331/4	*
				NFC 97/6/80	*
				LP 282/70	*
				CWB 240/187/5	*
				CEBECO 7927	*
				NFC 69/2/80	*
				G4114	*
				BR 481 B42	*
				MMG 7809/79	*
	Low	Nairn		Marinka	
		Celt		Pipkin	F
		Themis	F	CWB 22/6/13	
		Klaxon		MMG 8053/1200	*
		Doublet	F	NFC 113/1/80	F
		Delta			
		Tweed	F		
		Lina	F		

F - cultivars where browning was significantly reduced with fungicide treatment

High - percentage leaf area (angular transformation) greater than 15

Moderate - angular transformation 9-15

Low - angular transformation less than 9

* - winter barley cultivars not treated with fungicide

In the case of cultivars in the high mildew grouping, both mildew and browning were substantially reduced upon treatment with fungicide, suggesting that browning was attributable to the effects of the mildew fungus. This type of browning is associated with the later stages of mildew infection of susceptible or poorly resistant

cultivars; it may be considered as mildew induced senescence (Yarwood, 1957), referred to previously, and assigned to category 6 (Section 2). The browning symptom is usually easily attributable to mildew because of the presence of mycelium, although with weathering of the leaves and the resulting removal of surface fungal growth the involvement may become less apparent. Thus where assessments followed heavy rain, there would tend to be a low ratio of mildew relative to browning.

Browning on most barley cultivars in the moderate and low mildew groupings was reduced by fungicide sprays. Cultivars in the moderate mildew group tended to vary in their responses, Kym and Igri tending to show senescent browning with Triumph, Tasman and CWB 117/5/9/5 showing more distinct lesions. Cultivars in the low mildew category tended to show either very distinct brown symptoms, e.g. Heriot, Acclaim, Javelin and Marinka, or showed only a slight tissue response to mildew infection. Browning on most cultivars exhibiting distinct lesions was reduced by fungicide treatment but never to the extent of that on cultivars in the high mildew group. Also, although the extent of browning was reduced the cultivar-specific responses were always evident, even if only 1 or 2 lesions per plant.

It could be argued that small levels of mildew present before fungicides were applied are enough to elicit these few distinct lesions or that the fungicide did not act quick enough to prevent a resistance response being induced. Most of the fungicides used in the survey, fenpropimorph, propiconazole, tridemorph, carbendazim and prochloraz, allow a few cellular divisions to occur before interfering with ergosterol biosynthesis, a process necessary for cell division and expansion in many fungi (Sisler and Ragsdale, 1984). The hypersensitive resistance response to mildew infection is generally elicited where fungal walls contact host cell membranes and it is possible that enough fungal development may occur for the necrogenic response to be initiated before the fungicides act. However, an exceptionally large number of

infection sites would be necessary to induce visible symptoms of browning under such circumstances and would be unlikely to occur; also, there was no evidence of a gradient of browning from plot margins which one would expect if a large amount of avirulent inoculum was being introduced into plots of such cultivars from neighbouring susceptible cultivars. Many of the cultivars were grown in small plot trials yet none showed the distinct lesions under these circumstances. Moreover, it has been suggested that active resistance responses are not induced, due to suppression of primary infection processes, where some systemic fungicides are applied (Kradel, Pommer and Effland, 1969).

Proportionally fewer winter barley cultivars tended to show a low incidence of browning, possibly due to the longer period of exposure in the field. Moreover, the tendency for poorer correlations between mildew and browning for winter barley (Table 3.14) than for spring barley may relate to the greater opportunity for weathering in the former case.

It is evident from the work carried out here that in some cultivars there is a positive relationship between mildew infection and the level of browning on leaves. In others the involvement of mildew is less clear or absent.

SECTION 4

FURTHER INVESTIGATIONS INTO THE INVOLVEMENT OF *ERYSIPHE GRAMINIS* INFECTION IN THE DEVELOPMENT OF NECROTIC LESIONS ON BARLEY LEAVES

4.1 INTRODUCTION

Erysiphe graminis, the fungal organism responsible for powdery mildew disease of barley, other cereals and grasses, is the most important barley leaf pathogen in Britain today, causing considerable yield losses (King, 1972; King, 1974; Cock, 1975). The essentials of the infection process and asexual life cycle of *E. graminis* have been known for many years (Yarwood, 1957). Briefly, conidiospores (conidia) are deposited on the leaf surface, germinate (under favourable conditions) and produce a germ tube at the end of which a club-like swelling, an appressorium, develops and is attached to the host surface through the action of an adhesive substance. A fine infection hypha, or infection peg, grows from the undersurface of the appressorium and penetrates the host cuticle and epidermal cell wall through the action of enzymatic digestion and physical pressure (Masri and Ellingboe, 1966b; Albersheim, Jones and English, 1969; Edwards and Allen, 1970). Once inside the host epidermal cell (but not penetrating the plasma-membrane) the infection peg swells into a round structure with digitate appendages (Wood, 1967; Jones and Clifford, 1978; Carver and Williams, 1980). This structure, the haustorium, is involved in food absorption from the host cytoplasm, across the extrahaustorial matrix, into the fungus (Bushnell, 1971; Manners and Gay, 1983). At the same time as haustorial development a secondary hypha is produced from the appressorium and grows and branches along the surface of the host leaf, at intervals attempting further penetrations (Masri and Ellingboe, 1966a; McCoy and Ellingboe, 1966; Yang and Ellingboe, 1972). Hyphae grow from the spore-mother cell in a radial pattern producing the typical rounded pustule. Three to four days after colony initiation, condiophores bearing chains of spores develop at right angles to the colony, giving the pustule its typical powdery appearance (Cole, 1976; Jones and Clifford, 1978). The necrosis of infected tissues occurs only at an advanced stage of fungal infection and 'green island' effects are common (Bushnell and Gay, 1978).

The above represents the susceptible (compatible) reaction between pathogen and host. However, the host plant can resist infection by several means. In the case of barley plants infected with *E. graminis* f. sp *hordei*, defence is generally initiated after germination and appressorial formation, although some workers have shown appressoria may be replaced by long distorted germ tubes (Masri and Ellingboe, 1966b; Edwards, 1970; McKeen and Bhattacharya, 1970). After formation of appressoria, but prior to infection peg development, host cytoplasm aggregates beneath the appressorium and an electron dense material is deposited on the inner surface of the epidermal cell wall (Edwards, 1970). This deposit forms a structure known as the papilla and consists of callose, basic staining material, inorganic elements and an auto-fluorescent substance (Edwards and Allen, 1970; Mayama and Shishiyama, 1978). The formation of the papilla during infection is, however, a common phenomenon and may or may not be involved in resistance (Munro, 1985).

Masri and Ellingboe (1966b) considered that the formation of a functional haustorium was essential for a compatible reaction between mildew and its barley host, very few (4-35%) being produced from germinated conidia in resistant reactions. McKeen and Bhattacharya (1970) described a host-pathogen relationship where functional haustoria and elongating secondary hyphae were produced and only then did degeneration of structures occur in the resistant reaction. Ellingboe (1972) observed a different reaction and reviews by Day (1974) and Bushnell (1982) indicated that different resistance genes condition different resistance responses.

The genetics of barley/*E. graminis* interactions follow the gene-for-gene hypothesis (Flor, 1946; Flor, 1955; Ellingboe, 1972; Ellingboe, 1979). Major resistance genes in barley include Mla, Mlg, Mlk and Mlp: at the Mla locus there are at least twelve alleles and two alleles for each of the other loci have been identified (Ellingboe, 1978). The presence of some minor genes may modify the expression of resistance of major genes (Russell, 1978). The Mlk and Mlp genes allow haustoria on

the pathogen similar to those of a compatible reaction whereas Mla and Mlg genes cause retardation of haustorial formation (Masri and Ellingboe, 1966b). The visible signs of resistance are also dependent on the genes present (McCoy and Ellingboe, 1966). Necrotic spotting indicates the resistance response of barley leaves to mildew infection, but spots can also be produced when conditions are unfavourable for fungal growth (Benada, 1965). Resistance may also be associated with chlorotic spotting or reduced vigour of fungal growth (McCoy and Ellingboe, 1966; Benada, 1969). In some cases colonies may develop as the result of one susceptible cell surrounded by several collapsed necrotic cells (Carver and Williams, 1980). In such an instance the hyphae 'leap-frog' the collapsed cells and attempt further penetrations.

The necrogenic, hypersensitive reaction may be involved in three types of resistance: seedling plant resistance, adult plant resistance and field resistance. Seedling resistance is very specific, with seedling leaves being more resistant than mature leaves (Jones and Hayes, 1971). Adult plant resistance can be effective against several races of the pathogen, being non-specific (Király, Klement, Solymosy and Voros, 1974). This type of resistance can, however, be affected by the environment (Jenkyn and Bainbridge, 1978). Field resistant plants may show susceptibility in experimental conditions but in the field they are consistently less severely infected (Jones and Hayes, 1971). Both seedling and adult plant resistance involve the incorporation of major genes into the host genotype (Jones and Clifford, 1978). However, due to its liability to be overcome on a general field scale by the pathogen within a few years, the use of major gene resistance has been criticised by several workers who claim partial resistance should be taken more into consideration (Jones and Clifford, 1978; McIntosh, 1978; Wolfe and Barrett, 1980; Habgood and Clifford, 1981).

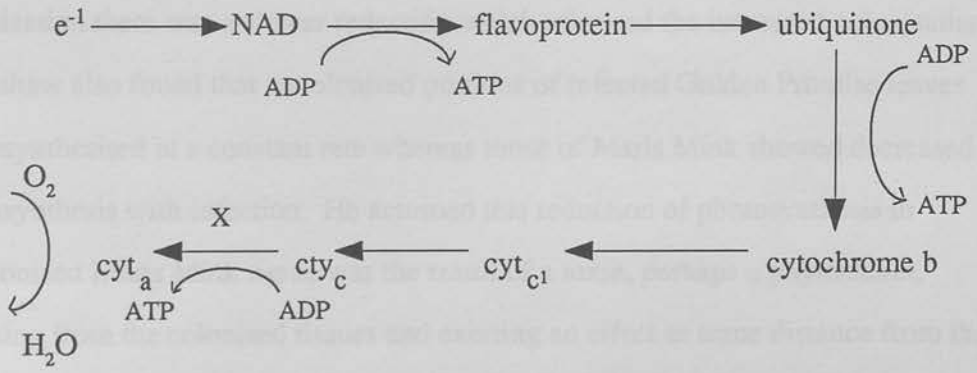
Partial resistance was thought not to occur until adult plant stages and may or may not involve an obvious hypersensitive response, but has now been shown in

seedling leaves, where the major effect is the reduction in the number of colonies, reduced sporulation or failure to produce elongating secondary hyphae (Asher, 1981; Bennett, 1981; Asher and Thomas, 1983; Asher, Thomas and Thomas, 1983). Asher and Thomas (1983) claimed such resistance was not always accompanied by death of the infected cell (non-hypersensitive resistance). However, White and Baker (1954) described semi-resistant cultivars which also showed reduced mycelial growth and sporulation but where mesophyll cell collapse and death was extensive.

Induced mutants of barley for resistance to powdery mildew infection have been produced, the resistance being conditioned by a series of allelic recessive genes at the mlo locus on chromosome 4: resistance genes at this locus have been shown to be present in a number of the Ethiopian barleys (Rubin and Artsikhovskaya, 1963; Russell, 1978). These alleles condition an unusual type of chlorotic and necrotic spotting on leaves (Jørgensen, 1975). Until recently, barleys containing this gene were considered to be resistant to all known races of *E. graminis* f. sp *hordei*, the gene preventing growth of the pathogen at all stages with reduced germination, reduced number of colonies and reduced sporulation (Schwarzbach, 1976). However, recent observations in Britain, Austria and Poland have detected mildew isolates with specific pathogenicity to the cultivar Atem which contains the mlo gene in combination with the MlLa gene (Mercer, 1987; Wolfe, Slater and Minchin, 1987).

The hypersensitive reaction involves changes in several complex biochemical pathways, one of the first being that of respiration. In almost all plant diseases invasion is associated with a rise in the rate of respiration (Allen, 1953; Cruickshanks, 1963; Daly, 1976; Uritani and Asahi, 1980). In hypersensitive reacting cells the rise is rapid, peaks within a few days then eventually falls (Shaw and Samborski, 1957). In mildew infected barley leaves respiration peaks at 48 hours, then falls to levels similar to, or below, levels in uninfected leaves after six days (Smedegaard-Petersen and Stølen, 1980). The peak represents a two-fold

increase in oxygen uptake compared with uptake in the healthy control. In susceptible plants the rise in respiration is slower, occurring 3 to 4 days after inoculation (Smedegaard-Petersen, 1980), coinciding with the visual appearance of mildew pustules. This rise in respiration with susceptibility remains high and culminates in senescence. That the early rise in respiration is involved in hypersensitivity was shown by the use of respiratory inhibitors such as phenol urethane, potassium cyanide or narcotics, which prevent or reduce the browning response (Müller, 1959; Tomiyama, 1982). The rapid rise in respiration is associated with an uncoupling of the electron transport chain in which the enzyme polyphenoloxidase plays a significant role (Wood, 1967). In the electron transport chain of healthy tissues cytochrome oxidase acts as the terminal oxidase (Figure 4.1).



X - cytochrome oxidase

FIGURE 4.1: The electron transport system in healthy plant cells (Yudkin and Offord, 1975).

In diseased tissues electrons are diverted from the cytochrome system to phenol biosynthesis (Section 5), with the enzymes polyphenoloxidase, peroxidase or ascorbic acid oxidase acting as the terminal oxidase (Fric̆, 1976). The rate-limiting steps of ATP formation are missed, allowing respiration to proceed at a much more rapid rate (Allen, 1953; Merret and Bayley, 1969). There have been a few reports that

uncoupling is not important in the respiratory rise (Daly, 1976), but with the amount of evidence to the contrary such reports are doubtful.

Accompanying the increase in respiration in host tissues infected with biotrophic organisms is a general decrease in photosynthesis which may be associated with the onset of premature senescence (Roberts and Boothroyd, 1972; Manners and Myers, 1975). The responses of the hypersensitive tissues may differ from non-hypersensitive tissues. Habeshaw (1979) found that on the barley cultivar Golden Promise infected with a compatible race of *E. graminis* f. sp *hordei* (non-hypersensitive reaction), as the percentage leaf area colonised increased so photosynthesis proportionally decreased. However, in Maris Mink infected with mildew, where some necrosis developed, increases, up to 30%, in colonisation gave a correspondingly more rapid reduction in the rate of photosynthesis: with further colonisation there was a slower reduction which mirrored the increased colonisation. Habeshaw also found that uncolonised portions of infected Golden Promise leaves photosynthesised at a constant rate whereas those of Maris Mink showed decreased photosynthesis with infection. He assumed this reduction of photosynthesis in uncolonised Maris Mink tissue was the result of a toxin, perhaps a phytoalexin, diffusing from the colonised tissues and exerting an effect at some distance from the infection court, as has been found by other workers (Dyer and Scott, 1972; Cartwright, Langcake and Ride, 1980). Thus death of cells can lead to reduced photosynthesis, or reduced photosynthesis can lead to chlorosis resulting in senescence, leaf drop and death.

The following section outlines studies carried out in the glasshouse and laboratory to investigate further the involvement of mildew infection and the resistance reaction in the development of brown lesions on barley leaves, the effects on yield and histological investigations into differences between compatible and incompatible reactions.

4.2 MATERIALS AND METHODS

4.2.1 Growth of barley plants (*Hordeum vulgare*)

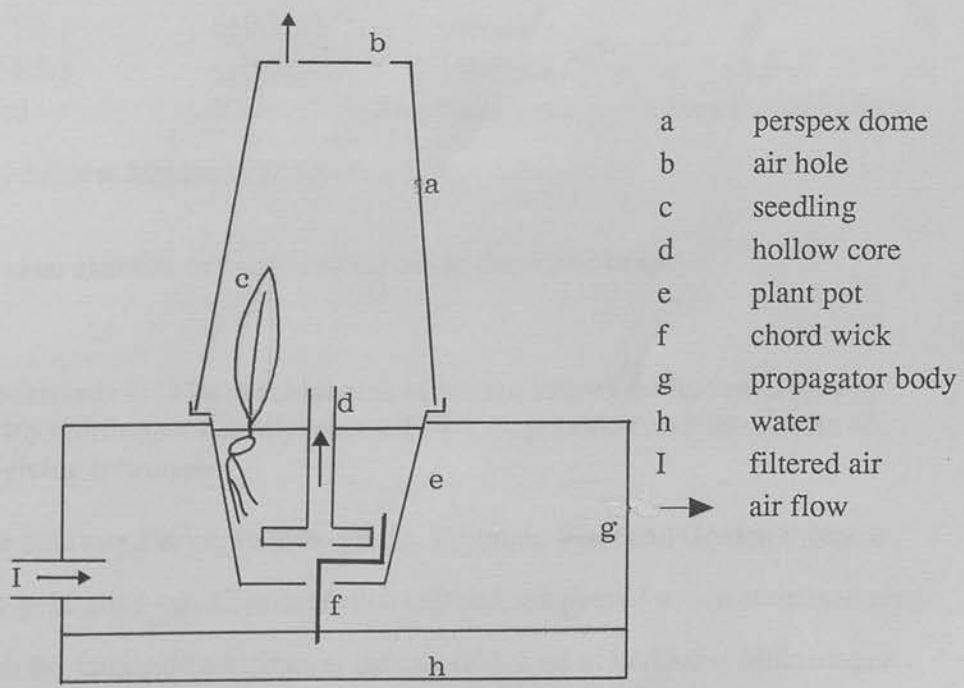
Plants were grown in 'Levington' or sphagnum peat based compost (Appendix 4.1). Plastic plant pots, 18 cm or 12.5 cm in diameter or 9 cm^2, and seed trays size 25 x 12 cm were used throughout. For the two larger sized pots, seeds were sown eight or nine per pot and thinned to six once seedlings were established. For small pots, four seeds were sown and thinned to one or two. Between 50 and 60 seeds were sown in seed trays. Pots were either placed into spore-free cabinets (Plate 4.1), a Burkard plant isolation propagator (Figure 4.2), in a large glasshouse or in a wire-mesh enclosed cage outside. All plants were watered regularly.

4.2.2 Maintenance of *Erysiphe graminis* f. sp *hordei* isolates and inoculation procedures

All mildew isolates, originally obtained from the Plant Breeding Institute, Cambridge, were maintained on detached barley leaf segments of Golden Promise supported on benzimidazole agar (Appendix 4.2) in petri dishes. Spores of the mildew isolates were transferred onto fresh leaf segments every 10 to 14 days using a small brush. Inoculations were carried out in a laminar flow cabinet to prevent cross contamination. The cabinet was allowed to run for several minutes before starting work to remove any spores from the air and all surfaces were wiped down with 70% ethanol. Leaves were removed from Golden Promise plants, cut into 2-3 cm segments using scissors, flamed in 70% ethanol, and placed onto benzimidazole agar plates with sterile forceps, using 10 segments per plate. For each isolate spores were transferred from old to new leaf segments in still air, petri-dish lids replaced and the cabinet switched on for 5 minutes. Between each different inoculation the brush was dipped in 70% ethanol and dried. All mildew isolates were incubated at 16-18°C and a 16 hour daylength in an illuminated incubator.

PLATE 4.1: Spore-free cabinets for growing mildew free barley plants, using filtered air to provide a positive air pressure.

FIGURE 4.2: Diagrammatic representation of the Burkard plant propagator.



4.2.3 Mildew isolates and spring barley cultivars

The isolates of *E. graminis* f. sp *hordei* used in controlled inoculation studies were as follows:

Isolate	Virulence factor	Reference
CC2	1, 4	P.B.I
CC9	1, 2, 6, 8	18/5/83
CC13	1, 2, 5	
CC21	1, 3, 4	
CC39	1, 2, 4, 5, 6	
CC40	1, 3, 4	
KB	unknown	

Details of the barley cultivars investigated in this section are listed below:

Cultivar	BMR*	Genes	Reference
Heriot	4+?	Mlv, ?	PBI, pers. comm.
Javelin	2+5	Mlg, Mla12	Wolfe *et al.* (1984)
Midas	3	Mla6	Wolfe *et al.* (1981)
Triumph	6bc	Mla7,Ab	Wolfe *et al.* (1986)
Vista	4+7	Mlv, Mla7	Wolfe *et al.* (1985)
Golden Promise	0	-	Wolfe *et al.* (1981)
WB 17E3-1	unknown	unknown	-
WB 26E5-3	unknown	unknown	-
Carnival	3	Mla6	Wolfe *et al.* (1982)

*barley mildew resistance group

Five individual experiments were carried out as described below.

4.2.4 Experiment 1: The development of brown lesions on leaves of spring barley cultivars naturally infected with *E. graminis* and the effects of fungicide treatment

Seed of the cultivars Heriot, Javelin, Midas, Triumph, Vista and Golden Promise were sown in 18 cm pots, 12 pots of each cultivar, six pots of which contained seed treated with the fungicide ethirimol at the rate of 1-2 ml of undiluted Milstem per 350-400 seed. Pots were placed in a glasshouse (with supplementary lighting) in six

replicate blocks, each block contained one seed treated and one untreated pot of each cultivar. Leaves were numbered in consecutive order from 1, representing the seedling leaf, 2 the first foliage leaf, 3 the second foliage leaf and so on. After the fourth leaf had emerged fungicide treated plants were further sprayed to run off with a 1:500 dilution of Calixin (tridemorph). One plant from each plot was labelled and scored weekly for percentage leaf area covered in mildew and brown lesions on all green leaves. The experiment was set up in a split-plot design with fungicide the main plot and cultivar the sub-plot, and analysed accordingly.

4.2.5 Experiment 2: The appearance of brown lesions on leaf segments of spring barley artificially inoculated with different mildew isolates in relation to cultivar and fungicide treatment

Seed of the same spring barley cultivars and treated or untreated with ethirimol as in the previous experiment were sown in 12.5 cm pots. The pots were covered in plastic domes and placed into the isolation propagator with supplementary lights. Once the second leaves, numbered as per previous experiment, had fully expanded the central 7 cm sections were removed and placed onto benzimidazole agar plates. Segments of each cultivar were assigned at random to each of six replicate plates for each fungicide treatment and isolate. The segments were inoculated with one of five mildew isolates or left as controls. Following incubation the leaf segments were assessed for percentage leaf area affected by mildew and browning over a 5 cm central area.

Plants, used to provide segments, were grown on until the fifth leaves had almost fully expanded when they were sprayed with tridemorph as in the previous experiment and 3 days later segments were removed from the fifth leaves and inoculation and assessment procedures repeated. However, as tridemorph caused severe scorching, only leaves of untreated plants were assessed.

The experimental lay-out thus consisted of six replicate blocks of six cultivars, six isolates and two fungicide treatments with fungicide forming the main plot, isolate the sub-plots and cultivar the sub-sub plots.

4.2.6 Experiment 3: The effect of leaf position on the development of brown lesions on leaf segments of spring barley cultivars inoculated with *E. graminis* f. sp *hordei*

Seed of spring barley cultivars used in the previous two experiments were sown in 12.5 cm pots, with four pots per cultivar, and places into spore free cabinets (two pots per cultivar into each of two cabinets). Once leaves were fully expanded they were removed from the plant and the central 7 cm portion excised, surface sterilised in 1% sodium hypochlorite solution for five minutes, rinsed in sterile water then segments of each cultivar randomly assigned to each of six replicate benzimidazole agar plates for inoculated and uninoculated treatments. The petri-dish lids were removed from plates and the leaves allowed to dry within the laminar flow cabinet before inoculation.

The day before inoculation, plants of Golden Promise infected with a mildew isolate of unknown virulence were shaken to remove spores so that only fresh spores were used in the experiment. On the day of inoculation spores were gently shaken into a sterile petri-dish and applied onto leaf segments with a brush. At the same time six Golden Promise segments were inoculated, one after each test plate, to determine numbers of spores inoculated onto test segments. Control segments were similarly treated but left uninoculated. Following incubation, leaf segments were scored for percentage leaf area covered in mildew and brown lesions over a 5 cm portion.

The experimental lay-out consisted of six replicate blocks of six cultivars and two treatments, inoculated and uninoculated, for each leaf position. A separate analysis of variance was carried out for each leaf position.

4.2.7 Experiment 4: The effect of fungicide treatment on the yield of spring barley in relation to mildew infection and browning

Untreated and ethirimol treated seed of Heriot, Javelin, Midas, Triumph, Carnival and Golden Promise were sown in 18 cm pots and placed in a caged area in a randomised block lay-out with a split-plot design, fungicide treatment forming main plots and cultivar sub-plots. There were six replicates. Plants were allowed to become naturally infected with mildew. Once they reached the fourth leaf stage fungicide treated plants were further sprayed to run off with ethirimol. Leaves were scored for percentage leaf area covered with mildew and browning. Plants were grown to maturity and harvested for yield assessments.

4.2.8 Experiment 5: Fungal development and host tissue responses for compatible and incompatible barley/mildew interactions

Seed of the spring barley cultivars used previously were sown in 12.5 cm pots, using three pots of each cultivar, and placed into spore free cabinets. A second set of seed was sown 3 weeks after the first. Both sets were allowed to grow until the fifth leaves of the first sown plants had fully expanded. The second and fifth leaves were removed from the plants and a 3 cm length excised from the central region of each. The segments of the different cultivars and leaf ages were placed randomly onto benzimidazole agar plates. The leaves were inoculated with one or other of three mildew isolates, CC21, CC39 or KB or left as uninoculated controls. Three replicates were used per treatment.

The leaves were incubated for 3 days then placed onto a leaf clearing solution of ethanol : chloroform + trichloroacetic acid (75 : 25 + 0.15%) overnight (Wolf and Fric, 1981). Leaves were then removed and placed into 0.1% aniline blue (water soluble) in 0.1M K_2HPO_4 buffer, pH 9.2, for 24 hours, counterstained in 0.1% trypan blue in glacial acetic acid : distilled water (45 : 55) for three minutes, rinsed in distilled water and mounted in the aniline blue solution on glass slides (Shimomura

and Dijkstra, 1975; Munro, 1985). Segments were observed for several factors using a Leitz Ortholux II microscope fitted with lamp housings for transmitted bright field and incident UV light. The microscope was also fitted with additional filter blocks:

	Exciting filter	Dichroic beam splitting mirror	Suppressing filter
Block 1 (blue)	2x2 mm UGI	TK400	K430
Block 2 (near UV)	3 mm BG12	TK510	K515

Observations on colony production were confined to colonies produced from single spores, clumps of spores producing colonies being ignored.

4.3 RESULTS

4.3.1 Experiment 1: The development of brown lesions on leaves of spring barley cultivars naturally infected with *E. graminis* and the effects of fungicide treatment

Mildew levels were always low but, on plants untreated with fungicide, progressively increased from 4-5 weeks after sowing to 12 weeks after sowing when there was a rapid senescence of plants (Figure 4.3; Appendix 4.3). Mildew infection did not appear on fungicide treated plants until 9-10 weeks after sowing and then at trace levels. Browning of untreated leaves did not appear until 2 weeks after mildew, between weeks 6 and 7, peaked at 10 weeks then decreased to low levels by week 12. The extent of mildew infection always exceeded that of brown lesions. Fungicide treatment prevented browning up until week 10, thereafter there were no differences between treated and untreated plants.

The assessment of infection on individual cultivars 12 weeks after sowing (Table 4.1) showed Golden Promise produced most mildew infection when untreated. Midas developed more infection than the remaining cultivars.

FIGURE 4.3: Development of mildew infection and browning on the leaves of glasshouse grown barley plants untreated (F-) or treated (F+) with fungicide (ethirimol seed treatment, tridemorph spray; average for cultivars).

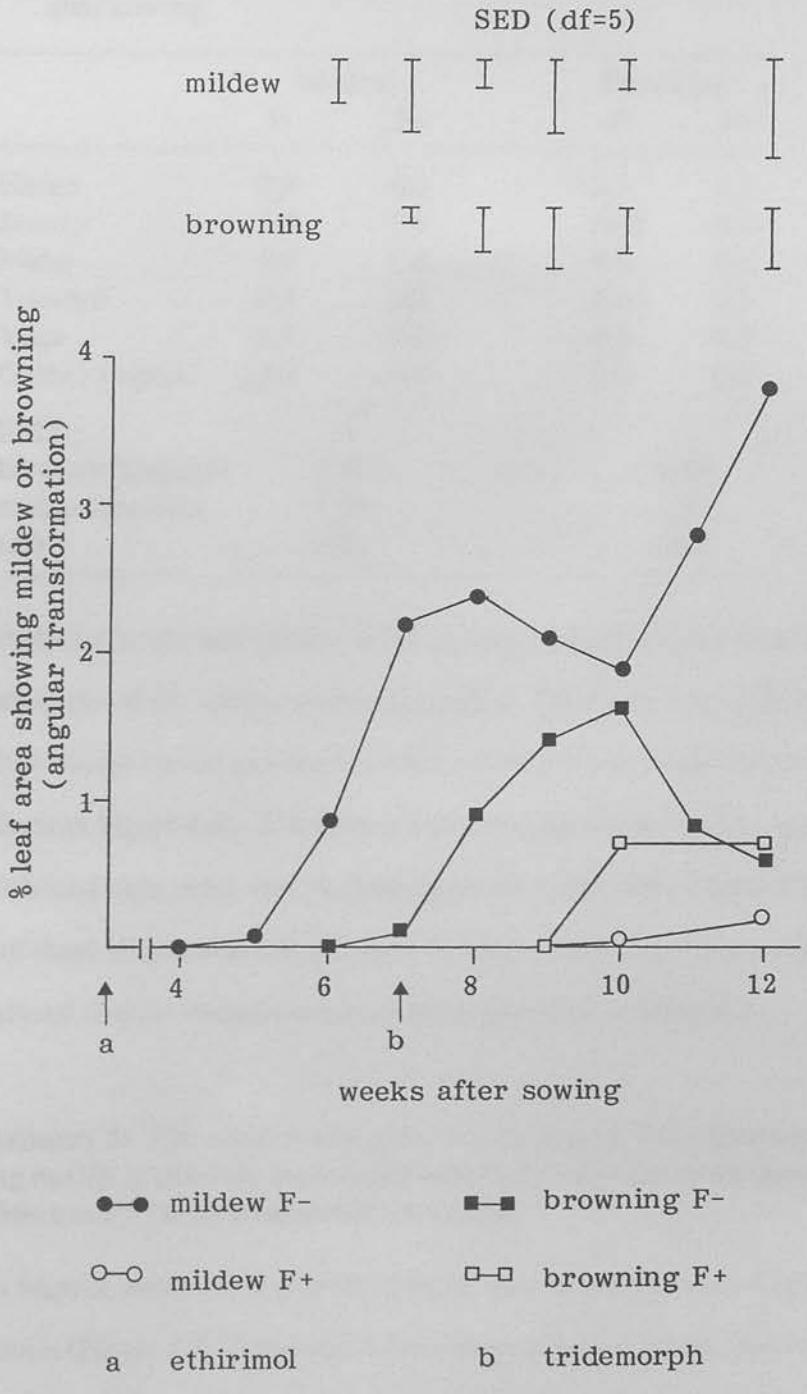


TABLE 4.1: Percentage leaf area (angular transformation) showing mildew and browning on the leaves of six glasshouse grown spring barley cultivars untreated (F-) or treated (F+) with ethirimol and tridemorph fungicides, naturally infected with *E. graminis*, assessed 12 weeks after sowing.

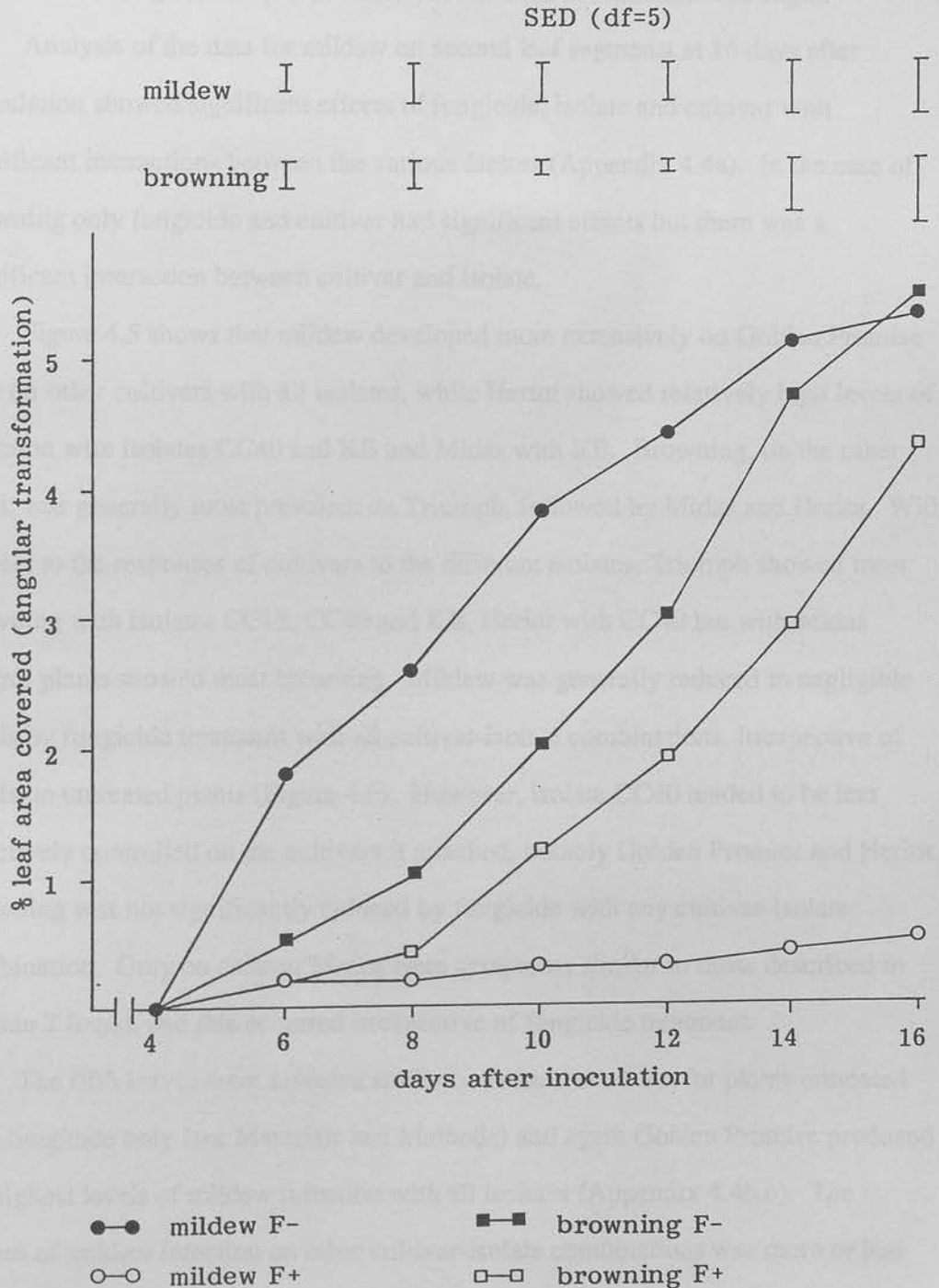
	Mildew		Browning	
	F-	F+	F-	F+
Heriot	2.4	0.0	3.3	3.7
Javelin	1.9	0.0	0.05	0.0
Midas	6.9	0.4	0.0	0.4
Triumph	0.1	0.0	0.05	0.0
Vista	1.7	0.0	0.5	0.2
Golden Promise	10.0	0.7	0.0	0.0
SED ±				
between fungicide	1.40		1.20	
within fungicide	1.32		1.23	
(df)	(49)		(49)	

Fungicide treatment eradicated mildew on all cultivars except Golden Promise and Midas, where traces of the disease were still present. There was very little browning on any cultivar except Heriot and no effect of fungicide at this stage (compare with the main effects in Figure 4.3). The brown lesions produced on Heriot, treated or untreated with fungicide, were very distinct, large chocolate brown spots (Plate 2.4). Production of these lesions was not the result of mildew infection and no other cultivar produced distinct lesions similar to those described in Section 2.

4.3.2 Experiment 2: The appearance of brown lesions on leaf segments of spring barley artificially inoculated with different mildew isolates in relation to cultivar and fungicide treatment

Mildew first became visible on segments from the second leaf between 4 and 6 days after inoculation (Figure 4.4; Appendix 4.4a) and progressively increased in subsequent observations. Significantly less mildew occurred on leaves from fungicide treated plants at all scoring times. Browning appeared at around the same

FIGURE 4.4: Development of mildew and browning on segments from the second leaves of spring barley plants untreated (F-) or seed treated (F+) with ethirimol following artificial inoculation with *E. graminis* (averaged for cultivars and isolates).



time as mildew but at lower levels. It progressively increased until, by day 16, browning reached levels similar to mildew. Ethirimol seed treatment significantly reduced browning from day 8 onwards but the level of reduction was slight.

Analysis of the data for mildew on second leaf segments at 16 days after inoculation showed significant effects of fungicide, isolate and cultivar with significant interactions between the various factors (Appendix 4.4a). In the case of browning only fungicide and cultivar had significant effects but there was a significant interaction between cultivar and isolate.

Figure 4.5 shows that mildew developed more extensively on Golden Promise than on other cultivars with all isolates, while Heriot showed relatively high levels of infection with isolates CC40 and KB and Midas with KB. Browning, on the other hand, was generally most prevalent on Triumph, followed by Midas and Heriot. With respect to the responses of cultivars to the different isolates, Triumph showed most browning with isolates CC13, CC40 and KB, Heriot with CC40 but with Midas control plants showed most browning. Mildew was generally reduced to negligible levels by fungicide treatment with all cultivar-isolate combinations, irrespective of levels on untreated plants (Figure 4.6). However, isolate CC40 tended to be less effectively controlled on the cultivars it attacked, notably Golden Promise and Heriot. Browning was not significantly reduced by fungicide with any cultivar-isolate combination. Only on cultivar Heriot were symptoms similar to those described in Section 2 found, and this occurred irrespective of fungicide treatment.

The fifth leaves were assessed similarly to the second but for plants untreated with fungicide only (see Materials and Methods) and again Golden Promise produced the highest levels of mildew infection with all isolates (Appendix 4.4b,c). The pattern of mildew infection on other cultivar-isolate combinations was more or less similar to that of the second leaf. Browning tended to be slightly higher than on leaf two, with Midas and CC40 showing most of the different cultivars and isolates respectively.

FIGURE 4.5: Mean levels of mildew and browning on segments from the second leaves of six spring barley cultivars inoculated with five *E. graminis* isolates, 16 days after inoculation (averaged for fungicide treatments).

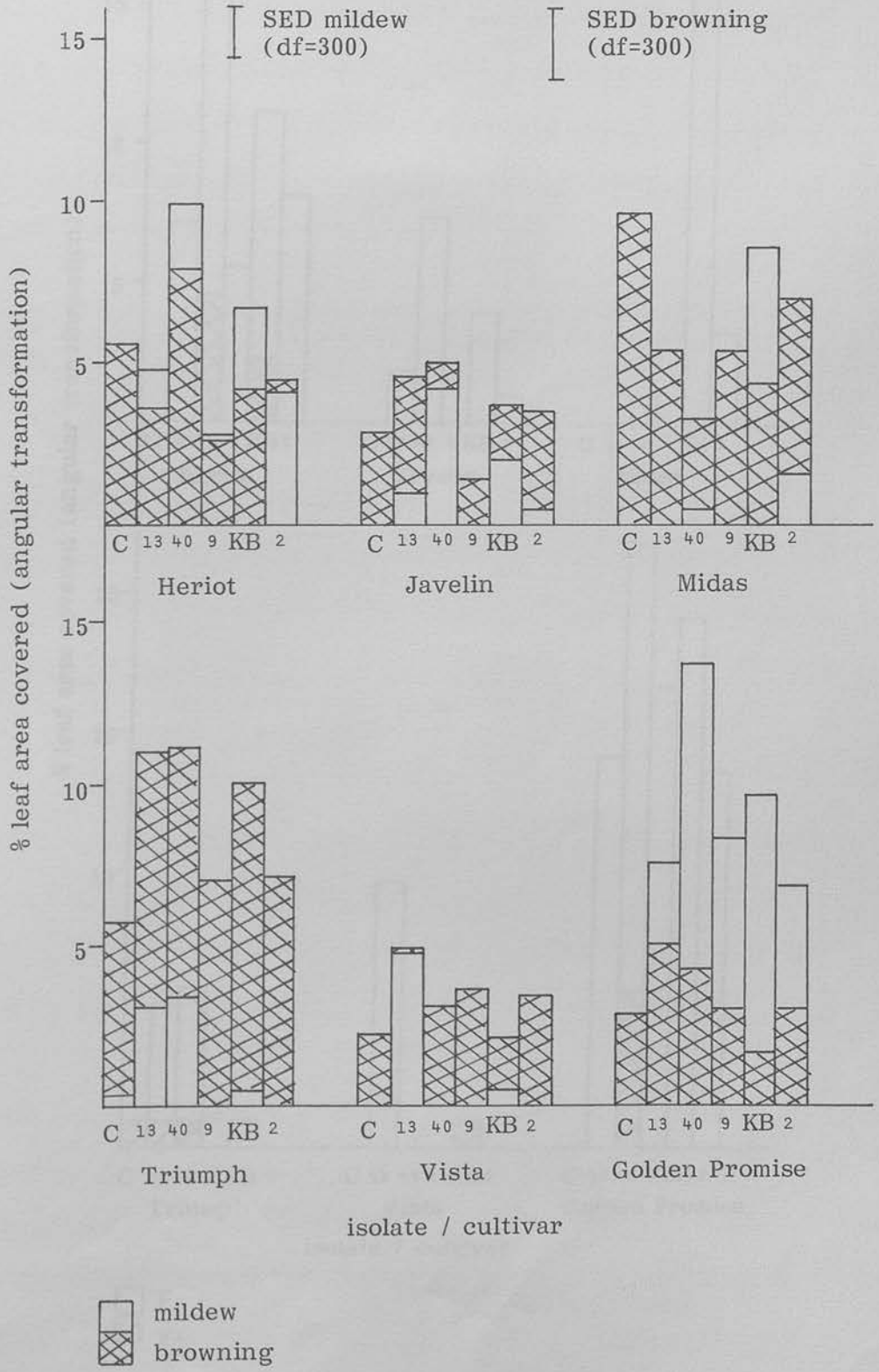
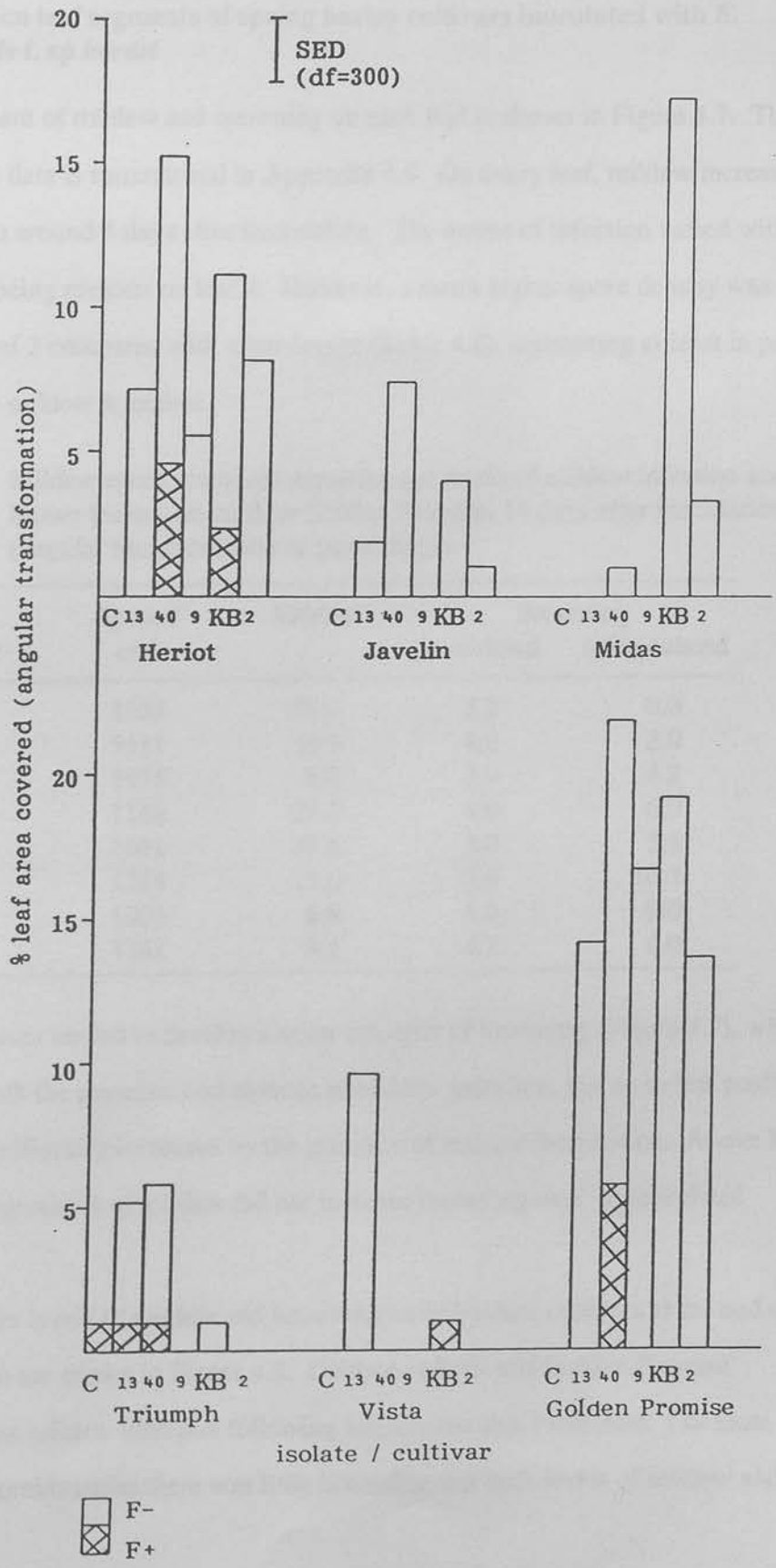


FIGURE 4.6: Mean levels of mildew on segments from the second leaves of untreated (F-) and ethirimol treated (F+) seed of six spring barley cultivars inoculated with five *E. graminis* isolates, 16 days after inoculation.



4.3.3 Experiment 3: The effect of leaf position on the development of brown lesions on leaf segments of spring barley cultivars inoculated with *E. graminis* f. sp *hordei*

The development of mildew and browning on each leaf is shown in Figure 4.7. The analysis of the data is summarised in Appendix 4.5. On every leaf, mildew increased with time from around 4 days after inoculation. The extent of infection varied with leaf position, being greatest on leaf 2. However, a much higher spore density was placed onto leaf 2 compared with other leaves (Table 4.2), accounting at least in part for this higher mildew infection.

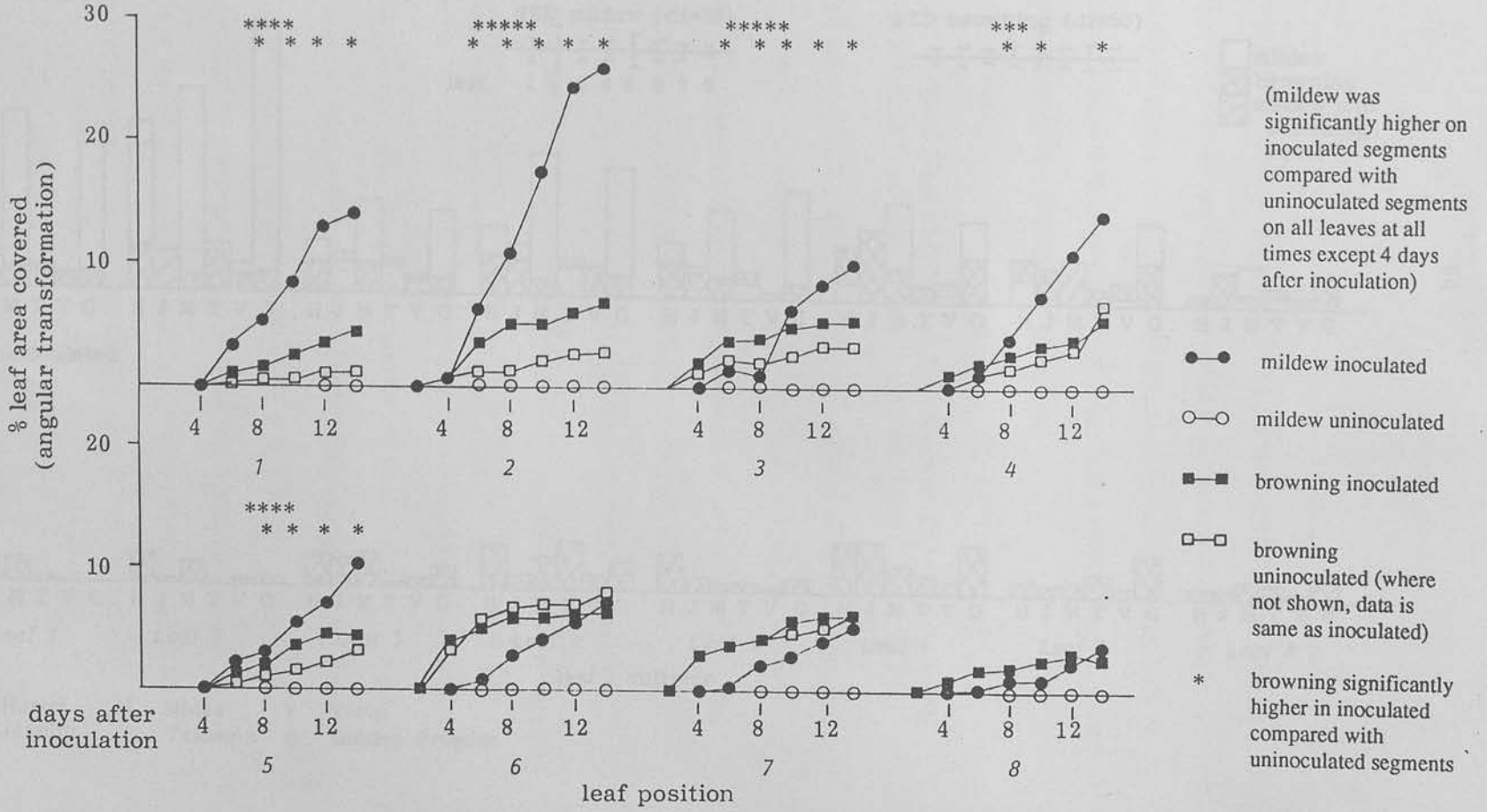
TABLE 4.2: Mildew spore inoculum densities and levels of mildew infection and brown lesions on cultivar Golden Promise, 14 days after inoculation (angular transformation of percentage).

Leaf number	Spores/ cm^2	Mildew	Browning	
			inoculated	uninoculated
1	1133	29.4	3.2	0.0
2	5511	54.3	4.0	2.0
3	1975	8.6	3.0	4.2
4	1168	22.3	4.0	6.0
5	1021	21.6	3.0	2.8
6	1284	13.0	9.0	10.2
7	1903	8.8	8.0	9.0
8	1361	4.1	4.7	6.0

Most leaves tended to develop similar amounts of browning (Figure 4.7), which occurred in both the presence and absence of mildew infection, but up to leaf position 4 or 5 was significantly increased by the presence of mildew inoculation. Above leaf position 5 the presence of mildew did not increase browning over uninoculated leaves.

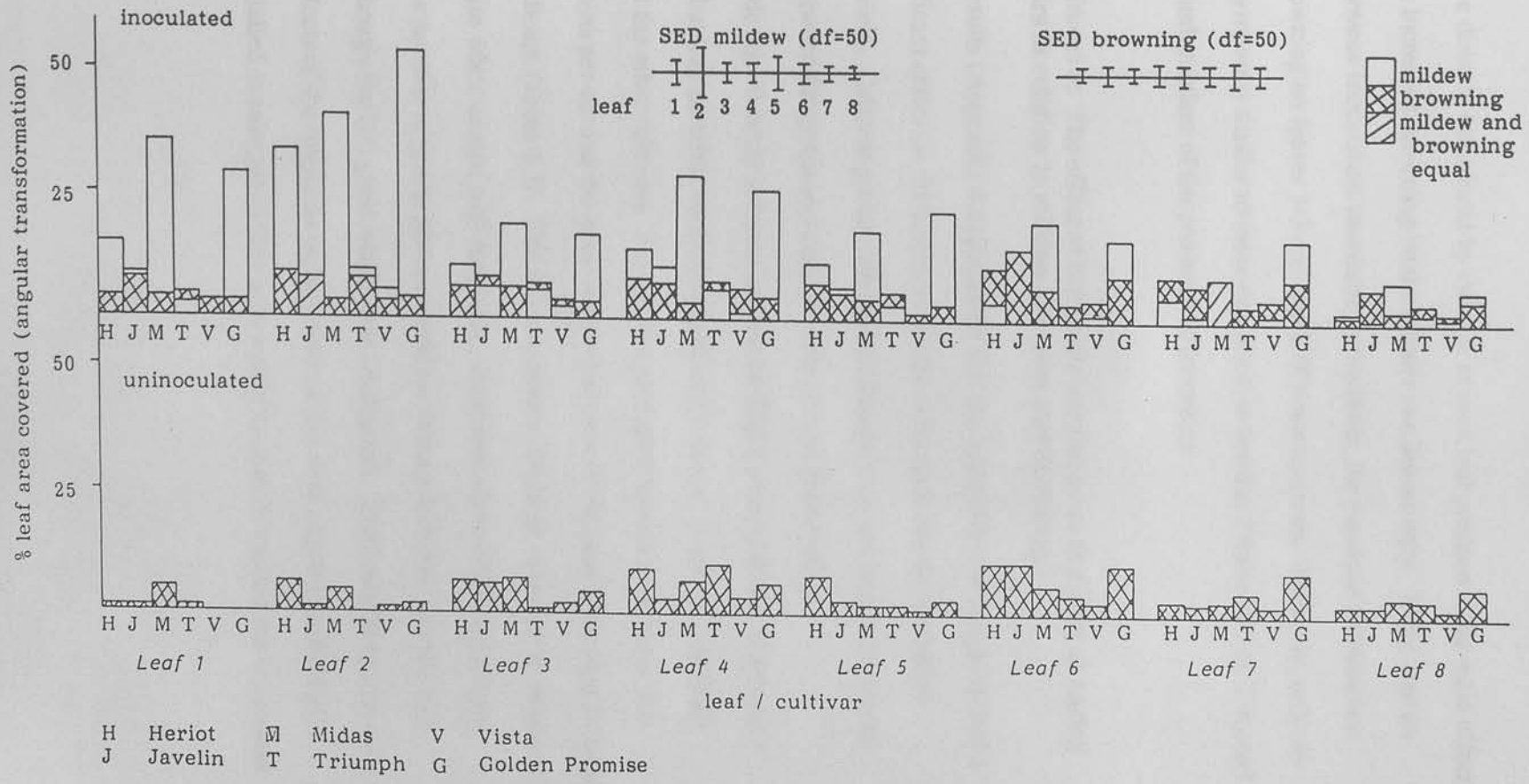
The mean levels of mildew and browning on individual cultivars at the end of the experiment are shown in Figure 4.8. Cultivars Midas and Golden Promise developed most mildew infection following inoculation and Vista least. For most leaf/cultivar combinations there was little browning, but both levels of mildew and

FIGURE 4.7: Development of mildew and browning with time on leaf segments from different leaf positions on spring barley plants, inoculated or uninoculated with a natural population of *E. graminis*.



(no SEDs shown)

FIGURE 4.8: Mean levels of mildew and browning on leaf segments from different leaf positions on six spring barley cultivars, inoculated or uninoculated with *E. graminis*, 14 days after inoculation.



browning were directly influenced by cultivar at each leaf position. The main effect of inoculation increased browning on the bottom two leaves only. There was an interaction between inoculation treatment and cultivar, the presence of inoculum increasing browning on leaves 1-3 and leaf 7 of some cultivars. However, only on Heriot were symptoms similar to those described in Section 2 found (Plate 2.4), and this occurred independent of the presence of inoculum.

4.3.4 Experiment 4: The effect of fungicide treatment on the yield of barley cultivars in relation to mildew infection and browning

Analysis of results (Appendix 4.6) indicated that the main effect of fungicide had a slightly significant effect on the levels of mildew infection, but did not affect browning or yield. Cultivar greatly affected all factors assessed and there was an interaction between fungicide and cultivar with all except browning.

Fungicide significantly reduced mildew on Golden Promise and Midas only (Figure 4.9), but had no effect on browning of any cultivar. Carnival had more browning that the other cultivars. Fungicide application tended to increase the number of grains per ear and the grain weight but only in the case of Golden Promise was this significant (Table 4.3). This tends to mirror the large decrease in mildew on Golden Promise when treated with fungicide. However, there was a significant decrease in the number of grains per ear found on cultivar Carnival treated with fungicide, although the 100 grain weight was unaffected. There would appear to be phytotoxic effects of the fungicide on this cultivar but with only a few replicates (many plants failed to emerge) further tests would have to be carried out to confirm this.

FIGURE 4.9: Levels of mildew and browning on leaves of spring barley cultivars grown for yield, untreated or treated with ethirimol fungicide.

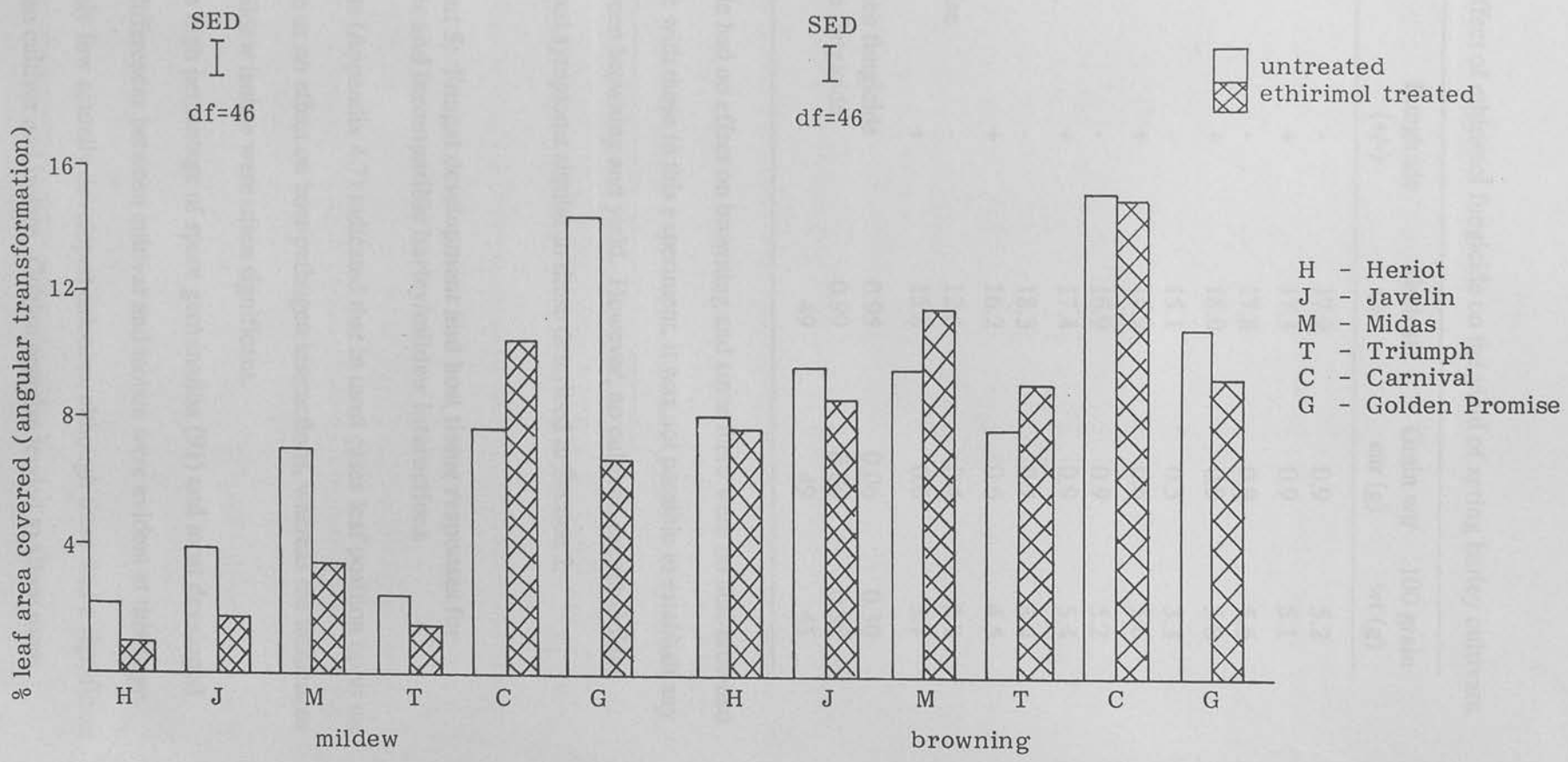


TABLE 4.3: Effect of ethirimol fungicide on the yield of spring barley cultivars.

Cultivar	Fungicide (+/-)	Grains/ ear	Grain wt/ ear (g)	100 grain wt (g)
Heriot	-	17.9	0.9	5.2
	+	17.3	0.9	5.1
Javelin	-	17.8	0.9	5.6
	+	18.0	0.9	5.0
Midas	-	15.1	0.5	3.3
	+	16.9	0.6	3.6
Triumph	-	16.9	0.9	5.2
	+	17.4	0.9	5.4
Carnival	-	18.3	0.7	3.9
	+	16.2	0.6	4.5
Golden Promise	-	12.6	0.4	2.9
	+	15.8	0.6	3.7
SED between fungicide		0.95	0.06	0.30
within fungicide		0.99	0.06	0.30
(df)		49	49	45

Because fungicide had no effect on browning and since there were no non-browned plants to compare with those in this experiment, it was not possible to establish any relationship between browning and yield. However, no cultivar, except Golden Promise, developed symptoms similar to those described in Section 2.

4.3.5 Experiment 5: Fungal development and host tissue responses for compatible and incompatible barley/mildew interactions

Analysis of results (Appendix 4.7) indicated that in most cases leaf position up to the fifth leaf had little or no effect on host-pathogen interactions, whereas the influences of cultivar and mildew isolate were often significant.

There was a high percentage of spore germination (91) and most developed appressoria: no differences between cultivar and isolate were evident at this stage. However, relatively few actually developed colonies, although there was a significant interaction between cultivar and isolate. Golden promise tended to allow more

colonies to develop than other cultivars whilst isolates CC21 and CC39 produced more colonies than the KB isolate (Table 4.4).

TABLE 4.4: Number of colonies produced as a percentage of germinated spores from three *E. graminis* isolates inoculated onto leaf segments from five barley cultivars.

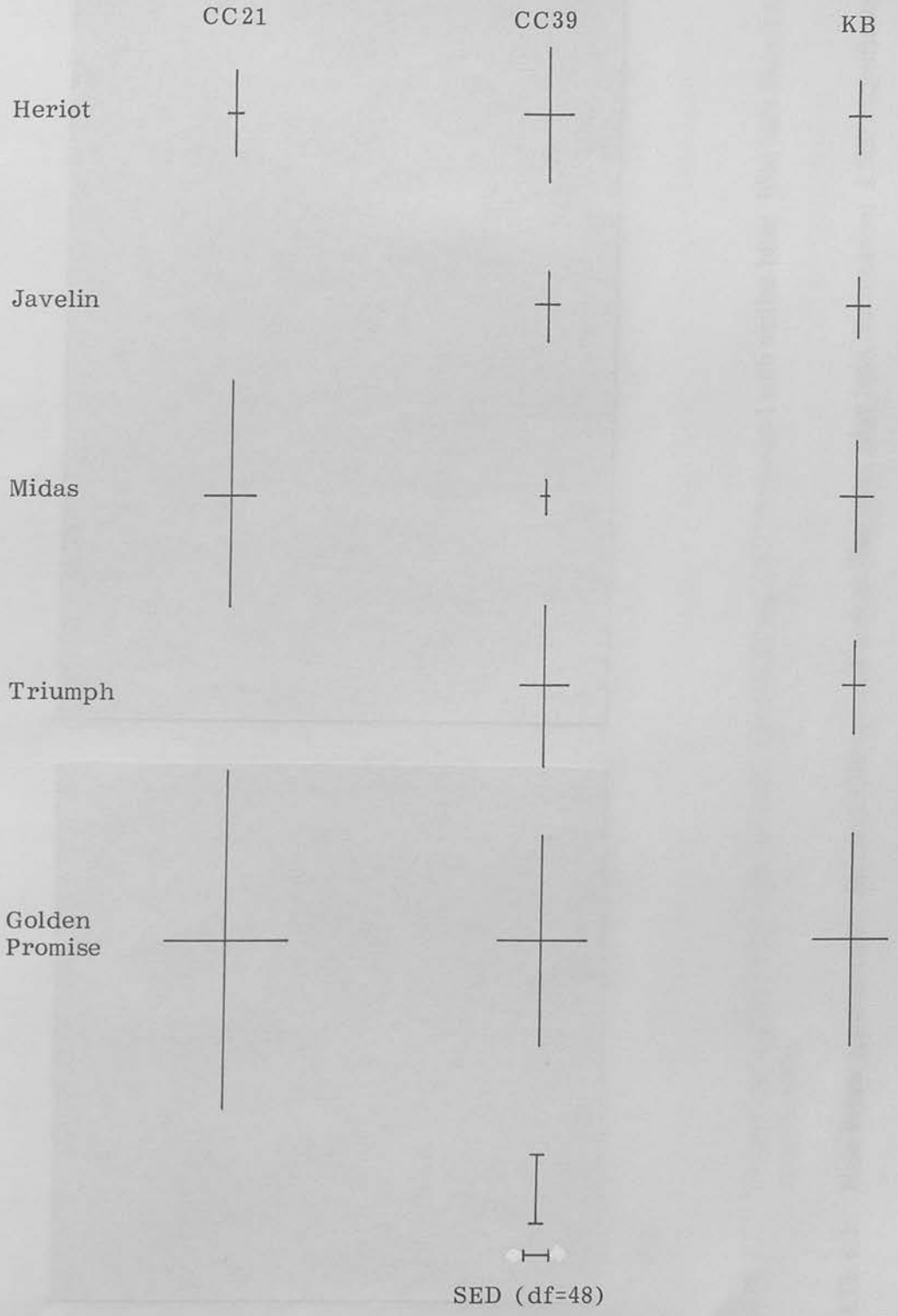
Isolate	Cultivar					Mean
	Hr	Jv	Ms	Tr	Gp	
CC21	0.7	0.0	6.0	0.0	10.7	3.5
CC39	3.6	1.3	1.0	2.9	15.0	4.8
KB	0.4	0.4	1.2	2.2	4.0	1.6
mean	1.6	0.6	2.7	1.7	9.9	
SED (df=48)	between isolates		2.35			
	within isolates		2.42			

On the cultivar Midas more colonies were produced by CC21 than by other isolates, while both CC21 and CC39 produced high numbers on Golden Promise. Heriot, Javelin and Triumph allowed few colonies with all isolates.

The average length of a colony was influenced only by the host cultivar but the breadth tended to be influenced to some extent by both cultivar and isolate. All isolates gave larger colonies on Golden Promise than on other cultivars (Figure 4.10). CC21 tended to develop relatively large colonies on Midas, but failed to develop colonies on Javelin and Triumph.

Observations on the cellular changes occurring showed that papillae were produced under an average 26% of appressoria. There were no overall significant effects on the number of fluorescent papillae (Plate 4.2) associated with cultivar or isolate, although Javelin tended to develop fewer when compared with the susceptible cultivar Golden Promise (Table 4.5). The number of germinated spores below which were found haloes (Plate 4.3) or papillae plus haloes were significantly influenced by cultivar (Appendix 4.7), with Javelin developing more haloes than other cultivars and Heriot and Triumph producing relatively few papillae plus haloes.

FIGURE 4.10: Mean length and breadth of *E. graminis* colonies from three isolates grown on five spring barley cultivars (µm).



4.2

4.3

PLATE 4.2: Fluorescent papilla below appressorium; Golden Promise/CC39 (stained with aniline blue; blue light illumination; x 500 magnification.

PLATE 4.3: Halo below appressorium; Javelin/CC40 (stained with aniline blue; blue light illumination; x 500 magnification).

4.4

4.5

PLATE 4.4: Fluorescent epidermal cell; Triumph/CC40 (stained with aniline blue; UV light; x 312.5 magnification).

PLATE 4.5: Fluorescent mesophyll cells; Midas/CC39 (stained with aniline blue; UV light; x 500 magnification).

TABLE 4.5: Percentage germinated *E. graminis* spores below which host barley leaf epidermal cells developed papillae, haloes or papillae and haloes.

	Cultivar						
	Hr	Jv	Ms	Tr	Gp	SED	df
Papillae	24.9	19.7	24.7	29.4	30.8	± 4.40	48
Haloes	4.2	13.8	6.9	6.8	5.9	± 2.15	47
Papillae + haloes	3.0	10.6	7.6	5.1	9.9	± 1.90	48

There were no differences between cultivars or isolates with respect to the percentage germinated spores below which were found fluorescent epidermal cell walls, fluorescent epidermal cells or fluorescent mesophyll cells (Plates 4.4 and 4.5), the percentages in all cases being very low (1.8, 1.0 and 0.6 respectively). The numbers of groups of fluorescent cells or brown cells per square centimetre of leaf were scored to determine if infected leaves differed from control, uninoculated leaves. In all cases there were little or no significant differences between controls and inoculated leaves, with the numbers of groups being mostly less than 1 per cm^2 of leaf.

4.4 DISCUSSION

The studies carried out in this section were aimed at determining whether the varietally distinct brown lesions found on the leaves of certain cultivars under field conditions were in any way associated with hypersensitive cell death in response to mildew infection. The results were in keeping with observations already found in field trial work (Section 3), that for some cultivars the development of brown lesions was independent of mildew infection, whereas in others browning was apparently linked with senescence induced by mildew. Of the cultivars investigated, only Heriot, grown in glasshouse conditions, showed cultivar-related symptoms of browning (Plate 2.4), but this occurred independently of mildew or fungicide treatment.

Ethirimol seed treatment controlled natural mildew infection up to the 6 leaf stage (7 weeks after sowing), even on the susceptible cultivar Golden Promise, where the infection pressure was low. Stoddart and Northwood (1984) found a similar degree of control of mildew by ethirimol up to 12 weeks after sowing. Ethirimol seed treatment also delayed early browning but there was no long-term control, any later relationships being possibly complicated by the confounding of natural senescence with mildew-induced senescence. The mode of action of ethirimol is that of spore germination inhibition (Sijpesteijn, 1972). Since ethirimol was applied as a seed dressing in experiments (Section 4.3.2), a large number of the spores would fail to germinate. The hypersensitive reaction is stated to occur at or after haustorial formation (McKeen and Bhattacharya, 1970), therefore treatment with ethirimol should reduce this response as fewer spores will develop to this stage. Since browning on leaves of mildew resistant cultivars known to exhibit severe browning under field conditions was not affected by treatment with ethirimol, it would appear that browning is not the consequence of a visible hypersensitive reaction to mildew: there was no evidence that mildew infection induced cultivar-related symptoms in more resistant cultivars.

There has been much debate as to the role of papillae in the resistance of host plants to mildew infection but it is now generally accepted that there are often no differences in this response between compatible and incompatible interactions (Bushnell and Bergquist, 1975; Aist and Israel, 1976; Kunoh and Ishizaki, 1976). Results from microscope studies (Section 4.3.5) would tend to agree with this. Cell fluorescence with aniline blue staining is often used as an indication of hypersensitive (necrotic) cells (Munro, 1985). However, although a large number of spores germinated in this present study and failed to produce colonies, few showed fluorescent epidermal or mesophyll cells.

Benada (1965) and Kiŕaly *et al.* (1974) stated that the development of brown lesions on the leaves of some barley cultivars did not occur until the stem extension or adult phase of development, this browning being associated with adult plant resistance; there was no evidence of such a response being the cause of distinct lesions on the cultivars used here.

If the brown lesions of barley leaves described in Section 2 comprised dead cells then such extensive loss of photosynthetic tissue would be expected to reduce yield. Studies showed a yield benefit being achieved by fungicide treatment of mildew susceptible cultivars. Such findings are common, having been shown by many workers (Martin, Morris and Chipper, 1981; Wale and Shipton, 1981). However, Smedegaard-Petersen and Stølen (1980) found that so-called resistant barley cultivars gave reduced yield when infected with mildew due to the rapid increase in respiration (Smedegaard-Petersen, 1980) and reduced photosynthesis due to leaf necrosis in some cultivars (Habeshaw, 1979). The mildew resistance genes at the ml-o locus of several Ethiopian barleys which condition chlorotic and necrotic spotting on leaves have been associated with low grain yield (Jorgensen, 1975). A true hypersensitive response can therefore give rise to reduced yields.

As already stated, a hypersensitive reaction to mildew infection is not the cause of browning on the more resistant cultivars used in these studies. Experiments failed to show if brown lesions reduced yield since no distinct lesions developed on plants. However, trials carried out by the Scottish Agricultural Colleges (SAC) showed that resistant cultivars such as Heriot, Javelin, Triumph and Tasman could give increased yields when treated with fungicides even although mildew incidence was only slight (Anon, 1984b, 1986a). If the brown lesions occurring on leaves of such cultivars are indeed necrotic, then it may be that even low levels of mildew infection on such reduced photosynthetic areas can result in yield losses. Hence the application of fungicides to these cultivars may be beneficial to yield. However, of the fungicides

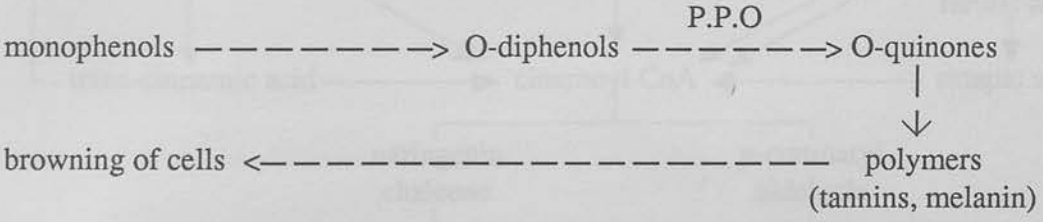
used some, such as propiconazole, fenpropimorph and propiconazole + tridemorph, are recognised to have also some effect in reducing levels of *Rhynchosporium*, net blotch and rust and the increase in yield may be the result of reduction of these or other pathogens than mildew.

SECTION 5

OBSERVATIONS ON THE HISTOLOGICAL CHANGES IN EPIDERMAL AND MESOPHYLL CELLS OF BARLEY LEAVES INFECTED WITH *ERYSIPHE GRAMINIS* OR SHOWING DISTINCT BROWN LESIONS

5.1 INTRODUCTION

The rise in respiration involved in the necrogenic or hypersensitive response of barley plants infected with incompatible races of *E. graminis* is associated with increased aromatic biosynthesis (Farkas and Kíraly, 1962; Kosuge, 1969). The aromatic compounds (phenolics) are formed via the shikimic acid pathway, with the first steps involving phospho-enoyl-pyruvate (PEP) from the glycolytic pathway and erythrose-4-phosphate from the pentose phosphate pathway, the latter also increasing (Müller, 1959; Tomiyama, 1963; Kosuge, 1969; Figure 5.1). PEP and erythrose-4-phosphate eventually give rise to phenylalanine and tyrosine which are monophenolic compounds (Davies, Giovanelli and Ap Rees, 1964). Through enzymatic action, the monophenols are polymerised to diphenols, polyphenols, quinones, melanins and tannins, the latter two of which are the brown/black pigments giving rise to the colour of necrotic cells (Kíraly, 1959; Farkas and Kíraly, 1962; Davies *et al.*, 1964; Figure 5.2).



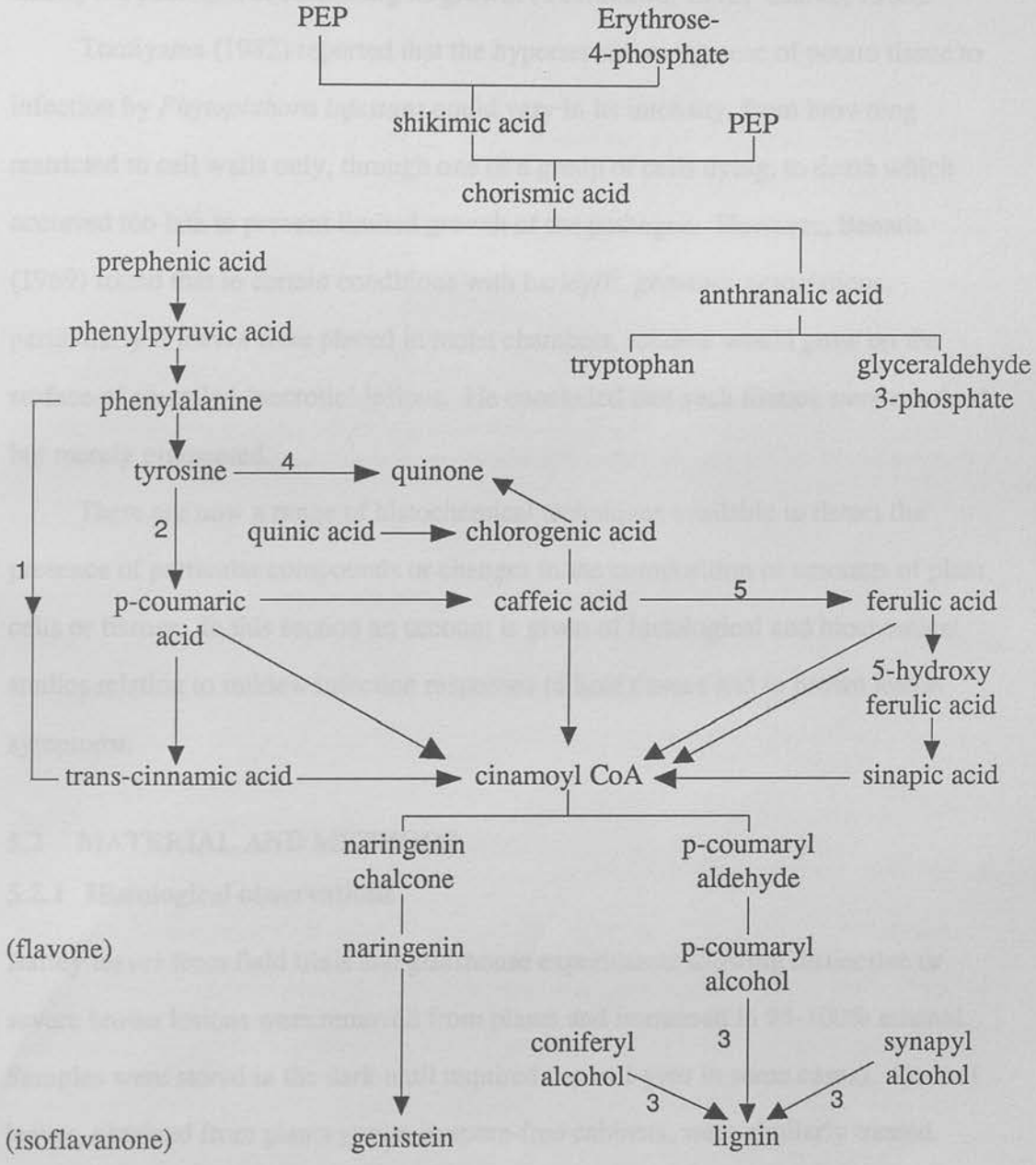
P.P.O. - polyphenol oxidase
(Oku, 1962; Butt, 1980)

FIGURE 5.2: Oxidation of phenolic compounds in cells reacting hypersensitively.

In general, the quinones are more toxic to pathogens than phenols and therefore may be involved in death of the pathogen (Le Tourneau, McLean and Guthrie, 1957). Phenols and quinones are produced, however, not only in response to infection but also to mechanical damage (Tomiyama, 1963) and possibly other factors.

Tyrosine gives rise to quinones but can also give lignin, isoflavanoids or other phytoalexins (Tomiyama, 1963; Figure 5.1). These products do not necessarily lead

FIGURE 5.1: Synthesis of aromatic compounds involved in necrosis of plant cells.



1 - phenylalanine ammonium lyase (PAL)
2 - tyrosine ammonium lyase (TAL)
3 - peroxidase
4 - polyphenoloxidase (PPO)
5 - O-diphenol O-methyl transferase (OMTS)

(Davies, Giovanelli and Ap Rees, 1964; Yudkin and Offord, 1975; Asada, Ohguchi and Matsumoto, 1976; Legrand, 1983; Ride, 1983)

to cell death, but are involved in the defence of the plant against invasion by either killing the pathogen or restricting its growth (Yoshikawa, 1978; Clarke, 1983).

Tomiyama (1982) reported that the hypersensitive response of potato tissue to infection by *Phytophthora infestans* could vary in its intensity, from browning restricted to cell walls only, through one or a group of cells dying, to death which occurred too late to prevent limited growth of the pathogen. However, Benada (1969) found that in certain conditions with barley/*E. graminis* associations, particularly if leaves were placed in moist chambers, mildew would grow on the surface of so-called 'necrotic' lesions. He concluded that such tissues were not dead but merely pigmented.

There are now a range of histochemical techniques available to detect the presence of particular compounds or changes in the composition or amounts of plant cells or tissues. In this section an account is given of histological and biochemical studies relating to mildew infection responses of host tissues and to brown lesion symptoms.

5.2 MATERIAL AND METHODS

5.2.1 Histological observations

Barley leaves from field trials and glasshouse experiments showing distinctive or severe brown lesions were removed from plants and immersed in 95-100% ethanol. Samples were stored in the dark until required (up to 1 year in some cases). Control leaves, obtained from plants grown in spore-free cabinets, were similarly treated.

Once required, small pieces of tissue (5-10 mm^2) were cut from leaves, placed between split pith, and cross-sections cut using a wet single-edged razor blade. The sections were floated off in a dish of distilled water, placed into fresh water using a small paint brush and observed directly or stained with one of the following:

A	-	Aniline blue	(O'Brien and McCully, 1972)
B	-	Calcofluor	"
C	-	FeCl	(Faulkner and Kimmins, 1975)
D	-	Maüle	"
E	-	Nitroso	(Jensen, 1962)
F	-	Phloroglucinol	(Faulkner and Kimmins, 1975)
G	-	Resorcinol blue	(O'Brien and McCully, 1972)
H	-	Thionin	(Sadik and Minges, 1964)
I	-	Toluidine blue O	(O'Brien and McCully, 1972)

Details of staining schedules are given in Appendix 5.1. Several sections were observed microscopically using normal bright field illumination or fluorescence.

5.2.2 Biochemical observations

These studies were carried out on the cultivar Javelin only. A series of extraction procedures were carried out on the assumption that the brown lesions were produced as the result of oxidation of phenolic compounds to melanins or tannins. The methods were modified from Harborne (1964). A sample of 0.75 g of leaf material showing thumb-print lesions (fixed in 70% ethanol : glycerol : water, 3:2:3) was cut into strips and placed into a 50 ml round-bottomed flask containing 50 ml of 2M hydrochloric acid plus anti-bumping chips. A reflux tube was attached to the flask, the water supply passing from bottom to top. Using an electromantle, the acid was boiled for 30 minutes then allowed to cool, the water left running to allow condensation of hydrochloric gas. Once cool, the hydrochloric acid was filtered off and placed into a 100 ml filtration column; 50 ml ether was added and the mixture shaken for 10 minutes, gas which built up being released at intervals. The bottom acid layer was removed and discarded. The upper ether layer was transferred to a 250 ml quickfit round-bottomed flask. This was attached to a vacuum rotavapor, the ether boiled off and the flask rinsed and evaporated three times. The phenols were redissolved in 2 ml ether and placed into a screw topped bottle for storage (Extraction

1). This procedure was repeated but tissue was ground in a mortar and pestle with a small quantity of 2M hydrochloric acid before boiling. The final extract was redissolved in 5 ml ether (Extraction 2). This was also carried out for healthy tissue (Extraction 3).

The above extracts were chromatographed one and two dimensionally on silica coated glass plates using acetic acid : chloroform, 1:9 (first run) and ethyl acetate : toluene, 9:11 (second run). After separation, plates were observed under long wave U.V light and any spots marked. Plates were then sprayed with either vanillin (1 g in 10 ml concentrated HCl) (Hathway, 1960) or full strength folin reagent (Procházka, 1966), for the detection of flavonoids or phloroglucinol-resorcinol phenols.

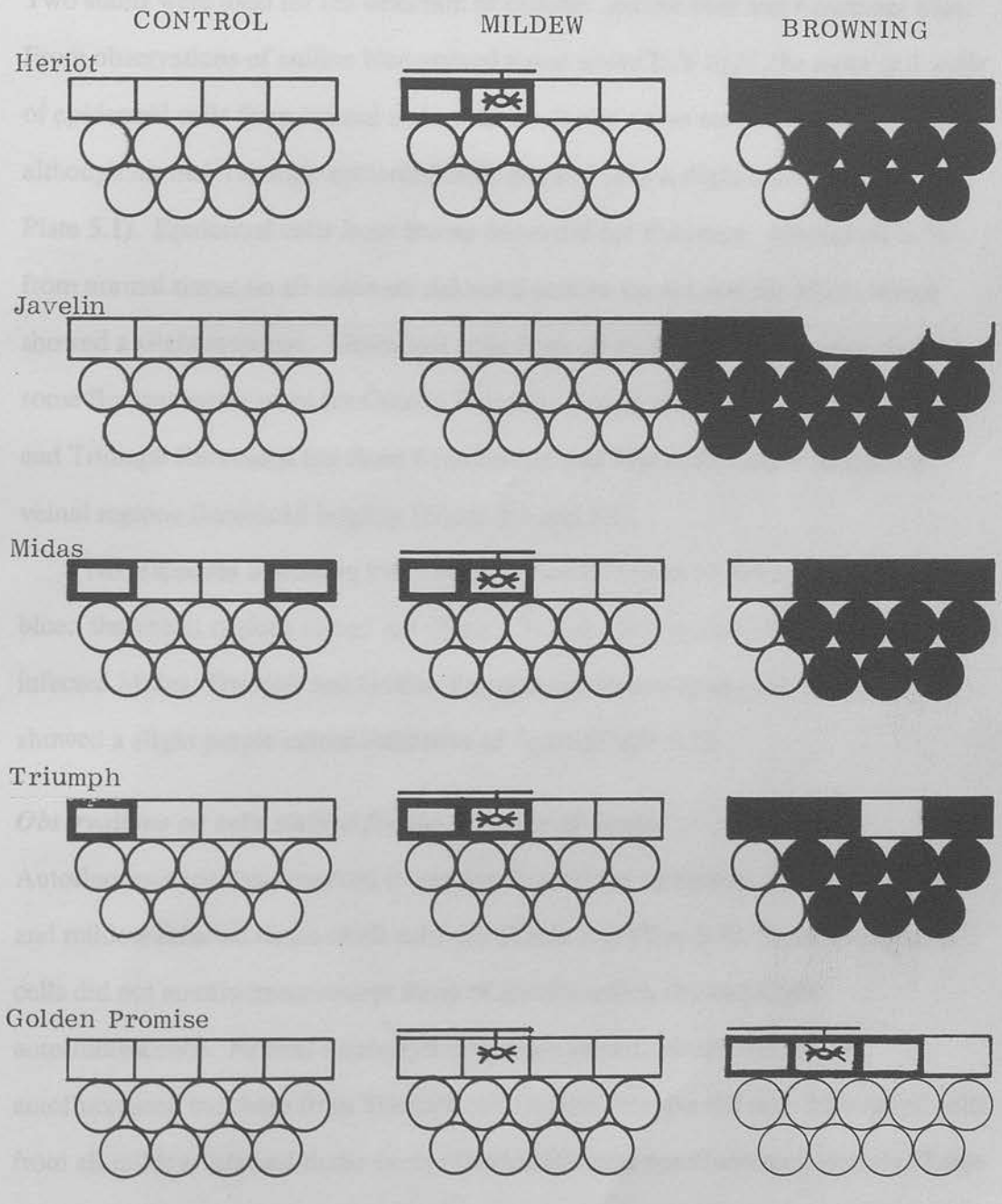
5.3 RESULTS

5.3.1 Histological observations

Observations of unstained sections under bright field illumination

Observation on unstained material indicated the various abnormal features of cells of different cultivars associated with established mildew infection or visual symptoms of browning. Figure 5.3 attempts to illustrate these features. In cultivars, except Midas and Triumph, tissue from control leaves free from visual symptoms comprised colourless epidermal and mesophyll cells: Midas, and to a lesser extent Triumph, tended to give one or two epidermal cells with brown walls. Where leaves were infected with *E. graminis*, the walls of epidermal cells were brown below the fungus, except for the early stages of infection on Golden Promise. Unfortunately, no mildew mycelium was found on Javelin. Of the cultivars showing distinctive brown lesions, Heriot, Midas and Triumph showed browning of epidermal and mesophyll cells (both cell walls and cytoplasm) within the lesion, while the surrounding tissue appeared normal. Javelin, which developed extensive thumb-print lesions, showed the same symptoms but with pockets of collapsed epidermal cells within lesions. The brown

FIGURE 5.3: Diagrammatic representation of 'brown' responses shown by normal, mildew infected and brown lesion regions of leaves from five spring barley cultivars.



lesions on all cultivars extended from the upper epidermis to, or almost to, the lower epidermis.

Observations on cells stained for the presence of callose

Two stains were used for the detection of callose: aniline blue and resorcinol blue. From observations of aniline blue stained tissue under U.V light, the outer cell walls of epidermal cells from normal and mildew infected tissue tended to fluoresce blue, although normal Triumph epidermal cells showed only a slight reaction (Table 5.1, Plate 5.1). Epidermal cells from brown tissue did not fluoresce. Mesophyll cells from normal tissue on all cultivars did not fluoresce except that for Midas which showed a slight response. Mesophyll cells from all mildew infected tissue showed some fluorescence except for Golden Promise. Brown mesophyll cells from Heriot and Triumph fluoresced but those from Javelin and Midas did not. Papillae and veinal regions fluoresced brightly (Plates 5.1 and 5.2).

No responses indicating the presence of callose were observed with resorcinol blue: the veinal regions turned red (Plate 5.3) and a few epidermal cells from mildew infected Midas, Triumph and Golden Promise and brown epidermal Triumph cells showed a slight purple colour indicative of lignin (Table 5.1).

Observations on cells stained for the presence of lignin

Autofluorescence was observed to varying degrees for epidermal cells from normal and mildew infected tissue of all cultivars (Table 5.1, Plate 5.4). Brown epidermal cells did not autofluoresce except those of Javelin which showed slight autofluorescence. Normal mesophyll cells from Heriot, Javelin and Midas autofluoresced but those from Triumph and Golden Promise did not. Mesophyll cells from all mildew infected tissue except Golden Promise autofluoresced slightly (Table 5.1). Only brown cells from Javelin showed traces of autofluorescence. Tissues from the veinal region autofluoresced brightly (Plate 5.4) indicative of high levels of lignin present.

TABLE 5.1: Reactions of normal, mildew infected and brown leaf tissue, from five spring barley cultivars, to histological stains specific for callose, lignin, phenols and necrotic cells.

Cultivar		Stain: Callose Ab	Callose Rs	Lignin Au	Lignin Ca	Lignin To	Lignin Ma	Lignin Ph	Phenol Ni	Phenol Fe	Dead Th
Epidermis											
Heriot	N	++	-	+/-	++	-	+/-	-	-	-	+
	M	+	-	+/-	++	-	+?	-	-	-	-/+
	B	-	-	-	+	-	+?	-/+	-	-	-
Javelin	N	+	-	++	++	-	+/-	-	-	-	-
	M	a									
	B	-	-	+/-	+	-	+?	-/+	-	-	-
Midas	N	++	-	+	++	-	+/-	-	-	-	-
	M	+/-	-/+	++	++	-	+?	-	-	-	-
	B	-	-	-	-	-	+?	-	-	-	-
Triumph	N	-/+	-	+	++	-	+/-	-	-	-	-/+
	M	+	-/+	+	++	-	+?	-	-	-	-
	B	-	-/+	-	+	-	+?	-	-	-	-
Golden	N	+	-	+	++	-	+/-	-	-	-	-
Promise	M	+	-/+	+/-	++	-	+/-	-	-	-	-
	B	a									
Mesophyll											
Heriot	N	-	-	+	+	-	+/-	-	-	-	-
	M	-/+	-	-/+	+	-	+?	-	-	-	-
	B	+	-	-	-/+	-	+	+/-	-	-	-
Javelin	N	-	-	+	+	-	+/-	-	-	-	-
	M	a									
	B	-	-	-/+	-	+/-	+/-	-	-	-	-
Midas	N	+/-	-	++	++	-	+/-	-	-	-	-
	M	+/-	-	+/-	++	-	+/-	-	-	-	-
	B	-	-	-	-	-	+/-	-	-	-	-
Triumph	N	-	-	-	+/-	-	+/-	-	-	-	-
	M	+/-	-	+/-	+/-	-	+/-	-	-	-	-
	B	+/-	-	-	+/-	+/-	+?	-	-	-	-
Golden	N	-	-	-	+	-	+/-	-	-	-	-
Promise	M	-	-	-	+	-	+/-	-	-	-	-
	B	a									

Key

Ab	aniline blue	Ma	Maüle	+	positive reaction
Rs	resorcinol	Ph	phloroglucinol	-	negative reaction
Au	autofluorescence	Ni	Nitroso	a	not observed
Ca	calcofluor	Fe	FeCl	+/-	slight reaction
To	toluidine blue O	Th	thionin	-/+	only one or two cells reacting

N - normal; M - mildew; B - brown

PLATE 5.1: TS of brown Javelin leaf stained with aniline blue showing bright blue fluorescence of veinal region but absence of fluorescence in brown mesophyll tissues (UV light; x 312.5 magnification).

PLATE 5.2: TS of mildew infected Heriot leaf stained with aniline blue showing bright yellow fluorescence of epidermal cell and papilla (arrowed). Note haustorium (H) within the epidermal cell (blue light; x 500 magnification).

PLATE 5.3: TS of mildew infected Heriot leaf stained with resorcinol blue (pH 3.2); note absence of blue staining for callose but red staining of thickened tissue of veinal region associated with lignin (bright field illumination; x 312.5 magnification).

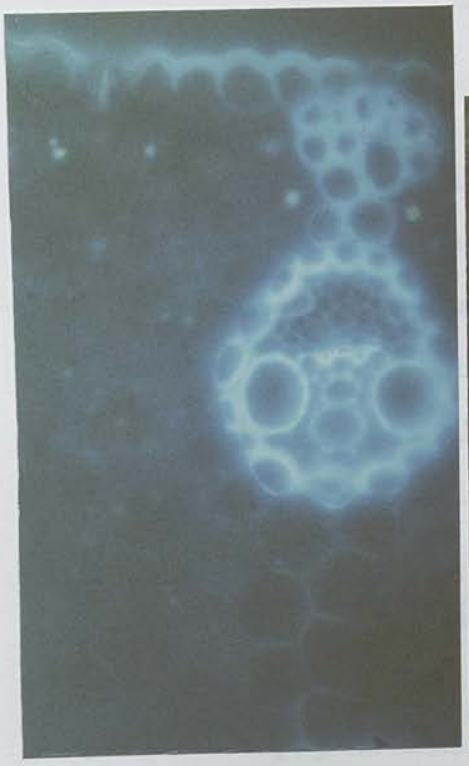


Plate 5.1

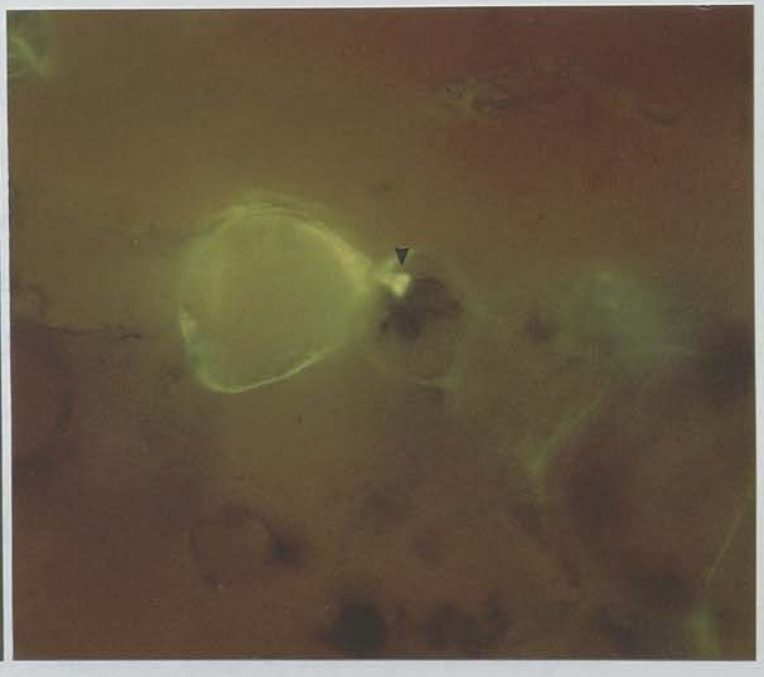


Plate 5.2

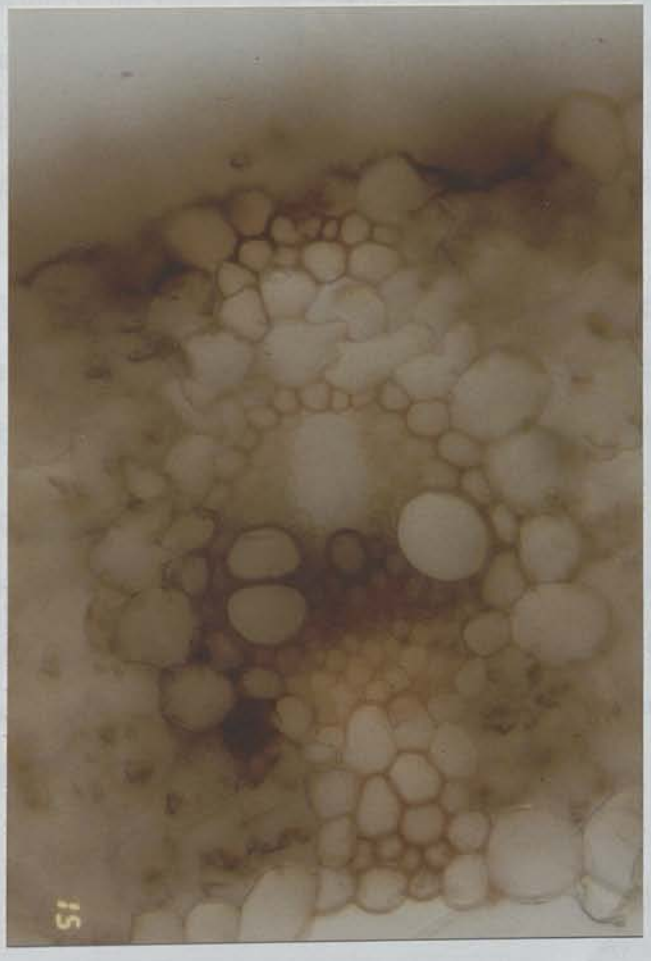


Plate 5.3

Calcofluor tended to stain all tissue bright yellow-green when observed under blue light (Table 5.1, Plate 5.5) and thus was unspecific in its action. However brown epidermal Midas cells and brown mesophyll cells from Javelin and Midas failed to react. Mesophyll cells from Triumph also reacted less. It must be noted that in the brown epidermal cells, especially Javelin, there was a masking of the fluorescence by the brown pigment in the cell wall. The large xylem vessels showed up bright yellow (Plate 5.5).

With toluidine blue a slight blue violet reaction was recorded in brown mesophyll cells of Javelin and Triumph only (Table 5.1), with most cells staining violet indicative of polysaccharides (Plate 5.6). Large xylem vessels stained green indicative of lignin.

Reaction to the Maüle stain was difficult to determine since the stain coloured all cells brown (Table 5.1, Plate 5.7). This was especially difficult with the brown tissue because of the pigment already present in cells. The colour of brown areas of tissue tended to be darker than non-brown tissue but compared with the veinal tissue, which was almost black, did not react to this stain (Plate 5.7).

Cells generally failed to react to phloroglucinol although a few brown Heriot (epidermal and mesophyll) and Javelin (epidermal) cells did have a slight red tinge to them (Table 5.1). Cells from the veinal region stained dark cherry red (Plate 5.8).

Observation on cells stained for the presence of phenols

Cells failed to react to either stain used for the detection of phenolic compounds (Nitroso turns tannins cherry red; FeCl turns phenols green) (Table 5.1). Large xylem vessels stained very pale yellow-orange with Nitroso stain (Plate 5.9) and green with FeCl (Plate 5.10).

PLATE 5.4: TS normal Javelin leaf showing blue autofluorescence of outer epidermal cell walls and bright blue autofluorescence of veinal region (UV plus bright field illumination; x 500 magnification).

PLATE 5.5: TS normal Golden Promise leaf stained with calcofluor showing general bright green fluorescence and yellowing of xylem walls (blue light; x 312.5 magnification).

PLATE 5.6: TS brown Javelin leaf stained with toluidine blue O showing general violet colour to epidermal and mesophyll cells; note collapse of epidermal cells (bright field illumination; x 500 magnification).

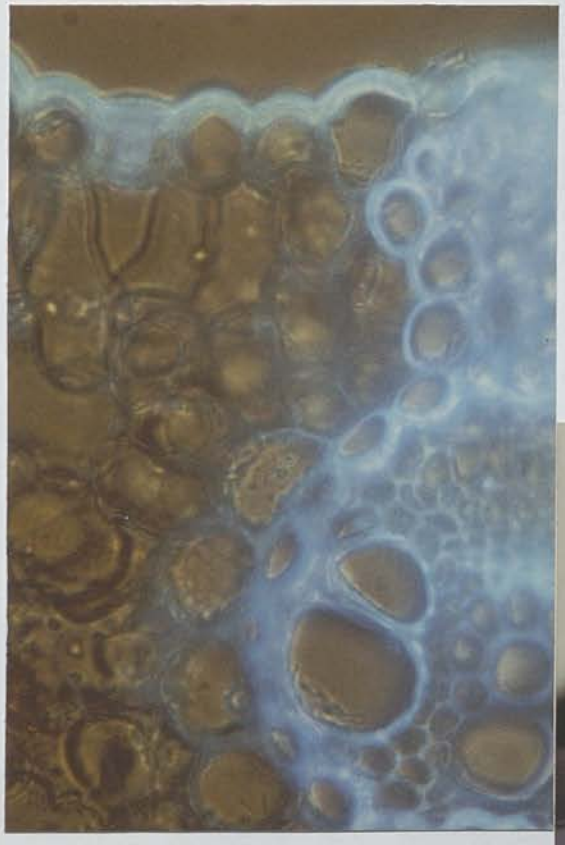


Plate 5.4

Plate 5.6

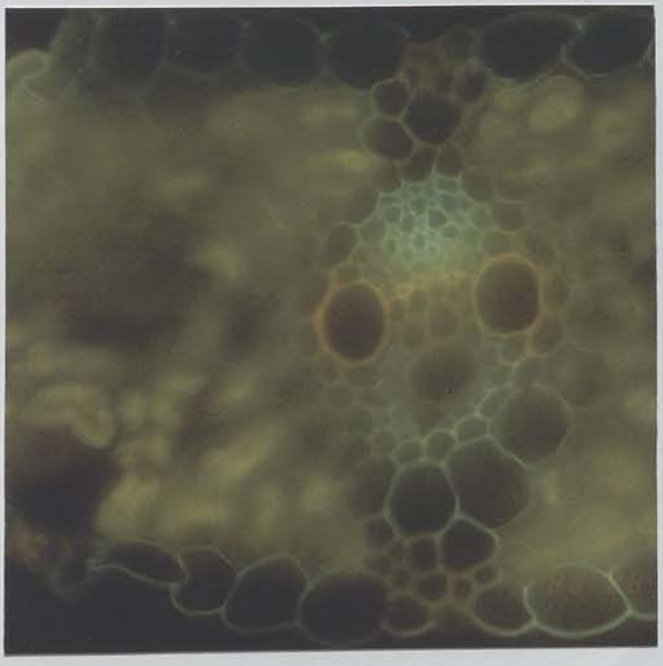


Plate 5.5

PLATE 5.7: TS brown Javelin leaf stained with Maüle stain, showing brown/black staining of thickened tissues associated with the vein but no such staining of epidermal/mesophyl cells (bright field illumination; x 312.5 magnification).

PLATE 5.8: TS brown Javelin leaf stained with phloroglucinol showing bright red staining of tissues associated with the vein; brown cells show no reaction (bright field illumination; x 312.5 magnification).

Plate 5.7

Plate 5.8

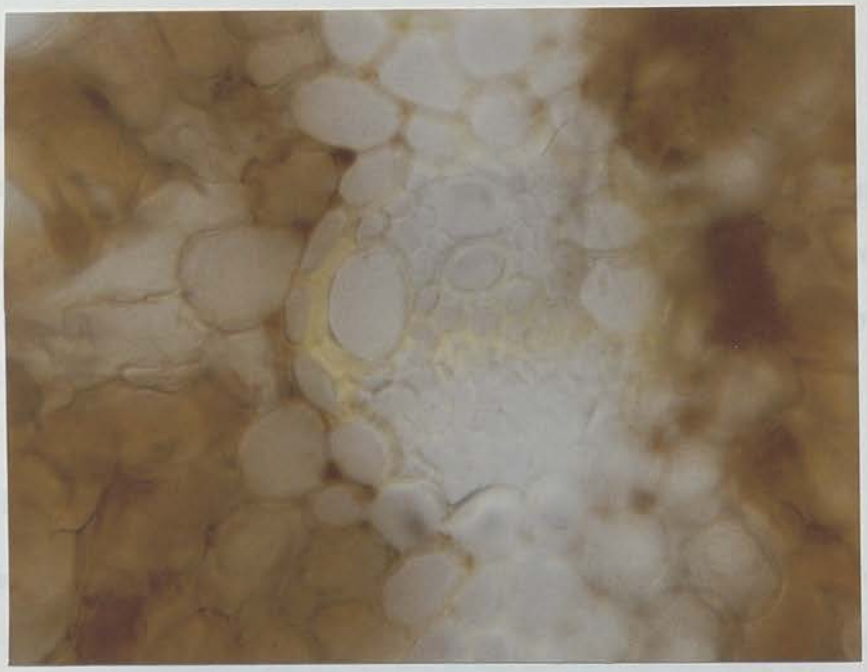
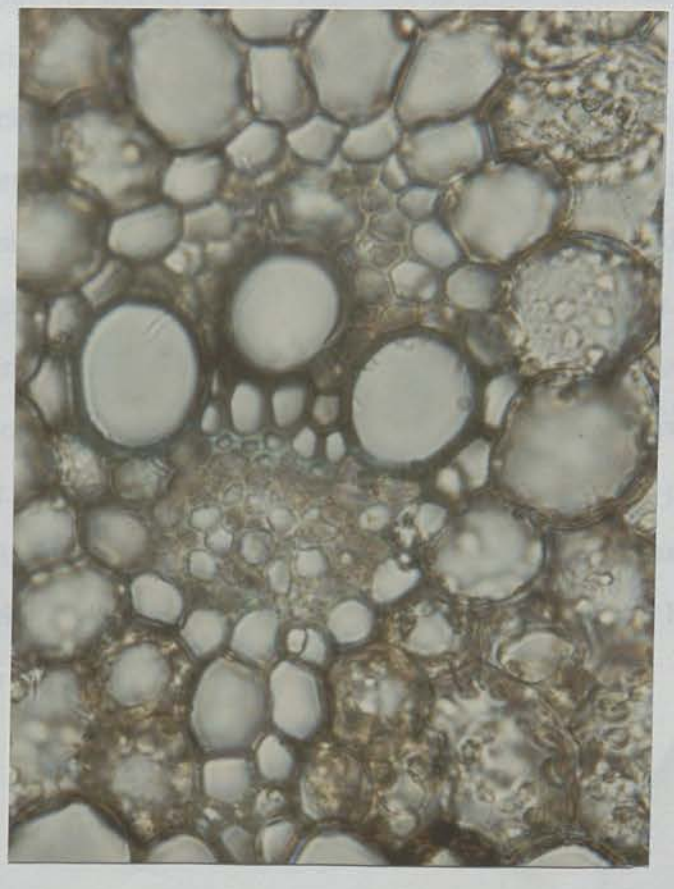


PLATE 5.9: TS of brown Heriot leaf stained with Nitroso showing pale orange-yellow colour of xylem tissues but no bright red colour associated with tannins (bright field illumination; x 500 magnification).

PLATE 5.10: TS of mildew infected Midas leaf stained with ferric chloride showing green staining of xylem tissues within the vein (bright field illumination; x 500 magnification).



Observations on cells stained for the presence of necrotic tissue

In general, cells failed to react to thionin (necrotic cells stain purple) although Heriot (normal and mildew infected) and Triumph (normal) epidermal cells reacted slightly (Table 5.1). Veinal regions tended to turn pale purple (Plate 5.11), possibly indicating some necrosis.

5.3.2 Biochemical observations

The one dimensional chromatography of extracts showed up several spots from both thumb-print and unaffected Javelin leaves (Figure 5.4). The reactions of individual spots to U.V light, vanillin and folin reagents are detailed in Table 5.2, along with Rf values. Figure 5.4 shows up one spot present in thumb-print leaves, spot 4/5 which was not present in normal leaves.

Extracts from control and thumb-print Javelin leaves were further separated two dimensionally and developed using folin reagent and fuming ammonia. Results are shown in Figure 5.5 and Table 5.3. There is a far greater difference between normal and thumb-print leaves, with many spots appearing on the thumb-print chromatography but few on the normal. Spots 7 and 15 correspond in position and Rf values as do spots 12 and 19 indicating common compounds. Spot 17 of normal leaves had a similar Rf value to spot 10 in thumb-print leaves but did not fluoresce under U.V light and only reacted to folin when fumed in ammonia. These two compounds are therefore most likely to be different. Spot 18 of normal leaves may be a mixture of 11, 13 and 14 of thumb-print leaves, separation having occurred to less an extent. Spot 16 of normal leaves did not appear on thumb-print leaves. Comparing Figure 5.4 with Figure 5.5 it is seen that the differences between normal and thumb-print leaves have occurred through separation of spots 1 and 4 of Figure 5.4 into spots 1-5 in Figure 5.5.

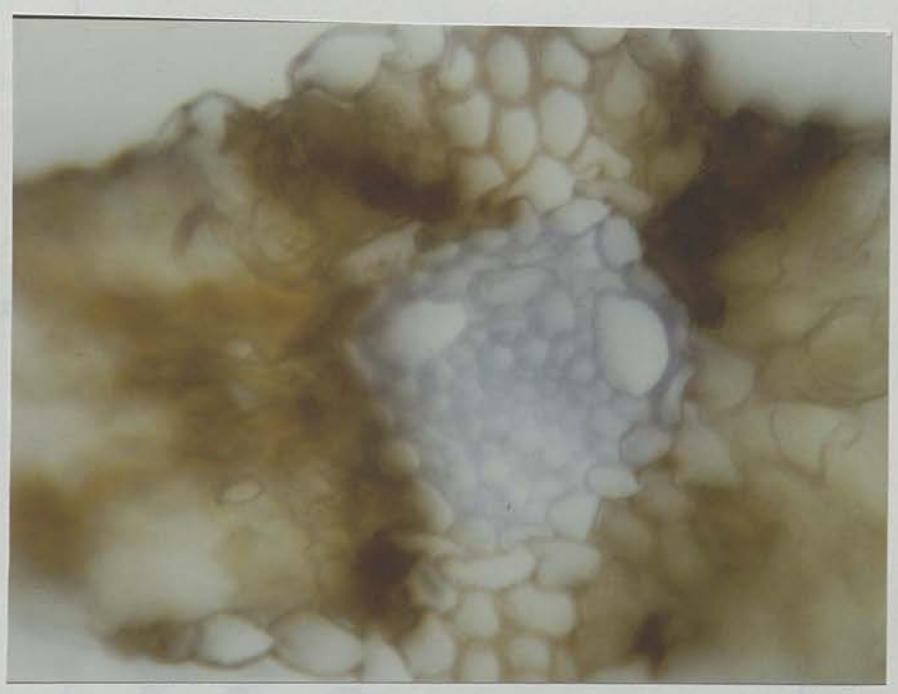
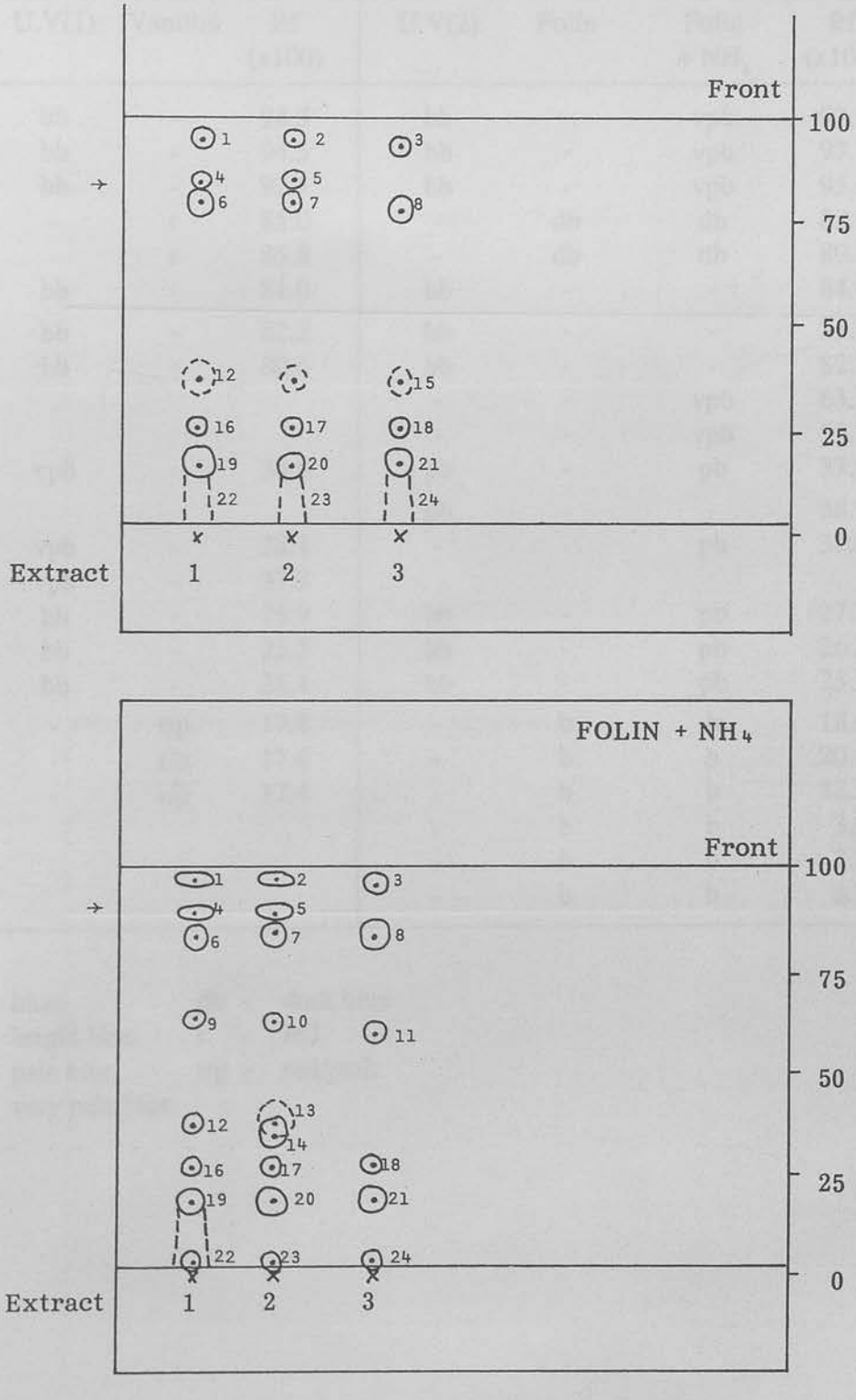


PLATE 5.11: TS of brown Heriot leaf stained with thionin showing pale purple colour of veinal tissues but no bright purple colour of brown cells associated with necrotic tissue (bright field illumination; x 500 magnification).

FIGURE 5.4: 1-D TLC of ether extracts from normal and thumb-print leaves of Javelin.



1 & 2 - thumb-print leaves
3 - normal leaves

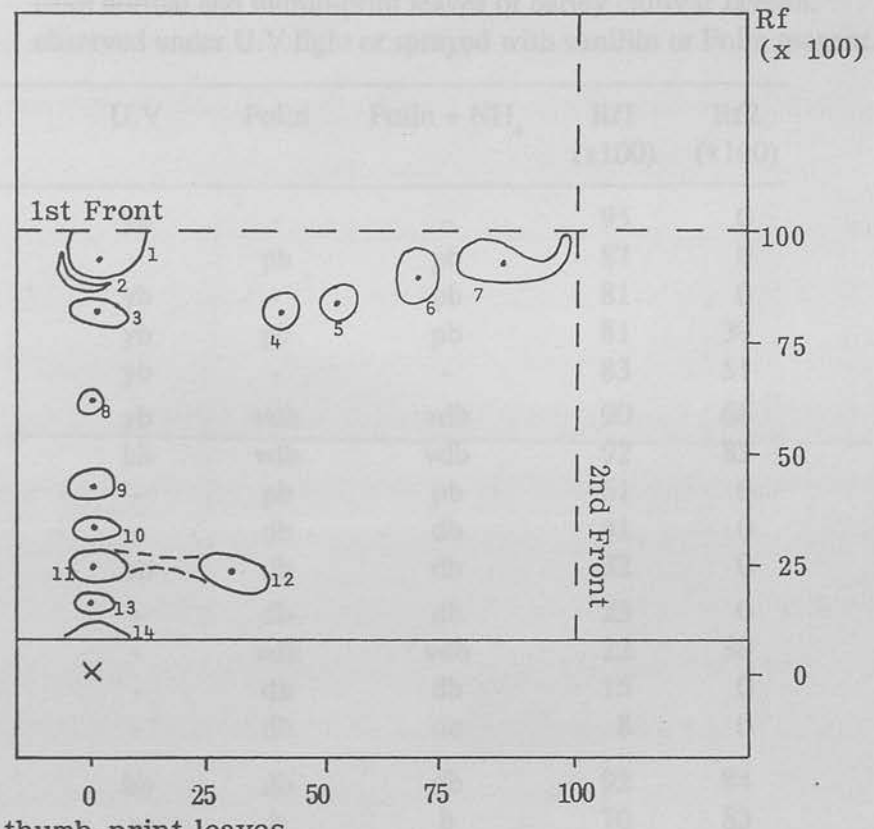
TABLE 5.2: Reaction of spots from 1-D TLC of phenol extractions from 'thumb-print' and normal Javelin leaves when observed under U.V light or sprayed with vanillin and Folin reagents.

Spot	U.V(1)	Vanillin	Rf (x100)	U.V(2)	Folin	Folin + NH_4	Rf (x100)
1	bb	-	94.3	bb	-	vpb	97.5
2	bb	-	94.3	bb	-	vpb	97.1
3	bb	-	92.7	bb	-	vpb	95.5
4	-	r	85.0	-	db	db	88.9
5	-	r	85.8	-	db	db	89.7
6	bb	-	81.0	bb	-	-	84.0
7	bb	-	82.2	bb	-	-	85.2
8	bb	-	80.6	bb	-	-	82.7
9				-	-	vpb	63.2
10				-	-	vpb	59.7
12	vpb	-	38.5	pb	-	pb	37.9
13				pb	-	-	38.7
14	vpb	-	38.1	-	-	pb	37.0
15	vpb	-	37.9				
16	bb	-	25.9	bb	-	pb	27.2
17	bb	-	25.7	bb	-	pb	26.3
18	bb	-	25.1	bb	-	pb	25.5
19	-	r/p	17.8	-	b	b	18.9
20	-	r/p	17.6	-	b	b	20.0
21	-	r/p	17.4	-	b	b	18.5
22				-	b	b	3.3
23				-	b	b	3.3
24				-	b	b	3.7

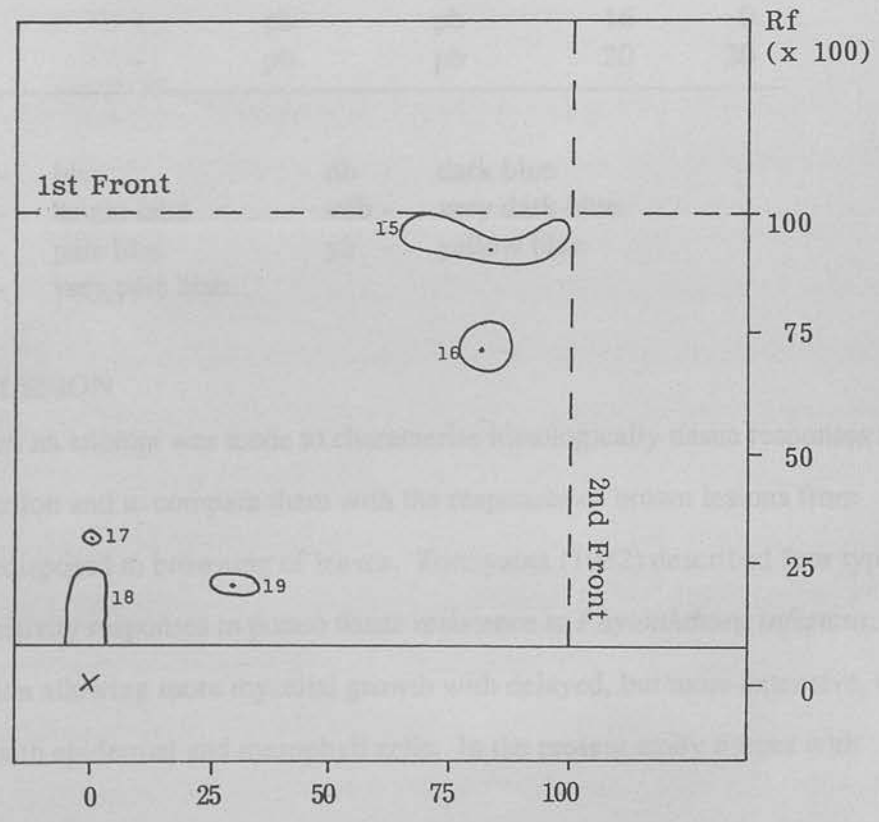
Key:

b - blue
bb - bright blue
pb - pale blue
vpb - very pale blue
db - dark blue
r - red
r/p - red/pink

FIGURE 5.5: 2-D TLC of ether extracts from normal and thumb-print lesion leaves of Javelin, developed using Folin reagent and ammonia.



thumb-print leaves



normal leaves

TABLE 5.3: Reaction of spots from 2-D chromatography of phenol extractions from normal and thumb-print leaves of barley cultivar Javelin, observed under U.V light or sprayed with vanillin or Folin reagent.

Spot	U.V	Folin	Folin + NH_4	Rf1 (x100)	Rf2 (x100)
1	yb	-	-	95	0
2	-	pb	pb	87	0
3	yb	-	pb	81	0
4	yb	pb	pb	81	39
5	yb	-	-	83	51
6	yb	vdb	vdb	90	68
7	bb	vdb	vdb	92	85
8	-	pb	pb	61	0
9	-	db	db	41	0
10	bb	db	db	32	0
11	-	db	db	23	0
12	-	vdb	vdb	22	30
13	-	db	db	15	0
14	-	db	db	8	0
15	bb	db	db	92	84
16	-	b	b	70	83
17	-	-	vpb	31	0
18	-	pb	pb	16	0
19	-	pb	pb	20	30

Key

b -	blue	db -	dark blue
bb -	bright blue	vdb -	very dark blue
pb -	pale blue	yb -	yellow blue
vpb -	very pale blue		

5.4 DISCUSSION

In this section an attempt was made to characterise histologically tissue responses to mildew infection and to compare them with the responses of brown lesions from cultivars predisposed to browning of leaves. Tomiyama (1982) described four types of hypersensitivity responses in potato tissue resistance to *Phytophthora infestans*, the type 4 reaction allowing more mycelial growth with delayed, but more extensive, cell necrosis in both epidermal and mesophyll cells. In the present study tissues with

cultivar related browning differed histologically from those infected with mildew. In the former browning occurred extensively through epidermal and mesophyll cells and involved the complete browning of cells whereas browning in mildew infected tissue was confined to the cell walls of epidermal cells below the mildew mycelium.

From the assessments of the tissues histochemically, results were inconsistent but some broad findings emerged. Callose was recorded occasionally in walls of normal or mildew infected epidermal cells or in brown mesophyll cells but was most frequently found at penetration sites of *E. graminis*. Aist and Israel (1976) observed that callose is often laid down in cells in response to pathogen invasion or mechanical injury, particularly within specialised structures or papillae. Skou (1985) working with barley cultivars containing the mlo gene, which exhibit brown spotting, could not detect callose on brown cells because of the intense colour but found large amounts of callose in the cell walls of cells surrounding necrotic spots. No such depositions were found in this study. Detailed studies carried out by Faulkner and Kimmins in 1975, to look at the staining reactions of tissues bordering necrotic lesions induced by wounding or by virus infection with Tobacco Mosaic Virus and Tobacco Necrotic Virus on bean, found no reaction to Maüle stain, phloroglucinol or ferric chloride, indicating the absence of lignification or phenols and in keeping with the findings in this study.

Staining with phloroglucinol, toluidine blue O and Maüle stain failed to provide conclusive evidence of lignification of brown cells even although lignification of vascular tissue was obvious. Lignification often accompanies the necrogenic response of cells to infection (Asada *et al.*, 1976; Pearce and Ride, 1978). Sadik and Minges (1964) claimed necrotic cells stained purple when treated with a solution of thionin but this effect was not found in the present study. Oku (1962) failed to get a reaction from strongly discoloured cells of rice infected with *Helminthosporium* due to the intense colour present, a similar response to that found here.

Lignin is said to autofluoresce bright yellow and its association with necrosis is

one reason for autofluorescence of necrotic cells (Preece, 1971). Rohringer, Kim, Samborski and Howes (1977) showed necrotic cells of an incompatible wheat/ *Puccinia graminis* relationship to autofluoresce bright orange-yellow and Munro (1985) found that aniline blue was specifically taken up by necrotic cells which fluoresced bright blue under U.V light. Although there was some autofluorescence and aniline blue-induced fluorescence of cells in this study, the intensity was very low. Toyoda, Mayama and Shishiyama (1978) considered that browning of cells occurred after fluorescence, the fluorescent material being converted to brown material. This could explain the general low intensity of fluorescence in brown mildew infected cells or brown lesions but the masking effect of the brown pigmentation is still not ruled out.

The tests for phenolics were also complicated by the presence of brown pigmentation. As shown in Figure 5.2, the browning of cells through infection involves conversion of phenolic compounds to the brown pigmented tannins and melanin (Farkas and Kifaly, 1962). Oku (1962) with *Helminthosporium* infection of rice and Suzuki, Doi and Toyoda (1953) with *Pyricularia oryzae* infection of rice demonstrated the presence of phenolic compounds within cells at an early stage of infection. Chromatographic analysis of extracts from normal tissues and brown lesions of leaves of Javelin indicated the presence of several phenolic compounds in tissues showing browning which were absent from normal tissue. It must be noted that the 2-D chromatographs were carried out only once and were only an indication of possible biochemical differences between normal and brown leaf tissues. For exact analysis and identification of phenolic compounds more extensive extractions and chromatography would need to be carried out.

SECTION 6

THE EFFECT OF DIFFERENT NUTRIENT SUPPLY TREATMENTS ON THE DEVELOPMENT OF BROWN LESIONS ON THE LEAVES OF SPRING BARLEY CULTIVARS

6.1 INTRODUCTION

Normal plant growth requires a satisfactory supply of essential mineral elements and a number of abiotic diseases can be attributed to such elements being deficient in the soil or unavailable to the plant (Anon, 1934). In field-grown cereal crops the most obvious deficiency symptoms are changes of colour and height of the plant (Hoffer, 1941). Chlorotic symptoms generally are attributed to the absence of elements essential for basic plant physiological processes. Brown or necrotic spots have been associated with deficiencies of magnesium, potassium and manganese (Mathre, 1982). Magnesium is an important component of chlorophyll and, in its absence, chlorophyll synthesis is inhibited, leading to reduced photosynthesis; magnesium is also required for activation of enzymes involved in the Calvin cycle. Potassium and manganese are involved in the activation of enzymes for photophosphorylation and protein synthesis, and potassium deficiency can also lead to increased respiration and decreased CO_2 assimilation (Zimmerman, 1947; Mengel and Kirkby, 1978; Sutcliffe and Baker, 1981; Bould, Hewitt and Needham, 1983). The more detailed symptoms of browning of leaves from barley plants grown under deficiency conditions have been described by several authors. Leaves of plants grown under magnesium or potassium deficiency show tip or edge scorch with some associated bleaching, while it has been reported that potassium deficiency can produce small purplish brown spots on barley leaves (Anon, 1934; Hoffer, 1941; Bould *et al.*, 1983). Manganese deficiency tends to produce interveinal necrotic spots on barley leaves (Wallace, 1961).

Since previous investigations into pathogen infection (Sections 3 and 4) did not conclusively reveal the cause of cultivar specific necrotic lesions, nutrient deficiency was considered as a possible factor.

6.2 MATERIALS AND METHODS

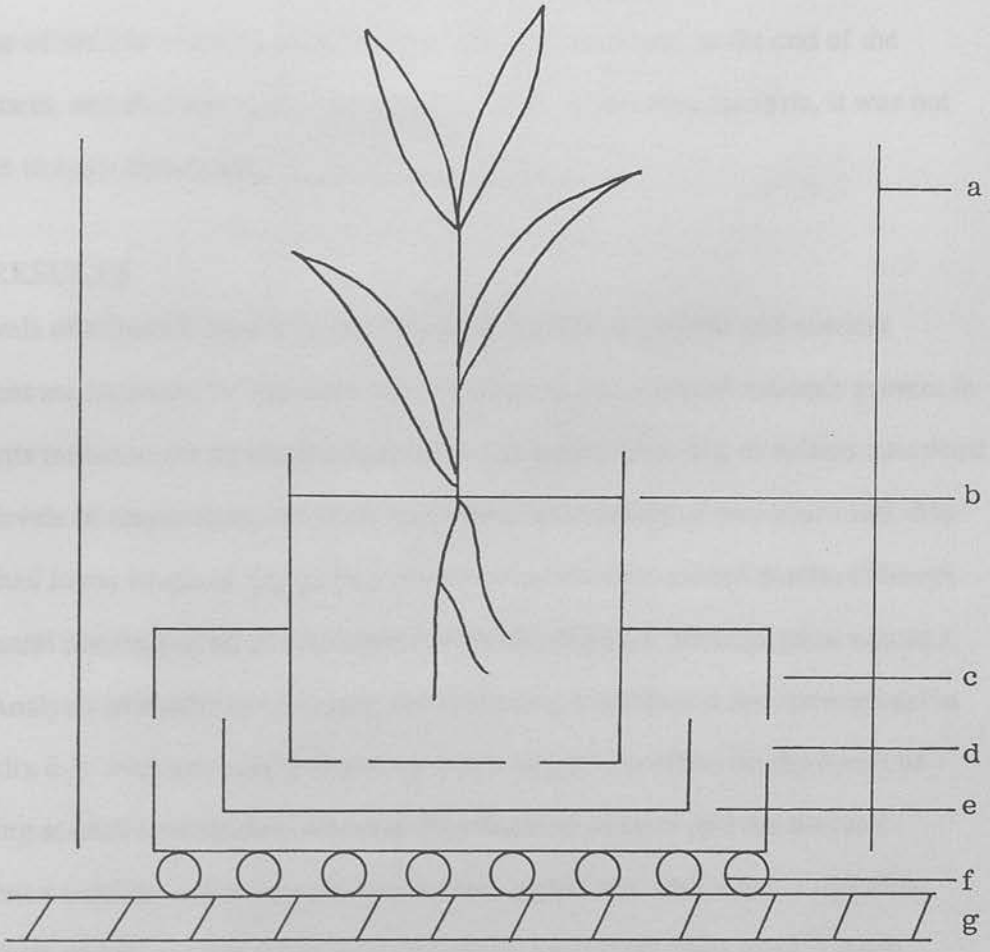
Seed of Heriot, Javelin, Midas, Triumph, Vista and Golden Promise were sown in Vermiculite in 9 cm^2 pots, using three seed, thinned to one, per pot and 16 pots per cultivar. Four pots of each cultivar were placed into each of four spore-free compartments (Plate 4.1), each compartment acting as a replicate. One pot of each cultivar was assigned to one of the following treatments: complete nutrient solution (C); complete nutrient minus magnesium (-Mg); complete nutrient minus potassium (-K) and complete nutrient minus manganese (-Mn). The experiment was set up in a split-plot design, with each compartment a block, nutrient treatment forming a main plot (randomised within blocks) and cultivar a sub-plot (randomised within plots). Each pot was placed onto a plastic petri-dish and covered with a polythene bag to prevent growth of roots into the gravel on the floor of each compartment. Holes were cut into the side of the bag to allow access of air to the roots. The whole system was surrounded with polythene sleeving (Figure 6.1).

An initial nutrient feed of 150 ml was added to each pot, thereafter 50 ml was given as required. Nutrient solutions were made by dissolving nutrient tablets from Griffin Plant Culture kits in the required amount of double distilled water. Tablets were available for all nutrient treatments except -Mn, which was prepared in the laboratory on the basis of similar specifications to those for the tablets (Appendix 6.1).

During this study it was essential to clean all equipment thoroughly. All plant pots, labels, petri-dishes and glassware were scrubbed with scouring powder, washed in hot 50% hydrochloric acid and rinsed in double distilled water (Hewitt and Smith, 1975).

At weekly intervals plants were scored for percentage leaf area covered in brown lesions or dead (withered) tissue. Statistical analysis was carried out on the means of all leaves from each plant. In the later stages of the experiment, when parts

FIGURE 6.1: Diagrammatic representation of experimental system used to investigate the effects of nutrient deficiency conditions on barley plants.



of leaves were withered, browning was scored as a percentage of the leaf area not withered.

Finally, plants were allowed to dry out, ground to a fine powder in a blender (Analysenmühle AIO, Janke and Kunkel, Ika-Werk) and analysed for mineral content by the Central Analytical Laboratory (CAL) at the Edinburgh School of Agriculture. Because of the low amounts of dried plant material remaining at the end of the experiment, and the large quantities required for each chemical analysis, it was not possible to have replication.

6.3 RESULTS

The levels of minerals present in the tissues in relation to cultivar and nutrient treatment are indicated in Appendix 6.2. In general, the levels of minerals present in the plants reflected the treatments applied in that plants with -Mg treatment contained lower levels of magnesium, -K plants contained lower levels of potassium and -Mn plants had lower levels of manganese when compared with control plants, although the mineral contents in all plants seemed relatively high (A. Sinclair, pers. comm.).

Analysis of results of browning and withering assessments are summarised in Appendix 6.3. Nutrient supply treatment had a significant effect on the levels of browning at each scoring date, whereas the effects of cultivar and the nutrient treatment x cultivar interaction did not become significant until week 5 and after. The effects of nutrient supply on withering were significant from week 3 when withering was first recorded, but cultivar effects did not become significant until week 6 and there was no interaction between nutrient treatment and cultivar.

The levels of browning and withering 6 weeks after sowing are shown in Table 6.1 as a general indication of nutrient and cultivar effects. With respect to browning symptoms, -Mn treated plants produced the most browning on leaves, with -Mg and -K producing levels similar to control plants (Figure 6.2). Browning first appeared on

-Mn plants two weeks after sowing, thereafter rapidly increasing with a corresponding increase in withering, which was significantly higher on -Mn plants than with other treatments. Javelin exhibited fewer brown lesions compared with other cultivars but, more particularly when grown under complete nutrient, magnesium deficient and potassium deficient conditions. Golden Promise also tended to show less than average amounts of browning. Vista had higher levels of necrosis under manganese deficient conditions than other cultivars. Individual cultivars differed in the extent of withering of tissues, Heriot showing higher than average levels and Golden Promise lower than average levels.

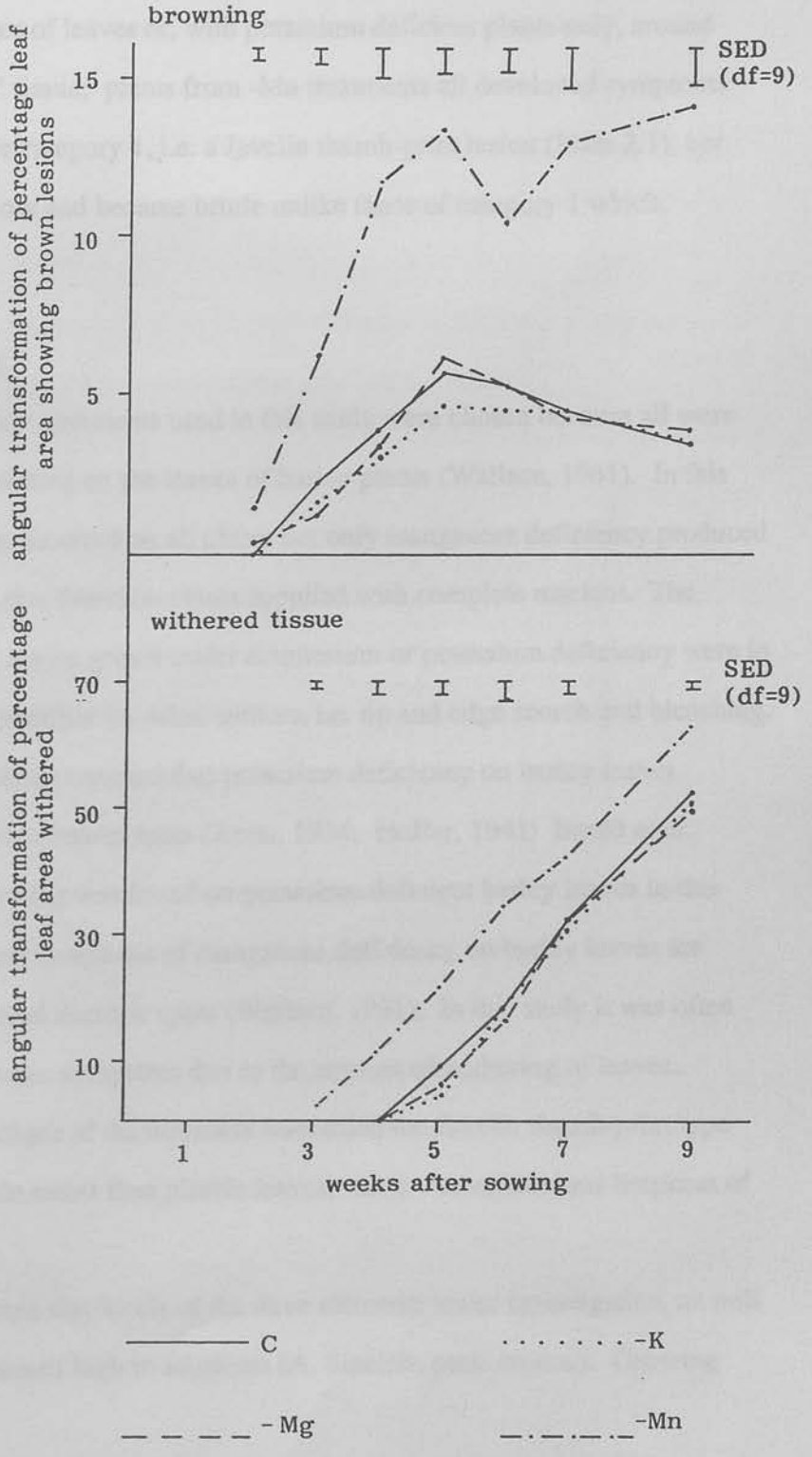
TABLE 6.1: Angle of percentage leaf area affected by brown lesions and withering for six spring barley cultivars grown under four nutrient conditions, 6 weeks after sowing.

Symptom	Nutrient treatment	Cultivar Hr	Jv	Ms	Tr	Vi	Gp	Mean
Brown lesions	C	7.5	1.6	5.1	6.2	6.0	4.8	5.2
	-Mg	6.2	2.8	5.1	5.8	7.2	3.5	5.1
	-K	4.6	2.6	6.5	5.0	4.2	4.1	4.5
	-Mn	8.7	9.6	9.5	10.8	14.3	9.4	10.4
mean		6.7	4.2	6.5	6.9	7.9	5.4	6.3
Withering	C	31.2	15.7	21.1	14.1	15.2	12.7	18.3
	-Mg	26.7	19.5	20.0	8.9	14.9	6.5	16.1
	-K	34.8	19.2	17.9	17.1	6.9	13.1	18.2
	-Mn	32.8	37.3	34.1	32.0	37.7	31.1	34.2
mean		31.4	22.9	23.3	18.0	18.7	15.8	21.7

SED (df)	Nutrient treatment (n) (9)	Cultivar (c) (60)	n x c (60)
Browning ±	0.75	0.67	(1) 1.34 (2)
Withering ±	3.14	3.02	6.35 (1) 6.05 (2)

(1) between nutrient treatment
(2) within nutrient treatment

FIGURE 6.2: Levels of brown and withered tissue on leaves of spring barley cultivars given for nutrient treatments



The expression of brown symptoms shown under the various nutrient treatments were the same for all cultivars: plants given complete nutrient developed browning mainly at the tips; plants from -K and -Mg treatments produced browning at the extreme tips and edges of leaves or, with potassium deficient plants only, around patches of 'bleached' tissue; plants from -Mn treatments all developed symptoms similar to those of the category 1, i.e. a Javelin thumb-print lesion (Plate 2.1), but leaves quickly dried out and became brittle unlike those of category 1 which remained pliable.

6.4 DISCUSSION

The nutrient deficiency treatments used in this study were chosen because all were reported to show browning on the leaves of barley plants (Wallace, 1961). In this experiment browning occurred on all plants but only manganese deficiency produced more browning than that found on plants supplied with complete nutrient. The symptoms shown on leaves grown under magnesium or potassium deficiency were in keeping with those described by other authors, i.e. tip and edge scorch and bleaching. Although it has also been reported that potassium deficiency on barley leaves produces small purplish-brown spots (Anon, 1934; Hoffer, 1941; Bould *et al.*, 1983), little or no spotting was found on potassium deficient barley leaves in this present study. Typical symptoms of manganese deficiency on barley leaves are limpness and interveinal necrotic spots (Wallace, 1961). In this study it was often difficult to observe exact symptoms due to the amount of withering of leaves. However, the early stages of the disorders resembled the Javelin thumb-print type lesions but with brittle rather than pliable leaves; there was no obvious limpness of leaves.

It should be noted that levels of the three elements under investigation, as well as other minerals, seemed high in all plants (A. Sinclair, pers. comm.). Growing

plants in glasshouses in pots may predispose them to accumulate high levels of mineral elements in general. However, plants in nutrient supply treatments deficient in either potassium, magnesium or manganese all showed lower contents of the respective elements than plants with complete nutrient, and in the case of -Mn treatment the incidence of brown lesions was higher than with other mineral supply treatments. It has been shown that barley plants grown under manganese deficiency are more prone to exhibit stress symptoms such as brown spotting when compared with plants grown under other nutrient conditions (A. Sinclair, pers. comm.). It is therefore possible in this present study that the manganese deficient plants are not simply responding to a lack of nutrient but also to other factors.

It has been claimed that some cultivar differences are shown by barley plants in strontium uptake and by oat leaves in magnesium and manganese content (Vose and Griffiths, 1961; Epstein, 1972). However, although different cultivars showed differences in extent of browning, or in the extent of browning in relation to particular nutrient treatments, no differences between cultivars in the type of symptoms shown in response to nutrient supply were found in the present study. Thus, different nutrient treatments led to variation in brown symptoms of barley leaf tissue but there was no evidence of a cultivar x nutrient supply interaction which would account for the cultivar symptom differences described in Table 2.1.

SECTION 7

THE OCCURRENCE OF BROWN LESIONS ON LEAVES OF SPRING BARLEY CULTIVARS IN RELATION TO THE APPLICATION OF HERBICIDES

7.1 INTRODUCTION

Herbicides form a diverse group of chemicals which may be considered in three main categories, soil-acting herbicides such as diuron, di-allate and simazine, the contact herbicides, including paraquat and diquat and the systemic herbicides, for example 2,4-D, MCPA, MCPB and CMPP (Evans, 1962).

The soil-acting herbicides kill germinating seedlings before they emerge through the soil surface (Evans, 1962). The contact herbicides produce a rapid local necrosis or scorching of tissues below herbicide droplets, usually within a few days but, in some cases, within a few hours (Klingman, 1961; Evans, 1962). Paraquat and diquat may produce a rapid bleaching, rather than browning, in sunny conditions followed by desiccation of leaves (Stephens, 1982). The contact herbicides elicit their effects on certain biochemical pathways within the plant cells. They inhibit or uncouple electron transport during the light reaction of photosynthesis (Stephens, 1982). However, chloroplasts continue to absorb light energy, which may lead to the destruction of membranes and release of hydrolytic enzymes into cell vacuoles, destruction of chlorophyll, loss of cellular integrity, desiccation and death (Klingman and Ashton, 1975). Paraquat destroys the cell plasma membrane then the chloroplast membrane, through the diversion of electrons to reduce the herbicide rather than passing down the electron transport chain: the herbicide is reoxidised to form organic radicals which further disrupt membranes and starch formation is prevented (Baur, Bovey, Baur and El-Seify, 1969). Dinoseb (2-sec-butyl-4,6-dinitrophenol), a contact herbicide which has now been withdrawn, stimulates respiration at low concentrations but inhibits it at high concentrations. As with photosynthesis, oxidative phosphorylation is uncoupled (Ashton and Crafts, 1981).

The systemic herbicides induce three possible responses, chlorosis, necrosis or abnormal growth, effects generally taking 2 to 3 weeks to appear (Evans, 1962). Dalapon, however, causes rapid scorching of grasses: the amino triazoles scorch

foliage but subsequent growth develops white patches within the green tissue. The auxin-like herbicides such as 2,4-D and 2,4,5-T affect the growing point of plants resulting in thin, twisted shoots and the development of adventitious roots and callus (Myers, 1953; Wooley, 1982). The thiocarbamates reduce leaf waxes so increasing transpiration, but also inhibit the synthesis of unsaturated fatty acids which are required for membrane formation. The compound 2,4-D interferes with the intermediary metabolism of photosynthesis and respiration (Klingman and Ashton, 1975). It has a similar effect on respiration as paraquat and causes stomatal closure, so curtailing transpiration, but also reducing CO_2 uptake (Klingman, 1961). This leads to reduced photosynthesis, starvation and eventual death.

The timing of application of herbicides to crops is critical to avoid crop injury, there being set guidelines as to growth stages and spraying times to reduce risk of economic damage. A further risk arises through spray drifting of contact herbicides onto a susceptible crop; this can cause the development of necrotic spots on leaves (Anon, 1981). Pyrazon, a soil-acting herbicide, is absorbed through the roots of bean plants and translocated to the leaves (Rodebush and Anderson, 1970). It causes failure of the leaves to expand, severe chlorosis with the chloroplasts failing to synthesise starch, epidermal collapse and necrosis (Anderson and Schaelling, 1970). Trifluralin can actually reduce wilt disease infection of tomato plants by inducing the accumulation of phytoalexins and phenolics leading to increased or rapid cell necrosis and death (Grinstein, Lisker, Katan and Eshel, 1984). Glyphosate has the opposite effect, inhibiting the conversion of shikimate to chorismate in the aromatic biosynthesis pathway (see Figure 5.1), so inhibiting phytoalexin production and resistance of soybeans to infection with *Pseudomonas syringae* pv *glycinea* (Johal and Rahe, 1984). Systemic herbicide damage of cereals produces abnormal growth patterns such as shrivelled grains, incompletely filled ears, blind or short ears (Tottman and Phillipson, 1974).

Glasshouse and laboratory studies failed to reproduce necrotic symptoms shown on leaves of certain barley cultivars grown under field conditions (Section 2). One difference between glasshouse and field plants was the presence of herbicides in field conditions. It was, therefore, decided to investigate the effects of certain herbicides on the leaf responses of barley cultivars grown in a glasshouse.

7.2 MATERIALS AND METHODS

Seed of Heriot, Javelin, Midas, Triumph, Golden Promise, WB17E3-1 and WB26E5-3 were sown in 18 cm pots as described in Section 4.2, using 48 pots per cultivar of which eight were placed into each of six replicate blocks on a glasshouse floor. Plants were grown to the 3-4 leaf stage (but before first node, G.S 31) and two pots of each cultivar per replicate given one of the following treatments:

A MCPA (methylchlorophenoxy acetic acid) at a rate of 2.5 litres of herbicide per 400 litres of water (Anon, 1983c);

B CMPP (chloromethylphenoxy proprionic acid) at a rate of 4.2 litres per 200 litres of water (Anon, 1983c);

C Deloxil (bromoxynil + ioxynil) at a rate of 1.5 litres per 225 litres of water (Anon, 1986b);

D Water.

The herbicides used were three of the most common in use on spring barley crops at the time of the experiment. Spraying was carried out using a knapsack sprayer. The treatments and cultivars were placed at random within each replicate block. Plants were scored for percentage leaf area exhibiting brown lesions at G.S 39.

7.3 RESULTS

From the analysis of variance of the data (angular transformations), both herbicide treatment and cultivar had highly significant effects on the levels of browning on leaves (Appendix 7.1) but with no interaction between the two factors. The mean

levels of browning found on leaves are shown in Figure 7.1. Browning was significantly increased if plants were sprayed with CMPP when compared with the control, but MCPA and Deloxil had no effect. With respect to the differences between individual cultivars, Javelin, WB17E3-1 and WB26E5-3 tended to show higher levels of browning compared with other cultivars.

Although Figure 7.1 shows the extent of browning, it gives no indication as to the actual appearance of symptoms, which varied in some instances between cultivars (Plates 7.1-7.4). Plate 7.1 shows three sets of leaves from cultivars Heriot, Javelin and Midas all sprayed with MCPA. Heriot is seen to show large dark spots, Javelin thumb-print lesions and Midas target spot lesions. When sprayed with CMPP (Plate 7.2), leaves of Heriot again developed distinctive lesions as did Triumph, although spots were smaller: the two cultivars WB17E3-1 and WB26E5-3 both showed thumb-print type lesions. Deloxil produced brown lesions on Heriot, target-spotting on Midas, small spots on Triumph and very distinct thumb-print lesions on WB26E5-3 (Plate 7.3). Most control plants developed only small spots or brown flecking (Plate 7.4), as did Golden Promise with all sprays.

The symptoms just described were more obvious or severe on particular cultivars sprayed with the various herbicides. In some instances only a few plants showed such symptoms, in others the symptoms were more extensive. When only a few plants exhibited distinct thumb-print symptoms (Javelin) or target-spotting (Midas) the remaining plants developed very dark, general browning, tending to follow the direction of veins.

7.4 DISCUSSION

Mayes and Marshall (1982) found mixtures of ioxynil/bromoxynil (Deloxil)/ mecoprop (CMPP)/linuron could produce severe damage to winter wheat, especially if night frost followed spraying. They also found mecoprop on its own could damage

FIGURE 7.1: Extent of brown lesions on the leaves of different spring barley cultivars sprayed with three herbicides before node 1. (a) herbicide effects, (b) cultivar effects.

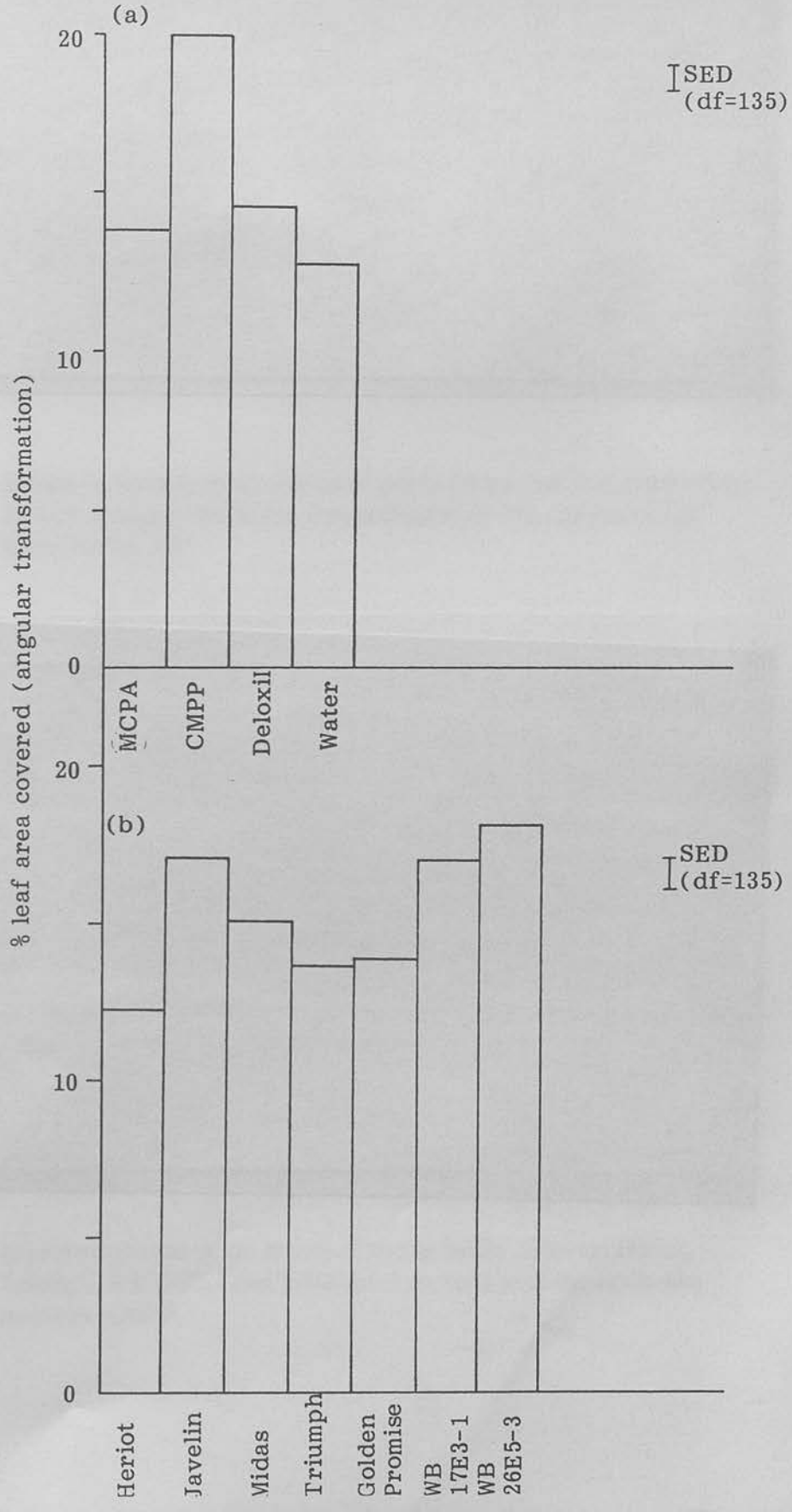


PLATE 7.1: Brown symptoms on the leaves of spring barley cultivars Heriot (top), Javelin (middle) and Midas (bottom) sprayed with the auxin-like herbicide MCPA.

PLATE 7.2: Brown symptoms on the leaves of spring barley cultivars Heriot, Triumph, WB17E3-1 and WB26E5-3 sprayed with the auxin-like herbicide CMPP.

PLATE 7.3: Brown symptoms on the leaves of spring barley cultivars Heriot, Midas, Triumph and WB17E3-1 sprayed with the contact herbicide Deloxil (bromoxynil + ioxynil).

PLATE 7.4: Brown flecking on leaves of the spring barley cultivars Heriot and WB26E5-3 sprayed with water.

leaves. Dichlorprop, a phenoxyproprionic acid similar to mecoprop, caused leaf scorch and lodging of winter wheat (Munroe, Hubbard and Scourey, 1973; Fletcher and Kirkwood, 1982). Robison and Fenster (1973) claimed herbicide damage could be reduced by replacing auxin-type herbicides with contact herbicides such as bromoxynil. Results from this study showed that the auxin type herbicide, CMPP, increased the levels of browning on barley leaves compared not only with the control but also when compared with the contact herbicide Deloxil: the auxin herbicide MCPA produced less browning than CMPP and showed no significant increase in browning over levels on Deloxil or control plants. However, Evans and Holroyd (1962) observed more severe scorching on cereal leaves treated with MCPA/2,3,6-TBA mixtures than with mecoprop (CMPP).

The time of application of herbicides can considerably affect the response of the crop. The ideal (safe) stages of growth for application vary with the herbicide concerned: MCPA from five leaf stage but before first node; mecoprop from leaf 1 to before first node; Deloxil from the two leaf stage but before first node (manufacturers recommendations). Lower doses of some herbicides can extend safe periods: for example, low doses of MCPA can be used before the five leaf stage (Evans, 1974). Elliot and Fryer (1954) found that although the recommended time of application for MCPA to spring oats was the sixth leaf stage, this plant was fairly resistant to MCPA and could be safely sprayed at the three leaf stage without adverse effects on the crop. Myers (1953) observed that spring barley plants showed growth abnormalities in leaves and ears if sprayed before the sixth leaf stage with MCPA or 2,4-D. Results from the present study, where all herbicides were sprayed at the three leaf stage, showed increased leaf necrosis with CMPP but only slight and non-significant increases with Deloxil and MCPA. If the safe stages mentioned above are to be believed, then little or no damage to any plant should have occurred regardless of herbicide applied. Plants were observed at G.S 39 (flag leaf emerged) and no leaf distortions were found.

Plants were not grown to the heading stage and the effects of herbicide application on ears and yield were not assessed. Several workers have indicated ear deformities, and grain yield reductions, associated with the application of auxin-like herbicides at critical stages in plant development (Elliot, 1953; Robison and Fenster, 1973; Evans, 1974; Mayes, 1980).

Results found here indicated cultivar differences in both the extent of browning and the types of browning shown on leaves of barley plants exposed to herbicide. Whitehead and Rea (1982) found no varietal sensitivity to herbicides in NIAB listed wheat or barley cultivars, although Robison and Fenster (1973) did find herbicide/ cultivar interactions with respect to yield reductions. There were no interactions between cultivar and herbicide treatment with respect to the extent of browning in the present study but there was with respect to symptom expression. Moreover, the symptom response generally conformed to the cultivar symptom classification outlined in Section 2.

SECTION 8

INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE OCCURRENCE OF NECROTIC LESIONS ON LEAVES OF BARLEY PLANTS

8.1 INTRODUCTION

Among the environmental factors associated with the development of necrotic lesions in the tissues of plants is temperature. An example of a physiological disorder related to temperature is that of apple scald in which brown lesions are found on the fruit skins of many apple cultivars following storage, the severity of symptoms becoming greater with increasing storage temperatures over the range 1-30°C (Pentzer and Heinze, 1954). The browning is associated with increased respiration within the fruit, the release of volatile substances and the production of a brown pigment within cells through the action of enzymes on phenolics. Also found in apples are brown lesions within the flesh which have a spongy texture and which can eventually collapse, the condition being associated with fruit grown at high temperatures of between 35 and 40°C (Brooks and Fisher, 1926).

Some tropical crops are prone to brown spotting on the leaves when exposed to short periods of low temperature between 1-4°C, due to the accumulation of toxins within cells (Pentzer and Heinze, 1954). Potato tubers kept continuously at low temperatures (0-5°C) show a build up of sugars within the tissues, followed by increased respiration (Walker, 1969). This can lead to oxygen stress in the internal tissues and internal discolouration. Larson and Albert (1945) described an internal necrosis of potato tubers which was attributed to a lack of oxygen diffusing into tissues and associated with temperatures above normal. Internal discolouration and browning of sweet potatoes has been described after 15 days or more storage at 0.1°C (Lauritzen, 1931).

A satisfactory supply of water is important for normal plant growth and water stress, in particular drought leading to permanent wilting, can cause death in many plants. Whatever the form of wilting, the closure of stomata to prevent further water loss through transpiration is involved (Laude, 1971). This can lead to a reduction in photosynthesis which, if left long enough, may result in chlorosis, premature

senescence and cell death (Levitt, 1980). The development of brown tissues in apple fruits grown at high temperatures is thought to be partly associated with high temperatures causing drought conditions (Levitt, 1980).

Gram and Weber (1952) described several abnormalities of leaves associated with excessive atmospheric humidity, more especially in inadequately ventilated glasshouses. These may take the form of oedematous eruptions in vines and tomatoes, and in potatoes the whole leaf tissue may turn mealy and fall away in patches. Cacti may react to excessive humidity by becoming in parts clear and glassy.

Two experiments were carried out to investigate the effects of various temperature and relative humidity treatments on the leaves of spring barley cultivars, with particular reference to the occurrence of browning responses.

8.2 MATERIALS AND METHODS

8.2.1 The effect of temperature on the levels of mildew and browning on the leaves of spring barley cultivars

The cultivars Heriot, Javelin, Midas, Triumph and Golden Promise were grown in 9 cm square pots as described in Section 4.2, with six pots of each cultivar assigned to each of three Fisons T5003 growth cabinets at either 26°C, 18°C or 10°C. In all cabinets the relative humidity was 75% and the daylength 16 hours. Once at the fourth leaf stage, or three leaf stage for plants grown at 10°C, the second and third leaves in three pots per cultivar were inoculated with a natural population (KB) of *E. graminis* spores using a brush (Section 4.2). The remaining three pots were left as controls. Plants were surrounded with polythene sleeving to prevent cross-infection and were watered every 2 days. The experiment was arranged in a split-split-plot design with temperature representing the block, replicate a plot, inoculum treatment a sub-plot randomised within plots and cultivar a sub-sub-plot randomised within sub-

plots. All second and third leaves were scored 7 and 13 days after inoculation for percentage leaf area covered in mildew, browning and chlorosis.

8.2.2 Effect of humidity on the incidence of browning on leaves of spring barley cultivars.

Seeds of Heriot, Javelin, Midas, Triumph and Golden Promise were sown in 9 cm square pots, using six pots for each cultivar. Half the number of pots were totally enclosed within polythene sleeving to create a humid atmosphere. The rest were left uncovered. Pots were placed onto plastic trays with a split-plot layout, each tray representing a block replicated three times, humidity treatment a plot randomised within the block, and cultivar a sub-plot randomised within plots. Trays were placed into a Fisons T5003 growth cabinet at 60% relative humidity, 18°C and continuous light. Plants were watered regularly. All plants were scored for percentage leaf area covered in brown lesions.

8.3 RESULTS

8.3.1 The effect of temperature on the levels of mildew and browning on the leaves of spring barley cultivars

The analyses of the data for the effects of temperature, inoculation treatment, cultivar and their interactions on levels of mildew, browning and chlorosis are summarised in Appendix 8.1. The results for the effects of inoculation treatment and temperature are given in Table 8.1.

Mildew developed most at 18°C and least at 26°C whereas browning development was greatest at 26°C. Chlorosis developed most at 18°C and 26°C on inoculated leaves but only appreciably at 26°C on uninoculated leaves. Inoculation slightly increased browning on average and markedly increased chlorosis.

TABLE 8.1: The effect of temperature and inoculation treatment on the percentage leaf area (angular transformation) affected by mildew, browning and chlorosis following 7 and 13 days incubation (averaged for the second and third foliage leaves).

Temperature °C	Mildew +*	Mildew -*	Browning +	Browning -	Chlorosis +	Chlorosis -
7 days incubation						
26	6.8	0	6.0	3.3	4.7	3.5
18	18.9	0	1.6	0.3	12.5	0.0
10	4.5	0	0.0	0.0	0.0	0.0
SED ±	2.80	-	1.03 (1.09)		2.34 (2.57)	
df	6	-	6		6	
13 days incubation						
26	5.6	0	16.2	10.1	42.5	34.1
18	29.8	0	3.4	1.8	43.8	1.2
10	13.7	0	2.7	0.0	6.6	0.0
SED ±	5.90	-	1.97 (0.99)		6.61 (7.38)	
df	6	-	6		6	

+ leaves inoculated with *E. graminis*

- uninoculated leaves

* only data for inoculated plants analysed

SED figures in brackets represent SEDs when comparing means at the same temperature.

With respect to the responses of cultivars, levels of mildew, browning and chlorosis for inoculated and uninoculated plants averaged for the different temperature treatments are given in Figure 8.1. Mildew developed extensively on leaves of all cultivars except Javelin while browning was more extensive on Heriot, Javelin and Midas than on Triumph or Golden Promise. The extent of chlorosis tended to reflect that of mildew for the cultivar. There was a slight interaction between inoculation treatment and cultivar with respect to levels of browning and chlorosis at 13 days, Midas, Triumph and Golden Promise showing a relatively small increase in browning but a very large increase in chlorosis in response to inoculation compared with the other two cultivars.

For all cultivars except Javelin mildew development was greatest at 18°C (Figure 8.2). The order of ranking for browning for different cultivars varied slightly at different temperatures, but in general all showed a marked increase in the extent of browning at 26°C compared with the levels at 18 and 10°C, with Heriot, Javelin and Midas always tending to give most browning. In the case of chlorosis there was a significant interaction between cultivar, inoculation treatment and temperature at 7 and 13 days (Figure 8.3). At 7 days chlorosis was highest at 18°C for inoculated plants of Heriot, Triumph and Golden Promise, while for Midas chlorosis was also high at 26°C: with uninoculated plants chlorosis was seen only at 26°C on these cultivars. Javelin showed no chlorosis at 7 days after inoculation with any treatment. At 13 days after inoculation chlorosis was most prevalent at the highest temperature on uninoculated plants, with most on Heriot and least on Javelin. On inoculated plants high levels of chlorosis occurred at both 18 and 26°C for all cultivars except Javelin which showed extensive chlorosis only at 26°C.

The brown symptoms shown by each cultivar, varied particularly at the highest temperature. All cultivars developed small brown spots at 10°C (Plates 8.1-8.5). At 18°C Heriot, Midas, Triumph and to a greater extent Javelin developed slightly larger brown spots (Plates 8.1-8.4), Golden Promise showing only small brown spots. However, at 26°C symptoms found on cultivar Heriot were very severe, leaves producing distinct chocolate brown spots (Plate 8.1) similar to those described in Section 2. Javelin exhibited a mixture of large and small lesions, the larger ones appearing similar to scorch marks (Plate 8.2). Midas and Triumph showed distinct spots, much smaller in size compared with those of Heriot (Plates 8.3 and 8.4). Golden Promise produced only speckling at the high temperature (Plate 8.5).

FIGURE 8.1: Percentage leaf area affected by mildew, browning and chlorosis for spring barley in relation to inoculation with *E. graminis* and cultivar; averaged for three temperatures (angular transformation), 7 and 13 days after inoculation.

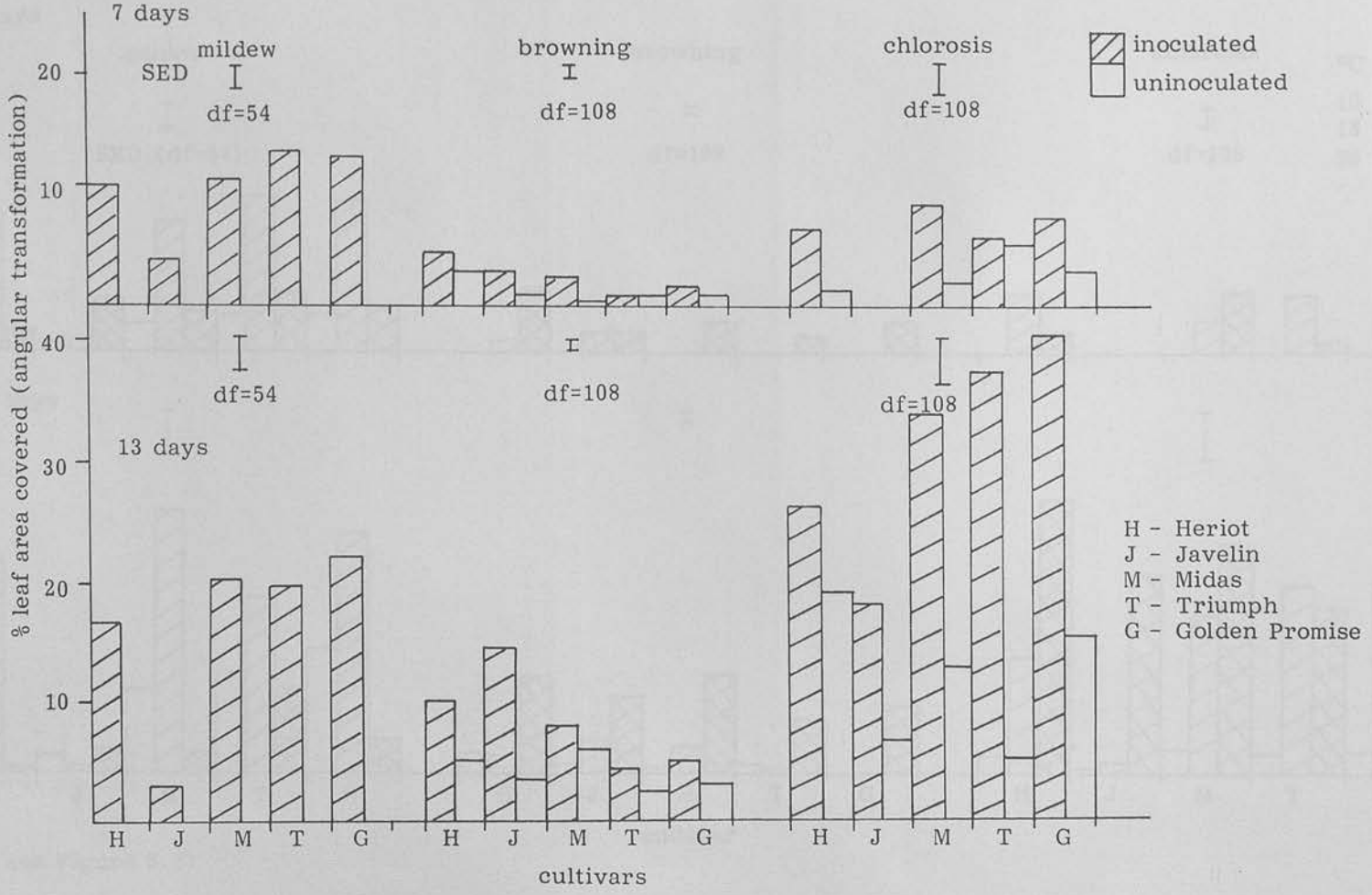
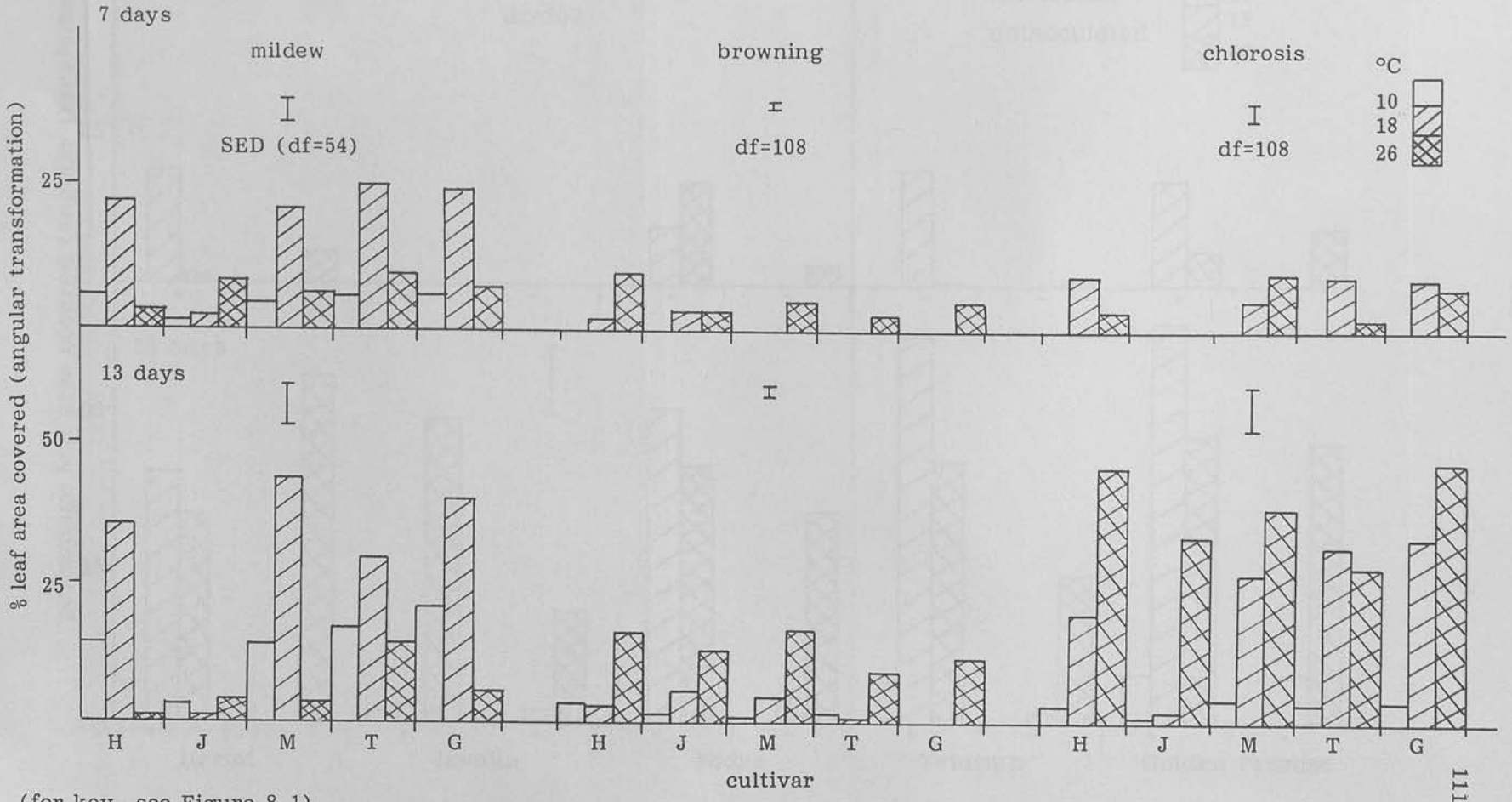


FIGURE 8.2: Percentage leaf area affected by mildew, browning and chlorosis in relation to cultivar and temperature 7 and 13 days after inoculation with *E. graminis*, isolate KB (averaged for inoculations; angular transformation).



(for key, see Figure 8.1)

FIGURE 8.3: Levels of chlorosis on leaves of five spring barley cultivars in relation to temperature and inoculation with *E. graminis*, 7 and 13 days after inoculation.

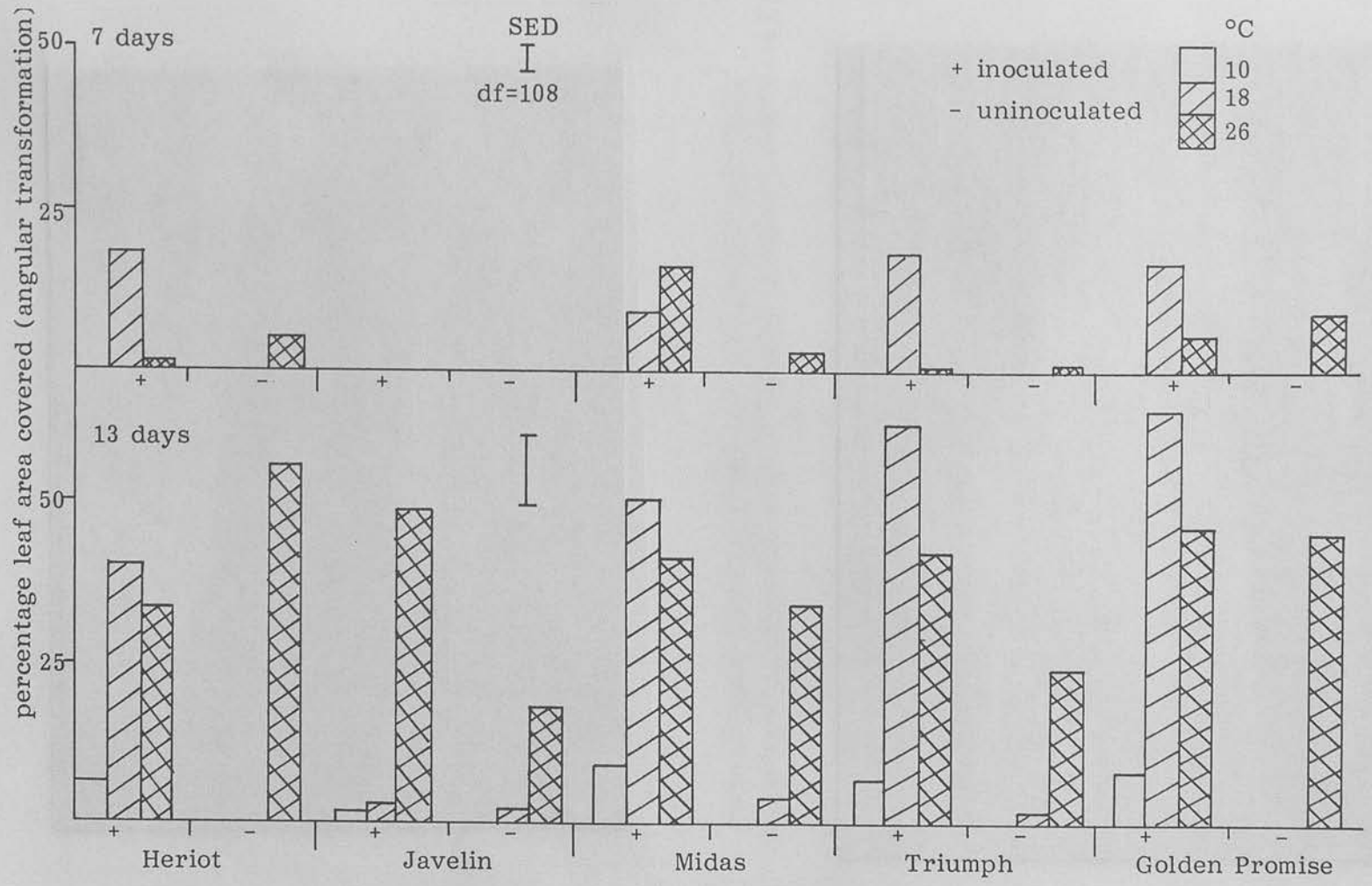


PLATE 8.1: Leaves from plants of the spring barley cultivar Heriot grown at 10°C (top), 18°C (x 2) and 26°C (x 2), leaves inoculated with *E. graminis*.

PLATE 8.2: Leaves from plants of the spring barley cultivar Javelin grown at 10°C (top), 18°C and 26°C, leaves inoculated with *E. graminis*.

PLATE 8.3: Leaves from plants of the spring barley cultivar Midas grown at 10°C (top), 18°C (x 2) and 26°C, leaves inoculated with *E. graminis*.

PLATE 8.4: Leaves from plants of the spring barley cultivar Triumph grown at 10°C (top), 18°C (x 2) and 26°C (x 2), leaves inoculated with *E. graminis*.

PLATE 8.5: Leaves from plants of the spring barley cultivar Golden Promise grown at 10°C (top), 18°C and 26°C (x 2), leaves inoculated with *E. graminis*.

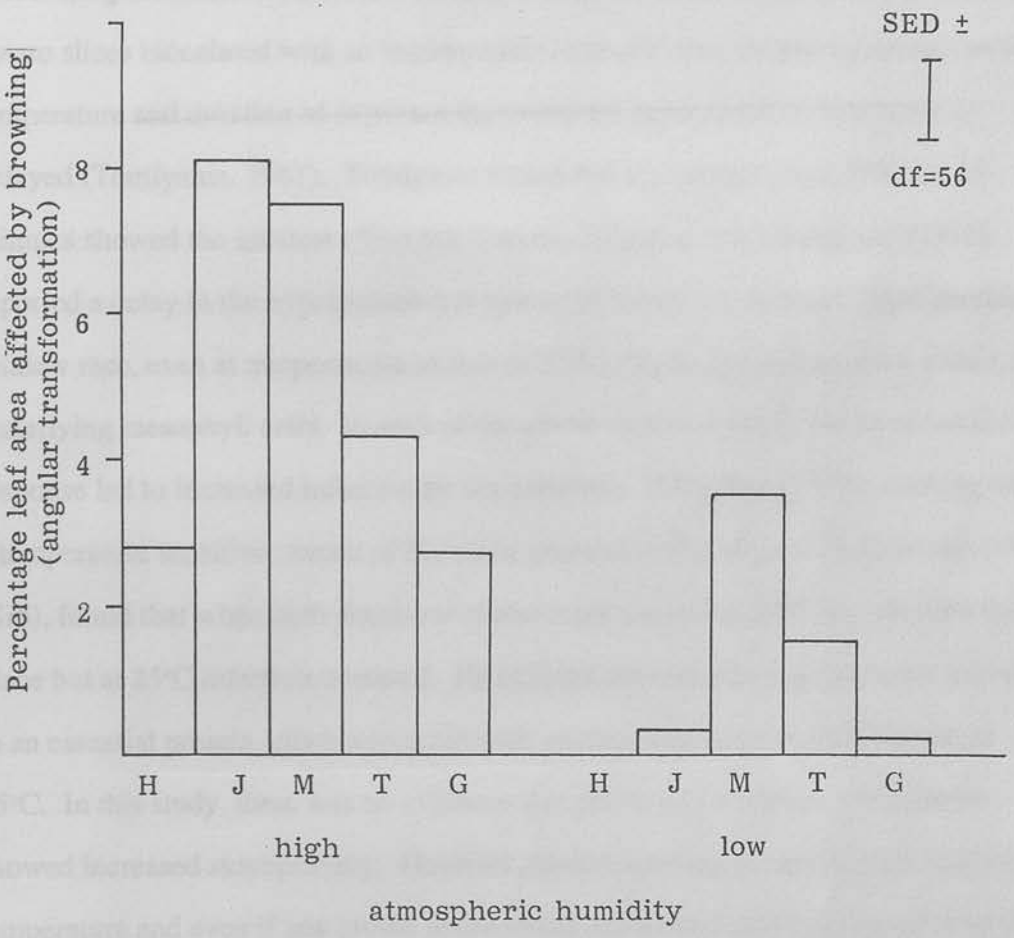
8.3.2 The effect of humidity on the incidence of browning on leaves of spring barley cultivars.

Analysis of results of the study of humidity effects showed humidity and cultivar both had an influence on levels of browning (Appendix 8.2) and there was a significant interaction between the two factors. Browning on leaves of plants grown at high humidity was more extensive than on those grown at the lower humidity (Figure 8.4). Javelin and Midas produced significantly more browning than the other cultivars at high humidity whereas at lower humidity Midas gave significantly more than Heriot, Javelin and Golden Promise. Although the individual cultivars differed in their degree of browning at high humidity, all developed similar symptoms in the form of very small (pinhead) brown spots.

8.4 DISCUSSION

When discussing the effects of environmental factors on plant growth and disease one should consider all factors that might be involved, since in field conditions each relates to the other (Burrage, 1971): however, only a single environmental factor was investigated in each of the two experiments. Much is known about environmental conditions required for growth of *E. graminis*, but little work has been carried out into the effects of changing environmental conditions on development of brown lesions on barley. *E. graminis* is known to grow over a large temperature range from 5°C to 30°C with the optimum around 18-20°C (Yarwood, 1957). Skou, Jørgensen and Lilholt (1984) claimed at temperatures below 13°C mildew development was slowed down. Last (1963) found the number of conidia which germinated within 24 hours was less at 12°C than at 17-20°C and least at 25°C. Results from this study agree with these findings, most infection occurring at 18°C, with reduced infection at 10°C and particularly 26°C. The relationship was observed not only with the susceptible cultivar Golden Promise but also with cultivars possessing major genes for resistance infected from a mixed inoculum.

FIGURE 8.4: Effect of high and low humidity on the levels of browning on the leaves of five spring barley cultivars.



key:

H Heriot
J Javelin
M Midas
T Triumph
G Golden Promise

The hypersensitive necrogenic resistance response of barley plants to an incompatible race of *E. graminis* can be delayed by a heat shock of 50°C before inoculating (Salmon, 1905 from Yarwood, 1978). A similar response was found in potato slices inoculated with an incompatible race of *Phytophthora infestans*: as the temperature and duration of exposure increased the hypersensitive reaction was delayed (Tomiyama, 1967). Tomiyama found that a temperature of 45°C for 25 minutes showed the greatest effect but Toyoda, Mayama and Shishiyama (1978) reported a delay in the hypersensitive response of barley leaves to an incompatible mildew race, even at temperatures as low as 25°C, due to delayed necrosis within the underlying mesophyll cells. In each of the above studies delay in the hypersensitive response led to increased infection by the pathogen. Ellingboe (1979), working with a temperature sensitive mutant of *Puccinia graminis tritici* (P_6) on *Triticum aestivum* (Sr6), found that when both dominant alleles were present at 20°C no infection took place but at 25°C infection occurred. He claimed the reduction in resistance was due to an essential protein which interacted with another substance at 20°C but not at 25°C. In this study, there was no evidence that plants at the highest temperature showed increased susceptibility. However, most browning occurred at the highest temperature and even if any brown lesions were associated with hypersensitivity the increase in browning with increased temperature occurred also in the absence of mildew on uninoculated plants. These results would suggest that the browning reaction in the cultivars under investigation was not substantially linked with a mildew hypersensitivity reaction but, more likely, linked with high temperature stress. Of the cultivars observed to produce distinctive brown symptoms (Table 2.1), Heriot was found to produce the cultivar-related form described in Section 2, developing large brown lesions with increased temperature. Such symptoms appeared in glasshouse conditions or detached leaves in petri-dishes in the absence of mildew infection. For other cultivars observed here, however, high temperature did not appear to be the cause of the cultivar-specific symptoms of Table 2.1.

Little is known about the effect of humidity on the development of lesions, but from this study it would appear small brown flecks, often seen in glasshouse grown plants, are the result of build up of a high humidity in the atmosphere. A similar response has been noted by other workers (Dr S.J. Wale, pers. comm.) and may be the result of localised collapse of cells around stomata. It may be that the very means of obtaining high humidities, by surrounding plants with polythene sleeving, could lead to build up of high temperatures. However, the symptoms seen in Experiment 8.1 associated with high temperature were not found. Whatever the reason for such symptoms it was obvious that high humidities were not responsible for the particular lesions found on leaves of cultivars grown under field conditions (Table 2.1).

SECTION 9

POLLEN DEPOSITION ON BARLEY LEAVES IN RELATION TO BROWNING SYMPTOMS

9.1 INTRODUCTION

It is well known that the phylloplane of a plant can be colonised by numerous organisms which are either pathogenic or non-pathogenic. It is most likely that such organisms interact with one another, if only in competing for nutrients, so influencing each other's growth and development. In some cases hyperparasitism may occur, for example *Alternaria* species can be parasitised by *Gonatobotrys simplex*, *Fusarium* by *Stephanoma phaeospora* and *Rhizoctonia solani* by *Verticillium* species (Whaley and Barnett, 1963; Butler and McCain, 1968). Such associations can result in reduced growth or death of the plant pathogenic fungi. The direct effects of saprophytic phyllosphere fungi on the leaf, however, have been little studied.

Pollen grains may form another component of the phyllosphere. They are produced in vast quantities, especially from wind pollinated plants. Rye (*Secale cereale*) produces in excess of four million pollen grains per inflorescence (Erdtman, 1943). Many of these pollen grains are carried by wind currents to the flowers of neighbouring plants, but many more fall to the ground or are deposited on leaves (Faegri and Iversen, 1975). Proteins, present in the intine layers of pollen grains, are easily leached out by water and may accumulate on the leaf surface (Knox and Heslop-Harrison, 1969). Several workers have reported that leachates from pollen grains prevent germination of fungal spores (Dubey and Manoharachary, 1984; Yamakawa, 1984) but they may also encourage epiphytic fungi or bacteria. Pollen grains are also capable of carrying fungal spores from one plant to another and under microscopic examination are often seen to have fungal mycelium associated with them (Rao and Manoharachary, 1985).

Preliminary studies with saprophytic micro-organisms isolated from barley leaves and inoculated onto leaf segments of different cultivars failed to induce any increase in leaf browning or cultivar-related necrosis but preliminary examination of barley leaves from field plots indicated that where there was an abundance of pollen clumps on leaves there was also extensive necrosis. This, together with the associated

fungal growth around pollen grains, led to the investigation of the possible role of pollen deposition on the development of browning on barley leaves.

9.2 MATERIALS AND METHODS

Seeds of the cultivars Javelin, Midas, Triumph and Golden Promise were sown in 18 cm plots, using 20 pots per cultivar in each of five replicate blocks, and grown to G.S 59 within a large glasshouse. Ten ears, with a few centimetres of stalk attached, were removed from mature plants of each replicate and placed into boiling tubes containing 10 ml of water agar (4 g agar/litre). Tubes were placed into a Fisons T5003 cabinet in continuous light at 16°C for 7 days. Ears were then removed from tubes and the washings observed for the number of pollen grains present using a haemocytometer slide.

Two weeks after anthesis, the flag leaves from ten plants/cultivar/replicate were removed and observed for the presence of pollen clumps.

Seeds of the above cultivars were sown in 12.5 cm pots and grown in spore-free cabinets (Section 4.2) so that the third leaf stage coincided with anthesis of the first set of plants. Anthers from mature plants were collected in glass petri-dishes and gently crushed to release the pollen. Leaf segments, 5 cm long, from the second leaves of seedling plants of each cultivar were placed onto 80 ppm benzimidazole agar and inoculated with pollen, using the brush method (Section 4.2) so that each cultivar was inoculated with the pollen from itself and all other cultivars. Leaf segments were incubated at 18°C for 14 days then scored for percentage leaf area with brown spotting.

9.3 RESULTS

The amounts of pollen released from the anthers of individual cultivars different significantly as shown in Table 9.1 (see also Appendix 9.1).

TABLE 9.1: Numbers of pollen grains released from the anthers of individual ears of four spring barley cultivars.

	Cultivar					
	Jv	Ms	Tr	Gp	SED	(df)
pollen/ear	8667	0	2000	667	2377.7	222

Javelin released higher numbers of pollen grains than other cultivars. Midas shed very little pollen and none was recorded in counting grids. It was noted that Javelin, and to a lesser extent Triumph, developed anthers which protruded from the ears, whereas those of Midas and Golden Promise were barely visible.

On observing pollen grains and browning on various portions of flag leaves removed from glasshouse grown plants it was found that pollen levels on cultivars differed: there were no differences in pollen levels deposited on the various parts of the leaves (Appendix 9.2). Browning severity varied with both cultivar and region of the leaf and there was a significant interaction between the factors (Appendix 9.2). Pollen deposition on leaves of Javelin and Triumph was seen to be very much higher than on leaves of Midas or Golden Promise (Table 9.2).

TABLE 9.2: Pollen numbers deposited on leaves of different barley cultivars (mean of three leaf regions).

	Cultivar					
	Jv	Ms	Tr	Gp	SED ±	(df)
pollen/cm^2 leaf	34.9	9.5	25.8	8.2	8.00	(44)

The distal portions of leaves had, on average, the greatest incidence of brown lesions, the middle and base having similar levels (Table 9.3). Javelin, on average, showed most browning, the level being significantly greater than that for Triumph, which showed the least. However, Javelin showed notably more browning at the tip than in any other region, this accounting for the high average incidence of browning of this cultivar. Other cultivars showed more or less similar levels of browning at the

different leaf positions. The symptoms which developed on all cultivars were the same, namely small brown spots.

TABLE 9.3: Percentage area of leaf browning (angular transformation) in relation to cultivar and leaf position.

Position	Cultivar				
	Jv	Ms	Tr	Gp	Mean
Tip	24.5	10.4	10.1	15.0	16.0
Middle	6.7	8.5	6.0	12.1	8.3
Base	6.7	13.6	7.1	11.2	9.6
Mean	14.0	10.8	7.7	12.8	

	CV	pos	CV x pos
(df)	(38)	(38)	(38)
SED ±	1.82	1.58	3.16

When pollen from different cultivars was artificially placed onto barley leaf segments, it had no significant effect on the extent of browning which developed over a 14 day period and did not increase browning over that of the control (Table 9.4, Appendix 9.3). In this experiment less browning occurred with Javelin than with other cultivars.

TABLE 9.4: Percentage area of leaf segments (angular transformation) affected by browning in relation to cultivar, 14 days after incubation (mean of five pollen deposition treatments).

Cultivar					
Jv	Ms	Tr	Gp	SED ±	(df)
6.9	13.2	11.0	10.9	2.17	(45)

9.4 DISCUSSION

Initial observations on leaf samples from the field indicated an association between deposition of pollen and browning of the underlying leaf tissues while experimental studies indicated that Javelin, a cultivar particularly prone to exhibit leaf browning symptoms in the field, released large quantities of pollen and had large depositions of pollen on its flag leaf relative to other test cultivars. Pollen can act not only as a carrier of fungi such as *Alternaria* spp, *Cladosporium* spp and *Fusarium* spp but can also be a vector for several plant viruses (Mandahar and Gill, 1984; Roa and Manharachary, 1985). As the pollen on the leaves would generally be derived from ears on the same plant, however, it is perhaps unlikely that the role of pollen in causing browning is in acting as a vector of pathogens.

There have been numerous reports that pollen suspensions or exudates can stimulate or inhibit fungal spore germination but there is a lack of information as to whether such exudates, for example sugars and amino acids, would themselves be capable of inducing brown lesion formation on barley leaves (Tripathi, Pandey, Tripathi and Dixit, 1984; Tripathi, Dubey and Dixit, 1985). The stimulation of the leaf surface microflora by pollen exudates may thus have been implicated in inducing browning, although preliminary studies (unpublished data) did not give evidence of any increase in browning from artificial inoculation with epiphytic microorganisms isolated from barley leaves. However, it is possible that barley cultivars with an open flower characteristic and likely to release pollen onto their leaves may be more likely to produce brown symptoms on their upper leaves following anthesis. *Cladosporium* spp, an organism which is common on the grains of barley and wheat, can often be isolated from leaves along with other saprophytes such as yeasts (King, Evers and Stewart, 1981; Tolstrup and Smedegaard-Petersen, 1984). There are reports (Zhdanova, Stepanichenko, Vasilevskaya, Navrezora, Tyshchenko, Mukhamedzhanov and Aslanov, 1986) that coloured mutants of *Cladosporium* can synthesise melanins, which if leached into the host tissues may lead to browning as described in Section 2.

Alternatively, the release of other metabolites, localised oxygen depletion due to microbial activity or the mere physical presence of pollen and associated micro-organisms may account for a disorder of the underlying tissues. It must be noted, however, that when brown symptoms occurred in association with pollen on leaves they were generally in the form of small spots and were not in the cultivar-related categories described in Section 2. Moreover, with Javelin, the cultivar in the present investigation which showed most heavy depositions of pollen on leaves, the distribution was concentrated at leaf tips, whereas brown symptoms associated with this cultivar are generally distributed over the whole leaf.

SECTION 10

BROWN LESION PRODUCTION ON BARLEY PLANTLETS GROWN ASEPTICALLY

10.1 INTRODUCTION

The use of plant tissue culture techniques for the large scale production of homologous populations of plants has been studied since the beginning of this century. The actual nutrient requirements and techniques used in tissue culture have been covered extensively by Reinert and Bajaj (1977). There are numerous uses for tissue culture techniques including their application as an experimental tool (Langhans, Horst and Earle, 1977; Grout and Short, 1979; Gamborg, Shyluk and Shahin, 1981; Yeung, Thorpe and Jensen, 1981). Only in the last decade have advances been made in the manipulation of such techniques for studies or use with cereal crops (Cummings, Green and Stuthman, 1976; Green, 1977; Dodds and Roberts, 1982). Several culture methods are available including callus, cell, protoplast, pollen, anther and embryo cultures (Gamborg and Shyluk, 1981). In the case of cereals it is the last technique which has most widely been used, although some cell, protoplast and pollen cultures have been produced from barley and maize (Green and Phillips, 1975; Evans, Sharp and Flick, 1981; Gamborg *et al.*, 1981). In the course of micropropagation or tissue culture work, difficulties have been encountered where certain plant groups have failed to show satisfactory plant establishment from aseptic tissue culture, often associated with browning of tissues and accumulation of phenolics (George and Sherrington, 1984). It was therefore decided to examine in tissue culture the behaviour of barley cultivars prone to give symptoms of browning in the field, in order to establish any link between cultivar-related field symptoms and the predisposition of their tissues to browning in aseptic culture.

10.2 MATERIALS AND METHODS

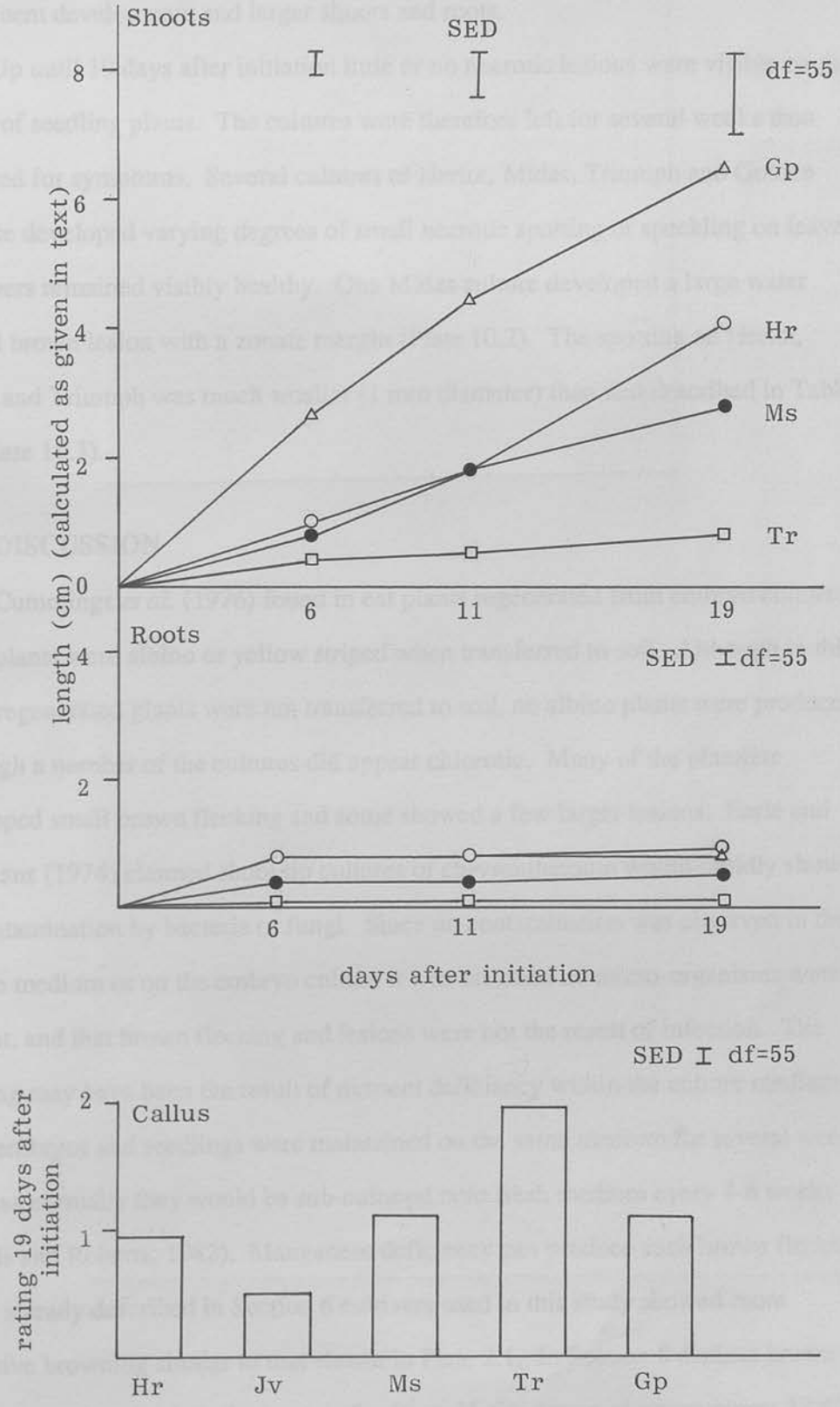
Seeds of Heriot, Javelin, Midas, Triumph and Golden Promise were placed into cooled 50% sulphuric acid for 2-3 hours, stirring every 10 minutes, until the grains turned dark brown. The seeds were washed under running water to remove the husks

and expose the embryo. Within a laminar flow cabinet the seeds were placed into 10% sodium hypochlorite solution containing 0.5% Tween 20 for 1 hour, rinsed in sterile water five to six times and the outer layers removed from around the embryo. The embryo was dissected out, excluding as much endosperm as was possible, and placed onto Schenk and Hildebrandt barley medium within 60 ml sterile Sterilin specimen bottles (Schenk and Hildebrandt, 1972). Fifteen bottles of each cultivar were placed into a growth room with constant light and a temperature of 20°C. At intervals the embryos were observed for callus, seedling production and brown spotting on leaves. Shoot and root lengths were measured by placing a centimetre rule on the outside of the culture vessel and measuring, approximately, the distance from emergence from the callus tissue to the tip of the shoot/root.

10.3 RESULTS

The analysis of the data from observations is summarised in Appendix 10.1. Callus production could not be measured accurately without contaminating the culture system but, by giving each culture a rating of 1, 2 or 3 (<5 mm, 5-10 mm, >10 mm diameter), the average callus production for the five cultivar cultures was established (Figure 10.1). Cultures of Triumph proliferated significantly more than others. Those of Javelin showed significantly less development than other cultivars and its callus eventually turned necrotic, with the brown colour leaching out into the growth medium (Plate 10.1). Javelin failed to produce seedling plants from the callus tissue by 105 days after the cultures were started. Of 15 replicate cultures established from the remaining cultivars, the numbers which produced shoots were 12, 8, 3 and 13 for Heriot, Midas, Triumph and Golden Promise respectively: the numbers which produced roots were 12, 9, 3 and 12 respectively. Golden Promise produced the largest shoots and Triumph the smallest (Figure 10.1). There were few differences in root lengths, although those from Triumph were barely detectable. Whereas Heriot,

FIGURE 10.1: Shoot, root and callus production on embryo cultures grown on Schenk and Hildebrandt medium.



Midas and Golden Promise produced less callus than Triumph they showed more subsequent development and larger shoots and roots.

Up until 19 days after initiation little or no necrotic lesions were visible on the leaves of seedling plants. The cultures were therefore left for several weeks then observed for symptoms. Several cultures of Heriot, Midas, Triumph and Golden Promise developed varying degrees of small necrotic spotting or speckling on leaves, but others remained visibly healthy. One Midas culture developed a large water soaked brown lesion with a zonate margin (Plate 10.2). The spotting on Heriot, Midas and Triumph was much smaller (1 mm diameter) than that described in Table 2.1 (Plate 10.3).

10.4 DISCUSSION

Cummings *et al.* (1976) found in oat plants regenerated from embryo cultures, some plants were albino or yellow striped when transferred to soil. Although in this study regenerated plants were not transferred to soil, no albino plants were produced although a number of the cultures did appear chlorotic. Many of the plantlets developed small brown flecking and some showed a few larger lesions. Earle and Langhans (1974) claimed shoot tip cultures of chrysanthemum would rapidly show up contamination by bacteria or fungi. Since no contamination was observed in the culture medium or on the embryo culture it was assumed no micro-organisms were present, and that brown flecking and lesions were not the result of infection. The flecking may have been the result of nutrient deficiency within the culture medium since embryos and seedlings were maintained on the same medium for several weeks, whereas normally they would be sub-cultured onto fresh medium every 4-6 weeks (Dodds and Roberts, 1982). Manganese deficiency can produce such brown flecking but as already described in Section 6 cultivars used in this study showed more extensive browning similar to that shown in Plate 2.1. In Section 8 distinct brown lesions were described on the leaves of cultivar Heriot grown at temperatures 18°C

PLATE 10.1: Necrotic callus tissue produced from embryo cultures of Javelin grown in aseptic conditions.

PLATE 10.2: Water soaked lesion on the leaf of Midas seedling shoot grown from embryo cultures produced under aseptic conditions.

PLATE 10.3: Small brown flecks on the leaves of Heriot, Midas and Triumph plantlets grown from embryo culture produced under aseptic conditions.

and 26°C. No such symptoms were found on the Heriot seedlings grown in culture yet these were grown at a constant temperature of 20°C. In most cases only one or two seedlings from each cultivar grown in culture which developed shoots showed browning, yet all were grown under the same conditions.

There was variation between cultivars in their ability to produce callus, shoots and roots (see Figure 10.1). Such variability in the development of tissue cultures has previously been described in maize and oat (Green, Phillips and Kleese, 1974; Cummings *et al.*, 1976).

In the case of Javelin, embryos failed to regenerate shoots and roots from the callus produced, the callus eventually becoming brown and the brown colour leaching into the growth medium. It is well known that cells in culture, for example those of *Acer pseudoplatanus*, can produce secondary metabolites such as polyphenols which can diffuse into the culture medium (Westcott and Henshaw, 1976; Crocomo, Aquarone and Gottlieb, 1981; Dodds and Roberts, 1982). As described in Section 5, polyphenols can give rise to brown pigments in intact leaves upon cell injury or pathogen invasion (Oku, 1962; Butt, 1980). The browning of the Javelin callus may be the result of an injury response within the cells peculiar to this cultivar. George and Sherrington (1984), in considering the problem of blackening or browning and subsequent growth inhibition with *in vitro* culture, indicated that tissue discolouration occurs through the action of copper-containing enzymes, such as polyphenoloxidase and tyrosinase, which are released or synthesised and presented with oxidative conditions when tissues are wounded. Growth inhibition is most severe in species that naturally contain high levels of tannins or other hydroxyphenols. In *Anigozanthus* the extent of discolouration in shoot tip explants depended on variety (George and Sherrington, 1984) and an equivalent cultivar variation may be associated with the necrosis of callus in Javelin. It is possible that Javelin has naturally higher levels of phenols or phenolic precursors within its tissues compared

with other cultivars used in this study. It may be that excision of the embryos induced a wound response, resulting in extensive production and release of toxic phenolic compounds into the growth medium, and death of the callus tissue which did not occur in other cultivars used. Or indeed it may be that conditions within culture vessels were not conducive to growth of the Javelin embryos, leading to stress and hence a response similar to that induced by wounding.

It is suggested that embryo culture may provide a potential technique for investigating stress effects associated with nutrient supply, temperature, other external factors or possible endogenous factors on the development of brown lesions on barley leaves, and assessing the risk of cultivars giving rise to certain types of browning in the field.

SECTION 11

GENERAL DISCUSSION AND CONCLUSION

This study has been concerned with the establishment of the causal nature of brown lesions which are liable to occur on leaves of certain barley cultivars in the absence of any obvious parasite or non-parasitic factor. In some cases these lesions have been associated with fungal infection and their incidence reduced by fungicide treatment: mildew has in particular been implicated although *Erysiphe graminis* is not usually regarded as a necrogenic pathogen. From the results of field trials on various cultivars treated or untreated with fungicide, there was a broad relationship between the occurrence of mildew and the extent of browning and, on average, a reduction of browning with fungicide. This browning may be associated mainly with the later stages of mildew disease and a stress-induced, premature senescence. The effect is generally easily attributed to mildew because of the presence of mycelium, although as previously discussed weathering may render the involvement of mildew less apparent. It was found that some barley cultivars showing intermediate levels of mildew tended to show varying ratios of browning to mildew: for example, the ratio is relatively high in Triumph. This higher incidence of browning may reflect a less compatible form of relationship associated with intermediate levels of host resistance. White and Baker (1954) described tissue responses of barley cultivars showing different degrees of resistance to mildew. In susceptible barley there was no mesophyll collapse and the mildew attained its maximum development, whereas in highly resistant cultivars mesophyll collapse began as soon as a single haustorium was formed in the infected epidermal cell. In resistant cultivars, mesophyll collapse was delayed until 6 to 10 haustoria were formed, old infections being visible and surrounded by necrotic tissue. Bushnell (1982) distinguished between determinant and indeterminant hypersensitivity in discussing cell necrosis associated with rust and powdery mildew diseases: the determinant hypersensitive response leads to a complete halt in fungal growth, whereas with indeterminant hypersensitivity cell necrosis is associated with retarded, but continuing, growth of the fungus. There was

no evidence from any of the studies, however, which would suggest effects analogous to those conditioned by the ml-o locus in several Ethiopian cultivars where an unusual type of chlorotic and necrotic spotting on leaves occurs.

For certain cultivars, e.g. Javelin, there was a high incidence of browning in the presence of low mildew levels and in these instances fungicide treatment seemed to have little effect. On these cultivars there was no obvious parasitic cause. However, in testing all possible biotic factors it was observed that Javelin tended to shed relatively large quantities of pollen onto flag leaves and pollen exudates may stimulate the leaf microflora in a way to cause browning, of the underlying tissues. Preliminary work did not provide evidence of such a relationship but further work requires to be carried out on this aspect.

In considering abiotic causes, certain stress factors were found to cause cultivar-related browning symptoms: certain cultivars, Midas, Triumph, Heriot and Javelin, gave characteristic lesions when sprayed with herbicides and Heriot grown at high temperatures or in glasshouses often produced the particular symptoms associated with this cultivar. In a survey of various types of non-parasitic leaf spotting commonly found on barley in Minnesota, many of the lesions described were characteristic for a particular cultivar (Christensen, 1934). The application of compounds containing boron was found to produce leaf spots, which could not be controlled by seed or soil treatment spraying with manganese sulphate and iron chloride or dusting with sulphur. Cultivars showed a stability in their behaviour in that they could be categorised as always severely spotted, remaining essentially free or intermediate in character from behaviour over a period of years. Christensen failed to find any effect of high temperature or humidity. Ahokas (1973) in Finland described a concentric leaf spot symptom and suggested that the ability to show this phenotype was based on the homozygosity of recessive complementary alleles in two unlinked loci. It was further suggested that the leaf spot might be caused by an

external inductor, possibly a hypersensitive reaction against toxins from an insect or an avirulent micro-organism. Clark, Seaman, Clough and Stirling (1979) described a leaf blotch on particular cultivars in Canada, but failed to identify a causal agent: observations suggested that symptoms were expressed by certain cultivars in conditions of variable temperature and high humidity.

Non-parasitic spotting associated with certain genotypes, occurring on barley leaves before and after heading, has been shown to have a considerable effect on grain yield, especially if the spotting was severe after heading (Faris, 1978). Jensen (1971) indicated that necrotic mutants occur commonly in mutation experiments and described the results of attempts to map eight recessive and two dominant necrotically spotted barley mutants isolated in his studies with mutagenically treated seed of the spring barley Carlsberg II. Thus, genetic factors which render leaves prone to necrotic responses to various stimuli may represent a significant source of yield loss. Javelin, the cultivar which initiated this study was removed from field trials after only 3 years despite its good yield potential (Blackett, 1983; Anon, 1984c), resistance to mildew and breeding as a malting cultivar (Dawson, 1985). Acclaim has also been removed (Anon, 1986c). These cultivars might be viewed as sensitive to certain stress factors, and thus the expression of symptoms in these cultivars in the field may be regarded as indications of a stress condition of concern in the environment.

The ability to identify genetic vulnerability to stress-induced necrosis in breeding material at an early stage is desirable and it may be possible to utilise an *in vitro* test for this purpose. It was noteworthy that Javelin failed to establish viable callus and this may be attributable to phenolic accumulation in response to stress and be indicative of vulnerability to cultivar-related browning due to stress factors during field growth.

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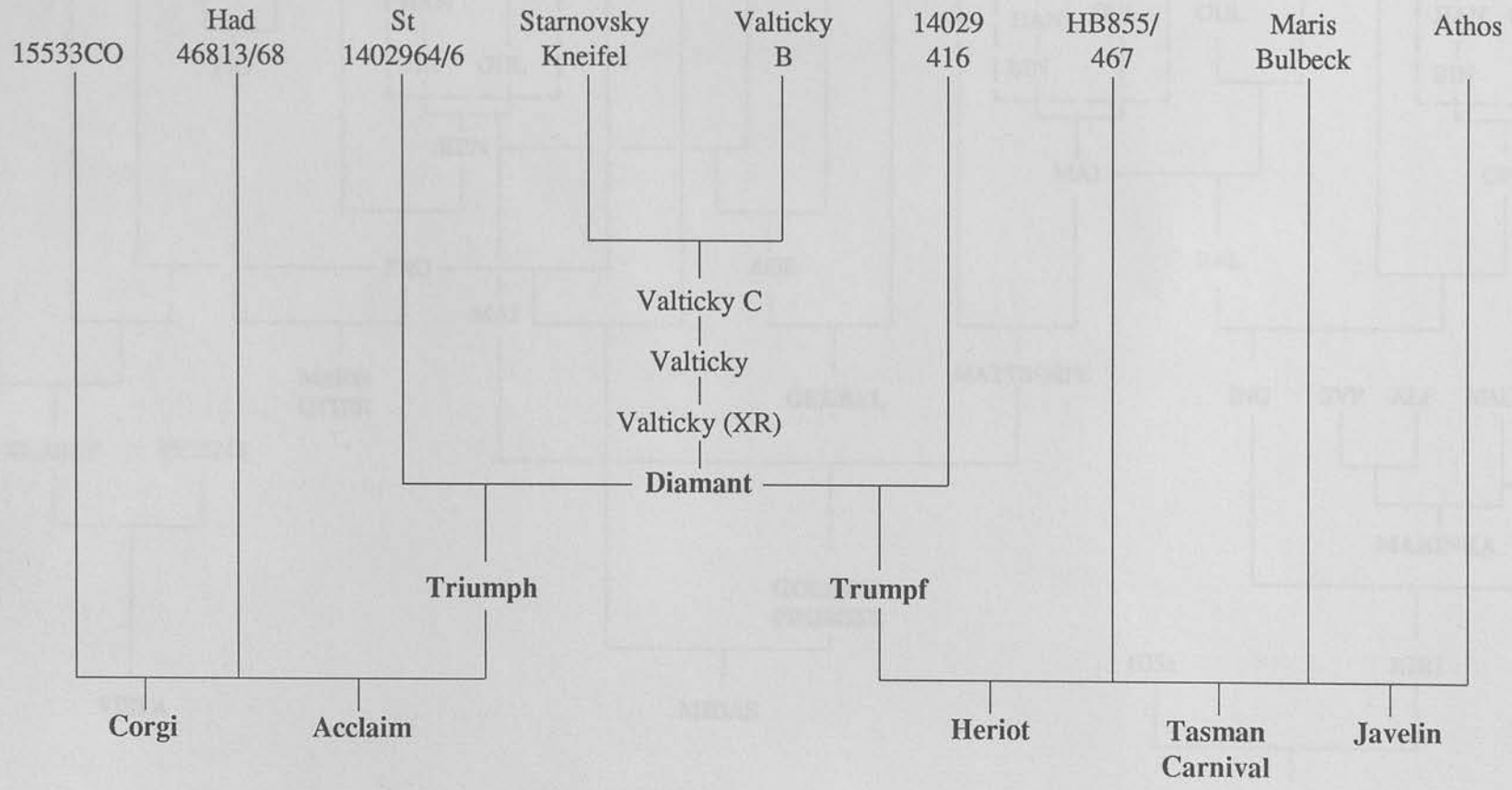
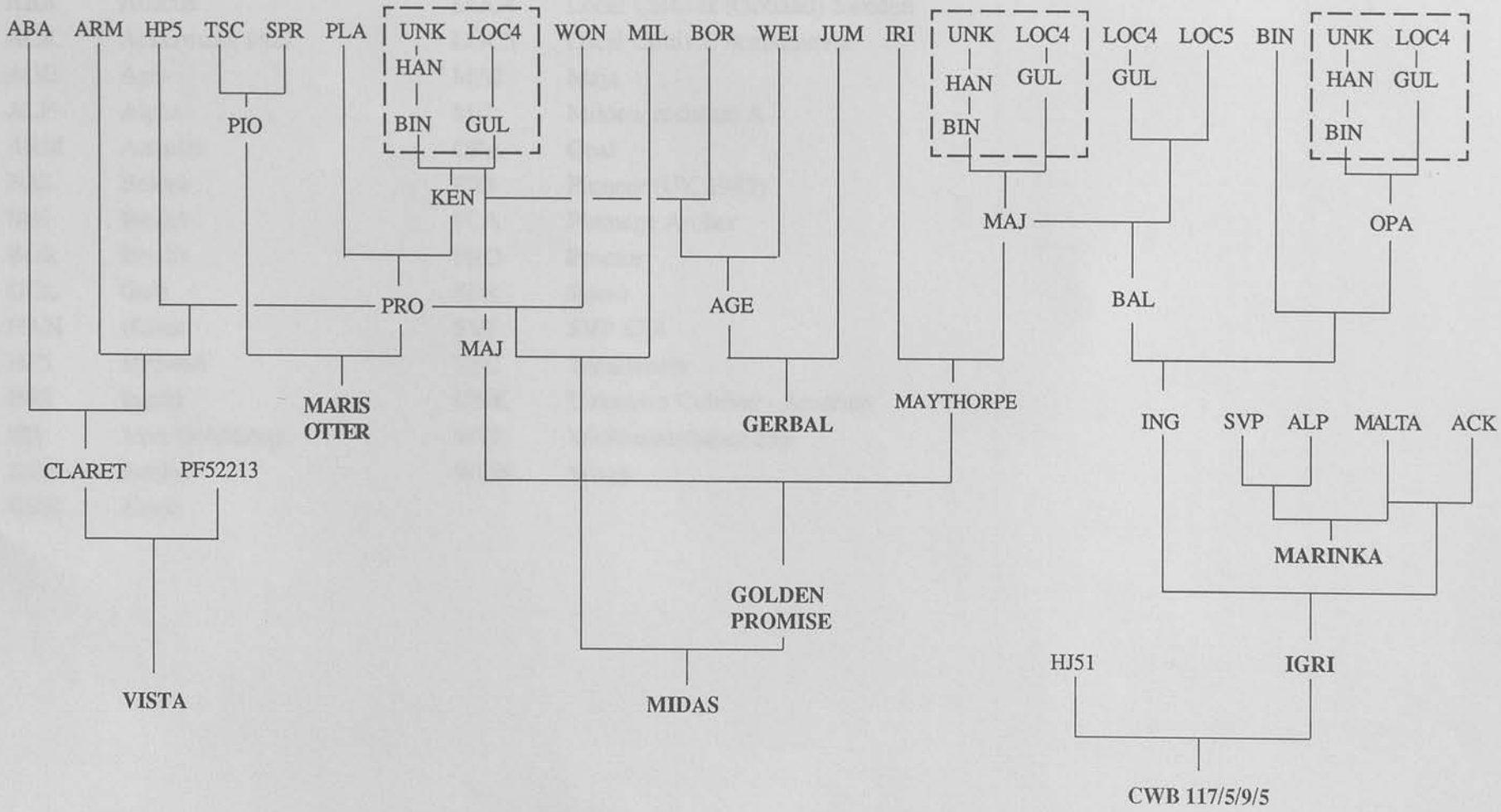
APPENDIX 2.1: **Abbreviated breeding pedigrees of the spring barley cultivars Acclaim, Carnival, Corgi, Heriot, Javelin, Tasman and Triumph (Anon., 1983a, 1984a; Baum *et al.*, 1985; Ellis, 1986).**



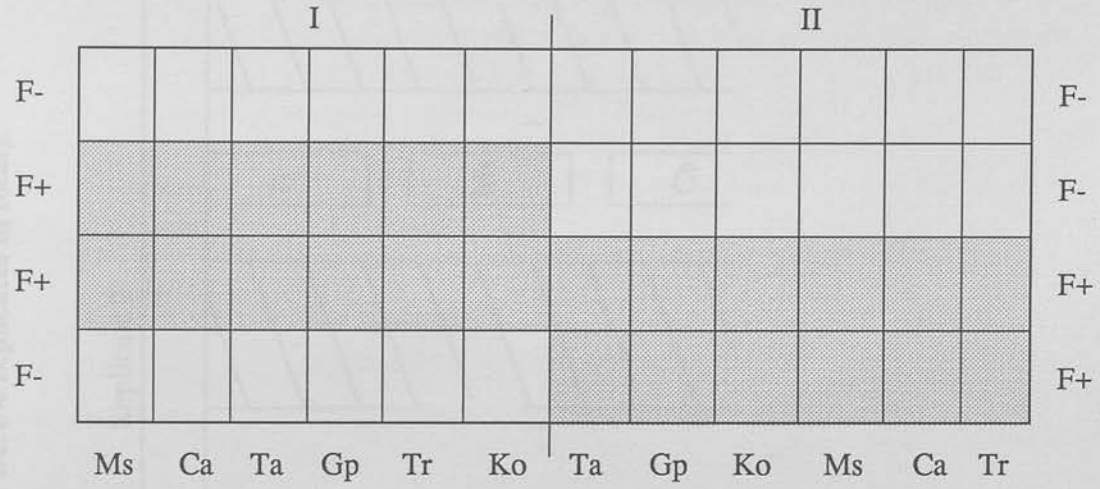
APPENDIX 2.2: **Abbreviated breeding pedigrees of the barley cultivars CWB 117/5/9/5, Gerbel, Golden Promise, Igri, Marinka, Maris Otter, Midas and Vista (Anon., 1983a, 1984a; Baum *et al.*, 1985).**

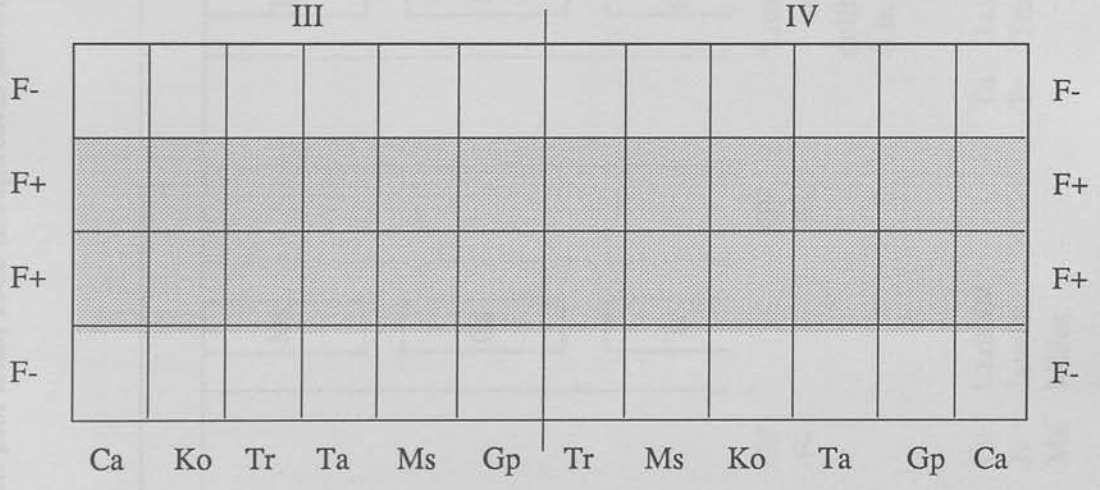


Key to Appendix 2.2

ABA	Abacus
ACK	Ackermans 1427
AGE	Ager
ALP	Alpha
ARM	Armelle
BAL	Balder
BIN	Binder
BOR	Bordia
GUL	Gull
HAN	Hanna
HP5	HP5466
ING	Ingrid
IRI	Irish Goldthorpe
JUM	Jumbo
KEN	Kenia
LOC4	Local Cultivar (Gotland) Sweden
LOC5	Local Cultivar Scandinavia
MAJ	Maja
MIL	Mildew resistant A
OPA	Opal
PIO	Pioneer (UK 1943)
PLA	Plumage Archer
PRO	Proctor
SPR	Spratt
SVP	SVP 67.4
TSC	Tschermark
UNK	Unknown Cultivar - Austrian
WEI	Weihenstephaner 259
WON	Wong

APPENDIX 3.1: Plan of trial site for small plot trial, 1985.

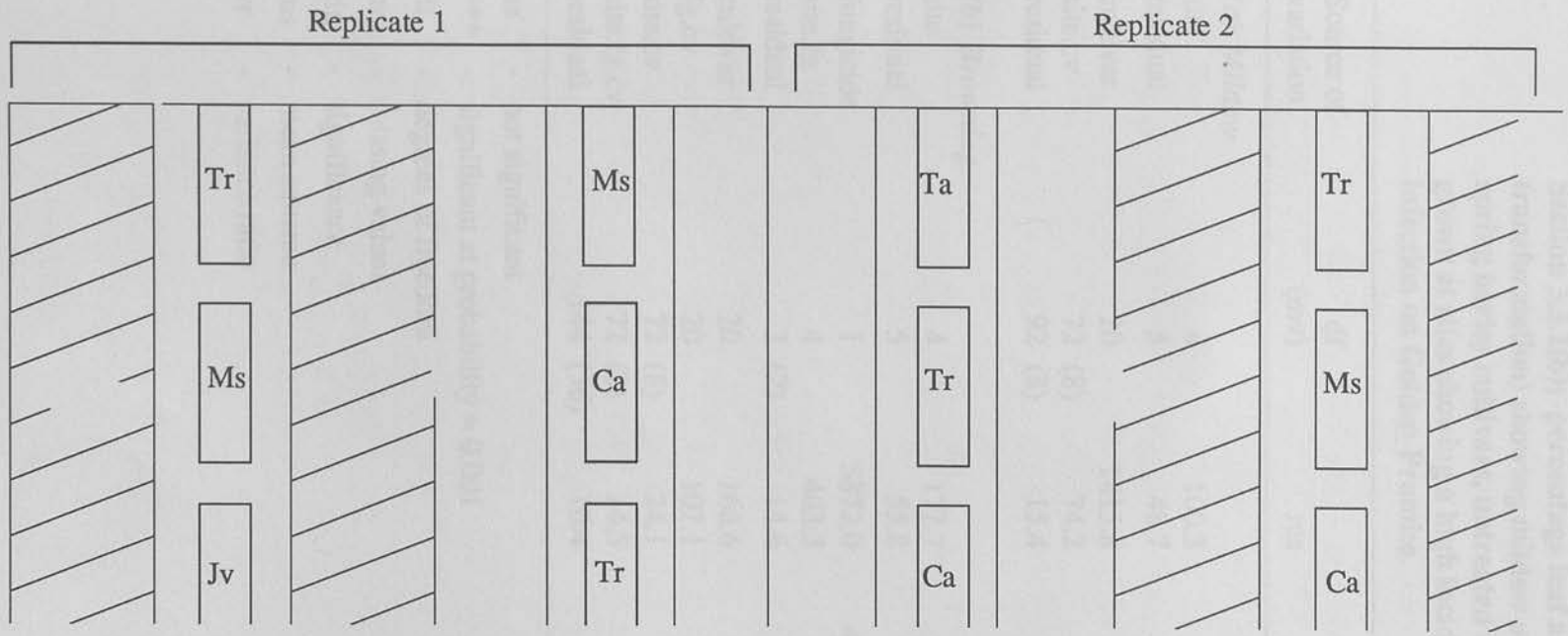




F- fungicide untreated
F+ fungicide treated

Ca Carnival
Gp Golden Promise
Ko Koru
Ms Midas
Ta Tasman
Tr Triumph

APPENDIX 3.2: Plan of trial site for small plot trial, 1986 (2 replicates shown; there were 4 replicates in total).



F- fungicide untreated
F+ fungicide treated

Ca Carnival
Jv Javelin
Ms Midas

Ta Tasman
Tr Triumph

APPENDIX 3.3: **Abbreviated analysis of variance (ANOVA) of results from Section 3.3.1(b); percentage leaf area (angular transformation) showing mildew and brown lesions for spring barley cultivars, untreated or treated with fungicide, grown at sites showing a high incidence of *E. graminis* infection on Golden Promise.**

Source of variation	df (mv)	ms	vr	sig
(a) Mildew				
site	4	100.3	2.0	ns
residual	5	48.7		
cultivar	20	1415.8	91.6	***
site.cv	72 (8)	74.2	4.8	***
residual	92 (8)	15.4		
(b) Browning				
site	4	177.7	3.2	ns
residual	5	55.8		
fungicide	1	5872.0	401.0	***
site.fg	4	403.3	27.5	***
residual	3 (2)	14.6		
cultivar	20	166.6	15.9	***
fg.cv	20	107.1	10.2	***
site.cv	72 (8)	24.1	2.3	***
site.fg.cv	72 (8)	14.5	1.4	***
residual	144 (56)	10.4		

ns - not significant

*** - significant at probability = 0.001

df - degrees of freedom

mv - missing values

sig - significance

ms - mean squares

vr - variance ratio

APPENDIX 3.4: **Abbreviated ANOVA of results from Section 3.3.2; percentage of leaf area (angular transformation) showing mildew or brown lesions for NL2 winter barley cultivars, untreated or treated with fungicide, grown at sites showing a high incidence of *E. graminis* infection on Gerbel.**

Source of variation	df (mv)	ms	vr	sig
(a) Mildew				
site	1	1236.3	81.5	*
residual	2	15.2		
fungicide	1	9052.8	173.3	**
site.fg	1	352.6	6.8	ns
residual	2	52.2		
cultivar	28	115.8	9.8	***
fg.cv	28	63.3	5.4	***
site.cv	28	24.6	2.1	**
site.fg.cv	28	23.5	2.0	**
residual	112	11.8		
(b) Browning				
site	1	391.9	16.3	ns
residual	2	24.0		
fungicide	1	2166.6	20.1	*
site.fg	1	121.7	1.1	ns
residual	2	107.7		
cultivar	28	61.0	10.4	***
fg.cv	28	12.3	2.1	**
site.cv	28	18.2	3.1	***
site.fg.cv	28	12.0	2.0	**
residual	112	5.9		

* - significant at p=0.05
** - significant at p=0.01
*** - significant at p=0.001

APPENDIX 3.5: Abbreviated ANOVA of results from Section 3.3.3; percentage leaf area (angular transformation) showing mildew and brown lesions for NL1 winter barley cultivars, 1985.

Source of variation	df (mv)	ms	vr	sig
(a) Mildew				
site	1	676.4	24.2	*
residual	2	27.9		
cultivar	27	143.3	9.4	***
site.cv	27	26.8	1.8	*
residual	54	15.2		
(b) Browning				
site	1	115.5	4.2	ns
residual	2	27.6		
cultivar	27	46.8	7.7	***
site.cv	27	23.6	3.9	***
residual	54	6.1		

APPENDIX 3.6: **Abbreviated ANOVA of results from Section 3.3.4. Percentage leaf area (angular transformation) showing mildew and brown lesions for spring barley cultivars, untreated or treated with fungicide, grown in small plot trial, 1985.**

	Source of variation	Weeks after sowing													
			6.5				10.5				12.5			15		
		df	ms	vr	sig	df	ms	vr	sig	ms	vr	sig	ms	vr	sig
Mildew	cultivar	5	1.8	2.7	ns	5	252.6	13.8	***	2559.3	89.2	***	4153.3	90.1	***
	residual	15	0.7			15	18.3			28.7			46.1		
	fungicide					1	53.5	11.4	**	1146.6	38.6	***	2241.5	56.4	***
	cv x fung					5	5.0	1.1	ns	89.9	3.0	*	95.6	2.4	ns
	residual					18	4.7			29.7			39.7		
Browning	cultivar	5	49.9	31.9	***	5	100.3	12.6	***	681.9	67.9	***	1552.6	56.6	***
	residual	15	1.6			15	7.9			10.0			27.4		
	fungicide					1	5.5	1.9	ns	42.1	3.0	ns	4.9	0.3	ns
	cv x fung					5	0.7	0.2	ns	50.8	3.6	*	73.1	4.9	**
	residual					18	2.8			14.0			14.8		

APPENDIX 3.7: Abbreviated ANOVA of results from Section 3.3.4; percentage leaf area (angular transformation) showing mildew and brown lesions of six spring barley cultivars grown in small plot trial, 1986.

Source of variation	df (mv)	ms	vr	sig
(a) Mildew				
fg	1	2.4	2.3	ns
residual	3	1.1		
cv	4	39.9	5.0	**
fg.cv	4	4.7	0.6	ns
residual	24			
(b) Browning				
fg	1	2.2	6.1	ns
residual	3	0.4		
cv	4	211.0	36.0	***
fg.cv	4	5.9	1.0	ns
residual	24	5.9		

ANOVA for Golden Promise inoculum source

Source of variation	df (mv)	ms	vr	sig
(a) Mildew				
fg	1	65.8	3.4	ns
residual	3	19.3		
(b) Browning				
fg	1	186.0	4.7	ns
residual	3	39.3		

APPENDIX 4.1: Formulation and method for preparation of sphagnum moss peat compost.

1 bale (340 l)	Sphagnum moss peat
140 g	Fritt (trace elements)
140 g	Potassium nitrate
140 g	Ammonium nitrate (Nitram)
500 g	Superphosphate
760 g	Ground limestone
760 g	Dolomitic limestone

The sphagnum peat was broken up and placed into a large mixing hopper, the hopper switched on to further break up the peat. The nutrients were thoroughly mixed and added to the hopper, the contents mixed for a few minutes. Water was added slowly to the hopper until the mixture had the consistency of coarse oatmeal. The compost was mixed for a further 5 to 10 minutes to allow even distributions of nutrients and moisture. The compost was removed and used immediately or stored in bags for use at a later date.

APPENDIX 4.2: Preparation of benzimidazole agar.

4 g	Standard Davis agar in 850 ml distilled water (in 1 litre conical flask)
150 ml	Distilled water in 250 ml conical flask
80 mg	Benzimidazole

The agar and distilled water were autoclaved for 15 minutes at 15 lb pressure and allowed to cool to hand hot. 80 mg benzimidazole was added to 150 ml sterile water, mixed and added to 850 ml sterile agar. The agar was poured into 10 cm square sterile plastic petri-dishes to half depth and allowed to set (all within a sterile laminar flow cabinet).

APPENDIX 4.3: **Abbreviated ANOVA of results from Experiment 4.1; percentage leaf area (angular transformation) showing mildew for six spring barley cultivars, untreated or treated with ethirimol and tridemorph fungicides, naturally infected with *E. graminis*.**

Source of variation	df	Weeks after sowing 5 ms	5 vr	5 sig	6 ms	6 vr	6 sig	7 ms	7 vr	7 sig	8 ms	8 vr	8 sig
Fungicide	1	0.08	1.0	ns	13.7	7.8	*	90.6	18.4	**	100.1	23.8	**
Residual	5	0.08	0.98		1.8			4.9			4.2		
Cultivar	5	0.08	0.98	ns	3.7	2.6	*	25.5	4.5	**	25.7	4.3	**
Fg.cv	5	0.08	0.98	ns	7.4	2.6	*	25.5	4.5	**	25.7	4.3	**
Residual	49	0.08			2.8			5.6			5.9		

Source of variation	df	9 ms	9 vr	9 sig	10 ms	10 vr	10 sig	11[a] ms	11[a] vr	11[a] sig	12 ms	12 vr	12 sig
Fungicide	1	80.1	20.7	**	63.2	30.3	***				236.2	26.8	**
Residual	5	3.9			0.5						8.8		
Cultivar	5	25.5	5.5	***	11.8	4.6	**	181.8	17.1	***	51.7	9.8	***
Fg.cv	5	25.5	5.5	***	12.6	5.0	***				37.5	7.1	***
Residual	49	4.6			2.5			10.6			5.3		

a - only fungicide untreated plants scored; df for residual = 25

APPENDIX 4.3: (continued): Browning

Source of variation	df	Weeks after sowing 5 ms	5 vr	5 sig	6 ms	6 vr	6 sig	7 ms	7 vr	7 sig	8 ms	8 vr	8 sig
Fungicide	1							0.4	2.5	ns	15.3	9.1	*
Residual	5							0.1			1.7		
Cultivar	5							0.1	0.8	ns	1.0	0.8	ns
Fg.cv	5							0.1	0.8	ns	1.0	0.8	ns
Residual	49							0.2			1.2		

Source of variation	df	9 ms	9 vr	9 sig	10 ms	10 vr	10 sig	11[a] ms	11[a] vr	11[a] sig	12 ms	12 vr	12 sig
Fungicide	1	33.6	13.8	*	13.7	6.6	*				0.03	0.01	ns
Residual	5	2.4			2.1						3.4		
Cultivar	5	1.8	1.0	ns	3.1	1.5	ns	13.9	7.6	***	24.1	5.3	***
Fg.cv	5	1.8	1.0	ns	3.3	1.6	ns				0.4	0.1	ns
Residual	49	1.8			2.1			1.8			4.5		

a - only fungicide untreated plants scored; df for residual = 25

APPENDIX 4.4a: Abbreviated ANOVA of results from Experiment 4.2; percentage leaf segment area (angular transformation) showing mildew and browning, untreated or treated with ethirimol, following artificial inoculation with *E. graminis* - leaf 2.

Source of variation	df	Days after inoculation: 6 ms	6 vr	6 sig	8 ms	8 vr	8 sig	10 ms	10 vr	10 sig	12 ms	12 vr	12 sig	14 ms	14 vr	14 sig	16 ms	16 vr	16 sig
Mildew																			
Fungicide	1	260.2	51.9	***	609.3	68.3	***	1275.3	103.8	***	1826.1	153.0	***	2339.3	157.4	***	2559.1	158.7	***
Residual	5	5.0			8.9			12.3			11.9			14.9			16.1		
Isolate	5	35.2	7.4	***	58.1	5.8	***	138.2	10.9	***	186.0	10.4	***	235.9	10.1	***	266.4	10.0	***
Fg.is	5	20.7	4.3	**	38.2	4.5	**	71.8	5.6	***	101.9	5.7	***	140.8	6.0	***	158.3	6.0	***
Residual	50	4.8			8.5			12.7			17.8			23.4			26.6		
Cultivar	5	97.2	31.6	***	142.6	42.3	***	317.1	41.7	***	398.9	38.7	***	494.9	42.1	***	516.8	39.9	***
Fg.cv	5	71.6	23.3	***	114.6	34.0	***	239.6	31.5	***	308.5	29.9	***	375.4	31.9	***	383.2	29.6	***
Is.cv	25	7.8	2.5	***	14.0	4.2	***	35.7	4.7	***	48.6	4.7	***	62.9	5.3	***	71.7	5.5	***
Fg.is.cv	25	8.3	2.7	***	9.6	2.8	***	22.8	3.0	***	34.0	3.3	***	45.5	3.9	***	51.8	4.0	***
Residual	300	3.1			3.4			7.6			10.3			11.8					

Source of variation	df	6 ms	6 vr	6 sig	8 ms	8 vr	8 sig	10 ms	10 vr	10 sig	12 ms	12 vr	12 sig	14 ms	14 vr	14 sig	16 ms	16 vr	16 sig
Browning																			
Fungicide	1	13.9	4.4	ns	48.2	7.0	*	69.6	102.6	***	126.9	156.5	***	169.3	12.2	*	172.5	7.2	*
Residual	5	3.1			6.9			0.7			0.8			13.9			23.9		
Isolate	5	1.9	0.4	ns	4.2	0.4	ns	27.2	1.2	ns	34.4	1.1	ns	71.7	1.8	ns	48.7	0.7	ns
Fg.is	5	3.5	0.7	ns	7.3	0.6	ns	18.4	0.8	ns	12.6	0.4	ns	11.4	0.3	ns	37.8	0.6	ns
Residual	50	5.2			12.1			22.1			30.0			40.0			69.6		
Cultivar	5	1.5	0.8	ns	8.0	2.2		41.6	4.4	***	75.3	5.3	***	176.1	9.6	***	320.9	13.8	***
Fg.cv	5	3.6	2.0	ns	9.2	2.5	*	20.4	2.1	ns	15.7	1.1	ns	34.3	1.9	ns	29.0	1.2	ns
Is.cv	25	1.7	1.0	ns	5.8	1.6	*	16.1	1.7	*	22.8	1.6	*	28.0	1.5	ns	32.7	1.4	**
Fg.is.cv	25	1.2	0.7	ns	4.0	1.1		5.0	0.5	ns	10.7	0.8	ns	23.8	1.3	ns	24.6	1.0	ns
Residual	300	1.7			3.7			9.5			14.1			18.2			23.3		

APPENDIX 4.4b: **Abbreviated ANOVA of results from Experiment 4.2; percentage leaf segment area (angular transformation) showing mildew and browning, untreated or treated with ethirimol, following artificial inoculation with *E. graminis* - leaf 5.**

Source of variation	df	Days after inoculation: 6 ms	6 vr	6 sig	8 ms	8 vr	8 sig	10 ms	10 vr	10 sig	12 ms	12 vr	12 sig	14 ms	14 vr	14 sig	16 ms	16 vr	16 sig
Mildew																			
Isolate	5	43.7	5.0	***	119.5	8.9	***	153.6	9.0	***	249.8	6.7	***	332.1	7.7	***	490.1	8.4	***
Residual	25	8.7			13.4			17.0			37.4			43.0			58.0		
Cultivar	5	184.5	28.8	***	619.8	45.1	***	734.7	38.6	***	1101.3	41.1	***	1271.1	39.0	***	1514.6	39.1	***
Is.cv	25	19.6	3.1	***	40.9	3.0	***	54.0	2.8	***	76.6	2.8	***	95.9	2.9	***	124.8	3.2	***
Residual	132	6.4			13.7			19.0			26.8			32.6			38.7		

Source of variation	df	6 ms	6 vr	6 sig	8 ms	8 vr	8 sig	10 ms	10 vr	10 sig	12 ms	12 vr	12 sig	14 ms	14 vr	14 sig	16 ms	16 vr	16 sig
Browning																			
Isolate	5	61.1	4.8	*	84.9	3.8	**	90.3	3.8	*	71.5	3.8	*	105.6	3.7	*	196.2	4.1	**
Residual	25	12.6			22.2			23.7			18.6			28.5			47.4		
Cultivar	5	4.0	0.4	ns	19.0	1.7	ns	23.9	2.0	ns	28.2	1.9	ns	21.3	1.1	ns	261.7	6.4	***
Is.cv	25	14.0	1.5	ns	15.0	1.3	ns	16.2	1.4	ns	16.9	1.2	ns	16.6	0.8	ns	27.8	0.7	ns
Residual	132	9.4			11.4			12.0			14.6			20.0			40.6		

APPENDIX 4.4c: Levels of mildew and browning on segments from the fifth leaves of six untreated (F-) spring barley cultivars inoculated with five *E. graminis* isolates, 16 days after inoculation.

Mildew

Cultivar	Hr	Jv	Ms	Tr	Vi	Gp	Mean
Isolate							
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CC13	6.2	6.5	0.0	2.3	2.3	13.9	5.1
CC40	10.2	5.6	0.0	5.0	0.0	25.3	7.5
CC9	7.7	1.0	5.0	0.0	0.0	19.3	5.5
KB	21.7	6.7	11.3	1.4	0.0	26.6	11.3
CC2	5.6	0.0	2.1	0.0	0.0	20.5	4.7
Mean	8.6	3.3	2.8	1.4	0.4	17.6	

SED	(df=25)	isolate	1.50
	(df=132)	cultivar	1.80
SED (is x cv) (df=132)		between isolate	3.74
		within isolate	3.59

Browning

Cultivar	Hr	Jv	Ms	Tr	Vi	Gp	Mean
Isolate							
Control	3.2	2.9	6.8	1.0	3.4	4.6	3.6
CC13	6.8	5.6	16.8	10.8	7.6	6.8	9.2
CC40	9.4	6.0	14.7	11.4	10.9	7.4	10.0
CC9	6.5	4.6	18.6	7.7	7.2	3.3	8.0
KB	6.3	5.6	9.7	8.7	4.2	4.4	6.5
CC2	5.2	5.2	6.9	6.9	5.0	4.7	5.6
Mean	6.2	5.0	12.2	7.7	6.4	5.2	

SED	(df=25)	isolate	1.62
	(df=132)	cultivar	1.50
SED (is x cv) (df=132)		between isolate	3.73
		within isolate	3.68

(is x cv) = interaction between isolate and cultivar

APPENDIX 4.5: **Abbreviated ANOVA of results from Experiment 4.3; percentage leaf segment area (angular transformation) from different leaf positions on spring barley plants, inoculated or uninoculated with a natural population of *E. graminis* , showing mildew and browning, 14 days after inoculation.**

Source of variation	df	Leaf number 1 ms	vr	sig	2 ms	vr	sig	3 ms	vr	sig	4 ms	vr	sig
Mildew													
Cultivar	5	1053.3	24.4	***	2321.3	22.5	***	265.5	12.3	***	754.9	39.5	***
Residual	35	43.2			103.3			21.6				19.1	
		5			6			7			8a		
Cultivar	5	458.1	6.4	***	491.4	16.1	***	248.0	17.5	***	79.4	9.5	***
Residual	25	71.0			30.5			14.2			8.4		
Browning		1			2			3			4		
Inoculum	1	204.5	32.6	**	287.8	37.0	**	58.6	4.4	ns	30.6	2.7	ns
Residual	5	6.3			7.8			13.2			11.4		
Cultivar	5	16.5	3.3	*	60.1	5.6	***	29.6	4.2	**	41.2	3.6	**
Inoc.cv	5	23.9	4.6	***	85.0	7.9	***	40.4	5.7	***	18.9	1.6	ns
Residual	50	5.0			10.8			7.0			11.5		
		5			6			7			8b		
Inoculum	1	17.2	2.7	ns	8.0	0.8	ns	1.7	0.2	ns	0.08	0.003	ns
Residual	5	6.4			10.6			7.1			27.3		
Cultivar	5	62.1	6.1	***	133.0	12.7	***	96.5	5.6	***	35.4	4.8	**
Inoc.cv	5	8.7	0.8	ns	1.4	0.1	ns	72.9	4.3	**	6.7	0.9	ns
Residual	50	10.2			10.5			17.1			7.3		

a - df: 4(1); 16(9) b - df: 1; 4(1); 4(1); 30(20

APPENDIX 4.6: Abbreviated ANOVA of results from Experiment 4.4; effect of fungicide treatment on the yield of spring barley cultivars in relation to mildew infection and browning.

Source of variation	df	Mildew			Browning			Grains/ear			Grain wt/ear			100 grain wt		
		ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig
Fungicide	1	80.6	8.6	*	<0.1	<0.1	ns	4.7	2.6	ns	0.03	2.8	ns	0.8	2.7	ns
Residual	5	9.4			7.2			1.8			0.01			0.3		
Cultivar	5	176.6	53.1	***	82.2	28.2	***	22.4	7.7	***	0.4	40.0	***	10.3	40.6	***
Fg.cv	5	36.5	11.0	***	6.2	2.1	ns	10.5	3.6	***	0.04	3.3	*	0.8	3.0	*
Residual	49	3.3			10.3			2.9			0.01			0.2		

APPENDIX 4.7: Abbreviated ANOVA of results from Experiment 4.5; fungal development and host tissue responses for compatible and incompatible barley/mildew interactions.

Source of variation		% Spores germinated			No. colonies as % of germ. spores			No. papp as % of germ. spores			No. haloes as % of germ. spores			Haloes + papp as % of germ. spores		
	df	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig
Isolate	2	150.2	5.5	ns	74.8	6.0	ns	67.1	0.4	ns	2.0	0.02	ns	22.1	0.9	ns
Residual	4	27.1			12.5			169.2			409.7			25.1		
Leaf	1	11.4	0.1	ns	20.2	1.5	ns	225.8	1.7	ns	130.0	1.3	ns	615.0	4.0	ns
Is.lf	2	61.9	0.7	ns	6.5	0.5	ns	197.4	1.5	ns	51.5	0.5	ns	81.6	0.5	ns
Residual	6	87.6			13.4			131.1			99.1			154.2		
Cultivar	4	73.8	1.1	ns	254.9	14.5	***	345.4	2.0	ns	243.2	5.8	***	186.8	5.8	***
Is.cv	8	39.7	0.6	ns	47.9	2.7	*	193.2	1.1	ns	41.0	1.0	ns	34.9	1.1	ns
Lf.cv	4	33.3	0.5	ns	5.1	0.3	ns	50.1	0.3	ns	55.2	1.3	ns	99.9	3.1	*
Is.lf.cv	8	57.3	0.8	ns	9.8	0.6	ns	149.5	0.8	ns	68.7	1.6	ns	38.6	1.2	ns
Residual	48	68.2			17.6			174.5			41.7			32.3		

Appendix 4.7 (continued)

Source of variation	df	Fluor. epid. cell walls as % germ. spores ms	vr	sig	Fluor. epid cells as % germ. spores ms	vr	sig	Fluor. meso. cells as % germl. spores ms	vr	sig	Length of colony (µm) ms	vr	sig
Isolate	2	0.2	0.0	ns	0.9	0.3	ns	1.7	1.1	ns	6979	0.5	ns
Residual	4	18.3			2.7			1.6			15115		
Leaf	1	39.1	7.3	*	8.2	1.3	ns	10.1	2.4	ns	7987	0.2	ns
Is.lf.cv	2	1.4	0.2	ns	1.5	0.2	ns	2.6	0.6	ns	64050	1.6	ns
Residual	6	5.3			6.4			4.3					
Cultivar	4	5.3	0.6	ns	4.2	1.4	ns	6.6	2.2	ns	299006	7.1	***
Is.cv	8	8.8	0.9	ns	2.6	0.9	ns	2.6	0.9	ns	87380	2.1	ns
Lf.cv	4	7.2	0.8	ns	7.3	2.5	ns	2.8	0.9	ns	22631	0.5	ns
Is.lf.cv	8	10.1	1.1	ns	1.1	0.4	ns	3.2	1.1	ns	33170	0.8	ns
Residual	48	9.3			3.0			3.0			42083		

	df	Breadth of colony (µm) ms	vr	sig	df	Groups fluor. epid./ meso. cells/cm² ms	vr	sig	Groups of brown cells/cm² ms	vr	sig
Isolate	2	2004	0.7	ns	3	1.0	3.3	ns	0.4	5.5	*
Residual	4	2955			6	0.3			0.1		
Leaf	1	709	0.1	ns	1	0.1	0.4	ns	4.4	26.7	***
Is.lf	2	5347	0.9	ns	3	0.3	1.5	ns	0.4	2.3	ns
Residual	6	5612			8	0.2			0.2		
Cultivar	4	48669	11.6	***	4	0.4	5.2	***	1.4	10.6	***
Is.cv	8	10309	2.4	*	12	0.1	1.3	ns	0.3	2.2	*
Lf.cv	4	5023	1.2	ns	4	0.2	2.8	*	1.3	9.5	***
Is.lf.cv	8	3173	0.8	ns	12	0.1	1.6	ns	0.3	2.4	**
Residual	48	4205	64			0.1			0.1		

APPENDIX 5: Staining schedules for histological observations.

Aniline blue: A staining solution of 0.05% w/v water soluble aniline blue in 0.067M phosphate buffer, pH 8.5, was prepared. Sections were mounted in the stain and observed under blue light and U.V light illumination (callose bright green or blue).
(O'Brien and McCully, 1972)

Calcofluor: A 0.01% calcofluor white solution was made up in distilled water. Sections were immersed in stain for approximately 2 minutes, rinsed then mounted in distilled water. Sections were observed under blue light and U.V light illumination (lignin bright green or blue).
(O'Brien and McCully, 1972)

FeCl: A few drops of a 10% ferric chloride solution (in distilled water) were added to sections for 2 hours. Sections were either mounted in this solution or rinsed and mounted in distilled water. Observed under bright field illumination (phenols dark green).
(Faulkner and Kimmins, 1975)

Maüle: Sections were rinsed, flooded with 1% KMnO (in distilled water) for 10 minutes, rinsed twice with distilled water, flooded with 2% HCl for 5 minutes and washed with distilled water. Sections were mounted in 2N NH Cl and observed under bright light illumination (lignin black).
(Faulkner and Kimmins, 1975)

Nitroso: To fresh sections add equal volumes of the following solutions in succession

1. 10% sodium nitrate
2. 20% urea
3. 10% acetic acid

Wait 3 to 4 minutes then add 2 volumes 2N sodium hydroxide. Observe under bright light illumination (tannins cherry red).
(Jensen, 1962)

Phloroglucinol: Sections were mounted in a few drops of 1% w/v phloroglucinol in 70% ethanol, covered with a cover-slide and left for a few minutes. A drop of HCl was placed at the edge of the cover-slide and drawn through. Observed under bright field illumination (lignin cherry red).
(Faulkner and Kimmins, 1975)

Resorcinol blue: 3 g of pure white resorcinol was dissolved in 200 ml distilled water. 3 ml 0.88 NH_4 was added and the solution heated without boiling for 10 minutes in a water bath. The red solution was stored in a cotton wool stoppered bottle until it turned blue then further heated for 30 minutes, filtered and evaporated off until no significant levels of ammonia were released. 3 drops of this stock solution were diluted in 10 ml distilled water and sections mounted in this dilute solution or in buffer at pH 3.2. Observed under bright light illumination (callose blue).
(O'Brien and McCully, 1972)

Thionin: Sections were washed in distilled water then soaked for 2 minutes in pH 4.0 acetate buffer. Sections were stained for 10 minutes in a 0.05% thionin solution made up in acetate buffer. Sections were rinsed in distilled water, passed through 5 minute soakings in 95% and 100% ethanol, stained for 30 seconds in a saturated solution of orange G (in 100% ethanol), washed briefly with 100% ethanol and mounted in distilled water. Observed under bright light illumination (necrotic cells purple).
(Sadik and Minges, 1964)

Toluidine blue O: Sections were immersed in a 0.05% solution (in water) toluidine blue O for 5 minutes, rinsed then mounted in distilled water. Observed under bright light illumination (lignin blue-green).
(O'Brien and McCully, 1972).

APPENDIX 6.1: Composition of nutrient solution (minus manganese).

	g	
$NH_4H_2PO_4$	50.0	made up to 2 litres (stock solution);
$Ca(NO_3)_2$	50.0	
KNO_3	50.0	
$MgSO_4$	50.0	
H_3BO_3	11.4	each made up to 1 litre
$ZnSO_4.7H_2O$	0.8	
$CuSO_4.5H_2O$	0.32	
$H_2MoO_4H_2O$	0.08	
$MnCl_2.4H_2O$	excluded	

To obtain a solution as near to that of complete medium less manganese, 20 ml stock solution plus 1 ml of each trace solution were made up to 1 litre with double distilled water. The pH was adjusted to 6.3 (as per complete nutrient solution) with 1M NaOH. The above composition was as specified in the manufacturers handbook for Griffin plant culture kits.

APPENDIX 6.2: Levels of mineral elements in six spring barley cultivars given various nutrient treatments.

Nutrient	Cultivar	% In dry matter						ppm
		N	P	Na	K	Ca	Mg	Mn
Complete	Heriot	2.8	0.3	0.3	5.8	0.2	0.6	60
	Javelin	2.0	0.2	0.1	4.9	0.1	0.4	47
	Midas	2.9	0.2	0.1	6.1	0.3	0.6	100
	Triumph	2.7	0.3	0.1	4.7	0.3	0.6	71
	Vista	2.8	0.2	0.2	5.6	0.2	0.5	62
	G.P	2.5	0.2	0.1	5.5	0.2	0.6	76
	mean	*2.6*	*0.2*	*0.2*	*5.4*	*0.2*	*0.6*	*69*
Minus	Heriot	3.1	0.2	0.5	5.3	0.2	0.4	101
Mg	Javelin	2.9	0.2	0.1	5.7	0.2	0.5	76
	Midas	2.8	0.2	0.1	5.4	0.2	0.4	112
	Triumph	2.8	0.2	0.1	5.0	0.2	0.4	80
	Vista	2.6	0.2	0.4	4.7	0.2	0.4	73
	G.P	2.5	0.2	0.1	5.1	0.2	0.4	104
	mean	*2.8*	*0.2*	*0.2*	*5.2*	*0.2*	*0.4*	*91*
Minus	Heriot	2.4	0.5	0.7	3.2	0.2	0.4	52
K	Javelin	2.3	0.1	0.5	4.0	0.2	0.4	78
	Midas	2.9	0.2	0.4	4.7	0.2	0.6	124
	Triumph	2.9	0.2	0.5	4.3	0.2	0.5	98
	Vista	2.6	0.2	0.7	4.2	0.2	0.6	80
	G.P	3.4	0.2	0.4	5.3	0.2	0.6	155
	mean	*2.8*	*0.2*	*0.5*	*4.3*	*0.2*	*0.5*	*98*
Minus	Heriot	2.3	1.0	0.2	3.0	0.1	0.4	34
Mn	Javelin	2.4	0.9	0.1	3.6	0.1	0.4	39
	Midas	2.7	1.0	0.1	3.7	0.1	0.4	43
	Triumph	2.9	0.9	0.1	3.6	0.2	0.4	38
	Vista	2.4	1.0	0.3	3.2	0.1	0.4	40
	G.P	2.8	1.3	0.1	4.3	0.1	0.5	51
	mean	*2.6*	*1.0*	*0.2*	*3.6*	*0.1*	*0.4*	*41*

APPENDIX 6.3: Abbreviated ANOVA of results from Experiment 6; the effect of nutrient treatment on the development of brown lesions and withering on leaves of spring barley cultivars.

(a) Browning

Source of variation	weeks	2			3			4			5			6			7			9		
	df	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig
Nutrient	3	10.1	4.6	*	119.8	33.8	***	413.8	31.8	***	395.3	44.9	***	179.0	26.8	***	448.2	22.1	***	664.5	47.1	***
Residual	9	2.2			3.5			13.0			8.8			6.7			20.3			14.1		
Cultivar	5	1.2	0.7	ns	4.8	1.0	ns	10.2	1.6	ns	44.0	10.6	***	27.5	7.6	***	16.1	2.6	*	22.6	2.6	*
N.cv	15	2.5	1.4	ns	6.7	1.5	ns	8.9	1.4	ns	11.0	2.6	**	7.6	2.1	*	11.8	1.9	*	6.8	0.8	ns
Residual	60	1.8			4.6			6.5			4.1			3.6			6.3			8.8		

(a) Withering

Source of variation	weeks	2			3			4			5			2			2			2		
	df	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig
Nutrient	3		-		28.6	5.1	*	721.6	20.3	***	1039.2	23.3	***	1687.6	14.3	***	647.6	20.9	***	973.6	34.3	***
Residual	9		-		5.6			35.6			56.1			118.2			31.0			28.4		
Cultivar	5		-		1.4	1.5	ns	9.5	0.9	ns	36.4	0.8	ns	494.3	6.7	***	474.5	7.9	***	382.0	5.1	***
N.cv	15		-		1.4	1.5	ns	9.5	0.9	ns	77.2	1.6	ns	100.7	1.4	ns	70.4	1.2	ns	56.0	0.8	ns
Residual	60		-		0.9			10.2			47.4			73.2			60.2			74.5		

APPENDIX 7.1: Abbreviated ANOVA results from Section 7, the occurrence of brown lesions on leaves of spring cultivars in relation to the application of herbicides.

Source of variation	df	ms	VR	sig
herbicide	3	429.3	30.2	***
cultivar	6	106.8	7.5	***
herb. cv	18	21.3	1.5	ns
residual	135	14.2		

APPENDIX 8.1: **Abbreviated ANOVA of results from Experiment 8.1, the influence of temperature on the development of mildew infection, browning and chlorosis on spring barley cultigvars inoculated or uninoculated with *E. graminis*.**

Source of variation	df	Day 7									Day 13								
		Mildew			Browning			Chlorosis			Mildew			Browning			Chlorosis		
		ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig	ms	vr	sig
Temperature	2	1793.0	14.7	**	370.2	26.6	***	605.0	9.3	*	4549.3	8.6	*	2514.4	24.7	***	18407.9	37.3	***
Residual	6	122.7			13.9			64.8			530.4			101.8			493.3		
Inoculum	1				81.6	4.6	ns	939.3	9.5	*				527.3	36.1	***	16543.6	20.2	**
Temp.inoc	2				27.6	1.5	ns	711.4	7.2	*				81.0	5.5	*	6165.0	7.5	*
Residual	6				17.9			99.2			14.6			817.0					
Cultivar	4	250.0	9.2	***	41.6	7.5	***	151.5	3.5	**	1068.8	16.2	***	124.8	10.4	***	1168.8	4.0	**
Temp.cv	8	178.3	6.6	***	35.7	6.4	***	118.2	2.7	**	513.5	7.8	***	36.0	3.0	**	729.8	2.5	*
Inoc.cv	4				7.4	1.3	ns	72.4	1.6	ns				31.7	2.6	*	907.4	3.1	*
Cv.leaf	5	190.5	7.0	***	11.5	2.1	ns	81.0	1.8	ns	143.8	2.2	ns	5.0	0.4	ns	820.6	2.8	*
Temp.inoc.cv	8				7.6	1.4	ns	134.9	3.1	**				19.4	1.6	ns	1223.2	4.1	***
Temp.cv.leaf	10	38.3	1.4	ns	9.1	1.6	ns	28.4	0.6	ns	140.0	2.1	*	18.8	1.6	ns	125.8	0.4	ns
Inoc.cv.leaf	5				3.9	0.7	ns	17.0	0.4	ns				9.1	0.8	ns	155.5	0.5	ns
Temp.inoc. cv.leaf	10				1.8	0.3	ns	16.6	0.4	ns				14.7	1.2	ns	212.1	0.7	ns
Residual	108	27.2	*a*		5.6			43.7			65.8	*b*		12.0	*c*		295.7	*d*	

Degrees of freedom:

a - 54 *c* - 105(3)

b - 50(4) *d* - 107(1)

Figures in brackets represent missing values

APPENDIX 8.2: Abbreviated ANOVA of results from Experiment 8.2, the influence of high and low humidity levels on browning of leaves from five spring barley cultivars.

Source of variation	df	ms	VR	sig
humidity	1	314.3	68.6	***
residual	7	4.6		
cultivar	4	49.8	10.2	***
hum. cv	4	19.5	4.0	**
residual	56	4.8		

APPENDIX 9

9.1 Abbreviated ANOVA of results of number of pollen grains released from ears of different barley cultivars (grains per ear).

Source of variation	df	ms	VR	sig
cultivar	3	1.2×10^9	5.6	***
residual	222	2.1×10^8		

9.2 Abbreviated ANOVA of results of number of pollen grains (grains/cm^3) on leaves and percentage leaf area affected by browning (angular transformation) in relation to cultivar and leaf position.

	Pollen				Browning			
Source of variation	df	ms	VR	sig	df	ms	Vr	sig
cultivar	3	2531.7	5.3	**	3	110.3	4.4	**
position	2	503.8	1.0	ns	2	337.6	13.5	***
cv. pos	6	503.9	1.0	ns	6	176.8	7.1	***
residual	44	478.9			38(6)	79.9		

9.3 Abbreviated ANOVA of the effect of artificial deposition of pollen on leaf segments, percentage leaf area affected by browning (angular transformation) in relation to barley cultivar.

Source of variation	df	ms	VR	sig
cultivar source of pollen (p)	4	129.6	2.8	ns
residual	12	46.7		
cultivar leaf segment (cv)	3	137.5	2.9	*
p.cv	12	41.8	0.9	ns
residual	45	47.2		

APPENDIX 10.1: Abbreviated ANOVA of results from Experiment 10, the development of shoot, root and callus tissue from embryo cultures of 5 spring barley cultivars.

Tissue	Days	Source of variation	df	ms	VR	sig
Shoot	6	cultivar	5	15.1	11.8	***
		residual	55(1)	1.3		
	11	cultivar	4	42.9	12.2	***
		residual	55(1)	3.5		
	19	cultivar	4	102.5	8.9	***
		residual	55(1)	11.5		
Root	6	cultivar	4	2.1	10.2	***
		residual	55(1)	0.2		
	11	cultivar	4	1.7	9.0	***
		residual	55(1)	0.2		
	19	cultivar	4	2.5	10.8	***
		residual	55(1)	0.2		
Callus	19	cultivar	4	4.0	12.4	***
		residual	55(1)	0.3		