INTERACTIONS OF ATMOSPHERIC SULPHUR DIOXIDE WITH FUNGAL DISEASES OF WINTER BARLEY

BY

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ABSTRACT

Fungal diseases were monitored through three consecutive seasons (1984-5, 1985-6 and 1986-7) on field plots of winter barley (Hordeum vulgare L.) cv. Igri growing in a range of sulphur dioxide (SO_2) concentrations (5-48 ppb SO_2, seasonal mean) in an open-air fumigation system. In each season the incidence and severity of powdery mildew (Erysiphe graminis f. sp. hordei) was increased and leaf blotch (Rhynchosporium secalis) was decreased in plots exposed to 14-48 ppb SO_2 compared with control plots exposed to 5-9 ppb SO_2. The apparent infection rate of powdery mildew was also greater in SO_2 - treated plots in the 1986-7 field season. The effects of SO_2 on eyespot (Pseudocercosporella herpotrichoides) and black ear moulds (Cladosporium and Alternaria)were variable while sharp eyespot (Rhizoctonia cerealis), Fusarium foot rot, brown rust (Puccinia hordei), septoria leaf spot (Septoria nodorum), net blotch (Pyrenophora teres) and Botrytis infection on the ear were all apparently unaffected by SO_2 concentration.

Further experiments into the effect of SO_2 concentration (0-5, 20, 40 or 60 ppb SO_2) on powdery mildew, leaf blotch and eyespot were done in fumigation chambers and in vitro. Conidia of E. graminis formed in SO_2 germinated better, and caused more severe mildew with increased conidial production when inoculated onto barley plants, than those formed in filtered air. Conidial production was also increased with increasing SO_2 concentration up to 60 ppb on plants fumigated for 7 days after inoculation with E. graminis and was increased on plants fumigated before inoculation, or before and after inoculation, with SO_2 up to 40 ppb but was decreased at 60 ppb SO_2. Conidial germination and initial mycelial growth of P. herpotrichoides

was reduced in SO_2 concentrations above 20 ppb. The severity of leaf blotch was significantly reduced in plants exposed to SO_2 for 14 days after inoculation with R. secalis. The addition of 0.1mM sulphite to solutions containing germinating conidia of R. secalis or P. herpotrichoides affected the percentage germination and initial mycelial growth of both pathogens, depending on the pH of solution, with sulphite tending to reduce fungal development at low pH values but to increase development at high pH values.

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GENERAL INTRODUCTION

In 1987 an area of 1.8 million hectares of the United Kingdom was planted to winter barley, with cv. Igri being the most widely grown variety, representing 48% of all winter barley crops. One of the main factors limiting yield of barley is fungal disease. The most common diseases on winter barley in England and Wales in 1987 were fusarium foot rot (Fusarium culmorum), net blotch (Pyrenophora teres) powdery mildew (Erysiphe graminis), eyespot (Pseudocercosporella herpotrichoides) brown rust (Puccinia hordei), leaf blotch (Rhynchosporium secalis), septoria (Septoria nodorum) and sharp eyespot (Rhizoctonia cerealis) which affected 89%, 82%, 78%, 75%, 69%, 67%, 48% and 42% of crops respectively. (Ministry of Agriculture, Fisheries and Food, 1987). The financial losses caused by disease in wheat and barley were estimated by Cook & King (1984) to average £150 million per year (based on 1982 grain values and production data) excluding the cost of control measures taken to reduce loss which was calculated at £60 million per year.

There is widespread concern over the impact of sulphur dioxide (SO_2) pollution on plant growth. Numerous reports have shown plants to be affected by high levels of ambient SO_2, either alone or in combination with other pollutants, and there is growing evidence that plant pathogens are also affected. Any influence of SO_2 on cereal pathogens, whether positive or negative, is thus potentially of considerable economic significance. However, there have been surprisingly few investigations into the effect of SO_2 on crop diseases of economic importance in the UK, particularly within the range of SO_2 concentrations currently experienced in arable areas. Observations have indicated that SO_2 can influence crop growth by modifying the

intensity of disease but it is unclear how frequently such effects occur and which factors determine the nature of the response of a particular disease to SO_2. Very little is known about the way in which SO_2 affects the epidemiology of diseases in the field.

The aims of this research were therefore firstly, to evaluate the effects of a range of SO_2 concentrations on the development of some major diseases of winter barley in the field over three seasons, using an open-air fumigation system and secondly, to examine in more detail the effects of SO_2 on particular stages in the disease cycles of three fungal pathogens of barley: powdery mildew (Erysiphe graminis), leaf blotch (Rhynchosporium secalis) and eyespot (Pseudocercosporella herpotrichoides). This second part of the programme was aimed at elucidating causal mechanisms for any effects observed in the field.

Sulphur dioxide: origin and distribution

Sulphur dioxide is a major air pollutant produced primarily from the combustion of fossil fuels, the refining of petroleum, the smelting of ores containing sulphur and the manufacture of paper and sulphuric acid. In 1985, 3.6 million tonnes of SO_2 were emmitted into the air in Britain.

SO_2 is a colourless gas, highly soluble in water. Atmospheric SO_2 is readily absorbed onto vegetation, soil and water surfaces in a process known as "dry deposition" and also dissolves in rainwater to be deposited on land surfaces by "wet deposition" (Garland, 1978). Dry deposition is the dominant atmospheric input of sulphur to England, except in the high rainfall areas of Cumbria, Devon and Cornwall, with dry deposition in much of Central and Eastern England exceeding wet deposition by a factor of three (Warren Spring Laboratory, 1986).

According to Garland, Clough & Fowler (1973), 40% of dry deposited SO_2 is sorbed onto plants.

Atmospheric concentrations of SO_2 are usually expressed in microgrammes of SO_2 per cubic metre ($\mu g/m^3$) or in parts per billion v/v (ppb). In this thesis SO_2 concentration will be given in ppb (1 $\mu g/m^3$ = 0.375 ppb at standard temperature and pressure).

Sulphur dioxide levels in the UK are monitored by the UK Smoke and Sulphur Dioxide Monitoring Network (Warren Spring Laboratory, 1986). Unfortunately there are relatively few rural monitoring sites. The highest annual mean SO_2 concentration of an urban survey site in 1983-4 was 37 ppb, recorded at Doncaster and for a rural site was 26 ppb, recorded at Ratcliffe-on-Soar near Nottingham. Concentrations in rural areas are generally lower than in urban areas, except in the vicinity of a low level source of pollution.

The annual mean SO_2 concentrations in most agricultural areas in the UK are estimated to be 4-8 ppb with the highest levels in the Midlands (15-25 ppb). However, an area of 2.8 million hectares of rural land in non-nordic Europe is exposed to annual mean SO_2 concentrations exceeding 8 ppb (D. Fowler, personal communication, 1988).

Sulphur dioxide levels fluctuate and much higher SO_2 concentrations than the annual mean can occur for short periods. Concentrations are higher in winter due to the increased combustion of fossil fuels for electricity generation and heating. SO_2 concentrations also vary diurnally with peak levels usually around 10am in Britain (Martin & Barber, 1981), depending on meteorological conditions.

Since the Clean Air Act of 1956, ground levels of SO_2 have been falling, but the decline in rural concentrations has been less than in urban areas (Roberts & Lane 1981). In order to meet the EEC directive restricting SO_2 emmissions, SO_2 levels are likely to fall further in the next decade.

Effects of SO_2 on plants

Possibly the first mention of air pollution damage to vegetation (Bell, 1983) is by Evelyn (1661) in "Fumigium or the Inconvienience of the Aer, and Smoake of London Dissipated", in which he observes that

'It kills our bees and flowers abroad, suffering nothing in our gardens to bud, display themselves, or ripen; our anenomies and many other choycest flowers, will by no industry be made to blow in London, or the precincts of it unlesse they be raised on a hot-bed and governed with extraordinary artifice to accellerate their springing, importing a bitter and ungrateful taste to those few wretched fruits, which never arriving to their desired maturity, seem, like the Apples of Sodome, to fall even unto dust, when they are but touched.'

Scientific investigation into air pollution did not begin until the mid 19th century. Since then there have been numerous reports of SO_2 affecting plant growth and influencing plant physiology and morphology. SO_2 has been shown to significantly affect photosynthesis (eg. Darral, 1986), glycollic acid metabolism (eg. Spedding & Thomas, 1973), stomatal function (eg. Biscoe *et al.* 1973), carbohydrate content (eg. Prasad & Rao, 1981), epicuticular wax structure (eg. Shelvey & Koziol, 1985) and various other metabolic processes (Wellburn *et al.*, 1976).

High concentrations of SO_2 can cause visible symptoms of plant damage and much of the early work on SO_2 effects on plants focussed on such acute injury. In cereals acute SO_2 injury is characterised by bleaching of the leaves usually from the tip down but sometimes in the central portion of the leaf blade at the point where the leaf

bends over. The occurrence of acute SO_2 injury is now uncommon due to the decline in atmospheric SO_2 concentrations, however, there is abundant evidence suggesting that SO_2 can affect plant growth in the absence of visible symptoms. (Crittenden & Read, 1979).

Several different techniques have been employed in investigations into the effects of SO_2 on plants. These include surveys comparing plants growing in areas experiencing different pollutant concentrations, often along a transect away from a source of pollution; filtration studies in which plants are grown in some form of enclosure supplied with air from which pollutants have been removed usually with an activated carbon filter; fumigation experiments in which controlled amounts of pollutants are added to filtered or non filtered air usually in an enclosure or wind tunnel, or more recently in the open air; and outdoor air exclusion systems in which positive pressure of filtered air is used to exclude polluted air from areas in which the experimental plants are growing.

There has been a progression towards the use of more realistic simulations of normal growth conditions by the use of open-top chambers and open-air fumigation systems in which field crops are exposed to controlled levels of SO_2. However, majority of studies carried out to date on arable crops have used much higher concentrations of SO_2 than would normally occur in rural UK.

In their review of research on the effects of gaseous air pollutants on agriculture and forestry in the UK, Roberts et al. (1983) concluded that levels of SO_2 exceeding 40 ppb seem to cause growth reductions. They pointed out that for fumigation experiments using SO_2 alone at concentrations below 65 ppb, the results were inconclusive. At concentrations between 15 and 38 ppb, 25% of the reviewed measurements showed yield reductions.

Considerable differences in SO_2 sensitivity exist between species and cultivars. Several attempts have been made to evaluate the response of cereals to SO_2.

Atmospheric SO_2 may contribute to the sulphur nutrition of plants growing in deficient soils as indicated by the work on Lolium perenne by Cowling & Lockyer (1978). Under conditions of adequate sulphur supply, SO_2 can still influence crop growth and a number of responses have been reported.

In a study on the effects of ambient levels of pollution in a primarily agricultural area of the Bedfordshire brickfields, Buckenham et al. (1982) demonstrated that by reducing pollutant concentrations by 60–70% using a charcoal filter, grain and straw yield of spring barley were increased. The experiments were carried out in open-top chambers above plots of field grown spring barley. The major pollutants in the area were SO_2 and hydrogen fluoride, and since the filtration was non-selective either or both of these may have caused the reductions in crop yield observed.

In a short term fumigation chamber experiment in which spring barley was fumigated with SO_2 alone (100 ppb for 20 days) significant reductions in crop growth were again observed when compared with plants grown in filtered air (Pande & Mansfield, 1985a). The main effects were on tiller number and root growth. When winter barley cv. Igri was fumigated for a longer period (September to July) with 62 ppb similar growth reductions were observed in winter but the plants recovered in spring and early summer and no significant effects on final grain yield were detected (Pande & Mansfield, 1985b). This finding highlights the importance of exposing a crop to SO_2 throughout the season in order to understand the ultimate effects of SO_2 on yield. Although these results suggest that yield reductions are

unlikely to occur at these high levels of SO_2, the reduction in root growth in SO_2 treated plants could significantly influence yield under certain conditions for example the plants may be more prone to drought stress.

Another important response demonstrated in the fumigation experiments by Pande & Mansfield (1985a; 1985b) was that when plants were exposed to a mixture of SO_2 + NO_2 the growth effects were significantly greater than the sum of the effects of the pollutants when they were applied singly. Since under field conditions pollutants invariably occur in mixtures it is important to consider the combined effects of pollutants in order to evaluate the impact of the air pollution on crop growth. Synergistic, antagonistic and additive effects of combinations of SO_2 with ozone (O_3) and SO_2 with NO_2 have all been demonstrated (Ormrod, 1982).

A similar pattern of response of barley to SO_2 was also found by Wiegel et al. (1987) at SO_2 exposures more similar to levels which would normally occur in rural UK: winter growth of potted barley plants in open-top chambers was significantly depressed in ambient air (fluctuating SO_2 concentrations 13-48 ppb in winter and 6-10 ppb in summer) compared with filtered air, mainly due to a reduction in number of plants/area and dry weight/plant, but grain yield was unaffected.

An alternative experimental approach was employed by Ashmore et al. (1988) in a recent study designed to assess the impact of SO_2, NO_2, O_3 and other environmental factors on growth of four spring barley varieties, Aramir, Claret, Dram and Golden Promise. Barley plants were grown in bags along a gradient of pollutant levels from Central London into the countryside. The advantage of this method is that the plants are exposed to ambient air under normal environmental

conditions, unmodified by enclosure within a chamber, although the growing environment would differ from a field crop and the plants may not be exposed to the same insect pest and disease pressures in urban areas as in rural areas. No significant correlation between plant growth and SO_2 deposition was found but there was a trend for straw weight in cv. Aramis and cv. Claret and ear weight in cv. Claret to decrease with increasing SO_2 deposition at mean annual SO_2 concentrations below 20 ppb.

Experiments using open-air fumigation systems, in which SO_2 is released into the air around an unenclosed field crop, have examined the effects of SO_2 on cereal crops growing under more realistic conditions. One such experiment was set up in Nottinghamshire where background SO_2 levels are relatively high for the UK (seasonal mean of 15 ppb), to expose winter barley cv. Igri to 15–200 ppb SO_2 from late December to harvest (Baker et al., 1986). At the two highest SO_2 concentrations in two seasons (fumigation period means of respectively 108 and 196 ppb in 1984 and 84 and 113 ppb in 1985) dry weight of plants was significantly reduced in late winter and early spring but later recovered. However, there was a stimulation in total plant weight at 63 ppb SO_2 in 1984 and at 51 ppb in 1985 from spring onwards although no corresponding yield increase was obtained from these treatments. All SO_2 treatments yielded lower than the control (ambient air) and a linear regression fitted to the data relating percentage yield reduction to SO_2 concentration produced a slope corresponding to a loss of 2.2% yield per 10 ppb above 15 ppb background for the site (seasonal mean).

Contrasting results were observed when winter barley cv. Sonja was exposed to 10–58 ppb SO_2 from November to harvest in an open-air fumigation system at Littlehampton where background SO_2 levels

(10 ppb mean over growing season) were lower (Mcleod, 1988). There was no winter depression in growth, but grain yield was reduced by 20% in the highest SO_2 treatment (58 ppb seasonal mean) but increased by about 20% in the low SO_2 treatment (23 ppb) compared with the control (ambient air). Grain size and grain weight were correspondingly affected and it was suggested that the increase in yield at 23 ppb was caused by a significant delay in leaf senescence during grain filling observed in the plot treated with 23 ppb SO_2.

It is clear from these experiments that effects of SO_2 at high concentrations may not apply at lower concentrations. Since investigations within the ambient range of SO_2 concentrations are so scarce, it is still very difficult to predict the effects of SO_2 on cereal crops in the UK, but on the basis of the evidence to date it seems unlikely that grain yield of barley is adversely affected by SO_2 concentrations currently experienced in arable areas and yield may be enhanced on certain cultivars in areas with relatively high SO_2 levels. A feature frequently observed in the investigations was a depression in crop growth during winter, followed by a recovery in spring. Seasonal changes in crop growth could alter the susceptibility of the crop to drought (Mansfield et al., 1986) cold stress (Davison & Barnes, 1986), or attack by pests (Alstad & Edmunds, 1982) and disease (Laurence, 1981), thereby modifying the response of a crop to SO_2.

Effects of SO_2 on plant pathogens

Fungi have long been known to be sensitive to SO_2 and high concentrations of the gas have been widely used in the food industry as a preservative (Cruess et al., 1931).

It is now well established that SO_2 can directly or indirectly affect the course of development of plant diseases and there are many reports in the literature (Tables 1&2). A wide range of pathogens are influenced by SO_2 and fungal diseases have been reported to be either stimulated, inhibited or unaffected. There are only a few reports of the effect of SO_2 on bacterial plant pathogens but these have consistantly shown a reduction in incidence of disease with SO_2 (Bell, 1987). Viral diseases have been shown to increase with SO_2 eg. Maize dwarf virus (Laurence et al., 1981).

In the main the performance of biotrophic fungal pathogens appears to be reduced by very high concentrations of SO_2 (above 100 ppb), whether this is assessed on the basis of lesion development, hyphal growth, sporulation or spore germination. However, at lower (but still high) concentrations of SO_2 the response may be reversed. For example, infection of bean by Uromyces viciae-fabae was decreased by post-inoculation fumigation with 137 ppb SO_2 but increased when fumigated at 70 ppb (Lorenzini & Panattoni, 1986). This highlights the danger of extrapolating responses found at high concentrations to lower field concentrations. The only other example of disease caused by a biotrophic pathogen increasing with SO_2 concentration is Erysiphe graminis f. sp. tritici which occurred on a higher proportion of tillers of wheat exposed to SO_2 concentrations in the range 24-57 ppb than 11 ppb SO_2 in an open-air fumigation system (Mcleod, 1988). Certain biotrophs have been found to be unaffected by SO_2 exposure even at high concentrations. Experiments on one such pathogen, Puccinia pelargonii-

Table 1: Reported effects of SO_2 on necrotrphic fungal pathogens

Pathogen and host	SO_2 exposure	Effects	References
Diplocarpon rosae on roses	1. Ambient SO_2 - field observations. 2. 0-220 ppb SO_2, 14d post-inoculation fumigation	1. Disease eliminated in areas exceeding 38 ppb 2. Increased disease severity at 16 ppb. Decreased disease severity above 38 ppb.	Saunders (1966)
Rhytisma acerinum on sycamore	1. Ambient - field observations 2. Ambient - pot plants exposed in May-June.	1. Eliminated above 34 ppb annual mean 2. Infection inhibited above 6-9 ppb.	1. Bevan & Greenhalgh (1976) 2. Greenhalgh & Bevan (1978)
Helminthosporium maydis on maize	150 ppb. $14hd^{-1}$ fumigation for 8d before inoculation	Decreased lesion number	Laurence et al. (1979)
Helminthosporium sativium on wheat	80-120 ppb SO2 for 6d pre-inoculation	Reduced disease severity at 80-100 ppb SO2 (control 10 ppb)	Fehrmann et al. (1986)
Helminthosporium sativium on barley	As above	Increased disease severity on cv Apex at 80-100 ppb No effect on cv Tapir	As above
Rhynchosporium secalis on barley	10-58 ppb seasonal mean Field fumigation	Incidence decreased at 23,38 and 58 ppb	McLeod (1988)
Scirrhia acicola on pine	200 ppb, 6h fumigation	Increased number of lesions when inoculated 5d before fumigation	Weidensaul & Darling (1979)

Continued overleaf .....

Table 1: Reported effects of SO_2 on necrotrphic fungal pathogens.... Continued

Pathogen and host	SO_2 exposure	Effects	References
Colletotrichum lindemuthianum on bean	187 ppb, 3d fumigation	Reduced disease severity	Krieg & Knösel (1983)
Ascochyta spp on cereals	80-120 ppb SO_2 for 6d pre-inoculation (control 10 ppb)	Increased severity on wheat at 80-100 ppb. No effect on barley	Fehrmann et al. (1986)
Pseudocercosporella herpotrichoides on cereals	10-58 ppb SO_2 seasonal mean Field fumigation	Reduced incidence on wheat at 46 and 57 ppb. Increased incidence on barley at 38 ppb.	McLeod (1988)
Fusarium spp. foot rot on wheat	11-57 ppb SO_2 seasonal mean Field fumigation	Reduced incidence at 24, 46 and 57 ppb	As above
Fusarium nivale on wheat	80-120 ppb SO_2 for 6d pre-inoculation (control 10 ppb)	Increased severity at 80-100 ppb on cv Diplomat No effect on cv Goetz. No effect at 100-120 ppb	Fehrmann et al. (1986)
Fusarium solani on peas	Air polluted with SO_2 and other pollutants in field	Increased incidence and severity in polluted regions	Szerzen (1981)
Fusarium oxysporum on peas	As above	As above	As above
Alternaria solani on tomatoes	1. 156-179 ppb, 7d fumigation pre-inoculation 2. 140-190 ppb, from 16h after inoculation for 1 or 2d	No effect on incidence or severity	Weinstein et al. (1975)

Continued overleaf .....

Table 1: Reported effects of SO_2 on necrotrphic fungal pathogens..... Continued

Pathogen and host	SO_2 exposure	Effects	References
Botrytis cinerea on grape vine	100-130 ppb SO_2, fumigation 6d at $7hd^{-1}$ pre-inoculation	Increased infection	von Triedemann & Fehrmann (1986)
Armillarea mellea on trees	Ambient air, up to 420 ppb.	Incidence increased up to 38 ppb, decreased between 40 and 420 ppb.	Grzywacz & Wazny (1973)
Heterobasidion annosum on trees	1. As above 2. $4-4x10^{5}$ ppb fumigation for 0.5h - 20d	1. Incidence increased up to 115 ppb, decreased above 115 ppb 2. Mycelial growth unaffected at 4 ppb, stimulated at 40 ppb when fumigation at least 5 days, reduced at 400 ppb	1. As above 2. Grzywacz (1973)

Table 2: Reported effects of SO_2 on biotrophic fungal pathogens

Pathogen and host	SO_2 exposure	Effects	References
Erysiphe graminis f. sp. hordei on barley	187, 562 and 936 ppb 8d post inoculation fumigation	Spore germination: reduced at 936 ppb, unaffected at 187 or 562 ppb. Decreased disease severity at 187, 562 and 936 ppb.	Krieg & Knösel (1983)
Erysiphe graminis f. sp. tritici on wheat	11-57 ppb seasonal mean Field fumigation	Incidence increased at 24, 46 and 57 ppb	McLeod (1988)
Microsphaera alni on lilac	1. Urban air: field observations. 2. 300-400 ppb and 1000 ppb 24h fumigation of conidia or leaves pre-inoculation	1. Less infection than in areas with clean air 2. Reduced spore germination at 300-400 ppb, no effect at 1000 ppb. No effect on initial mycelial growth. No effect on pre-fumigated plants.	1. Hibben & Walker (1965) 2. Hibben & Taylor (1975)
Microsphaera alni var. quercina on oak	Ambient SO_2: field observations	Absence from vicinity of point sources of SO_2 pollution (and other pollutants)	Köck (1935)
Uromyces viciae-fabae on field bean	1. 70 ppb fumigation for 9d pre-inoculation and 34d from 1d after inoculation. 2. 131 ppb fumigation for 14d pre-inoculation and 28d from 1d after inoculation.	1. Increased infection 2. Decreased infection	Lorenzini & Panattoni (1986)
Uromyces phaseoli on beans	1. 130 ppb, 8d post-inoculation 2. 130 ppb, 9d pre-inoculation fumigation.	1. Reduced incidence and severity of disease and reduced spore germination. 2. Reduction from pre-inoculation fumigation.	Weinstein et al. (1975)

Continued overleaf .....

Table 2: Reported effects of SO_2 on biotrophic fungal pathogens .....Continued

Pathogen and host	SO_2 exposure	Effects	References
Puccinia graminis f. sp. tritici on wheat	100 ppb SO_2, 100 h fumigation from 2d after inoculation	Decreased lesion numbers on wheat cv Thatcher. No effect on cv Prelude	Laurence et al. (1979)
Puccinia pelargonii-zonalis on pelargonium	562 and 936 ppb, 2-20d fumigation from 2d after inoculation	Disease severity unaffected	Krieg & Knösel (1983)
Puccinia hordei on barley	10-58 ppb seasonal mean Field fumigation	Reduced incidence at 23, 38 and 58 ppb	McLeod (1988)
Melampsorella cerastii on conifers	Ambient SO_2: field observations	Absent from areas with high SO_2 injury on conifers around smelters	Scheffer & Hedgecock (1975)
Melampsora albotensis on conifers	As above	As above	As above
Melampsora pinitorqua on trees	Ambient SO_2, up to 420 ppb Field observations	Incidence increased up to 38 ppb, decreased between 38 and 115 ppb.	Gryzwacz & Wazny (1973)
Peridermium coloradense on conifers	Ambient SO_2: field observations	Absent from areas with high SO_2 injury on conifers around smelters	Scheffer & Hedgecock (1975)
Peronospora spinaceae on spinach	187 ppb, 48 h fumigation	Decreased spore germination	Krieg & Knösel (1983)

zonalis, showed that neither spore germination nor disease severity was affected by 562 or 936 ppb fumigations post-inoculation (Krieg & Knosel, 1983).

In the case of necrotrophs, the effects are very variable. Many weakly pathogenic fungi have been reported to increase in incidence or severity at high SO_2 concentrations eg. Fusarium nivale, Helminthosporium sativum and Ascochyta spp. (Fehrmann et al., 1986). It has been suggested that this was caused by SO_2 weakening the host for infection, although stimulation of disease with SO_2 has also been observed in direct fungal fumigation of Heterobasidion annosum (Grzywacz, 1973).

There does not appear to be any consistent association between fungal Class and sensitivity to SO_2. For example, the reported responses of rusts to SO_2 ranges from stimulation to insensitivity to inhibition.

There have been many field observations which indicate changes in incidence of diseases in polluted areas compared with clean areas. Most of these investigations have been made around point sources of SO_2, where this pollutant is often accompanied by other phytotoxic gases and/or particulates. The studies have mainly focussed on woody plants which, because they are long lived perennials, could be exposed to pollutants for very long periods and the effects of cumulative SO_2 concentration could be more significant than for annual crop plants.

Sycamore tar spot was found to be absent from parts of Liverpool where the annual mean SO_2 concentration exceeded 34 ppb (Bevan & Greenhalgh 1976). By placing potted plants in the clean and polluted areas during different periods of the disease cycle, Greenhalgh & Bevan (1978) were able to show that tar spot infection was completely inhibited in May and June when mean SO_2 concentrations were only

6–9 ppb in the polluted area, but once established the disease will develop in the polluted areas. Recently it has been suggested that the different incidences of disease found in the survey could have been due to the practise of sweeping up leaves in the city area (polluted) but not in the country areas ("clean") thereby reducing the amount of inoculum in city areas (Leith & Fowler, 1987). It is a problem with all surveys that any effects of SO_2 may be confounded with factors other than the pollutant concentration which will vary between sites, including topography, density of host populations, growing systems and weather conditions.

Another disease which has been absent from urban areas is black-spot of roses, but with falling levels of SO_2 in UK cities this disease has re-appeared even in Central London. Saunders (1966) identified a mean of 38 ppb SO_2 during the growing season as being the threshold concentration for disease elimination and was able to confirm the causal relationship with SO_2 by fumigation experiments in which disease severity was reduced by post-inoculation fumigation with 38 ppb SO_2 for 14 days, although disease was increased at 16 ppb SO_2 compared with the control (0 ppb - filtered air).

Many other investigations have used fumigation chambers for SO_2 treatment. Most of the fumigations have been at very high SO_2 concentrations over short periods of time and although short term peak concentrations may be important if they occur at critical times in the disease cycle, such as during spore germination, caution should be exercised in extrapolating these results to ambient conditions in this country.

Fumigation chambers do provide a useful technique for examining the sensitivity of the pathogen or host at different stages in the disease cycle. When designing fumigation experiments many workers

have endeavoured to separate out direct effects on the disease complex from indirect effects on disease mediated via the host by exposing pathogens *in vitro* or plants before and/or after inoculation. It should be remembered that effects seen in fumigation chambers may be modified in the field due to alterations in environmental conditions and constraints on pathogen dispersal and interactions with other components of the ecosystem caused by the enclosure.

Most attempts of pathogens at host invasion, fail at the pre-invasion stage when the pathogen is uniquely vulnerable to environmental conditions (Dowding, 1988). Pathogens are probably most exposed to SO_2 during spore germination and there are several examples of SO_2 influencing spore germination of biotrophs, usually causing inhibition (Table 2 .).

Germination of *Botrytis cinerea* and *Alternaria* spores have been shown to be inhibited at extremely high concentrations of SO_2 (above 50,000 ppb), with the toxicity of SO_2 being proportional to SO_2 concentration and exposure time, and increasing with temperature and relative humidity indicating that the hydrated form of SO_2 (sulphurous acid) is the active agent (Couey & Uota, 1961; Couey, 1965). Conidial germination and appresorium formation have been found to be the developmental phases at which *Microsphaera alni* is most sensitive to SO_2 (Hibben & Taylor, 1975). Although acute doses of 1000 ppb SO_2 for 1-6h had no effect on spore germination, exposure of leaf discs to 300-400 ppb SO_2 often reduced germination by 50-60%.

Fumigations during the germ tube stage or after the formation of primary, secondary and tertiary hyphae had no effect. Detached conidia of *Microsphaera alni* were more sensitive than those exposed on conidiophores. Conidia treated with SO_2 that did not germinate, or germinated but failed to progress beyond the appresorium stage, did

not resume normal development when transferred into clean air. By commencing SO_2 fumigation of barley at different intervals after inoculation with E. graminis, Krieg and Knosel (1983) were able to show that spore germination on the leaf surface is reduced by 936 ppb but unaffected at 187 or 562 ppb. Powdery mildew spores are normally relatively insensitive to toxic substances due to their impermeability (Gay et al., 1985).

Fungal hyphae are quite resistant to SO_2, especially when protected by host tissues (Heagle, 1973). The direct effects of SO_2 on mycelial growth of Heterobasidion annosum and Schizophyllum commune in culture were examined by Grzywacz (1973) and no inhibition of growth was observed below 4000 ppb but there was a slight stimulation by 40 ppb compared with 4 ppb SO_2.

The effect of SO_2 on the lesion development stage (after establishment until symptom development) varies between pathogens, host cultivar and with SO_2 concentration. For example, severity of Puccinia pelargonii-zonalis was unaffected after exposure to 562 or 936 ppb SO_2 from 2-20 d after inoculation (Krieg & Knosel, 1983). An interesting effect of host cultivar was observed by Laurence et al., (1979): lesion numbers of Puccinia graminis were reduced by 100 h fumigation with 100 ppb SO_2 from 2 d after inoculation on the wheat cultivar Thatcher but not affected on cv. Prelude. This indicates that the effect was host mediated and could be related to host resistance.

In a number of fumigation experiments, plants have been fumigated with SO_2 before inoculation with a pathogen to determine the effect of SO_2 on host susceptibility to disease. Various responses have been demonstrated (Tables 1&2). In the case of Uromyces phaseoli, reduction in disease severity was greater from pre-inoculation than post-inoculation fumigation (Weintstein et al., 1975). Even within

the same genus, Helminthosporium, contrasting responses have been observed: disease severity was decreased by high SO_2 on maize and wheat, increased on barley cv. Apex but unaffected on barley cv. Tapir (Laurence et al., 1979; Fehrmann et al., 1986). SO_2 (80-120 ppb) was shown to pre-dispose cereals to infection by a range of weak pathogens (Fehrmann et al., 1986).

It is very difficult to make generalisations about the response of different pathogens to SO_2 or the mechanism by which disease is affected as the effects reported are so variable. Certainly, SO_2 affects the pathogen both directly and indirectly via the host and also affects the disease complex. The sensitivity to SO_2 of saprophytic microorganisms on the leaf surface demonstrated by Dowding & Carvill (1980) and Hibben & Taylor (1975) could have important implications on disease development in the field since complex competitive interactions occur between pathogens and other phylloplane microorganisms.

The development of open-air fumigation systems has allowed natural infections of fungal diseases to be studied in normal field conditions without the problems of variability encountered in survey work. Interactions between phylloplane microorganisms or pests and pathogens are less restricted than in chamber experiments. More realistic SO_2 concentrations have been used in more recent experiments and significant responses have been observed (Mcleod, 1988).

It is very difficult on the basis of current knowledge to estimate the importance to agriculture of the impact of air pollution on plant disease. Both positive and negative effects on disease have been demonstrated but factors governing these effects are poorly understood and the repeatability of the responses needs to be demonstrated. There still remain numerous crop diseases of economic

importance in Europe for which the effect of SO_2 levels currently occurring in agricultural areas have not been investigated.

This thesis sets out to investigate the interaction between ambient atmospheric sulphur dioxide concentrations and fungal pathogens of winter barley. This crop was chosen because of its significance in European agriculture and the study focuses on diseases which regularly cause large yield losses in barley crops. The response of the barley diseases to SO_2 levels in the field is compared in different seasons and the way in which SO_2 influences pathogen development is examined.



Plate 1. Barley infected with powdery mildew caused by *Erysiphe graminis* f. sp. *hordei*.



Plate 2. Symptoms of leaf blotch caused by Rhynchosporium secalis on barley



Plate 3. Stem-base lesions of eyespot caused by Pseudocercosporella herpotrichoides infection of barley.

SECTION I

FIELD EXPERIMENTS AT THE CERL OPEN AIR FUMIGATION SITE

INTRODUCTION

Atmospheric sulphur dioxide has been shown to affect the growth and yield of barley in field experiments (Baker et al., 1986; Mcleod et al., 1988). It is well established that many plant pathogens are also sensitive to SO_2. The majority of barley crops become infected with several diseases over a season which can cause significant reductions in crop growth and yield.

Most investigations into the effects of SO_2 on plant pathogens or crop growth have used some form of enclosure within which SO_2 concentrations were controlled. Such enclosures create an artificial environment which may differ from the field environment in various respects. For example, air movement, humidity, temperature, water availability and solar radiation could be altered and constraints on the dispersal of pathogens, pests and other biota would be introduced. Modifications of these factors can influence disease development.

In order to evaluate the significance to agriculture of the responses of crops and diseases to SO_2, the effects of realistic SO_2 concentrations should be examined under normal field conditions. It is also important to measure the responses of a crop to SO_2 exposures over the entire growing season since there is evidence of seasonal variability in SO_2 sensitivity of cereals (Baker et al., 1986).

One way in which this can be done is by comparing disease levels in areas experiencing different SO_2 concentrations, usually along a gradient away from a source of pollution as in the studies by Bevan & Greenhalgh (1976); Saunders (1966) and Szerszen (1981). The main disadvantage of this method is that other factors such as climate,

soil or inoculum density may also differ making it difficult to define the cause of variation between sites.

An alternative method, first devised by de Cormis et al., (1975), is to produce different SO_2 concentrations in field plots by releasing controlled amounts of SO_2 through a network of pipes into the air above a crop growing under normal agricultural conditions, without any form of enclosure. Thus sulphur dioxide levels in a crop may be increased with minimal alteration of other environmental conditions. This technique of open-air fumigation has been used by several researchers for experiments on pollutant effects on field crops using a range of designs attempting to achieve a realistic spatial and temporal distribution of gas within the crop (eg. Baker et al., 1982; Mcleod et al., 1985; Miller et al., 1980; Laurence et al., 1982).

A new open-air fumigation system was set up by the Central Electricity Research Laboratories (CERL) in 1980, designed to simulate ambient exposures of crops to a range of fluctuating SO_2 concentrations, including values typical of the UK, in order to evaluate the effect of SO_2 on crop growth (Mcleod et al., 1988). During the fumigation of a winter barley cv. Sonja crop in 1982-3 and a winter wheat cv Rapier crop in 1983-4, the occurrence of a range of fungal pathogens differed significantly between plots exposed to different SO_2 concentrations within the range 0-60ppb (Mcleod, 1988). These observations raised the questions of how SO_2 influences the epidemiology of a disease, whether these effects can be obtained consistently and how they may influence the response of the crop to SO_2. The experiments described in this section aimed to address these topics.

Disease measured in crops over several seasons enables us to plot progress curves of the epidemics. This helps us determine some of

the factors influencing the epidemics. If measurements are also taken of yields then it is possible to evaluate disease severity yield relationships and thus potential crop loss through disease.

These guidelines were followed in the field experiments with the objective of evaluating the effects of a range of SO_2 concentrations typical of the UK on the development of some important fungal diseases on winter barley growing under normal agricultural conditions. To this end, the incidence and severity of natural infections of fungal diseases occurring in the field plots in the CERL open-air fumigation system was monitored over three consecutive field seasons: 1984-5, 1985-6 and 1986-7. Throughout these seasons different plots were exposed to four different SO_2 regimes: control (5-10ppb SO_2), low SO_2 (14-21ppb), medium SO_2 (28-32ppb) and high SO_2 (38-48ppb), which represented concentrations typical of rural, rural fringe, urban fringe and urban areas of the UK in the year previous to the start of the experiments (Warren Spring Laboratory, 1984).

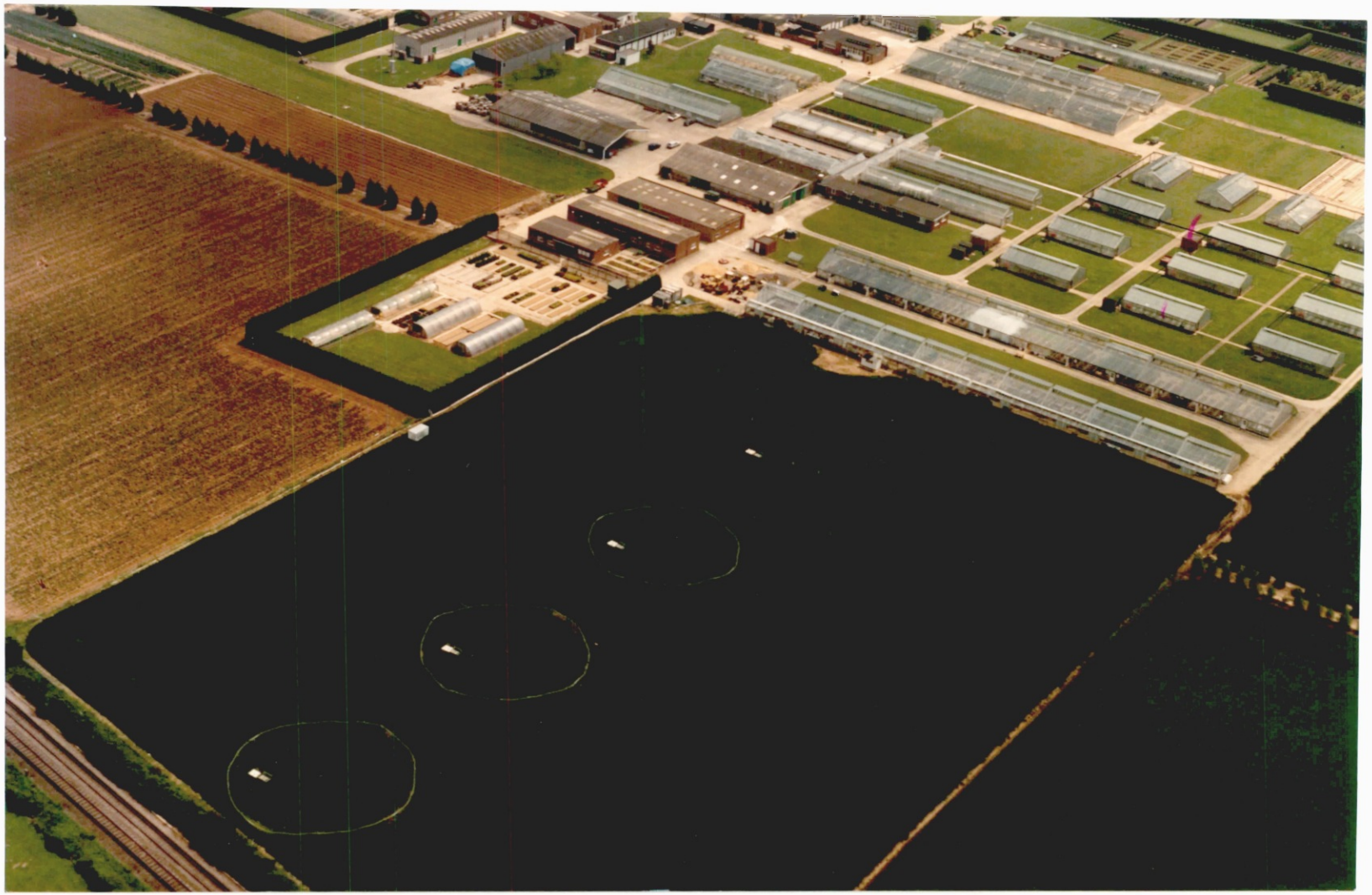


Plate 4. Aerial view of the experimental field of winter barley cv. Igri with the open-air fumigation system in place.

MATERIALS AND METHODS

A. Description of site and Experimental Design

The field experiments were conducted at the site of the Central Electricity Research Laboratories open-air fumigation system at the Glasshouse Crops Research Institute, Littlehampton. The soil type at this site is a brickearth of the Hamble Series (Hodgson, 1957). The experimental field had been planted to winter cereals for three years previously. The field was located in an area of low background concentrations of sulphur dioxide, usually below 5 ppb. Details of crop management are given in Table 3 .

The open-air fumigation system was used to expose winter barley cv. Igri to a range of atmospheric SO_2 concentrations throughout the growing seasons of 1984-5, 1985-6 and 1986-7. In each season there were four different SO_2 treatments: control, low, medium and high SO_2, details of which area given in part C.

A 2.6 hectare area was drilled with barley and the plots marked out after drilling as shown in Fig 1 . Four 9m x 9m main plots P1, P2, P3 and P4 were positioned 30m apart in a south-north line across the field. Each main plot received a different SO_2 treatment. In the 1985-6 and 1986-7 seasons four additional control plots were marked out in a line adjacent to the main plots. These 'companion' control plots C1, C2, C3 and C4 were positioned 30m to the east of the main plots P1, P2, P3 and P4 respectively.

B. Fumigation system

(i) Gas release

The design, construction and operation of the open air fumigation system was carried out by staff from CERL as described in detail by McLeod and Fackrell (1983) and McLeod Alexander and Hatcher (1983)

Table 3 . Details of crop management operations during each growing season.

Operation	1984-5	1985-6	1986-7
Sowing date	5th Nov	11th Oct	29th Sept
Seed rate	190 Kg ha^{-1}	190 Kg ha^{-1}	190 Kg ha^{-1}
Row spacing	12.5cm	12.5cm	12.5cm
Seed treatment	Mergamma 30*	Mergamma 30	Mergamma 30
Fertiliser applications			
1) Date	15th Oct	30th Sept	25th Sept
Type	NPK,5-20-25	NPK,5-20-25	NPK,5-20-25
Rate	250 Kg ha^{-1}	250 Kg ha^{-1}	250 Kg ha^{-1}
2) Date	10th April	24th Feb	12th March
Type	Nitram 24.5N*	Nitram 34.5N	Nitram 34.5N
Rate	375 Kg ha^{-1}	190 Kg ha^{-1}	375 Kg ha^{-1}
3) Date	None	9th May	None
Type		Nitram 34.5N	
Rate		250 Kg ha^{-1}	
Herbicide applications			
Date	10th Nov **	23rd Sept	2nd Oct
Type	Dicurane500FW	Dicurane500FW	Dicurane500FW
Rate	7 l ha^{-1}	7 l ha^{-1}	7 l ha^{-1}
Fungicide applications			
Date	20th May	29th May	None
Type	Bayleton	Bayleton	
Rate	500g ha^{-1}	500g ha^{-1}	
Insecticide applications			
Date	None	None	31st Oct ***
Type			"Metasystox 55"
Rate			0.4 l ha^{-1}
Harvest date	23rd July	21st July	14th July

* ICI PLC
** Ciba - Geigy Agrochemicals
*** Bayer A.G.

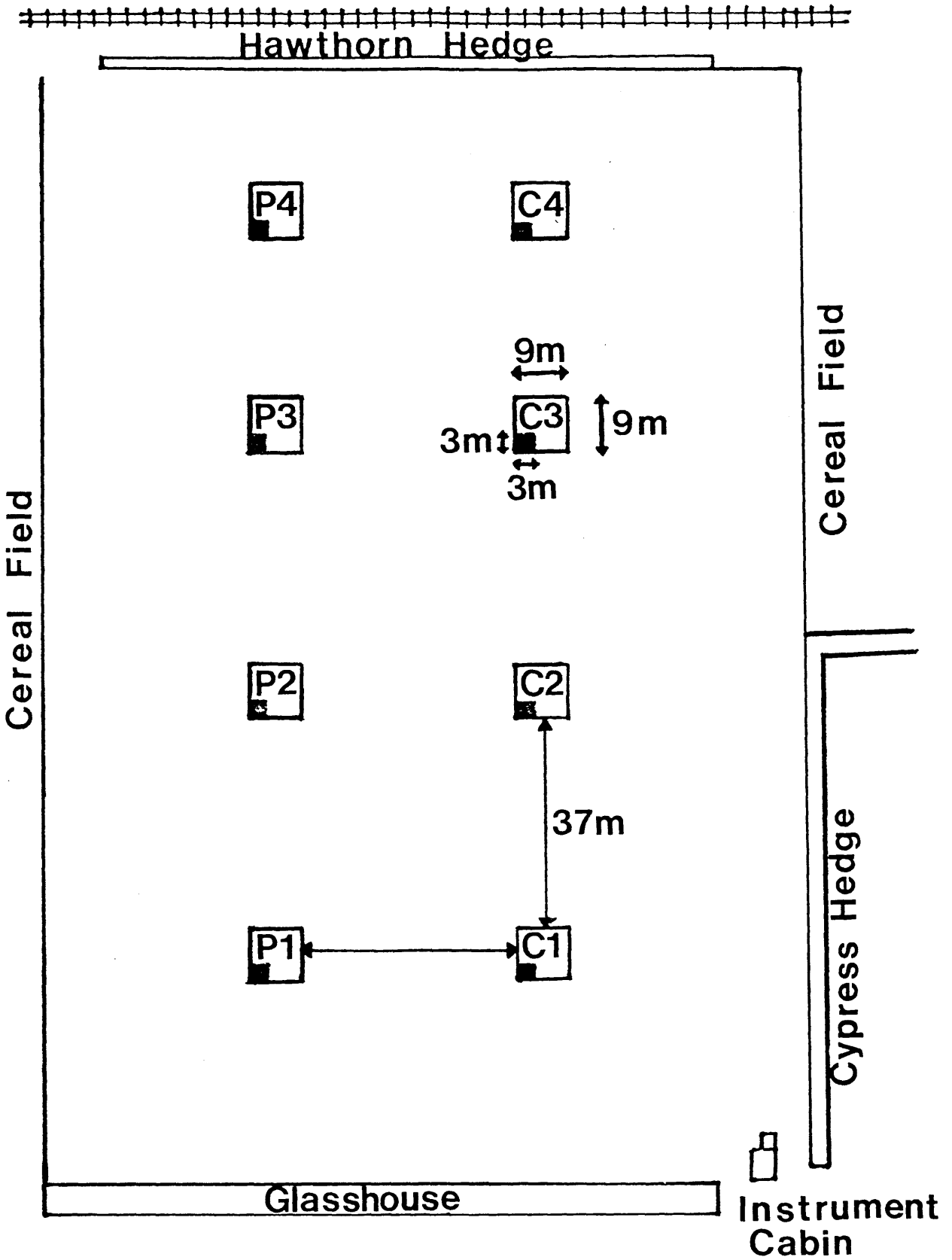


Fig. 1. Plan of field plots at Littlehampton, showing 9m x 9m sample area of the main plots P1, P2, P3 and P4 and the companion control plots C1, C2, C3 and C4. Marked plants assessed for leaf and ear diseases were randomly distributed in the shaded 3m x 3m area of each plot.

The fumigation system consisted of a system of pipes through which air containing controlled amounts of SO_2 was pumped into the atmosphere above the crop, thereby increasing the concentration of SO_2 in the air in which the plants were growing.

Air was pumped by a centrifugal fan from the instrument cabin in the corner of the field to each of the fumigated plots through two polypropylene pipelines. Pure gaseous SO_2 was injected into the start of each pipeline at a rate controlled by means of mass flow controllers (Fig 2). One pipeline supplied the SO_2-air mixture at a rate of 270 l min^{-1} to 24 vertical pipes through which the mixture was released at a height of 0.5m above the crop (low level sources). These "low level source" pipes were positioned in a circle of radius 13.5m from the centre of the plot. The second pipeline supplied the SO_2-air mixture at 400 l min^{-1} to 64 vertical pipes through which it was released at a height of 1.5m above the crop ("high level sources"). The high level sources were positioned within the circle of the low level sources (Fig 3). The pattern of the two types of release pipes was designed to achieve a uniform SO_2 exposure to the plants growing in the central 9m x 9m area of the plot (McLeod, Alexander and Hatcher, 1983). The height of the gas release above the crop was maintained by fitting extensions to the release pipes as the crop height increased.

The supply of SO_2-air mixture to the low and high level sources in each quadrant of the circle of pipes was controlled separately by solenoid valves.

(ii) Control of sulphur dioxide concentrations

The concentration of SO_2 in the fumigated plots was controlled using a feedback system operated by a Macsym 2 microcomputer

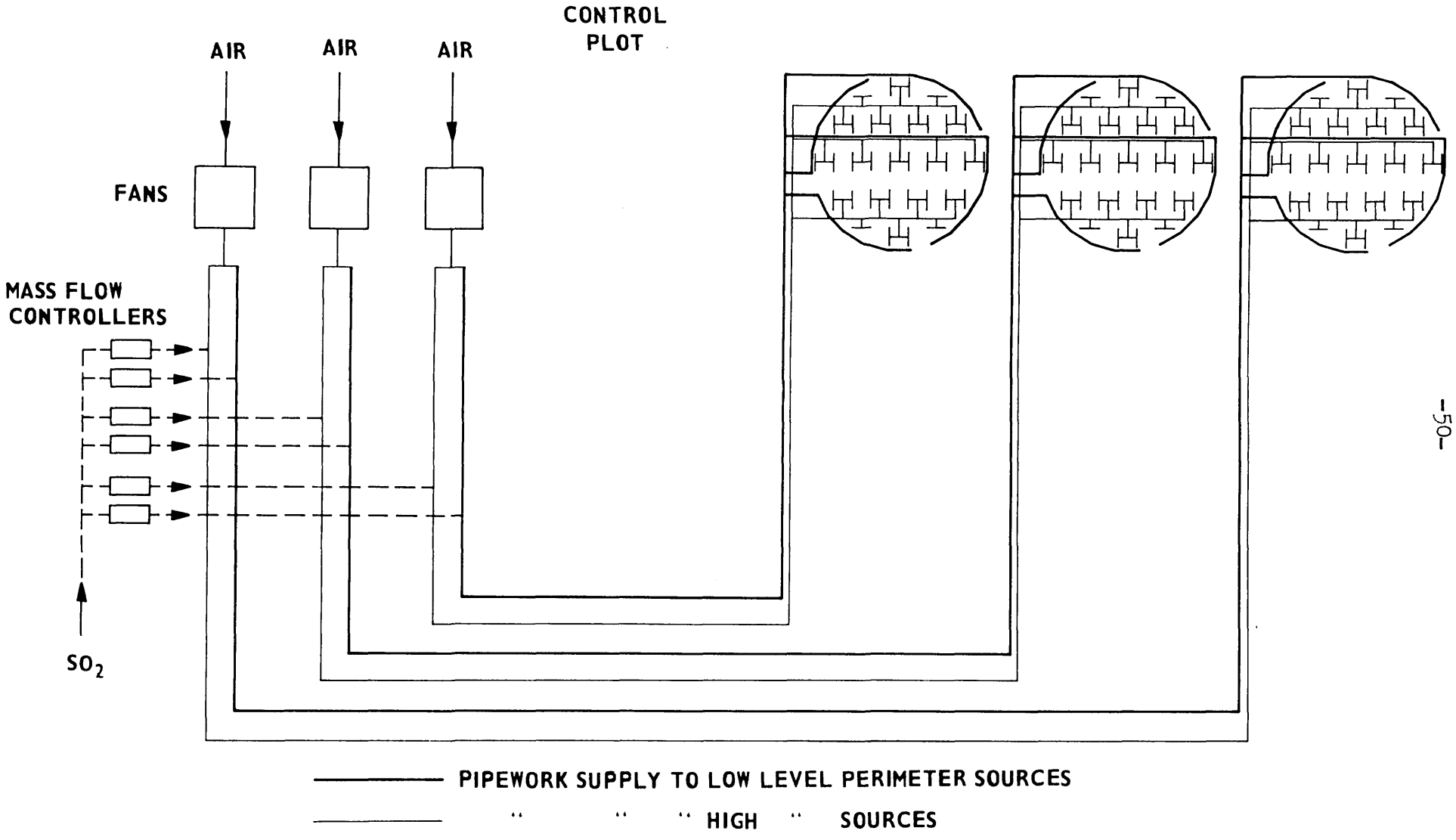


FIG. 2 DIAGRAM SHOWING THE GAS PIPEWORK SUPPLY OF THE FUMIGATION SYSTEM

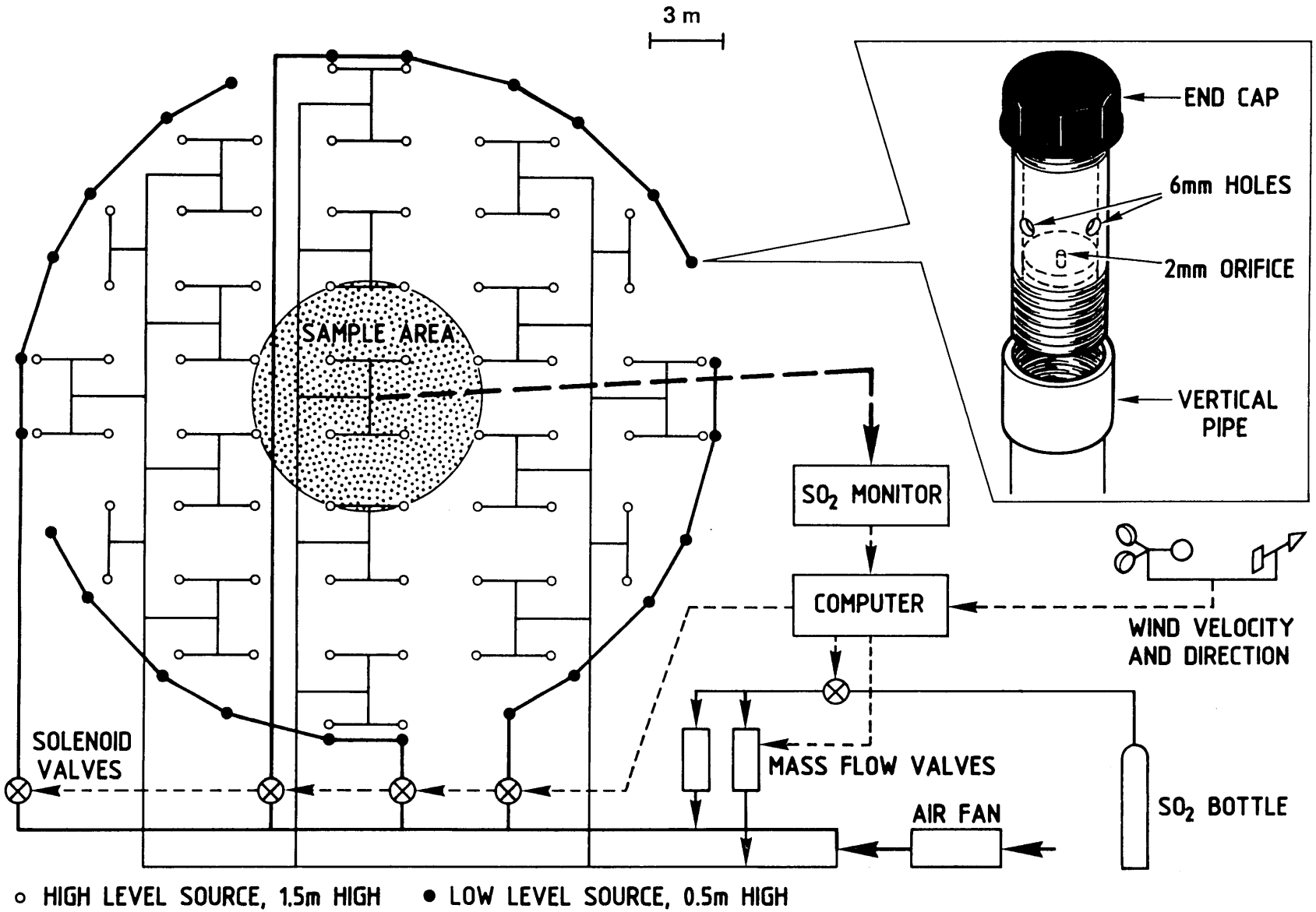


FIGURE 3. DIAGRAM OF THE PIPEWORK IN A FUMIGATION PLOT AND ITS CONTROL SYSTEM

(Analog Devices Ltd.) The computer stored hourly target SO_2 concentrations for each fumigated plot. Measurements of SO_2 concentration in the plot and wind velocity were fed into the computer and the rate of SO_2 injection adjusted to achieve the target concentration. Wind direction measurements were also fed into the computer and the supply of the SO_2-air mixture to the different plot quadrants controlled accordingly.

Air was sampled continuously from the centre of each main plot at 10cm above the top of the canopy. The air sample was sucked through a teflon tube, fitted with a plastic funnel at the inlet to prevent rain entering the tubing. The concentration of SO_2 in the air sample was measured with a Meloy Laboratories Inc. flame photometric sulphur dioxide analyser (Model SA 285E) in the instrument cabin, which was calibrated using a Monitor Laboratories $SO_2/NO_2/O_3$ calibrator (Model 8500).

The sampled air in the four main plots was switched sequentially to the SO_2 analyser at five minute intervals. After a waiting period of one minute to allow the sample lines to clear, the output from the analyser was integrated to calculate a mean SO_2 concentration for the last four minutes of the sample period. The four minute mean is then compared with the hourly target for that plot and adjustment made to the SO_2 injection rate as follows:

$$F = Fo + (T-O).(Fo/O)$$

where F = new SO_2 injection rate

Fo = Original SO_2 injection rate

T = target SO_2 concentration

O = Observed SO_2 concentration

Wind velocity and direction are monitored continuously by a Vector Instruments anemometer mounted on a mast at 2.5 metres above ground level in the centre of the field.

The wind velocity was checked at the beginning of each five minute air sample period. If the velocity fell below $1ms^{-1}$ the SO_2 supply automatically turned off at the cylinder to prevent the exposure of the plants to excessively high concentrations, and turned on again when the velocity exceeded $1ms^{-1}$. If the velocity was greater than $6ms^{-1}$ the SO_2 supply was no longer adjusted to attempt to maintain the target concentrations but remained at its current rate. The feedback control function resumed automatically when the wind velocity fell below $6ms^{-1}$.

Wind direction was checked at the beginning of each sample period and the solenoid valves controlling the supply of the SO_2-air mixture to each quadrant of the fumigated plots (Fig 3) were opened or closed so that at any one time the solenoid valves of the two up-wind quadrants only were open, so releasing SO_2 in air, while the solenoid valves of the two down-wind quadrants were closed.

C. Sulphur dioxide treatments

The hourly target concentrations of SO_2 in the fumigated plots for the whole growing season were adapted from hourly measurements taken during 1979-80 at Bottesford, Central England, a rural site 20km from towns or industrial sources (Martin & Barber, 1981). These values were used directly as target concentrations for the low SO_2 treatment and for the medium and high SO_2 treatment target concentrations, a further 30 and 60 ppb SO_2 were added to the values, respectively. The control plots were exposed to background SO_2 concentrations only. The four SO_2 treatments were re-allocated at

random each season to the four main plots P1, P2, P3 and P4. The exposure system operated from mid November continuously except for periods of equipment failure, instrument calibration, low or high wind velocity and when fungicide, herbicide or insecticide was applied, until the final harvest.

The arithmetic mean, minimum and maximum concentrations of SO_2 on the main plots for the duration of the exposure in each growing season are given in Table 4. and means up to each disease assessment date are shown in Table 5. The 24 hourly means of SO_2 concentration in the main plots on each day of the exposure periods are presented in Figs 4, 5 and 6.

The cumulative frequency distributions of the hourly mean SO_2 concentrations (Fig 7) show the percentage of time a given SO_2 concentration is exceeded in each main plot for the three growing seasons.

D. Background nitrogen oxides and ozone levels

Air was sampled continuously from just outside the instrument cabin for measurement of nitrogen monoxide (NO), nitrogen dioxide (NO_2) and ozone (O_3) concentration using a Monitor Laboratories Dual Channel Chemiluminescent NO - NOX analyser (model 8840) and a Dasibi ultra-violet absorption ozone monitor (Model 1003-PC), both of which were calibrated against a Monitor Labs. $SO_2/NO_2/O_3$ calibrator (Model 8500).

The seasonal arithmetic mean concentrations of NO, NO_2 and O_3, 24 hourly means and cumulative frequency distributions of hourly means on the main plots in each growing season are given in Table 6 and Figs 8-10 & 7 respectively.

Table 4 : Arithmetic mean, minimum and maximum concentrations of SO_2, over the whole exposure period, in the main plots for each field season

SO_2 treatment	Plot	SO_2 concentration (ppb) Mean ± Standard error	Minimum	Maximum
(a) 1984-5 growing season (fumigation began on 20/11/84)				
Control	P1	9 ± 0.19	0	144
Low	P2	21 ± 0.29	0	219
Medium	P4	32 ± 0.34	0	164
High	P3	43 ± 0.45	0	194
(b) 1985-6 growing season (fumigation began on 25/11/85)				
Control	P4	7 ± 0.19	0	87
Low	P1	14 ± 0.29	0	140
Medium	P3	29 ± 0.44	0	154
High	P2	48 ± 0.44	0	178
(c) 1986-7 growing season (fumigation began on 10/11/86)				
Control	P4	5 ± 0.09	0	62
Low	P3	14 ± 0.19	0	232
Medium	P1	28 ± 0.33	0	252
High	P2	38 ± 0.43	0	172

Table 5 : Arithmetic mean concentrations ($\pm$ standard error) of SO_2 (ppb) in the main plots from start of fumigation up to each disease assessment date for the three growing seasons 1984-5, 1985-6 and 1986-7.

(a) 1984-5 growing season

Assessment date	SO_2 treatment			
	Control	Low	Medium	High
30/12/84	5.1 $\pm$ 0.1	9.5 $\pm$ 0.4	20.7 $\pm$ 0.7	28.4 $\pm$ 1.0
31/1/85	13.1 $\pm$ 0.5	24.9 $\pm$ 0.7	34.2 $\pm$ 0.9	40.2 $\pm$ 0.7
12/3/85	13.3 $\pm$ 0.4	23.5 $\pm$ 0.5	34.2 $\pm$ 0.6	42.6 $\pm$ 0.7
10/4/85	11.4 $\pm$ 0.3	21.9 $\pm$ 0.4	34.1 $\pm$ 0.6	44.0 $\pm$ 0.5
23/4/85	11.1 $\pm$ 0.3	21.7 $\pm$ 0.4	33.8 $\pm$ 0.4	44.2 $\pm$ 0.6
15/5/85	11.3 $\pm$ 0.3	22.4 $\pm$ 0.4	35.0 $\pm$ 0.4	46.1 $\pm$ 0.5
30/5/85	10.9 $\pm$ 0.2	21.9 $\pm$ 0.3	34.6 $\pm$ 0.4	44.8 $\pm$ 0.5
11/6/85	10.5 $\pm$ 0.2	21.7 $\pm$ 0.3	33.9 $\pm$ 0.4	44.5 $\pm$ 0.5
27/6/85	9.9 $\pm$ 0.2	21.5 $\pm$ 0.3	33.5 $\pm$ 0.4	44.5 $\pm$ 0.5
5/7/85	9.7 $\pm$ 0.2	21.6 $\pm$ 0.3	33.2 $\pm$ 0.4	44.3 $\pm$ 0.5
12/7/85	9.5 $\pm$ 0.2	21.3 $\pm$ 0.3	32.6 $\pm$ 0.4	43.7 $\pm$ 0.5
19/7/85	9.2 $\pm$ 0.2	21.1 $\pm$ 0.3	32.4 $\pm$ 0.4	43.6 $\pm$ 0.5
31/7/85	9.0 $\pm$ 0.2	20.9 $\pm$ 0.3	32.1 $\pm$ 0.4	43.4 $\pm$ 0.5

Table 5 : cont.

(b) 1985-6 growing season

Assessment date	SO_2 treatment			
	Control	Low	Medium	High
9/1/86	8.3 ± 0.3	15.7 ± 0.6	32.0 ± 0.7	53.0 ± 1.1
30/1/86	7.9 ± 0.3	16.7 ± 0.4	34.9 ± 0.6	56.3 ± 0.9
27/2/86	9.4 ± 0.2	17.8 ± 0.4	32.3 ± 0.5	52.4 ± 0.7
27/3/86	9.0 ± 0.2	16.5 ± 0.3	29.7 ± 0.4	47.6 ± 0.6
10/4/86	8.7 ± 0.1	16.3 ± 0.3	30.5 ± 0.4	49.0 ± 0.6
24/4/86	8.5 ± 0.2	16.1 ± 0.3	31.0 ± 0.4	49.6 ± 0.6
8/5/86	8.3 ± 0.2	15.8 ± 0.3	30.9 ± 0.4	49.7 ± 0.6
22/5/86	7.9 ± 0.1	15.4 ± 0.2	31.2 ± 0.3	50.6 ± 0.5
29/5/86	7.7 ± 0.1	15.3 ± 0.2	31.3 ± 0.3	50.9 ± 0.5
12/6/86	7.4 ± 0.1	15.0 ± 0.2	30.7 ± 0.3	50.2 ± 0.5
19/6/86	7.3 ± 0.1	14.9 ± 0.2	30.3 ± 0.3	49.6 ± 0.5
2/7/86	7.1 ± 0.1	14.7 ± 0.2	30.0 ± 0.3	49.1 ± 0.5
10/7/86	7.0 ± 0.1	14.6 ± 0.2	29.8 ± 0.3	48.8 ± 0.5
17/7/86	6.9 ± 0.1	14.4 ± 0.2	29.4 ± 0.3	48.1 ± 0.5
23/7/86	6.8 ± 0.1	14.4 ± 0.2	29.3 ± 0.3	47.8 ± 0.4

Table 5 : cont.

1986-7 Growing season

Assessment date	SO_2 treatment			
	Control	Low	Medium	High
11/12/86	3.7 ± 0.2	15.2 ± 0.6	29.0 ± 0.9	40.5 ± 1.2
11/2/87	6.4 ± 0.2	16.7 ± 0.3	29.2 ± 0.6	36.9 ± 0.7
16/3/87	6.6 ± 0.2	17.3 ± 0.3	29.6 ± 0.5	37.8 ± 0.6
16/4/87	5.9 ± 0.1	16.4 ± 0.3	29.3 ± 0.4	38.4 ± 0.5
30/4/87	5.7 ± 0.1	16.0 ± 0.3	28.9 ± 0.4	38.3 ± 0.5
14/5/87	5.5 ± 0.1	15.9 ± 0.2	29.1 ± 0.4	39.0 ± 0.5
21/5/87	5.4 ± 0.1	15.7 ± 0.2	29.1 ± 0.4	39.3 ± 0.5
28/5/87	5.4 ± 0.1	15.7 ± 0.2	29.1 ± 0.4	39.3 ± 0.5
4/6/87	5.3 ± 0.1	15.5 ± 0.2	29.1 ± 0.4	39.4 ± 0.5
11/6/87	5.1 ± 0.1	15.4 ± 0.2	28.9 ± 0.4	39.5 ± 0.5
17/6/87	5.1 ± 0.1	15.2 ± 0.2	28.7 ± 0.4	39.2 ± 0.5
25/6/87	4.9 ± 0.1	15.0 ± 0.2	28.5 ± 0.4	39.3 ± 0.5
2/7/87	4.9 ± 0.1	14.8 ± 0.2	28.2 ± 0.3	39.1 ± 0.5
9/7/87	4.9 ± 0.1	14.7 ± 0.2	27.9 ± 0.3	38.6 ± 0.4

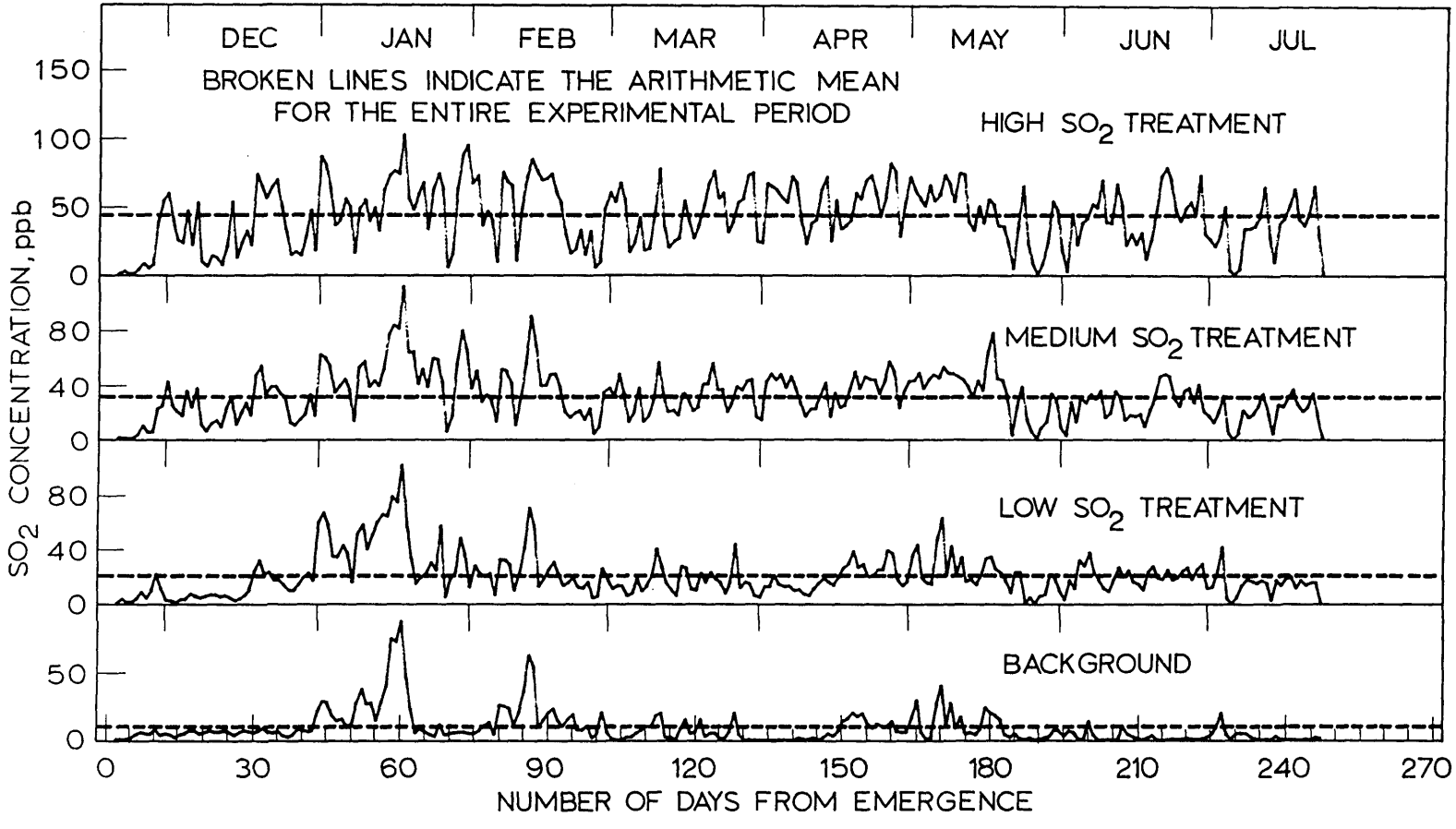


Fig 4. THE 24-HOUR MEAN SO_2 CONCENTRATION ON EACH TREATMENT PLOT FOR EACH DAY OF THE EXPERIMENTAL PERIOD NOVEMBER 1984-JULY 1985

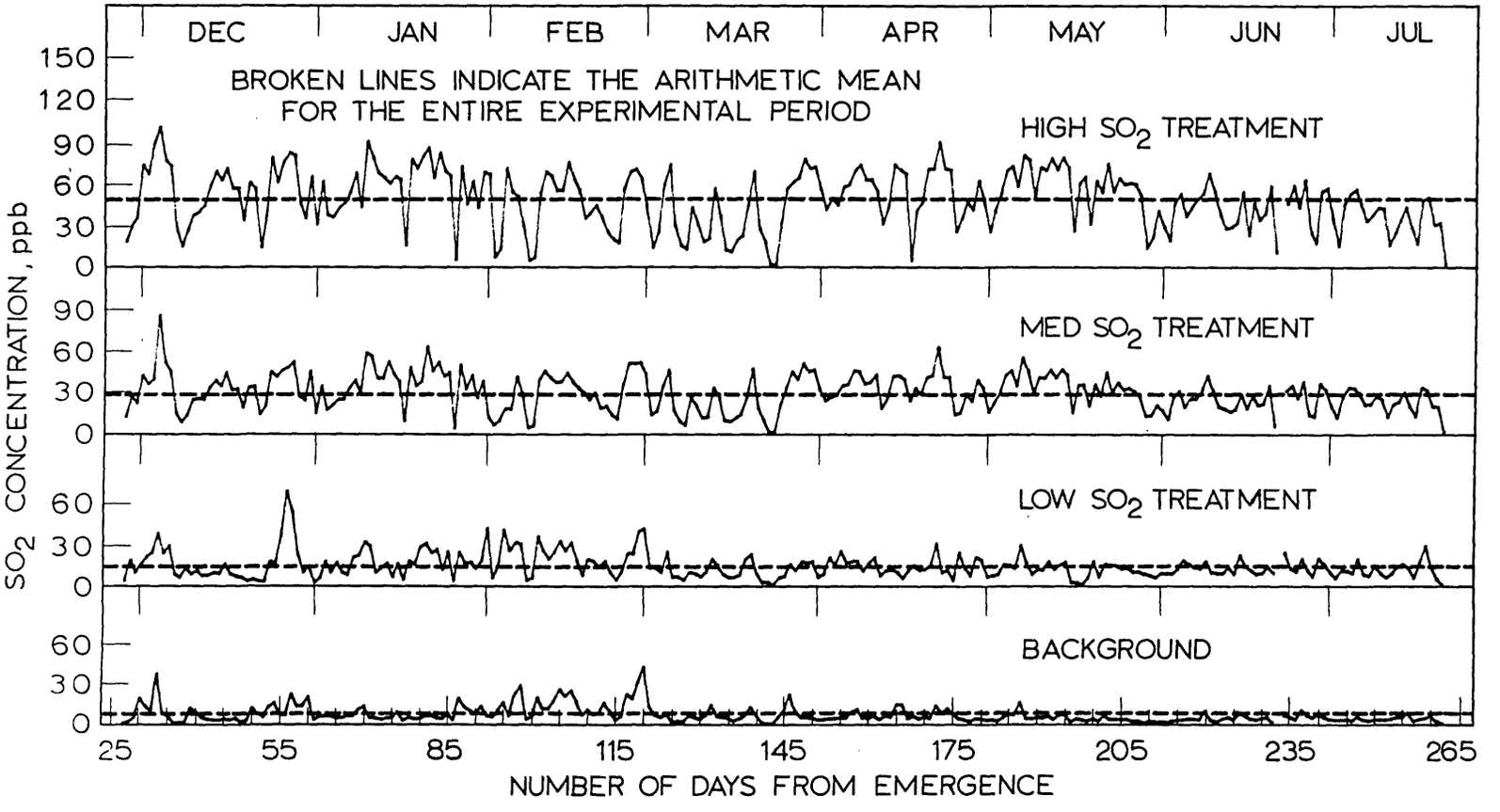


Fig 5. THE 24-HOUR MEAN SO_2 CONCENTRATION ON EACH TREATMENT PLOT FOR EACH DAY OF THE EXPERIMENTAL PERIOD NOVEMBER 1985-JULY 1986

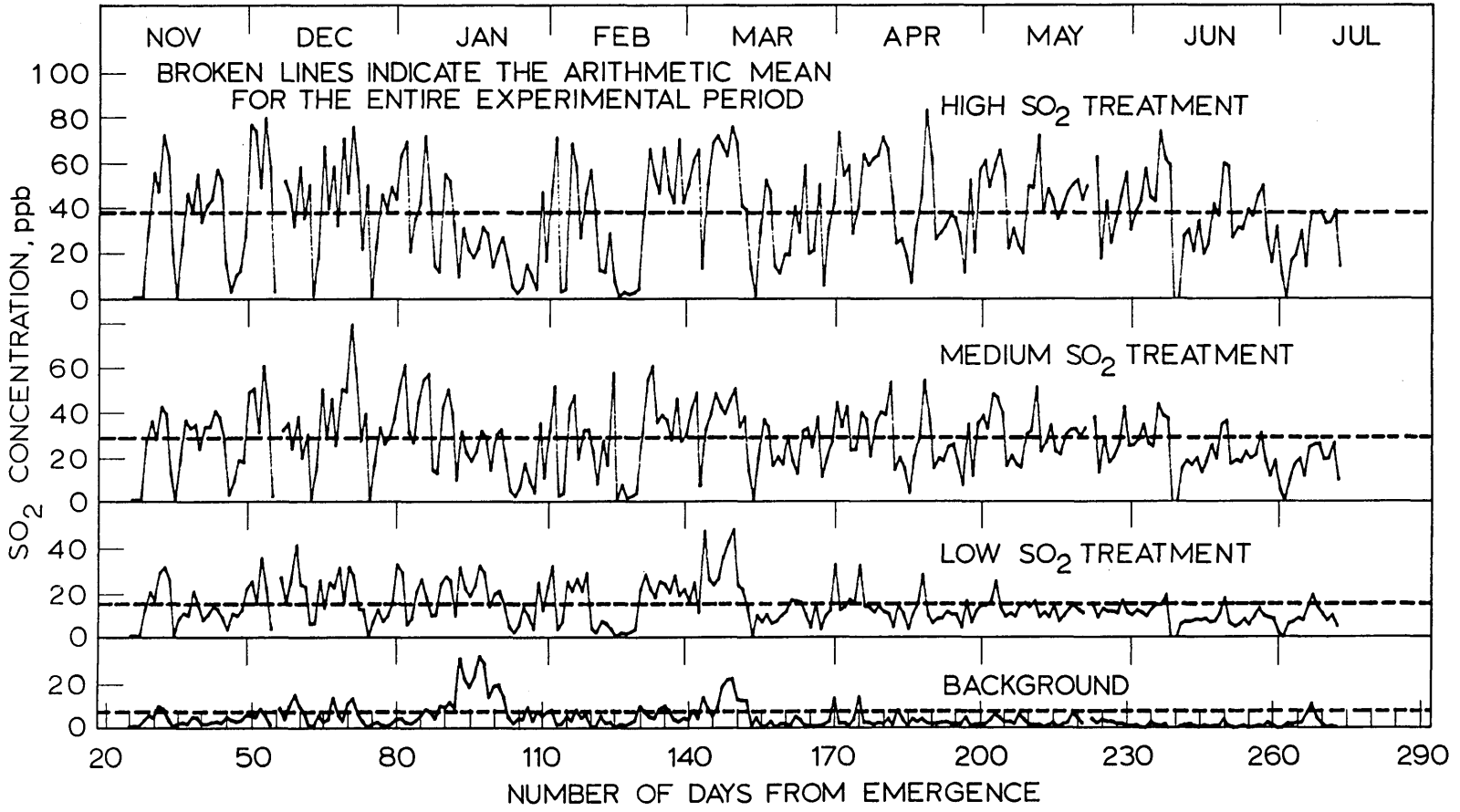


Fig 6. THE 24-HOUR MEAN SO_2 CONCENTRATION ON EACH TREATMENT PLOT FOR EACH DAY OF THE EXPERIMENTAL PERIOD NOVEMBER 1986-JULY 1987

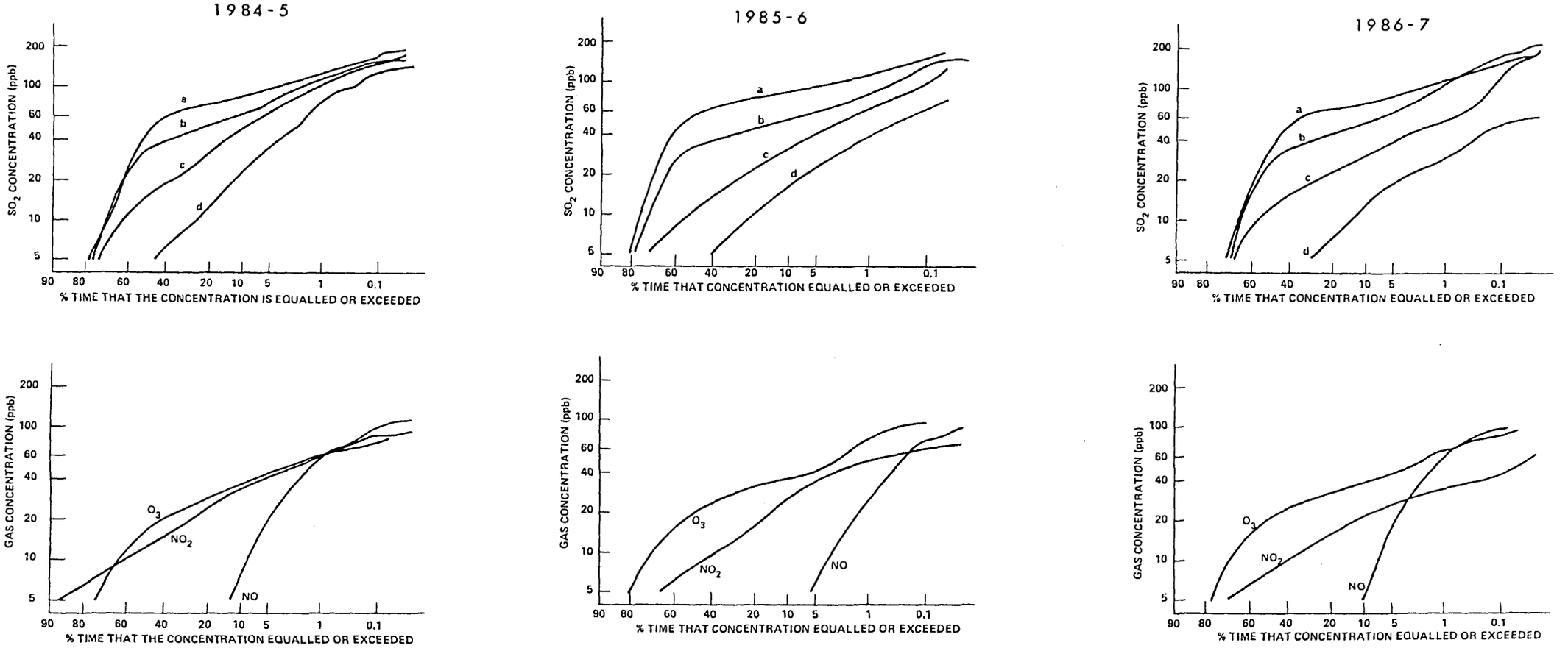


Fig 7. Cumulative frequency distributions of SO_2 concentrations in the high SO_2 (a), medium SO_2 (b), low SO_2 (c) and control (d) plots and of background O_3, NO_2 and NO concentrations in the experimental field during the 1984-5, 1985-6 and 1986-7 seasons.

Table 6 : Mean and maximum concentrations of nitrogen monoxide, nitrogen dioxide and ozone over the whole exposure period in the main plots for each growing season

Gas concentrations (ppb)		1984-5	1985-6	1986-7
NO:	Mean ± SE	3.0 ± 10.2	1.2 ± 5.4	3.2 ± 0.13
	Maximum	192.3	108	118
NO_2:	Mean ± SE	16.1 ± 12.4	10.7 ± 10.5	10.1 ± 0.12
	Maximum	96.3	67.5	320
O_3:	Mean ± SE	17.3 ± 14.1	19.7 ± 14.7	19.8 ± 0.2
	Maximum	82.7	112.1	93

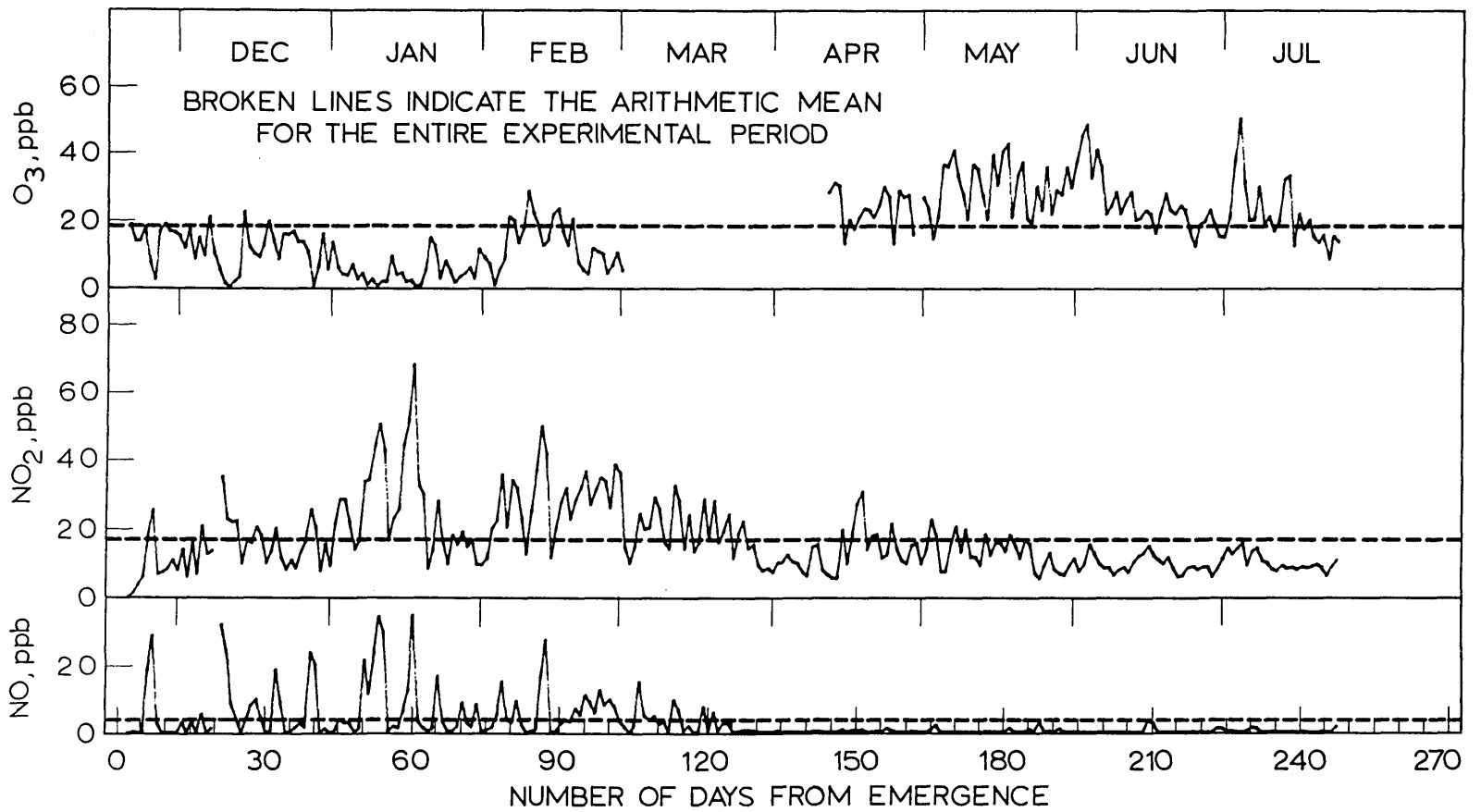


Fig 8. THE 24-HOUR MEAN BACKGROUND O_3, NO AND NO_2 CONCENTRATION AT THE LITTLEHAMPTON EXPERIMENTAL SITE FROM NOVEMBER 1984-JULY 1985

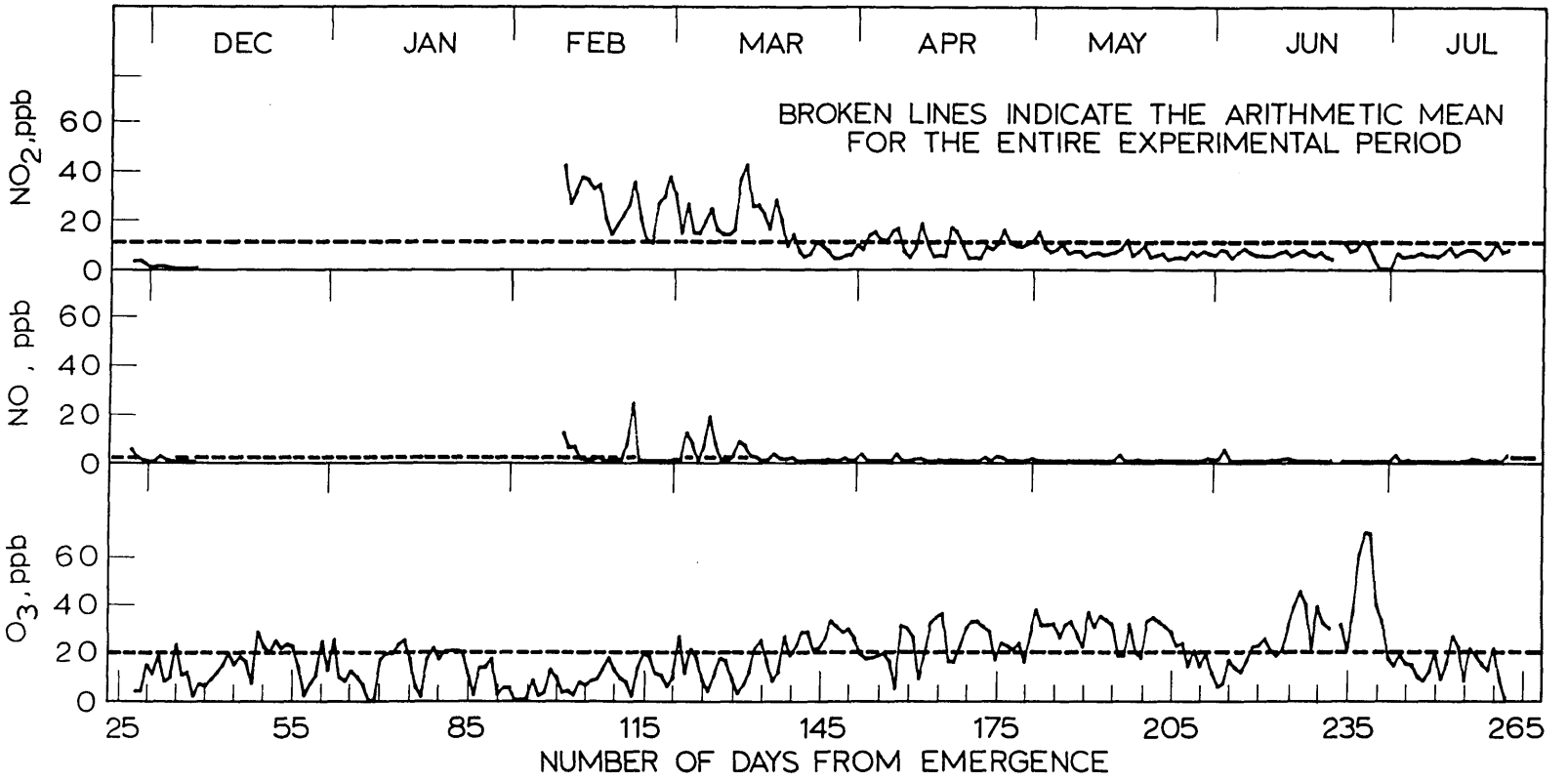


Fig 9. THE 24-HOUR MEAN BACKGROUND O_3, NO AND NO_2 CONCENTRATION AT THE LITTLEHAMPTON EXPERIMENTAL SITE FROM NOVEMBER 1985-JULY 1986

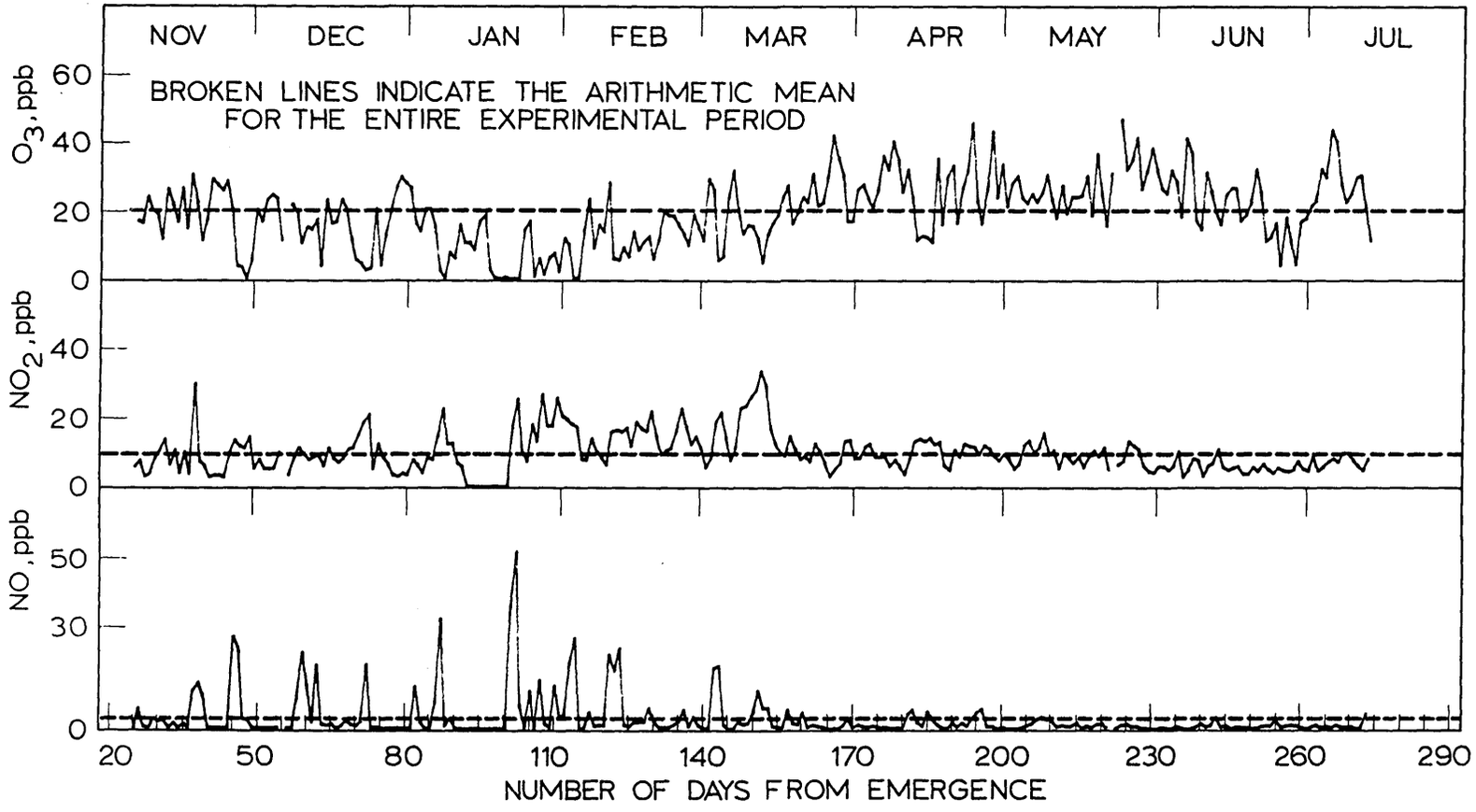


Fig 10. THE 24-HOUR MEAN BACKGROUND O_3, NO AND NO_2 CONCENTRATION AT THE LITTLEHAMPTON EXPERIMENTAL SITE FROM NOVEMBER 1986-JULY 1987

E. Disease Assessments

(i) 1984-5

Assessments were made of natural infections of fungal diseases occurring on the leaves, ears and stem bases of plants growing in the four main plots: P1, P2, P3 and P4.

Leaf disease assessments were made at monthly intervals during seedling growth until the beginning of tillering (GS13-23) and fortnightly until complete inflorescence emergence (GS23-59). Ten plants randomly distributed along the diagonals of each 9m x 9m main plot were examined at each visit. The Growth Stage (Zadoks et al., 1974) of each plant assessed was noted, and the incidence of disease symptoms on individual leaves was recorded.

From GS60-85 weekly leaf disease assessments were made on twelve marked tillers randomly distributed within a 3m x 3m area in the south-west corner of each main plot (Fig 1). The incidence of disease symptoms on individual leaves of each tiller was recorded. The severity of powdery mildew (Erysiphe graminis), leaf blotch (Rhynchosporium secalis) and brown rust (Puccinia hordei) on individual leaves was assessed using standard diagrams of percentage leaf area affected by disease (Ministry of Agriculture, Fisheries and Food, 1976; James, 1967).

The marked tillers were also examined for the incidence of ear disease at each visit until harvest. The severity of black ear moulds (Cladosporium and Altemaria spp.) was assessed by recording those ears on which infection was visible on the glumes only (not severe) and those on which both the glumes and lemmae were infected (severe).

During tillering an assessment of the incidence of eyespot (Pseudocercosporella herpotrichoides) was made on samples of ten

plants taken at random along a transect of each main plot. The percentage of plants infected per plot and the number of leaf sheaths infected per plant was recorded.

From anthesis until harvest weekly samples of ten tillers per plot were taken from the main plots for assessment of the incidence and severity of eyespot (Pseudocercosporella herpotrichoides) on the stem base. At the final sample, 10 tillers were also taken from the companion control plots. The severity of eyespot was assessed using a modified version of the scale devised by Scott & Hollins (1974):

Not Severe - uninfected or slight infection (one or more lesions occupying in total less than half the circumference of the stem base)

Severe - one or more lesions occupying at least half the circumference of the stem base

The sample tillers were also examined for the incidence of Fusarium foot rot and sharp eyespot (Rhizoctonia cerealis) on the stem bases from late dough development (GS 86) until harvest.

(ii) 1985-6

Assessments were made of fungal diseases occurring on plants growing in the main plots P1, P2, P3 and P4 and in the companion control plots C1, C2, C3 and C4 (Fig 1).

Leaf diseases were assessed on all expanded green leaves at monthly intervals during seedling growth and the beginning of tillering (GS 13-26), and fortnightly until the start of stem elongation (GS 26-31), on twelve marked plants randomly distributed in a 3m x 3m area in the south-west corner of each plot. At first node stage a further three plants were marked in the area and one tiller per marked plant was chosen at random and labelled. The

fifteen labelled tillers per plot were assessed weekly for leaf diseases until leaf senescence (GS31-85). The incidence and severity of the leaf diseases was assessed as described in part (i).

The incidence of ear diseases on the marked tillers was assessed weekly from anthesis until harvest.

During seedling growth and tillering (GS13-30) monthly samples of ten plants were taken at random along a transect of each plot. The percentage of plants infected with eyespot and the number of leaf sheaths infected per plant was assessed. From stem elongation until harvest, samples of twenty tillers per plot were taken fortnightly. The leaf sheaths were removed from the sampled tillers and the incidence of eyespot, Fusarium foot rot and sharp eyespot on the stem base was assessed as described in part (i).

An assessment of the degree of lodging per plot was made at the end of the season using a simple key showing the percentage area of the plot in which lodging had occurred.

(iii) 1986-7

Assessments were made of fungal diseases occurring on plants growing in the main plots P1, P2, P3 and P4 and in the companion plots C1, C2, C3 and C4. Leaf diseases were assessed monthly during tillering (GS20-29) on 15 marked plants randomly distributed in a 3m x 3m area in the south-west corner of each plot, then fortnightly during stem elongation and booting (GS30-49) and weekly until leaf senescence (GS87) on one labelled tiller per marked plant. The incidence and severity of disease of the fully expanded green leaves was assessed as described in part (i), on 20 plants per plot sampled monthly during tillering stem eleongation and booting (GS22-49) then on 20 tillers per plot sampled fortnightly until harvest.

F. Meteorological Data

Measurements of maximum and minimum air temperature, solar radiation, daily sunshine hours, rainfall and wind for each season were made at the Glasshouse Crops Research Institute and are presented in Table 7, (GCRI Annual Reports 1985; 1986; 1987).

Table 7 . Meteorological records at the Glasshouse Crops Research Institute, Littlehampton, (Lat. 50°49'N, Long. 0°31'W), for the duration of the open-air fumigation experiments.

(a) 1984-5 field season

Month	Air[a] temperature°C		Rainfall[b]	Sunshine[c]	Solar[d] Radiation	Wind[e]
	Max.	Min.	(mm.)	(hours)	integral (MJm^{-2})	($Kmday^{-1}$)
Nov	12.6	6.2	93.4	2.40	99	169.0
Dec	9.8	3.0	99.2	1.89	66	137.0
Jan	4.5	-2.0	50.8	2.00	88	93.8
Feb	5.6	-0.2	33.5	3.66	167	93.2
March	8.6	0.8	54.1	3.84	279	120.9
April	13.0	4.9	42.2	6.32	443	186.5
May	16.6	8.3	41.5	7.00	564	115.5
June	18.1	9.6	65.0	6.43	547	141.3
July	21.3	12.0	56.9	8.55	629	155.8

(b) 1985-6 field season

Oct	15.6	8.1	26.3	4.6	222	91.0
Nov	8.8	1.4	76.8	2.95	112	118.5
Dec	10.0	4.9	67.4	1.46	60	185.1
Jan	7.5	1.2	117.1	2.23	80	206.7
Feb	2.4	-3.7	10.6	3.24	149	81.6
March	8.4	1.7	77.0	3.84	279	203.2
April	10.2	2.8	46.4	5.60	382	175.2
May	14.3	7.3	57.8	6.20	521	250.4
June	20.5	10.3	25.9	8.16	602	104.8
July	20.6	12.1	26.5	6.96	559	127.2

Table 7 . cont.

(c) 1986-7 field season

Month	Air[a] temperature°C		Rainfall[b]	Sunshine[c]	Solar[d] Radiation	Wind[e]
	Max.	Min.	(mm.)	(hours)	integral (MJm^{-2})	($Kmday^{-1}$)
Sept	16.8	6.7	28.3	5.64	337	76.3
Oct	15.9	8.2	93.2	3.97	206	140.7
Nov	12.4	5.6	111.5	2.95	104	196.9
Dec	9.8	3.2	107.9	2.26	69	205.9
Jan	3.6	-1.4	14.4	2.10	80	62.0
Feb	7.3	1.2	41.4	3.26	147	100.8
March	8.5	1.3	103.5	3.83	260	158.3
April	13.4	5.5	73.3	5.90	427	141.3
May	15.8	5.9	30.8	7.40	553	101.0
June	17.2	9.9	93.8	6.30	513	145.3
July	20.8	12	51.3	7.12	533	89.5

[a] Mean screen

[b] Monthly total

[c] Daily means recorded by Campbell-Stokes instrument

[d] Total shortwave radiation received by Kipp Solarimeter at 4.61m above ground level

[e] Mean daily kilometres run, recorded at 3.048m (10ft.) above ground level.

RESULTS

A. Crop growth

(i) Seasonal growth

The growth stage (Zadoks et al., 1974) of the plants assessed for disease was recorded at each visit.

Crop growth measurements were carried out by CERL on plants sampled approximately monthly through each season. On each date five samples were taken per plot, each sample consisting of all the plants growing in four adjacent half-metre row lengths. Measurements of tiller density, total above ground dry weight, leaf weight and leaf area index were made. The results were compared by analysis of variance on a planned comparison between each treatment plot and companion control plot.

Tiller density

There were significant differences in tiller density between the SO_2 treated plots and the controls (Table 8) but the response changed through the season and differed between the years. There were no significant differences between the paired control plots in either 1985-6 or 1986-7.

In the 1984-5 season tiller density was significantly lower in the high SO_2 plot (P3) than the corresponding control plot (P1) from December to March but by April this position was reversed and from May onwards there were no significant differences between these plots. The rate of tiller production was high in the medium SO_2 plot (P4) and tiller density was significantly higher than in the control plot (P1) from March until June, however tiller death was also greater and by harvest tiller density was similar to that in the control plot. Tiller density in the low SO_2 plot (P2) was significantly higher than in the control plot (P1) in April, June and July (final harvest).

Table 8 . Seasonal changes in tiller density in plots of Igri barley exposed to different SO_2 concentrations throughout three growing seasons.

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	Number of tillers per m^2 (mean ± standard error)[a] on Dec 6	Jan 24	Mar 6	Apr 17	May 22	Jun 26	Jul 23
P1	9 (Control)	268(14)	273(24)	530(40)	743(28)	828(37)	740(52)	843(70)
P2	21 (Low)	253(22)	267(14)	470(28)	952(117**)	924(101)	890(29*)	1025(61**)
P4	32 (Medium)	264(36)	288(18)	621(19*)	1115(84**)	1088(45**)	908(39*)	846(23)
P3	43 (High)	211(16*)	219(26*)	427(25*)	841(14*)	924(52)	838(47)	819(50)

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	Number of tillers per m^2 (mean ± standard error)[a] on Nov 13	Jan 2	Feb 18	Apr 3	May 7	Jun 4	Jul 7
P4	7 (Control)	285(12)	434(31)	700(31)	1914(109)	2037(76)	737(40)	641(26)
C4	(Control)	304(15)	490(41)	645(41)	1927(52)	1977(72)	755(32)	746(34)
P1	14 (Low)	306(13	570(38)	746(34)	2366(100)	1917(67)	1012(56)	821(110)
C1	(Control)	298(11)	549(22)	738(44)	2240(36)	2113(92)	914(49)	797(41)
P3	29 (Medium)	327(17)	601(36)	879(51*)	2097(170)	1878(50)	824(4)	810(71)
C3	(Control)	284(17)	682(24)	720(46)	1994(167)	1784(72)	890(59)	845(78)
P2	48 (High)	280(10)	562(42)	791(26)	2026(94)	1962(99)	809(78)	839(65)
C2	(Control)	294(19)	596(26)	771(60)	2281(26)	1790(102)	922(74)	873(92)

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	Number of tillers per m^2 (mean ± standard error)a on							
		Oct 29	Dec 8	Jan 28	Mar 11	Apr 8	May 12	Jun 15	Jul 14
P4	5 (Control)	315(12)	899(87)	926(119)	1306(39)	1662(66)	855(36)	653(53)	699(32)
C4	(Control)	308(17)	825(50)	1006(59)	1237(64)	1940(62)	778(21)	747(19)	631(28)
P3	14 (Low)	313(7)*	747(71)	786(93)**	1090(145)*	2064(146)*	858(25)	642(29)	699(47)
C3	(Control)	366(19)	938(131)	1272(96)	1487(42)	1892(130)	952(72)	770(59)	822(59)
P1	28 (Medium)	347(18)	811(77)	974(49)	1142(97)**	1686(56)	914(37)	666(49)**	683(41)
C1	(Control)	321(10)	958(29)	1119(72)	1545(58)	1895(118)	975(77)	778(68)	849(210)
P2	38 (High)	364(21)	664(21)***	804(85)**	1012(71)***	1638(37)	831(61)	610(13)	721(53)
C2	(Control)	338(25)	1066(36)	1229(79)	1578(57)	1701(65)	878(50)	755(38)	657(46)

[a] Tiller density based on five replicate samples of tillers from four half-metre row lengths on each date.

*, **, *** Values of tiller density differ significantly from corresponding control plot at $P < 0.05$, 0.01 or 0.001 respectively.

In the 1985-6 season tiller densities were similar in all plots and only on one occasion in February was there a significant difference when there were more tillers in the medium SO_2 plot (P3) than the control plot (C3).

In 1986-7 tiller density was significantly lower in the high SO_2 plot during December to March, as in 1984-5, but thereafter was similar. In the low SO_2 plot (P3) tiller density was also lower than in the control plot (C3) in October, January and March but significantly higher in April and similar thereafter. Tiller density in the medium SO_2 plot (P1) was significantly lower than the control plot (C1) on two occasions through the season in March and in June, in contrast to the response in the first two seasons. There were no significant differences in tiller density by the final harvest 1987.

Total dry weight

Significant differences in total dry weight per m^2 were apparent between SO_2 treated plots and companion control plots on numerous occasions but there was no consistent trend in the responses, (Table 9). Paired control plots in the last two seasons did not differ significantly on any date.

In the 1984-5 season, total dry weight was initially lower in the low and high SO_2 plots (P2 and P3) than the control plot (P1) but thereafter total dry weight in the SO_2 treated plots was frequently higher than in the control plot. (In the low SO_2 plot in March, April, May and July; in the medium SO_2 plot in March, April, May and June; in the high SO_2 plot in April and May).

In 1985-6, total dry weight was significantly lower in the medium SO_2 plot (P3) than the companion control plot (C3) in January and in the high SO_2 plot (P2) than control plot (C2) in April but there were no other significant differences.

Table 9 . Seasonal changes in total above ground dry weight of Igri barley in plots exposed to different SO_2 concentrations throughout three field seasons.

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	Total dry weight (g) per m^2 (mean ± standard error)[a] on						
		Dec 6	Jan 24	Mar 6	Apr 17	May 22	Jun 26	Jul 23
P1	9 (Control)	7.6(0.4)	13.8(1.1)	16.0(2.1)	48.7(3.0)	345(28)	1082(101)	1420(110)
P2	21 (Low)	6.5(0.6*)	14.3(1.0)	**20.1(1.4*)**	71.1(8.7**)	453(51*)	1168(39)	1661(106*)
P4	32 (Medium)	7.1(0.6)	16.7(0.6*)	23.0(1.3**)	87.7(8.6**)	601(27**)	1474(109*)	1524(42)
P3	43 (High)	5.9(0.3**)	12.0(2.1)	14.3(1.0)	55.6(2.4*)	457(37*)	1204(83)	1385(82)

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	Total dry weight (g) per m^2 (mean ± standard error)[a] on Nov 13	Jan 2	Feb 18	Apr 3	May 7	Jun 4	Jul 7	Jul 21
P4	7 (Control)	6.1(0.4)	30.9(2.8)	38.5(1.4)	84.9(6.6)	526(37)	905(72)	1561(88)	2124(73)
C4	(Control)	6.9(0.4)	25.6(2.5)	33.2(2.4)	84.1(2.3)	543(20)	967(46)	1693(113)	1960(58)
P1	14 (Low)	7.3(0.3)	27.2(2.4)	40.2(1.8)	94.6(4.8)	428(17)	950(93)	1457(151)	1425(59)
C1	(Control)	7.2(0.4)	24.5(1.3)	33.1(3.0)	91.2(3.6)	388(29)	952(42)	1546(30)	1518(90)
P3	29 (Medium)	7.6(0.5)	27.5(1.6)**	45.1(2.3)	97.7(10.5)	500(23)	905(43)	1514(129)	1813(71)
C3	(Control)	7.4(0.6)	35.9(1.4)	38.2(2.8)	87.5(7.7)	493(20)	968(52)	1513(64)	1677(78)
P2	48 (High)	7.0(0.2)	26.5(1.7)	44.2(1.6)	83.2(3.6)	389(28)	811(79)	1448(67)	1577(81)
C2	(Control)	7.5(0.6)	28.8(1.4)	50.0(3.3)	100.3(4.8)	463(23)	943(71)	1444(98)	1407(26)

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	Total dry weight (g) per m^2 (mean ± standard error)[a] on Oct 29	Dec 8	Jan 28	Mar 11	Apr 8	May 12	Jun 15	Jul 14
P4	5 (Control)	11.6(0.9)	32.6(2.9)	47.8(7.6)	82.3(3.6)	136(18)	779(42)	1434(102)	1445(88)
C4	(Control)	11.5(1.1)	30.7(1.9)	44.5(3.9)	74.0(3.5)	135(6)	719(46)	1503(56)	1443(34)
P3	14 (Low)	12.7(0.8)**	29.9(2.9)	35.1(6.8)*	51.4(10.2)**	137(12)*	687(22)	1395(84)	1513(97)
C3	(Control)	18.5(1.1)	44.4(7.9)	57.6(5.0)	107.6(3.3)	201(15)	815(58)	1378(88)	1619(88)
P1	28 (Medium)	14.0(1.3)	34.5(3.8)*	47.1(4.1)	65.2(11.7)*	124(18)	801(42)	1268(93)	1315(103)
C1	(Control)	16.0(1.5)	45.0(1.5)	60.4(6.0)	97.1(5.6)	154(10)	220(25)	1494(113)	1576(159)
P2	38 (High)	12.6(0.7)	27.4(0.9)***	34.7(4.5)***	48.3(6.7)***	82(4)***	660(31)	1147(65)	1362(94)
C2	(Control)	16.1(1.5)	47.6(3.1)	68.1(7.1)	116.4(4.3)	196(6)	810(63)	1358(69)	1399(87)

[a] Mean oven dry weight of total above ground crop (g/m^2) based on five replicate samples of four half metre row lengths on each date.

*, **, *** Total dry weight differs significantly from that in corresponding control plot at $P < 0.05$, 0.1 or 0.01 respecitively.

In contrast to the first season, in 1986-7 total dry weight was generally lower in the SO_2 treated plots than the corresponding control plots with significant differences between the low SO_2 plot (P3) and control (C3) in October, January, March and April; between the medium SO_2 plot (P1) and control (C1) in December and March, between the high SO_2 plot (P2) and control (C2) in December, January, March and April. However, from May until the final harvest total dry weight was similar in all plots.

Leaf weight

The response of leaf weight in the different seasons followed a similar pattern to total weight (Table 10).

In 1984-5 leaf weight was significantly lower in the high SO_2 plot (P3) than the control plot (P1) in December but from April onwards was significantly higher. Leaf weight was also significantly higher in the medium SO_2 plot (P4) than control plot (P1) from January onwards and in the low SO_2 plot (P2) in April and May.

In 1985-6, leaf weight was lower in the medium SO_2 plot (P3) than the companion control plot (C3) in January and in the high SO_2 plot (P2) than control plot (C2) in April, with no other significant differences.

In 1986-7, leaf weight was usually lower in the SO_2 treated plots than the control plots with significant differences between the low SO_2 plot (P3) and control plot (C1) in October, January, March and April; between the medium SO_2 plot (P1) and control plot (C1) in December; between the high SO_2 plot (P2) and control plot (C2) in December, January, March and April. By May and June there were no significant differences in leaf weight between the plots.

Table 10 . Seasonal changes in leaf weight of Igri barley growing in plots subjected to different SO_2 concentrations through three field seasons.

(a) 1984-5)

Plot No.	Mean annual ppb SO_2 (Treatment)	Leaf weight (g) per m^2 (mean ± standard error)[a] on Dec 6	Jan 24	Mar 6	Apr 17	May 22	Jun 26
P1	9(Control)	4.5(0.3)	6.9(0.4)	7.3(0.9)	23.6(1.2)	90.2(7.4)	44.1(6.2)
P2	21(Low)	4.1(0.3)	7.2(0.5)	8.1(0.9)	32.7(4.3*)	122.0(10.5*)	53.6(4.2)
P4	32(Medium)	4.4(0.4)	9.1(0.5**)	10.5(0.4*)	41.8(3.4**)	138.5(1.8)	72.2(3.0**)
P3	43(High)	3.8(0.2*)	6.5(1.2)	6.2(0.3)	27.1(1.6*)	117.8(6.7*)	65.5(7.8*)

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	Leaf weight (g) per m^2 (mean ± standard error)[a] on Nov 13	Jan 2	Feb 18	Apr 3	May 7	Jun 4
P4	7(Control)	3.7(0.2)	18.7(2.3)	22.0(0.8)	53.5(3.7)	194(11)	136(8)
C4	(Control)	4.1(0.2)	15.1(1.4)	19.1(1.5)	51.6(1.3)	201(5)	157(8)
P1	14(Low)	4.4(0.1)	17.2(1.5)	21.0(1.0)	59.5(3.0)	159(4)	130(13)
C1	(Control)	4.4(0.2)	16.1(0.7)	17.7(1.5)	56.8(1.7)	149(9)	139(6)
P3	29(Medium)	4.5(0.3)	16.7(1.1**)	25.5(1.7)	59.5(5.8)	177(8)	125(4)
C3	(Control)	4.4(0.3)	22.2(0.8)	22.2(1.6)	53.3(4.4)	170(6)	129(8)
P2	48(High)	4.3(0.1)	16.8(1.1)	24.6(1.0)	51.2(2.2*)	159(10)	125(13)
C2	(Control)	4.5(0.4)	17.9(0.9)	25.4(1.9)	60.4(2.8)	163(7)	122(11)

(c) 1986–7

Plot No.	Mean annual ppb SO_2 (Treatment)	Leaf weight (g) per m^2 (mean ± standard error)[a] on Oct 29	Dec 8	Jan 28	Mar 11	Apr 8	May 12	Jul 14
P4	5 (Control)	8.2(0.8)	18.9(1.7)	23.0(3.7)	35.2(2.1)	63.9(7.4)	141(13)	45.4(6.4)
C4	(Control)	7.7(0.8)	18.1(1.1)	22.6(1.8)	30.7(0.8)	65.0(2.3)	139(3)	52.7(6.4)
P3	14 (Low)	8.7(0.5)**	17.4(1.9)	16.8(3.6)***	23.7(3.6)***	69.8(5.2)*	125(7)	35.2(7.0)
C3	(Control)	12.8(0.7)	26.6(5.0)	28.0(2.9)	46.5(2.4)	90.7(6.4)	148(15)	23.2(5.0)
P1	28 (Medium)	10.0(0.9)	21.0(2.5)*	21.9(3.0)	41.1(2.4)	62.7(9.4)	143(11)	42.2(6.4)
C1	(Control)	11.6(1.0)	28.3(0.7)	27.0(3.1)	31.2(5.4)	72.1(3.8)	149(15)	58.0(4.4)
P2	38 (High)	8.9(0.7)	17.1(0.6)***	15.2(2.3)**	24.4(3.3)***	43.6(2.0)***	129(10)	36.2(5.5)
C2	(Control)	11.3(1.1)	29.4(2.2)	31.5(2.7)	52.4(2.0)	86.8(2.6)	133(8)	26.4(3.1)

[a] Oven dry weight of leaves, based on five replicate samples of tillers from four half metre row lengths on each date.

*, **, *** Values of leaf weight differ significantly from corresponding control at $P < 0.05$, 0.01 or 0.001 respectively.

Leaf Area Index

Leaf area index (LAI) followed a similar pattern to leaf weight and total weight in each season (Table 11).

In 1984-5, the LAI was similar in all plots in December and January but was significantly higher in at least one of the SO_2 treated plots compared with their companion control plot on each sample date between early March and late June (in the low SO_2 plot (P2) in April and May; in the medium SO_2 plot (P4) in March, April, May and June; in the high SO_2 plot (P3) in April, May and June) LAI did not differ significantly between any main plot and its companion control on any sample date in 1985-6.

On several occasions in 1986-7, LAI was significantly lower in the SO_2 treated plots than their companion control plots (in the low SO_2 plots, P3, in October, January, March and April; in the medium SO_2 plot, P1, in December; in the high SO_2 plot, P2, in December, January, March and April). LAI did not differ significantly in May or July.

(ii) Final Yield

Measurements of grain yield, thousand grain weight, ear weight, ear number and Harvest Index were made by CERL on the final sample of plants taken as described in part (i) (Table 12).

Grain yield (tonnes/hectare) did not differ significantly between any of the SO_2 treated plots and control plots in 1985 or 1986. However, in 1987 the final grain yield was significantly lower in the medium SO_2 plot, P1, than the control plot, C1. Grain yield was lowest in plot P1 (medium SO_2), but was highest in plot C1 (control) and was also significantly higher in this control plot than in two of the other control plots (C2 and C4).

Thousand grain weight was lower in the low SO_2 plot (P2) than the control plot (P1) in 1985 but there were no other significant differences between SO_2 plots and control plots in any season.

Ear weight and ear number were higher in the low SO_2 plot, P2, than control plot, C1, in 1985 but did not differ significantly between the other SO_2 plots and control plots in that year or in 1986 or 1987.

Harvest Index values (ear weight/total weight) were similar in all plots in each season.

Table 11 . Seasonal changes in Leaf Area Index in plots of Igri barley growing in different SO_2 concentrations throughout three field seasons.

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	LAI (mean ± standard error)[a] on Dec 6	Jan 24	Mar 6	Apr 17	May 22	Jun 26
P1	9 (Control)	0.10(0.01)	0.14(0.01)	0.11(0.01)	0.34(0.02)	1.7(0.2)	1.2(0.2)
P2	21 (Low)	0.10(0.01)	0.14(0.01)	0.13(0.02)	0.45(0.07*)	2.5(0.3*)	1.4(0.1)
P4	32 (Medium)	0.10(0.01)	0.16(0.01)	0.16(0.01*)	0.65(0.06**)	3.4(0.1**)	1.6(0.2*)
P3	43 (High)	0.09(0.00)	0.12(0.02)	0.10(0.01)	0.41(0.03*)	2.4(0.2)	1.6(0.2*)

(b) 1985–6

Plot No.	Mean annual ppb SO_2 (Treatment)	LAI (mean ± standard error)[a] on Nov 13	Jan 2	Feb 18	Apr 3	May 7	Jun 4
P4	7 (Control)	0.14(0.01)	0.44(0.01)	0.35(0.02)	0.90(0.06)	4.5(0.4)	3.7(0.3)
C4	(Control)	0.16(0.00)	0.44(0.05)	0.30(0.03)	0.89(0.04)	4.0(0.2)	4.5(0.3)
P1	14 (Low)	0.16(0.00)	0.45(0.03)	0.40(0.02)	0.82(0.06)	2.8(0.1)	3.5(0.4)
C1	(Control)	0.16(0.00)	0.45(0.02)	0.34(0.03)	0.90(0.06)	2.7(0.1)	3.8(0.2)
P3	29 (Medium)	0.16(0.01)	0.54(0.04)	0.42(0.03)	1.04(0.11)	3.7(0.2)	3.3(0.1)
C3	(Control)	0.16(0.01)	0.52(0.02)	0.35(0.03)	0.92(0.10)	3.4(0.2)	3.3(0.3)
P2	48 (High)	0.16(0.00)	0.44(0.03)	0.41(0.02)	0.94(0.07)	3.4(0.3)	3.4(0.4)
C2	(Control)	0.16(0.01)	0.48(0.03)	0.47(0.03)	1.06(0.05)	3.0(0.1)	3.2(0.3)

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	LAI (mean ± standard error)[a] on Oct 29	Dec 8	Jan 28	Mar 11	Apr 8	May 12	Jul 14
P4	5 (Control)	0.20(0.02)	0.41(0.04)	0.35(0.04)	0.51(0.03)	1.2(0.1)	3.6(0.3)	1.0(0.2)
C4	(Control)	0.18(0.02)	0.39(0.02)	0.31(0.04)	0.44(0.02)	1.2(0.1)	4.1(0.1)	1.1(0.2)
P3	14 (Low)	0.21(0.02**)	0.37(0.05)	0.26(0.06*)	0.33(0.04***)	1.2(0.1***)	3.2(0.2)	0.6(0.1)
C3	(Control)	0.31(0.02)	0.62(0.12)	0.42(0.03)	0.60(0.02)	1.7(0.1)	4.0(0.3)	0.4(0.1)
P1	28 (Medium)	0.29(0.03)	0.43(0.05*)	0.30(0.03)	0.37(0.06)	1.1(0.2)	3.8(0.3)	0.8(0.1)
C1	(Control)	0.27(0.02)	0.61(0.03)	0.32(0.05)	0.52(0.03)	1.3(0.1)	4.2(0.4)	1.2(0.1)
P2	38 (High)	0.23(0.02)	0.31(0.01***)	0.23(0.04**)	0.37(0.05***)	0.7(0.0***)	3.4(0.4)	0.7(0.1)
C2	(Control)	0.27(0.04)	0.66(0.06)	0.48(0.04)	0.74(0.02)	1.5(0.1)	3.7(0.2)	0.5(0.1)

[a] Leaf area (m^2) per m^2 ground, based on five replicate samples of leaves from four half metre row lengths on each date.

*, **, *** Values of LAI differ significantly from corresponding control plot at $P < 0.05$, 0.01 or 0.001 respectively.

Table 12 . Final yields of Igri barley in field plots after exposure to different sulphur dioxide levels throughout three seasons

(a) 1985 (Harvested on 23 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Grain yield (t/ha)	Thousand grain weight(g)	Ear weight (g/m^2)	Number of ears per m^2	Harvest Index[b]
P1	9 (Control)	8.0(0.6)	51.9(0.3)	858(58)	834(71)	0.61(0.01)
P2	21 (Low)	9.1(0.6)	49.1(0.2*)	984(60*)	990(62*)	0.61(0.01)
P4	32 (Medium)	8.3(0.3)	52.6(0.7)	897(29)	841(23)	0.59(0.01)
P3	43 (High)	7.8(0.4)	49.6(1.0)	846(49)	782(44)	0.61(0.00)

(b) 1986 (Harvested on 21 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Grain yield (t/ha)	Thousand grain weight(g)	Ear weight (g/m^2)	Number of ears per m^2	Harvest Index[b]
P4	7 (Control)	10.3(0.3)	50.1(1.1)	1082(27)	940(44)	0.51(0.01)
C4	(Control)	9.7(0.3)	54.2(0.8)	1009(48)	784(30)	0.48(0.03)
P1	14 (Low)	7.7(0.3)	45.9(0.8*)	775(30)	876(37)	0.54(0.01)
C1	(Control)	7.8(0.4)	45.6(1.1)	789(42)	869(75)	0.52(0.00)
P3	29 (Medium)	9.1(0.3)	48.5(0.5)	944(34)	952(54)	0.52(0.01)
C3	(Control)	8.7(0.5)	46.6(1.3)	899(51)	977(64)	0.53(0.01)
P2	48 (High)	8.2(0.4)	48.0(0.9)	837(38)	897(46)	0.53(0.00)
C2	(Control)	7.1(0.3)	45.3(0.6)	755(9)	861(33)	0.54(0.01)

(c) 1987 (Harvested on 14 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Grain yield (t/ha)	Thousand grain weight(g)	Ear weight (g/m^2)	Number of ears per m^2	Harvest Index[b]
P4	5 (Control)	7.4(0.5)	50.2(0.3)	723(47)	689(34)	0.50(0.01)
C4	(Control)	7.3(0.1)	52.7(1.1)	718(14)	602(26)	0.50(0.01)
P3	14 (Low)	7.9(0.5)	54.0(0.7)	753(45)	690(45)	0.50(0.02)
C3	(Control)	8.3(0.5)	50.6(1.5)	806(47)	797(68)	0.50(0.01)
P1	28 (Medium)	6.8(0.6)	52.3(0.9)	657(53*)	655(39)	0.50(0.01)
C1	(Control)	8.9(1.2)	50.6(1.1)	871(116)	783(55)	0.50(0.05)
P2	38 (High)	7.1(0.5)	50.9(0.5)	688(45)	708(54)	0.51(0.01)
C2	(Control)	7.1(0.4)	48.8(1.1)	688(42)	738(42)	0.49(0.01)

[a] Mean values of five replicate samples given, with standard error of mean in parenthesis. All weights at 15% moisture.

[b] Harvest Index = ear weight (g/m^2) / total above ground dry wt (g/m^2).

B. Leaf Diseases

(i) Powdery mildew (Erysiphe graminis)

The development of powdery mildew was compared on barley plants growing in plots treated with different levels of sulphur dioxide throughout three growing seasons.

Incidence of disease

In 1985 mildew was first recorded on 15 May and spread rapidly during late May so that most tillers were infected by early June (Table 13). At the end of May significantly ($P < 0.001$) more tillers were infected in the low SO_2 plot (P2) than in the untreated plot (P1) but from June onwards there was no marked difference in mildew incidence between the untreated plot (P1) and those subjected to elevated SO_2 levels (P2, P3 and P4).

In 1986 mildew was first recorded at trace levels in one plot only (C3) in late March. Affected leaves senesced and subsequently mildew did not become established within plots until mid-May. In this season each SO_2 treated plot (P1, P2 and P3) had a corresponding control (C1, C2 and C3) receiving background SO_2 only which enabled direct comparisons to be made of the effect of SO_2 on disease incidence.

Additionally two adjacent plots (P4 and C4), which received only background SO_2, provided some information on variation in disease incidence which could be used as a check on the validity of comparisons between SO_2 treated plots and their corresponding controls. In 1986 disease incidence did not differ significantly between the companion plots P4 and C4 but on five instances there was a significantly greater incidence of mildew on plots treated with SO_2 (22 May, 29 May and 12 June in P1 (low SO_2); 5 June in P3 (medium SO_2); 19 June in P2 (high SO_2)).

Table 13 . Incidence of powdery mildew in field plots of Igri barley subjected to different levels of sulphur dioxide.

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on May 15	May 30	June 11	June 27	July 5	July 12
P1	9 (Control)	0	8	75	100	83	100
P2	21 (Low)	0	67***	100	100	92	86
P4	32 (Medium)	17	25	100	83	100	100
P3	48 (High)	8	50	100	100	83	71
Growth Stage		37	59	75	83	85	87

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on Mar 27	Apr 24	May 8	May 22	May 29	June 5	June 12	June 19
P4	7 (Control)	0	0	7	33	53	80	53	67
C4	(Control)	0	0	0	27	20	67	40	33
P1	14 (Low)	0	0	13	67*	100**	80	87*	93
C1	(Control)	0	0	13	20	40	33	40	60
P3	29 (Medium)	0	0	0	40	40	87*	73	93
C3	(Control)	8	0	0	33	40	40	60	60
P2	48 (High)	0	0	0	40	47	73	67	100**
C2	(Control)	0	0	0	53	80	87	73	47
Growth Stage		26	30	31	47	49	61	65	83

Table 13. cont.

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on										
		Feb	Mar	Apr			May			June		
		11	16	2	12	30	14	21	28	4	11	18
P4	5 (Control)	33	87*	7	0	0	0	7	7	7	7	0
C4	(Control)	20	47	33	13	13	0	0	13	20	20	13
P3	14 (Low)	27	13**	53	33	13	20	20	53**	53**	40	20
C3	(Control)	33	73	27	40	27	0	13	13	13	13	1
P1	28 (Medium)	13*	47*	33	0*	0	13	20	80	73	60	45
C1	(Control)	53	87	47	40	27	27	33	47	40	33	25
P2	38 (High)	7**	33	13*	13	7	13	27	73	87***	86***	92***
C2	(Control)	53	87	53	20	20	20	13	40	27	20	0
Growth Stage [b]		27	29	29	30	31	47	53	65	83	83	85

*, **, *** value differs significantly from that of corresponding control plot at $P < 0.05$, 0.01 or 0.001 respectively

[a] Incidence based on 12 plants in 1985 on 15 May, thereafter on 12 tillers per plot; in 1986 on 12 plants to 24 April, thereafter on 15 tillers per plot; in 1987 on 15 plants to 11 Feb, thereafter on 15 tillers per plot.

[b] Approximate growth stage (Zadoks *et al.*, 1974) of all plants

In 1987 mildew was recorded early (11 Feb). On 6 March the main plots P4, P3, P1 and P2 all had fewer infected tillers than their corresponding plots C4, C3, C1 and C2 but since the companion control plots P4 and C4 differed significantly comparisons between other companion plots are dubious on this occasion. Apart from 6 March, disease incidence was similar in plots P4 and C4 throughout the season. Before stem extension, significant reductions in mildew incidence were also found in the medium SO_2 plot (P1) on 11 Feb and 16 April and in the high SO_2 plot on 11 Feb and 2 April. However, from the end of May onwards there were five instances when the incidence of mildew on SO_2-treated plots was significantly greater than on the corresponding controls (28 May and 4 June on P3 (low SO_2); 4, 11 and 18 June on P2 (high SO_2)).

Overall, there is some indication that mildew incidence from May onwards is higher in SO_2-treated plots but significant effects on specific sampling dates are relatively infrequent. Mildew occurring before stem extension may be affected differently by SO_2.

The possible effects of SO_2 were examined further by comparing mildew incidence on <u>leaves</u> from stem extension until leaf senescence in each of the three seasons. The analysis of those data (Fig 11) does give further support to the hypothesis that SO_2 increases mildew incidence especially in the low (14-21ppb) and high (38-48ppb) SO_2 treatments. Regretfully in 1986, a significant difference between plots C4 and P4 make conclusions for that year of doubtful validity.

<u>Incidence on leaves at different positions on tiller</u>

The apparent effect of SO_2 was then examined in relation to leaves at different positions (Table 14). The incidence of mildew on leaves at corresponding positions (and therefore of similar

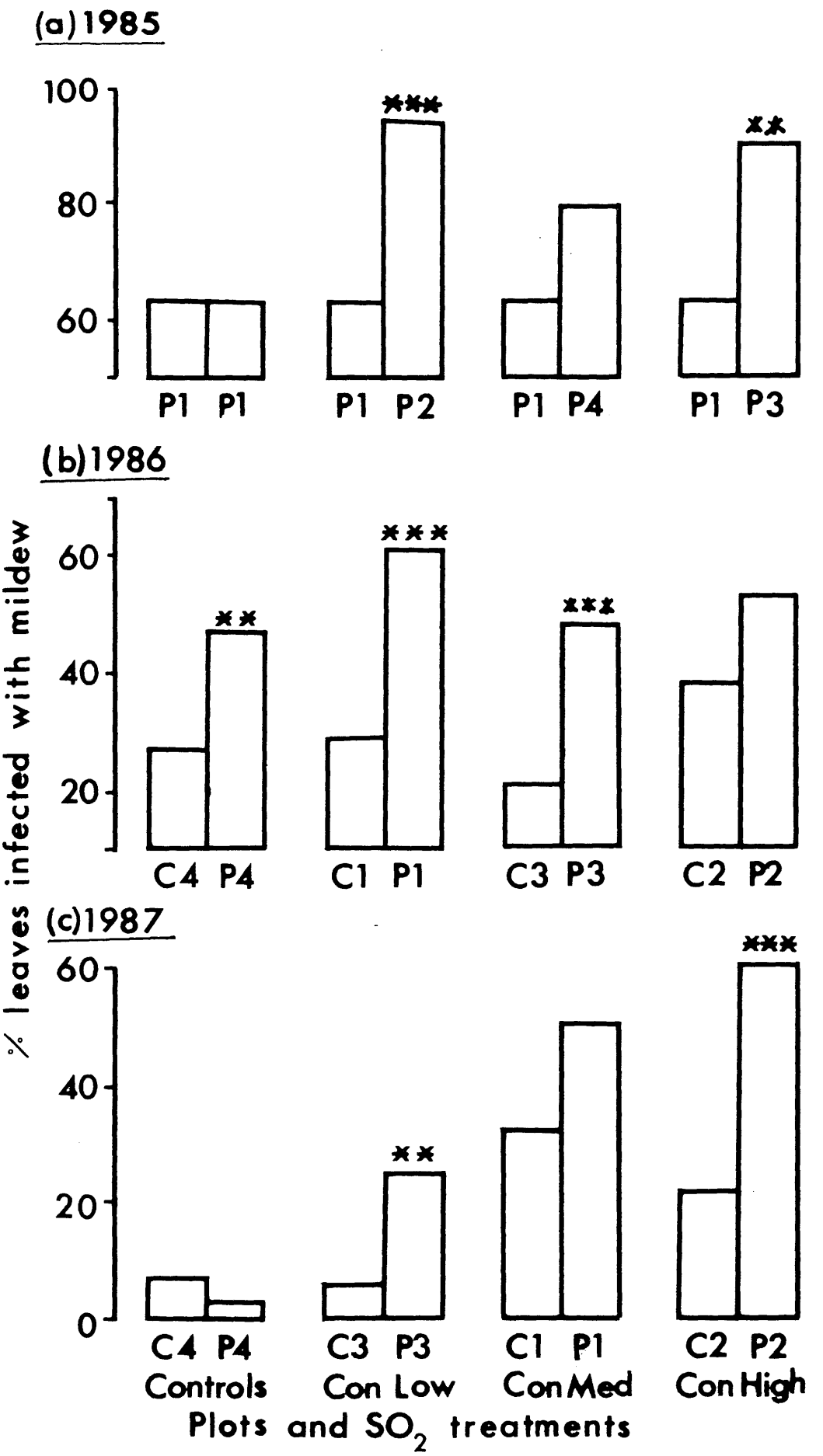

(a) 1985
100
80
60
***
**
P1 P1
P1 P2
P1 P4
P1 P3
(b) 1986
% leaves infected with mildew
60
40
20
***
**
***
C4 P4
C1 P1
C3 P3
C2 P2
(c) 1987
60
40
20
0
***
**
C4 P4
Controls
C3 P3
Con Low
C1 P1
Con Med
C2 P2
Con High
Plots and SO_2 treatments

Table 14 . Incidence of powdery mildew on leaves at different positions on Igri barley growing in field plots subjected to different levels of sulphur dioxide.

(a) 1985 (11 June - 12 July)

Plot	Mean annual		% Incidence [a] on leaf				
No.	ppb SO_2 (Treatment)		1 (flag)	2	3	4	5
P1	9	(Control)	75	92	67	25	0
P2	21	(Low)	92	100	100	92**	38
P4	32	(Medium)	83	100	100	83**	10
P3	43	(High)	67	100	100	92**	33

(b) 1986 (8 May - 19 June)

Plot	Mean annual		% Incidence [a] on leaf					
No.	ppb SO_2 (Treatment)		1 (flag)	2	3	4	5	6
P4	7	(Control)	20	53	87	73	40	7
C4		(Control)	7	27	60	40	60	7
P1	14	(Low)	47	80*	87	93**	53*	7
C1		(Control)	27	33	53	33	13	13
P3	29	(Medium)	27	80**	93	67**	20	0
C3		(Control)	0	27	60	20	20	0
P2	48	(High)	20	87*	87	80	53	0
C2		(Control)	7	47	93	60	27	0

Table 14 . cont.

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on leaf				
		1 (flag)	2	3	4	5
P4	5 (Control)	0	13	0	0	0
C4	(Control)	7	7	7	13	0
P3	14 (Low)	7	27	47**	29	14
C3	(Control)	7	13	0	0	0
P1	28 (Medium)	33	67	73	13	25
C1	(Control)	20	33	47	25	0
P2	38 (High)	60***	80***	67	42	20
C2	(Control)	0	20	53	11	0

*, **, *** Values differ significantly from corresponding control at $P < 0.05$, 0.01, 0.001 respectively.

[a] Incidence assessed on 12 tillers (1985) or 15 tillers (1986 and 1987) per plot.

physiological age) in different plots was compared. In this analysis no significant differences between companion control plots P4 and C4 were found and all significant differences that occur relate to increases in incidence of mildew over corresponding controls (in 1985 on leaf 4 in all SO_2 treatments; in 1986 on leaf 2 in all SO_2 treatments, on leaf 4 in the low and medium SO_2 plots and on leaf 5 in the low SO_2 plot; in 1987 on leaf 1 and leaf 2 in the high SO_2 plot and on leaf 3 in the low SO_2 plot).

Rate of increase in mildew incidence

The rate of increase in the number of leaves infected with mildew, from the start of the main epidemic after stem extension in each season, was compared in different plots. Table 15 shows the cumulative total number of infected leaves at each assessment date and the infection rates (Van der Plank, 1963), during the periods shown, for each plot. The infection rates calculated for 1985 and 1986 did not differ significantly between plots although the rate in the high SO_2 plot appeared higher in both years (Appendix 1). However, in 1987 the infection rates in both the low and the high SO_2 plots were significantly higher than in the companion control plots, indicating that the disease is spreading to new leaves more rapidly at 14 and 38 ppb SO_2 than at 5 ppb SO_2 (Fig 12).

Severity of powdery mildew

The severity of mildew on the leaves which became infected in different plots was compared firstly at each assessment date using the Mann-Whitney U Test. Mildew severity on leaves in the main plots was compared with the companion control plots, where present, or with the main control plot (Table 16).

At medium milk, in 1985 mildew was significantly more severe on infected leaves growing in the low and high SO_2 plots than in the

Table 15 . Cumulative totals of leaves infected with powdery mildew and overall infection rates in field plots of Igri barley subjected to different levels of sulphur dioxide.

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Total No. leaves[a] with mildew on: June 11	June 27	July 5	July 12	Apparent Infection rate[b] (r)
P1	9 (Control)	16	29	32	32	0.032
P2	21 (Low)	37	43	49	49	0.064
P4	32 (Medium)	28	32	44	44	0.048
P3	48 (High)	36	42	46	50	0.095

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Total No. leaves[a] with mildew on: May 8	May 22	May 29	June 5	June 12	June 19	Apparent infection rate[b] (r)
P4	7 (Control)	1	6	13	33	34	41	0.097
C4	7 (Control)	1	6	8	18	19	23	0.071
P1	14 (Low)	2	21	39	42	47	55	0.084
C1	7 (Control)	2	5	12	18	21	27	0.070
P3	29 (Medium)	0	7	11	28	33	43	0.086
C3	7 (Control)	0	6	9	14	20	25	0.060
P2	48 (High)	0	9	15	26	32	48	0.170
C2	7 (Control)	0	11	19	29	32	34	0.053

Table 15 . Cont.

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Total No. leaves[a] with mildew on						Apparent infection rate[b] (r)
		May			June			
		14	21	28	4	11	18	
P4	5 (Control)	0	1	1	1	1	1	0
C4	(Control)	0	0	3	4	5	5	0.026
P3	14 (Low)	5	5	11	14	16	17	0.041*
C3	(Control)	0	2	2	2	3	4	0.026
P1	28 (Medium)	3	8	21	23	27	29	0.081
C1	(Control)	4	8	13	13	14	16	0.047
P2	38 (High)	4	8	23	28	31	37	0.088*
C2	(Control)	3	3	8	10	12	12	0.045

* Value of r differs significantly from that for corresponding control plot at $P < 0.05$

[a] Total no. infected leaves on 12 tillers (1985), or 15 tillers (1986, 1987) per plot.

[b] Mean increase in number of infected leaves per day (Van der Plank, 1963).

Fig 12. Comparison of the rates of increase (r) in number of leaves infected with powdery mildew in SO_2-treated plots of Igri barley and in control plots during the summer of 1987.

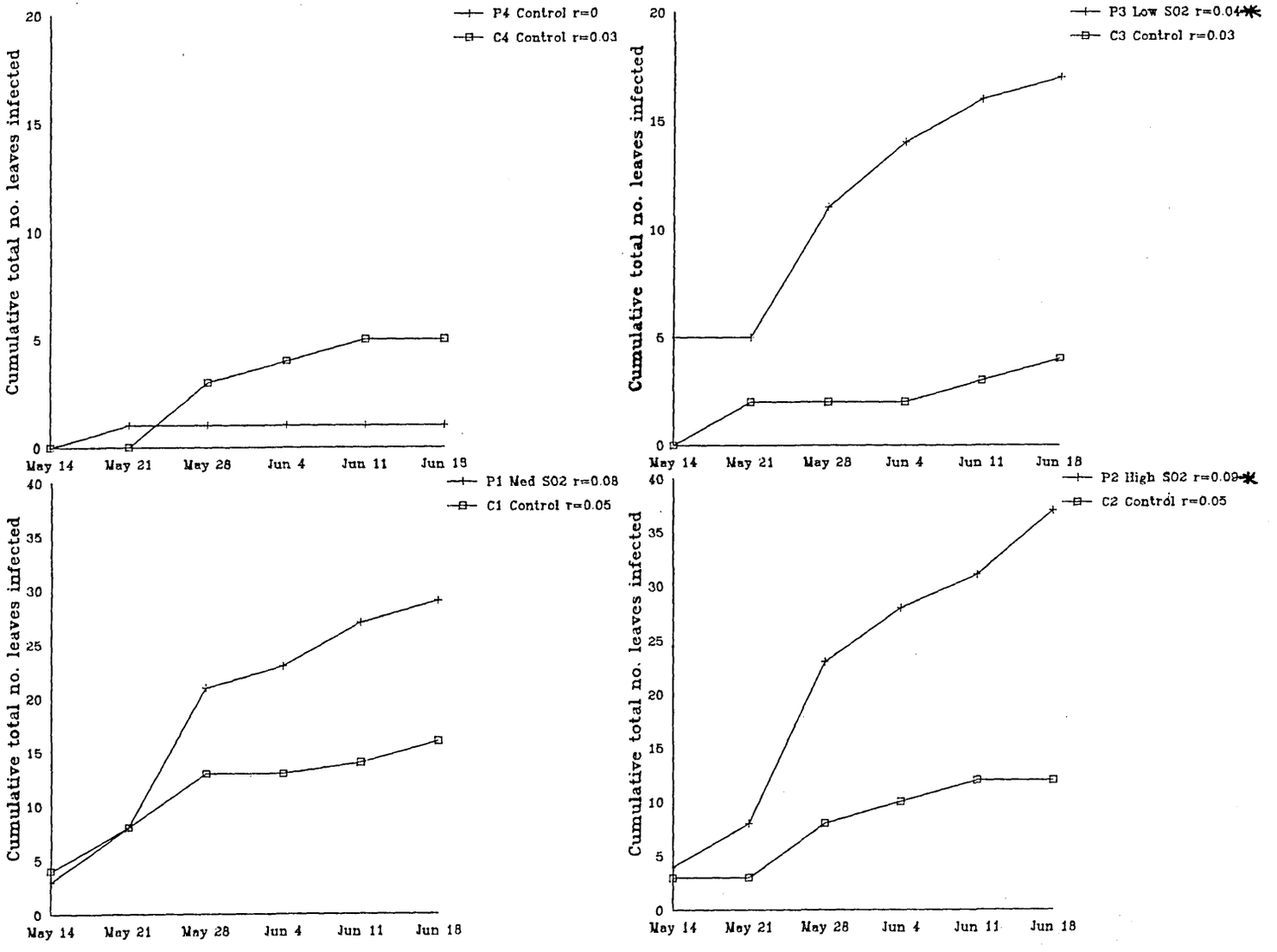


Table 16. Leaf area affected by powdery mildew in field plots of Igri barley subjected to different levels of sulphur dioxide

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	% Leaf area [a] with mildew on			
		June		July	
		11	27	5	12
P1	9 (Control)	3.9	7.3	9.9	5.0
P2	21 (Low)	11.3**	7.9	10.5	21.7
P4	32 (Medium)	5.5	4.0	5.8	19.8
P3	48 (High)	11.5**	14.3*	16.3	37.0
Growth Stage		75	83	85	87

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	% Leaf area [a] with mildew on					
		May			June		
		8	22	29	5	12	19
P4	7 (Control)	0.7	0.3	0.6	1.6*	1.1	1.3
C4	(Control)	0.3	0.3	0.3	0.5	0.5	0.7
P1	14 (Low)	0.3	0.7	2.4**	1.5	2.2	4.0**
C1	(Control)	0.5	0.3	0.6	1.2	1.0	0.7
P3	29 (Medium)	0	0.3	0.5	1.5	1.1	1.7
P3	(Control)	0	0.2	0.5	0.7	0.6	1.1
P2	48 (High)	0	0.4	0.6	0.8	1.3	6.0**
C2	(Control)	0	0.4	1.0	0.9	1.3	1.3
Growth Stage		31	47	49	61	65	83

Table 16 . Cont.

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Leaf area [a] with mildew on: Mar 6	Apr 2	Apr 16	Apr 30	May 14	May 21	May 28	June 4	June 11	June 18
P4	5 (Control)	4.6	1.7	-	-	-	0.3	0.3	0.3	2.5	-
C4	(Control)	3.6	1.6	2.1	1.5	-	-	1.4	0.8	0.8	0.8
P3	14 (Low)	1.7	0.9	1.0	1.0	1.0	2.0	1.0	2.0	1.0	1.5
C3	(Control)	4.7	2.0	6.0	3.1	-	0.3	0.5	0.7	-	2.0
P1	28 (Medium)	1.5	0.6	-	-	1.5	1.0	2.3	4.7	4.2	4.0
C1	(Control)	7.4**	1.0	1.9	10.3	1.5	2.5	3.0	5.4	4.2	5.3
P2	38 (High)	4.8	2.3	1.0	1.7	2.5	1.8	2.6	3.7	3.4	2.5
C2	(Control)	6.6**	0.4	2.6	2.8	0.8	0.8	1.3	2.8	0.9	-
Growth Stage		29	29	30	31	47	53	65	83	83	85

*, **, *** Severity significantly higher than on corresponding control (or paired) plot at $P < 0.05$, 0.02, 0.002 respectively.

[a] Mean severity per leaf on infected tillers assessed on 12 tillers (1985) or 15 tillers (1986, 1987) per plot.

control or the medium SO_2 plot and was still more severe in the high SO_2 plot than the control plot at early dough. As leaves senesced during July this trend in mildew severity continued but too few leaves remained on the tillers sampled for valid comparisons with the Mann Whitney U test.

Mildew was much less severe in 1986, but there was a similar pattern of response to SO_2 as seen in 1985. Mildew was significantly more severe in the low SO_2 plot (14 ppb) at awn appearance and again at early dough stage than in the companion control plot and was also more severe in the high SO_2 plot at early dough than in the companion control plot. However, at the beginning of anthesis severity of mildew in the control plots P4 and C4 differed significantly making comparisons between SO_2 treatments invalid at this stage.

The pattern of mildew severity was very different in 1987. The epidemic started much earlier in the season and when mildew was first recorded in March it was significantly more severe in two of the control plots C1 and C2 than in any other plot. Mildew severity remained low in all plots throughout the season and did not differ significantly between SO_2 treatments.

Distribution of mildew severities

The maximum percentage leaf area affected by mildew on each leaf assessed, during the main epidemic period of each season, is shown in Table 17 (i). The number of leaves in the different severity categories was compared using a chi^2 test (Table 17 (ii)). There were more leaves severely affected by mildew in 1985 than in the other years. Significantly more leaves had 5% or more 10% or more and 15% or more of the leaf area affected by mildew in the low and high SO_2 plots than in the control plot. The distribution of mildew

Table 17 (i) Distribution of maxiumum percentage mildew recorded on leaves of Igri barley in field plots subjected to different levels of SO_2

(a) 1985 (11 June - 12 July)

Plot	Mean annual	No. leaves per plot with % mildew [a]			
No.	ppb SO_2 (Treatment)	≤2	5	10	≥15
P1	9 (Control)	12	2	8	9
P2	21 (Low)	5	5	12	26
P4	32 (Medium)	12	11	7	14
P3	48 (High)	4	3	7	32

(b) 1986 (8 May - 19 June)

Plot	Mean annual	No. leaves per plot with % mildew [a]			
No.	ppb SO_2 (Treatment)	≤2	5	10	≥15
P4	7 (Control)	28	12	2	0
C4	(Control)	21	3	0	0
P1	14 (Low)	22	18	12	2
C1	(Control)	22	4	0	0
P3	29 (Medium)	23	18	2	0
C3	(Control)	14	5	0	0
P2	48 (High)	22	12	7	7
C2	(Control)	19	13	2	0

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	No. leaves per plot with % mildew [a]			
		⩽2	5	10	⩾15
P4	5 (Control)	1	1	0	0
C4	(Control)	3	2	0	0
P3	14 (Low)	6	9	1	1
C3	(Control)	1	2	0	0
P1	28 (Medium)	8	10	7	4
C1	(Control)	3	6	3	5
P2	38 (High)	8	2	1	1
C2	(Control)	14	10	10	3

[a] Severity assessed on 12 tillers (1985) or 15 tillers (1986, 1987) per plot.

Table 17 (ii) Contingency tables (and X^2 values) for number of leaves per plot in different % mildew categories.

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2	No. leaves per plot with % mildew					
		≤2	≥5	≤5	≥10	≤10	≥15
P1	9 (Control)	12	19	14	17	22	9
P2	21 (Low)	5	43	10	38	22	26
		(7.33**)		(4.18*)		(3.86**)	
P4	32 (Medium)	12	31	22	21	29	14
		(0.53)		(0.08)		(0.01)	
P3	48 (High)	4	42	7	39	14	32
		(8.39**)		(6.93**)		(10.65**)	

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2	No. leaves per plot with % mildew			
		≤2	≥5	≤5	≥10
P4	7 (Control)	28	14	40	2
C4	(Control)	21	3	24	0
		(2.46)		(-)	
P1	14 (Low)	22	32	40	14
C1	(Control)	32	4	26	0
		(11.93***)		(-)	
P3	29 (Medium)	23	20	41	2
C3	(Control)	14	5	19	0
		(1.47)		(-)	
P2	48 (High)	22	26	34	14
C2	(Control)	19	15	32	2
		(0.45)		(5.47*)	

Table 17 (ii) Cont.

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2	No. leaves per plot with % mildew			
		$\leqslant 2$	$\geqslant 5$	$\leqslant 5$	$\geqslant 10$
P4	5 (Control)	1	1	2	0
C4	(Control)	3	2	5	0
		(-)		(-)	
P3	14 (Low)	6	11	15	2
	(Control)	1	2	3	0
		(0.35)		(-)	
P1	28 (Medium)	8	21	18	11
C1	(Control)	3	14	9	8
		(0.16)		(0.09)	
P2	38 (High)	14	23	10	13
C2	(Control)	8	4	24	2
		(2.0)		(0.72)	

*, **, *** X^2 values for comparisons with appropriate controls significant at $P<0.05$, 0.01 and 0.001 respectively.

severity in the control and the medium SO_2 plots did not differ significantly.

In 1986, there were significantly more leaves in the low SO_2 plot with at least 5% leaf area affected by mildew than in the companion control plot, but there were no significant differences between the control plot and the medium or high SO_2 plots. There were significantly more leaves with at least 10% leaf area affected in the high SO_2 plot compared with the companion control plot. There were also markedly more leaves in the low SO_2 plot with at least 10% mildew, but a X^2 value could not be derived from the contingency table since it contained a zero.

No significant differences in the distribution of mildew severity was found in 1987.

Mildew severity on leaves at different positions

The maximum percentage leaf area affected by mildew recorded on individual leaves during the main epidemic periods is shown in Table 18. In 1985 mildew severity on the flag leaf was significantly greater in the high SO_2 plot than in the control plot, according to the Mann Whitney U test. In 1986 mildew severity on leaf 3 was significantly higher in all the SO_2 fumigated plots than in their companion control plots and mildew severity on leaf 4 was significantly higher in the medium and high SO_2 plots than their companion controls. There were no significant differences in maximum percentage mildew recorded on any of the leaf numbers in 1987.

Table 18 . Maximum % powdery mildew recorded on leaves at different positions on Igri barley tillers growing in field plots subjected to different levels of sulphur dioxide.

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Maximum % mildew [a] on leaf 1 (flag)	2	3	4	5
P1	9 (Control)	2.9	19.0	14.6	10.7	-
P2	21 (Low)	6.5	16.3	18.3	30.0	35.0
P4	32 (Medium)	2.2	9.8	11.1	18.0	50.0
P3	43 (High)	23.1	29.8	22.5	30.0	38.3

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Maximum % mildew [a] on leaf 1 (flag)	2	3	4	5	6
P4	7 (Control)	4.0	1.5	2.5	4.2	3.5	2.0
C4	(Control)	1.0	2.5	2.2	1.5	1.0	1.0
P1	14 (Low)	1.3	7.3	5.7***	6.2	4.8	2.0
C1	(Control)	1.8	1.6	2.0	3.8	1.5	1.5
P3	29 (Medium)	2.0	2.6	4.1**	4.4*	3.7	-
C3	(Control)	-	2.3	2.3	2.7	2.7	-
P2	48 (High)	2.7	2.5	10.7*	14.4**	1.9	-
C2	(Control)	-	2.7	4.6	2.7	2.5	-

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Maximum % mildew [a] on leaf				
		1 (flag)	2	3	4	5
P4	5 (Control)	-	3.0	-	-	-
C4	(Control	1.0	1.0	5.0	3.0	-
P3	14 (Low)	1.0	2.3	5.6	6.3	5.0
C3	(Control)	1.0	5.0	-	-	-
P1	28 (Medium)	4.0	7.9	10.2	3.5	0.5
C1	(Control)	5.3	7.4	14.3	8.5	-
P2	38 (High)	2.2	6.4	9.8	6.0	5.0
C2	(Control)	-	4.7	3.5	5.0	-

*, **, *** Values differ significantly from corresponding control at $P<0.05$, 0.01 and 0.001.

[a] Mean of 12 tillers (1985) or 15 tillers (1986, 1987) per plot.

B. (ii) Leaf blotch (Rhynchosporium secalis)

Leaf blotch caused by Rhynchosporium secalis was widespread in the field plots in each season. Comparisons of disease development in plots subjected to elevated levels of SO_2 (14-48ppb) and those exposed to background levels only (5-9ppb) were made as for mildew (part (i)).

Incidence of disease (Table 19)

In 1985, leaf blotch first appeared during tillering (10 April). By flag leaf emergence (mid May) there was a high incidence of disease in plots P1 (Control) and P2 (Low SO_2) but a significantly lower incidence in plots P4 (Medium SO_2) and P3 (High SO_2). From the end of May onwards incidence was similar in all plots.

In 1986, leaf blotch was again first recorded during tillering (27 March). Throughout the season disease incidence in the two paired control plots P4 and C4 did not differ markedly, however, significant differences did occur between each SO_2 treated plot and its corresponding control on several occasions. In all cases the incidence of leaf blotch was lower in the SO_2 treated plot than in the control plot and this was particularly evident at the highest SO_2 level.

In 1987, crop growth was more forward and leaf blotch became established during tillering in early February. By March the majority of tillers in all plots were affected and incidence remained high until leaf senescence. Disease incidence in the paired control plots P4 and C4 was similar. However, there were significant differences between each of the SO_2 treated plots and its corresponding control plot on five occasions. As in 1986, incidence of leaf blotch was lower in the SO_2 treated plots than in the control plots exposed to background levels of SO_2 only.

Table 19 . Incidence of Rhynchosporium leaf blotch in field plots of Igri barley subjected to different levels of sulphur dioxide.

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on April 10	April 23	May 15	May 30	June 11	June 27	July 5	July 12
P1	9 (Control)	0	10	75	67	67	100	100	100
P2	21 (Low)	0	0	50	75	58	50	92	86
P4	32 (Medium)	0	0	0***	67	25	75	100	100
P3	48 (High)	10	10	8***	58	58	58	83	100
Growth Stage [b]		25	25	37	59	75	83	85	87

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on Mar 27	Apr 10	Apr 24	May 8	May 22	May 29	June 5	June 12	June 19
P4	7 (Control)	0	42	50	47	53	47	73	93	93
C4	(Control)	8	8	8	20	27	40	60	60	73
P1	14 (Low)	8	50	58	7***	7***	13**	47*	80	93
C1	(Control)	17	58	92	73	80	73	93	93	87
P3	29 (Medium)	8	33	0*	20	27*	40	80	100	87
C3	(Control)	8	25	42	47	73	47	53	93	93
P2	48 (High)	8	8**	17**	7**	0***	13***	53*	67*	73
C2	(Control)	0	67	75	53	93	80	93	100	93
Growth Stage [b]		26	27	30	31	47	49	61	65	83

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on										
		Feb	Mar	Apr			May			June		
		11	16	2	16	30	14	21	28	4	11	18
P4	5 (Control)	7	73	100	93	100	87	100	87	100	87	87
C4	(Control)	13	87	100	87	93	93	100	93	93	100	93
P3	14 (Low)	7	73	73	73	67	47**	53	80	93	100	60
C3	(Control)	7	73	93	100	80	93	87	87	80	93	98
P1	28 (Medium)	7	60	60***	73	20***	47	53	80	67	73	36
C1	(Control)	13	80	100	100	87	100	80	93	80	93	92
P2	38 (High)	7	60	33***	80	47**	60	67	53	73	86	67
C2	(Control)	20	73	93	100	93	73	67	67	87	93	100
Growth Stage [b]		27	29	29	30	31	47	53	65	83	83	85

*, **, *** Values differ significantly from that of corresponding control plot at $P<0.05$, 0.1, 0.01 respectively.

[a] Incidence based in 1985 on 12 plants to 15 May, thereafter on 12 tillers per plot; in 1986 on 12 plants to 24 April, thereafter on 15 tillers per plot; in 1987 15 plants to 11 Feb, thereafter on 15 tillers per plot.

[b] Approximate growth stage (Zadoks et al., 1974) of all plants.

This evidence indicates that the number of tillers affected by leaf blotch is reduced at elevated SO_2 levels. The overall incidence of leaf blotch on leaves from stem extension onwards was then examined (Fig 13). This analysis also demonstrated a significant reduction in the incidence of leaf blotch in SO_2 treated plots compared with their corresponding control plots during 1986 and 1987, however, in 1986 the paired control plots P4 and C4 also differed significantly so the effects in this year may not necessarily be attributable to SO_2 concentration.

Incidence on leaves at different positions on tiller (Table 20).

The possible effects of SO_2 on leaves at different positions on the stem was examined next. There were significant reductions in incidence of leaf blotch in the SO_2-treated plots compared with their corresponding control plots on the lower leaves (Leaf 3, 4, 5 and 6) in 1986 and on the upper four leaves in 1987. However, in 1986 incidence on leaf 3 also differed significantly between the control plots P4 and C4 so conclusions should be cautious. In addition, in 1985 incidence of leaf blotch on the flag leaf was higher in the medium SO_2 plot than in the control plot. Unfortunately in this year no comparisons with adjacent control plots are possible as in the last two seasons to check the validity of this difference.

In general, there are apparent reductions in disease incidence in SO_2 treated plots although variability within the field makes conclusions difficult.

Rates of increase in incidence of leaf blotch

The rate of increase in the number of leaves infected with leaf blotch from stem extension was compared in different plots, as for mildew (Table 21 , Appendix 3). No consistent effects on infection

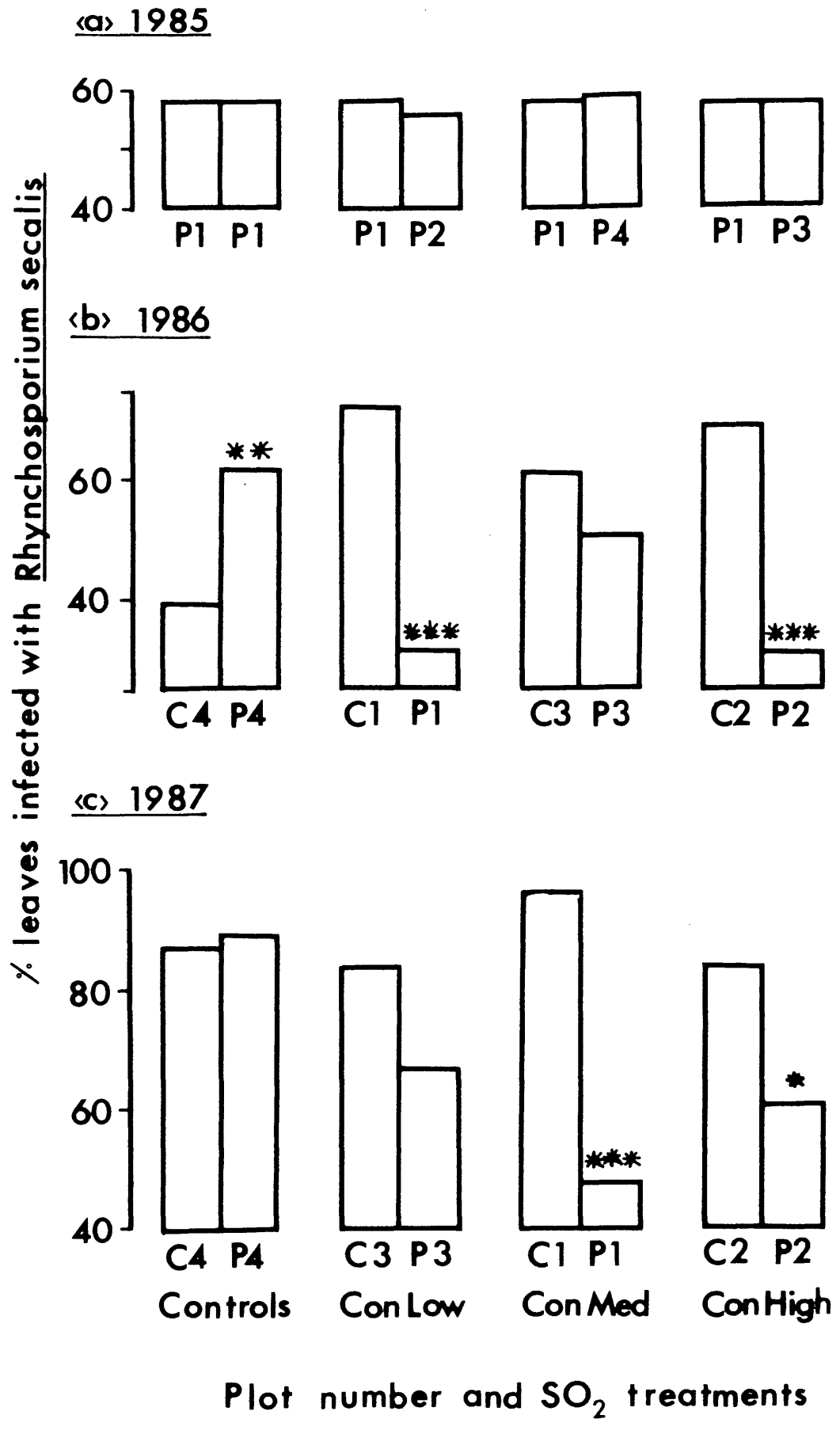

‹a› 1985
60
40
P1 P1
P1 P2
P1 P4
P1 P3
‹b› 1986
**
***
***
C4 P4
C1 P1
C3 P3
C2 P2
‹c› 1987
100
80
60
40
***
*
C4 P4
C3 P3
C1 P1
C2 P2
Controls
Con Low
Con Med
Con High
% leaves infected with Rhynchosporium secalis
Plot number and SO_2 treatments

Table 20 . Incidence of Rhynchosporium leaf blotch on leaves at different positions on Igri barley growing in field plots subjected to different levels of sulphur dioxide

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on leaf 1 (flag)	2	3	4	5
P1	9 (Control)	58	92	67	33	8
P2	21 (Low)	75	67	67	33	0
P4	32 (Medium)	100*	92	67	8	8
P3	43 (High)	92	75	33	42	8

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on leaf 1 (flag)	2	3	4	5	6
P4	7 (Control)	13	33	93**	100	87	53
C4	(Control)	0	27	40	80	53	33
P1	14 (Low)	7	27	73	67	0***	13***
C1	(Control)	20	60	100	80	80	67
P3	29 (Medium)	7	60	100	93	40	20
C3	(Control)	7	40	100	87	80	47
P2	48 (High)	0	20	67*	60*	33	7**
C2	(Control)	27	60	100	100	60	53

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on leaf 1 (flag)	2	3	4	5
P4	5 (Control)	80	87	93	92	100
C4	(Control)	73	100	93	86	86
P3	14 (Low)	60	87	67	57	63
C3	(Control)	87	73	87	100	-
P1	28 (Medium)	53*	53*	40*	47*	33
C1	(Control)	93	93	100	100	-
P2	38 (High)	53*	93	53	50	40
C2	(Control)	93	93	67	80	-

*, **, *** Values differ significantly from corresponding control at $P<0.05$, 0.01, 0.001 respectively.

[a] Incidence assessed on 12 tillers (1985) or 15 tillers (1986, 1987) per plot.

Table 21 . Cumulative totals of leaves infected with Rhynchosporium leaf blotch and overall infection rates in field plots of Igri barley subjected to different levels of sulphur dioxide.

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Total No. infected leaves [a] on June 11	June 29	July 5	July 12	Apparent Infection Rate [b] (r)
P1	9 (Control)	13	29	29	29	0.028
P2	21 (Low)	10	17	29	30	0.058
P4	32 (Medium)	4	12	32	33	0.096
P3	48 (High)	11	18	27	29	0.051

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Total No infected leaves [a] on May 8	May 22	May 29	June 5	June 12	June 19	Apparent Infection rate (r)
P4	7 (Control)	7	14	21	37	46	57	0.075
C4	(Control)	3	7	12	19	26	35	0.071
P1	14 (Low)	1	3	5	10	20	26	0.086**
C1	(Control)	18	31	39	47	58	60	0.048
P3	29 (Medium)	3	7	11	26	39	46	0.085
C3	(Control)	7	20	23	32	47	54	0.065
P2	48 (High)	1	1	4	14	19	28	0.106
C2	(Control)	11	27	39	48	55	60	0.076

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Total No infected leaves [a] on May 14	May 21	May 28	June 4	June 11	June 18	Apparent Infection Rate (r)
P4	5 (Control)	23	36	43	50	53	53	0.066
C4	(Control)	22	30	38	42	55	58	0.071
P3	14 (Low)	9	17	26	34	45	45	0.078
C3	(Control	14	22	29	33	41	43	0.071
P1	28 (Medium)	8	10	20	22	26	30	0.052
C1	(Control)	19	31	40	42	45	47	0.076
P2	38 (High)	7	16	19	26	36	38	0.072
C2	(Control)	11	24	30	38	45	45	0.083

** Value of r significantly different from that for corresponding control plot at $P<0.01$

[a] Total number of infected leaves on 12 tillers (1985) or 15 tillers (1986, 1987) per plot.

[b] Mean increase in number of infected leaves per day (Van der Plank, 1963).

rate were found. In 1986, the apparent infection rate in P1 (Low SO_2) was significantly higher than in the corresponding control plot C1 but it should be noted that the incidence in C1 was markedly greater than in P1 at the start of the infection period examined.

Severity of leaf blotch (Table 22.)

The severity of leaf blotch on the tillers which became infected was compared by analysis of the percentage leaf area affected on tillers in different plots on several occasions during each season (using the Mann-Whitney U Test).

No differences in severity were significant in 1985. In both 1986 and 1987 the severity of leaf blotch was significantly lower in SO_2 treated plots when compared with their controls on several occasions (1986, P1 on 5 and 19 June, P2 on 5, 12 and 19 June; 1987 P3 on 30 April, P1 on 2 and 16 April, 14, 21 and 28 May and on the 4 and 11 June). However, in each year on one occasion in June the control plots P4 and C4 also differed significantly.

Distribution of leaf blotch severity

Further evidence of SO_2 reducing the severity of leaf blotch can be seen when comparing the frequency distribution of leaves in different categories of maximum percentage leaf blotch recorded (Table 23).

In 1985, significantly fewer leaves in plot P4 (Medium SO_2) had a maximum % leaf blotch greater than 5% (10 leaves) than in the control plot P1 (20 leaves). In 1986 there were significantly fewer with more than 5% leaf blotch in plot P2 (High SO_2, 17 leaves) than in the corresponding control plot C2 (38 leaves) and in 1987

Table 22 . Leaf area affected by Rhynchosporium leaf blotch in field plots of Igri barley subjected to different SO_2 levels.

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	% leaf area [a] affected on June 11	June 27	July 5	July 12
P1	9 (Control)	2.6	2.3	4.0	10.0
P2	21 (Low)	1.9	1.3	4.4	14.2
P4	32 (Medium)	1.3	1.2	2.3	4.3
P3	48 (High)	1.9	1.8	3.2	6.0
Growth Stage		75	83	85	87

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	% leaf area [a] affected on May 8	May 22	May 29	June 5	June 12	June 19
P4	7 (Control)	1.2	2.4	1.0	2.1	3.6*	7.8
C4	(Control)	0.5	0.4	0.8	1.3	1.5	4.3
P1	14 (Low)	1.3	0.4	1.1	0.9**	3.7	2.4*
C1	(Control)	5.3	1.4	2.9	4.1	8.3	8.8
P3	29 (Medium)	0.7	0.9	1.7	1.6	4.9	2.7
C3	(Control)	0.4	0.7	1.5	1.5	3.6	3.9
P2	48 (High)	1.3	-	0.8	0.8**	1.5***	3.3*
C2	(Control)	3.4	1.7	1.9	3.8	7.2	8.2
Growth Stage		31	47	49	61	65	83

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Leaf area [a] affected on									
		Mar	April			May			June		
		6	2	16	30	14	21	28	4	11	18
P4	5 (Control)	7.4	7.1	6.6	9.2	8.2	6.7	11.2	18.0	25.1**	27.9
C4	(Control)	4.2	11.2	4.5	6.8	6.0	5.3	8.7	10.2	13.5	18.5
P3	14 (Low)	8.1	2.8	3.6	3.0**	5.2	4.4	3.2	5.9	13.4	17.3
C3	(Control)	4.3	4.9	8.2	9.8	6.2	4.8	6.4	7.9	13.1	20.0
P1	28 (Medium)	4.4	1.8***	7.2*	3.9	1.4***	1.6**	2.3**	3.1***	7.6*	14.4
C1	(Control)	4.8	11.4	14.5	12.1	8.9	5.3	7.2	11.4	16.3	27.7
P2	38 (High)	7.1	3.6	8.3	5.9	5.0	4.5	3.9	5.3	13.6	9.1
C2	(Control)	10.4	7.4	14.2	10.9	6.5	6.4	5.6	9.0	18.9	25.8
Growth Stage		29	29	30	31	47	53	65	83	83	85

*, **, *** Severity differs significantly from that in corresponding control plot at $P<0.05$, 0.02, 0.002 respectively.

[a] Mean severity per leaf on infected tillers, assessed on 12 tillers (1985) and 15 tillers (1986, 1987) per plot.

Table 23 . (i) Distribution of maximum percentage leaf blotch recorded on leaves of Igri barley growing in field plot subjected to different levels of sulphur dioxide.

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	No. leaves per plot with % leaf blotch [a]			
		⩽2	5	10	⩾15
P1	9 (Control)	10	10	9	1
P2	21 (Low)	11	7	6	6
P4	32 (Medium)	23	8	2	0
P3	43 (High)	8	15	6	1

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	No. leaves per plot with % leaf blotch [a]			
		⩽2	5	10	⩾15
P4	7 (Control)	17	13	11	14
C4	(Control)	17	10	5	3
P1	14 (Low)	9	6	10	3
C1	(Control)	12	15	17	16
P3	29 (Medium)	13	13	12	8
C3	(Control)	21	8	17	8
P2	48 (High)	11	9	4	4
C2	(Control)	7	23	16	15

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	No. leaves with % Rhynchosporium [a]			
		≤2	5	10	≥15
P4	5 (Control)	2	5	6	38
C4	(Control)	4	2	14	37
P3	14 (Low)	5	7	6	24
C3	(Control)	1	7	14	20
P1	28 (Medium)	5	7	10	8
C1	(Control)	0	11	10	29
P2	38 (High)	3	13	6	16
C2	(Control)	3	6	9	28

[a] Severity assessed on each leaf of 12 tillers (1985) or 15 tillers (1986, 1987) per plot.

Table 23 . (ii) Contingency tables (and X^2 values) for number of leaves per plot in different % leaf blotch categories.

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2	No. leaves per plot with % leaf blotch					
		⩽2	⩾5	⩽5	⩾10	⩽10	⩾15
P1	9 (Control)	10	20	20	10	29	1
P2	21 (Low)	11	19	18	12	24	6
		(0.00008)		(0.07)		(2.6)	
P4	32 (Medium)	23	10	31	2	33	0
		(6.94^{**})		(5.91^{*})		(0.002)	
P3	48 (High)	8	22	23	7	29	1
		(0.08)		(0.3)		(0)	

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual SO_2 ppb	No. leaves per plot with % leaf blotch							
		⩽2	⩾5	⩽5	⩾10	⩽10	⩾15	⩽20	⩾25
P4	7 (Control)	17	38	30	25	41	14	49	16
C4	(Control)	17	18	27	8	32	3	33	2
		(2.1)		(3.781)		(2.95)		(0.2)	
P1	14 (Low)	9	19	15	13	25	3	25	3
C1	(Control)	12	48	27	33	44	16	49	11
		(0.95)		(0.3)		(2.0)		(0.4)	
P3	29 (Medium)	13	33	26	20	38	8	42	4
C3	(Control)	21	33	29	25	46	8	51	3
		(0.8)		(0.007)		(0.006)		(0.1)	
P2	48 (High)	11	17	20	8	24	4	27	1
C2	(Control)	7	54	30	31	46	15	54	7
		(7.56^{**})		(3.01)		(0.7)		(0.7)	

Table 23 . (ii) cont.

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual SO_2 ppb	No. leaves per plot with % leaf blotch							
		⩽5	⩾10	⩽10	⩾15	⩽20	⩾25	⩽40	⩾50
P4	5 (Control)	7	44	13	38	27	24	32	19
C4	(Control)	6	51	20	37	34	23	35	24
		(0.05)		(0.8)		(0.3)		(0.03)	
P3	14 (Low)	12	30	18	24	30	12	38	4
C3	(Control)	8	34	22	20	33	9	38	4
		(0.6)		(0.4)		(0.3)		(0)	
P1	28 (Medium)	12	18	22	8	28	2	30	0
C1	(Control)	11	39	21	29	31	19	33	0
		(2.2)		(6.198*)		(-)		(-)	
P2	38 (High)	16	22	22	16	32	6	32	6
C2	(Control)	9	35	18	28	29	17	42	4
		(3.45)		(7.799**)		(3.68)		(0.4)	

*, ** X^2 Values for comparisons with appropriate controls significant at $\underline{P} < 0.05$, 0.01 respectively.

there were significantly fewer leaves with severities greater than 15% in plots P1 (Medium SO_2, 3 leaves) or P2 (High SO_2, 4 leaves) than each of the corresponding control plots (16 leaves and 15 leaves respectively). In neither 1986 nor 1987 were there any marked differences between the paired control plots P4 and C4.

Leaf blotch severity on leaves at different positions

The apparent reduction in severity of leaf blotch in SO_2 treated plots was further supported by comparing the maximum % leaf blotch recorded on leaves at different positions down the plant from stem extension onwards (Table 24). In all three seasons significant reductions in % leaf blotch in SO_2 treated plots were detected when compared with the appropriate control plot. (In 1985 on the flag leaf in the Medium SO_2 plot P4; in 1986 on leaf 2 in the High SO_2 plot P2 and on leaf 3 in the Low SO_2 plot P1 and plot P2; in 1987 on leaf 4 in the Medium SO_2 plot P1).

(iii) Brown rust (Puccinia hordei)

Brown rust occured at trace levels only in 1985 and did not occur in 1986 or 1987. No significant differences in incidence in different plots were detected (Table 25).

(iii) Septoria (Septoria nodorum)

Septoria infected plants in all plots late in the 1985 and 1987 seasons. Neither incidence or severity differed significantly in different plots (Table 26).

(iv) Net blotch (Pyrenophora teres)

A trace of net blotch was recorded on a few plants in 1986 (on plots P4, C4 and P3), but incidence and severity were too low to make any valid comparisons between plots.

Table 24. Maximum % leaf blotch recorded on leaves at different positions on Igri barley tillers growing in field plots subjected to different levels of sulphur dioxide

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Maximum % leaf blotch[a] on leaf 1 (flag)	2	3	4	5
P1	9 (Control)	5.4	5.5	4.0	4.3	50.0
P2	21 (Low)	8.9	6.8	3.4	8.0	-
P4	32 (Medium)	1.6**	3.8	3.1	5.0	5.0
P3	43 (High)	4.9	5.3	4.5	5.4	20.0

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Maximum % leaf blotch[a] on leaf 1	2	3	4	5	6
P4	7 (Control)	3.5	10.8	12.4	13.2	10.1	4.0
C4	(Control)	-	4.5	2.3	14.0	5.9	1.6
P1	14 (Low)	10.0	6.8	4.7**	12.7	-	3.5
C1	(Control)	1.7	12.1	20.3	16.4	8.5	11.5
P3	29 (Medium)	1.0	5.0	8.1	13.1	8.2	2.7
C3	(Control)	2.0	4.5	8.1	13.6	9.0	1.4
P2	48 (High)	-	1.7**	6.2*	11.0	3.8	5.0
C2	(Control)	5.5	11.5	18.7	11.4	4.6	8.8

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Maximum % leaf blotch [a] on leaf 1 (flag)	2	3	4	5
P4	5 (Control)	29.6	35.0	25.5	25.2	35.0
C4	(Control)	19.7	23.7	27.1	19.1	27.5
P3	14 (Low)	19.4	20.9	11.9	24.3	14.0
C3	(Control)	19.4	16.8	14.2	29.2	-
P1	28 (Medium)	14.6	14.3	8.3	9.1***	2.0
C1	(Control)	26.8	15.4	17.3	42.5	-
P2	38 (High)	23.8	12.8	7.1	18.3	27.5
C2	(Control)	26.9	19.4	11.7	30.0	-

*, **, *** Values differ significantly from corresponding control at $P < 0.05$, 0.02, 0.002 respectively.

[a] Mean of 12 tillers (1985) or 15 tillers (1986, 1987) per plot.

Table 25 . Incidence of brown rust in field plots of Igri barley subjected to different levels of SO_2 during the 1984-5 growing season

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 11	June 27	July 5	July 12
P1	9 (Control)	17	25	50	50
P2	21 (Low)	0	25	42	75
P4	32 (Medium)	25	9	25	50
P3	48 (High)	25	9	17	57

[a] % tillers infected with brown rust, assessed on 12 tillers per plot.

Table 26 . Incidence and severity of Septoria in field plots of Igri barley subjected to different levels of sulphur dioxide

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 27	July 5	July 12
P1	9 (Control)	8	50	50
P2	21 (Low)	17	50	-
P4	32 (Medium)	42	50	-
P3	48 (High)	42	25	-

(b) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 4	June 11	June 18	% leaf area [b] affected on July 4	July 11	July 18
P4	5 (Control)	53	67	60	3.4	7.4	14.5
C4	(Control)	47	60	40	3.4	7.3	10.0
P3	14 (Low)	27	27	7	3.5	7.5	10.0
C3	(Control)	40	27	13	3.9	8.8	10.0
P1	28 (Medium)	0	27	0	-	5.0	-
C1	(Control)	13	20	17	3.8	10.7	20.0
P2	38 (High)	20	7	0	2.0	10.0	-
C2	(Control)	33	33	0	2.6	7.0	-

[a] % tillers infected with Septoria, assessed on 12 tillers (1985) or 15 tillers (1987) per plot.

[b] % leaf area affected by Septoria (mean per infected leaf), assessed on 15 tillers per plot.

C. Stem base diseases

(i) Eyespot (Pseudocercosporella herpotrichoides)

Eyespot was present in the crops in all three seasons. The proportion of infected plants or tillers in different plots at each assessment were compared using Fisher's exact probability test, with main plots compared directly with their companion control plots in the second and third season (Table 27). The incidence of severe eyespot (lesion/s occupying more than half the stem circumference) was analysed similarly (Table 28).

In the 1984-5 season eyespot incidence was high while severity was low in the Medium and High SO_2 plots during tillering but by harvest incidence in these plots was lowest and no differences between plots were significant ($P < 0.05$) at any time during the season. Clearly, the sample size was insufficient to detect any differences accurately, and this was doubled in the final two seasons. In 1985-6 neither incidence nor severity differed significantly between the plots ($P < 0.05$). In 1986-7 eyespot incidence and severity in the High SO_2 plot (P2) was significantly ($P < 0.05$) lower than in the companion control plot C2 at GS65. However, incidence and severity in C2 were also significantly higher than in the other control plots. The effect was transitory. There were no significant ($P < 0.05$) differences in the extent of lodging in the plots at the end of the 1985-6 season (Appendix 5) and lodging was not assessed in the other seasons.

(ii) Sharp eyespot (Rhizoctonia cerealis)

Sharp eyespot infection developed in each season and was particularly common in 1987 (Appendix 6), but SO_2 concentration did not appear to affect the incidence significantly ($P < 0.05$) and no clear trends were seen.

Table 27 . Incidence of eyespot in field plots of Igri barley subjected to different levels of sulphur dioxide

(a) 1985

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on Apr 10	June 11	June 27	July 5	July 12	July 19	July 31
P1	9 (Control)	50	80	40	60	20	30	50 (50)[c]
P2	21 (Low)	40	70	30	30	10	30	20 (40)
P4	32 (Medium)	80	70	50	40	20	20	10 (40)
P3	43 (High)	80	40	20	30	20	20	10 (40)
Growth Stage		25	75	83	85	87	91	92

(b) 1986

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on Jan 9	Feb 26	Mar 27	Apr 24	May 22	June 5	June 19	July 2	July 17
P4	7 (Control)	8	20	20	60	0	35	45	35	50
C4	(Control)	17	0	0	50	20	20	30	25	50
P1	14 (Low)	17	0	0	70	10	25	25	30	35
C1	(Control)	0	10	20	60	10	25	55	60	55
P3	29 (Medium)	0	0	10	50	25	15	35	40	35
C3	(Control)	8	10	10	30	5	20	20	15	45
P2	48 (High)	0	20	10	50	15	10	35	30	50
C2	(Control)	8	10	30	30	15	25	45	40	55
Growth Stage [b]		21	23	26	30	47	61	83	87	91

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on							
		Dec	Feb	Apr	May		June		July
		11	11	16	14	28	11	25	9
P4	5 (Control)	10	25	5	0	5	25	25	50
C4	(Control)	20	5	10	5	0	10	25	60
P3	14 (Low)	10	5	10	0	5	20	15	50
C3	(Control)	10	10	0	0	5	10	20	55
P1	28 (Medium)	5	15	15	5	15	15	30	35
C1	(Control)	10	5	0	10	15	35	30	40
P2	38 (High)	0	15	0	10	0**	35	30	60
C2	(Control)	15	5	10	5	35	55	30	40
Growth Stage [b]		24	27	30	47	65	83	87	93

** Values differ significantly ($P<0.01$) from corresponding control.

a Incidence based in 1985 on 10 plants to April, thereafter on 10 tillers per plot; in 1986 on 12 plants to April, thereafter on 20 tillers per plot; in 1987 on 20 plants to February, thereafter on 20 tillers per plot.

b Approximate growth stage (Zadoks *et al.*, 1974) of all plants.

c Values in brackets are incidences in companion control plots on 31/7/85.

Table 28 . Incidence of severe eyespot on tillers in field plots subjected to different SO_2 levels.

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence of severe eyespot [a] on June 11	June 27	July 5	July 12	July 19	July 31	
P1	9 (Control)	40	40	30	10	10	20	(40)[c]
P2	21 (Low)	10	0	20	30	10	10	(20)
P4	32 (Medium)	20	10	40	20	10	10	(20)
P3	48 (High)	0	0	10	20	0	0	(10)
Growth Stage [b]		75	83	85	87	91	92	

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence of severe eyespot [a] on May 22	June 5	June 19	July 2	July 17
P4	7 (Control)	0	20	30	25	10
C4	(Control)	5	10	10	10	20
P1	14 (Low)	0	10	15	10	20
C1	(Control)	5	15	25	40	40
P3	29 (Medium)	5	5	10	20	25
C3	(Control)	5	15	10	10	25
P2	48 (High)	10	0	10	15	45
C2	(Control)	0	5	30	25	45
Growth Stage [b]		47	61	83	87	91

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence of severe eyespot [a] on Apr 16	May 14	May 28	June 11	June 25	July 9
P4	5 (Control)	5	0	5	20	20	50
C4	(Control)	0	5	0	5	10	60
P3	14 (Low)	10	0	5	15	15	45
C3	(Control)	0	0	0	10	15	50
P1	28 (Medium)	10	0	0	5	30	35
C1	(Control)	0	10	10	25	25	35
P2	38 (High)	0	5	0*	20	25	55
C2	(Control)	0	5	25	50	25	40
Growth Stage [b]		30	47	65	83	87	93

* Value differs significantly ($P<0.05$) from corresponding control plot.

[a] % tillers with eyespot lesion/s occupying more than half the stem circumference, based on 10 tillers per plot in 1985 and 20 tillers per plot in 1986 and 1987.

[b] Approximate growth stage (Zadoks *et al.*, 1974) of all plants.

[c] Values in brackets are % incidence in companion control plots, sampled on 31/7/85.

(iii) Brown foot rot (Fusarium spp.)

Brown foot rot occurred in all plots in each season on up to 60% of tillers but incidence did not differ significantly ($P < 0.05$) between plots, (Appendix 7.)

D. Ear Diseases

(i) Black ear moulds (Cladosporium and Alternaria spp.)

There was a high incidence of black ear moulds in all plots by the end of each season, but ears were only severely affected in 1985 (Table 29). When black ear moulds first appeared in 1985, significantly fewer ($P < 0.05$) ears in the High SO_2 plot (43 ppb) were infected than in the control plot. However, the number of infected tillers in the SO_2 plots appeared to increase more rapidly than in the control plot and two weeks later (GS 87) there were significantly more ($P < 0.05$) infected ears in the Medium SO_2 plot than in the control plot. By the next assessment (GS 91) all ears in all plots were infected and no significant differences in severity were detected. Black ear moulds did not occur until later (GS 87) in 1986 and there were no significant differences ($P < 0.05$) in incidence in different plots. In 1987, significantly fewer ($P < 0.01$) ears in the Medium SO_2 plot (28 ppb) were infected than in the companion control plot at GS 87, 91 or 93. Also, significantly fewer ($P < 0.01$) ears in the High SO_2 plot (38 ppb SO_2) were infected with black ear moulds than in the companion control plot at GS 87.

Table 29(i). Incidence of black ear moulds in field plots of Igri barley subjected to different SO_2 levels in three seasons.

(a) 1985

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 27	July 5	July 12	July 19	July 31	
P1	9 (Control)	42	67	58	100	100	(100)[c]
P2	21 (Low)	17	58	83	100	100	(100)
P4	32 (Medium)	17	58	100*	100	100	(100)
P3	43 (High)	0*	33	83	100	100	(100)
Growth Stage [b]		83	85	87	91	92	

(b) 1986

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on July 10	July 17	July 23
P4	7 (Control)	0	40	67
C4	(Control)	0	80	87
P1	14 (Low)	7	53	60
C1	(Control)	7	73	80
P3	29 (Medium)	0	53	67
C3	(Control)	0	47	80
P2	48 (High)	7	40	53
C2	(Control)	20	67	80
Growth Stage [b]		87	91	93

(c) 1987

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 18	June 25	July 2	July 9
P4	5 (Control)	0	13	60	60
C4	(Control)	0	0	47	47
P3	14 (Low)	0	7	60	80
C3	(Control)	7	13	73	80
P1	28 (Medium)	0	7**	27**	33**
C1	(Control)	7	73	87	93
P2	38 (High)	0	7**	67	67
C2	(Control)	7	53	67	67
Growth Stage [b]		83	87	91	93

*, **, *** Values differ significantly from corresponding control plot at $P<0.05$, 0.01, 0.001 respectively.

[a] Incidence based on 12 tillers per plot in 1985 and 15 tillers per plot in 1986 and 1987.

[b] Approximate growth stage (Zadoks *et al.*, 1974) of all plants sampled.

[c] Values in brackets are incidences in companion control plots on 31/7/85.

Table 29(ii). Incidence of severe black ear moulds [a] in field plots of Igri barley subjected to different levels of sulphur dioxide during the 1984-5 growing season

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [b] on July 19 GS 91	% Incidence [b] on July 31 GS 92 [c]
P1	9 (Control)	52	66
C1	(Control)	-	47
P2	21 (Low)	36	92
C2	(Control)	-	47
P4	32 (Medium)	56	83
C4	(Control)	-	66
P3	43 (High)	56	83
C3	(Control)	-	75

[a] Ears with symptoms of black ear moulds on both the glumes and the lemmae were assessed as suffering from severe black ear moulds.

[b] Incidence assessed on 12 tillers per plot.

[c] GS = approximate growth stage (Zadoks et al., 1974) of all plants sampled.

(ii) Ear Botrytis (Botrytis cineria)

There was a fairly high incidence of ear botrytis in 1985 and 1986 and a low incidence in 1987 (Appendix 8). No significant differences ($P < 0.05$) in incidence in the different plots were detected using the Fisher's Exact Probability Test, in any season.

DISCUSSION

The field trials described in this section aimed to establish the relationship between sulphur dioxide concentration, within the range of 5-50 ppb, and disease development in winter barley growing under normal field conditions. Assessments were made of natural infections of diseases in the field plots. Over the course of three seasons the following diseases affected the plants:-

Leaf diseases: Powdery mildew (*Erysiphe graminis*)
Leaf blotch (*Rhynchosporium secalis*)
Brown rust (*Puccinia hordei*)
Net blotch (*Pyrenophora teres*)
Septoria (*Septoria nodorum*)

Stem base diseases: Eyespot (*Pseudocercosporella herpotrichoides*)
Sharp eyespot (*Rhizoctonia cerealis*)
Brown foot rot (*Fusarium* spp.)

Ear diseases: Black ear moulds (*Cladosporium* and *Alternaria* spp.)
Ear botrytis (*Botrytis* spp.)

The response of the diseases to SO_2 fumigation varied. The most severe leaf diseases in each year, mildew and leaf blotch, both appeared to be sensitive to SO_2 concentration. Incidence and severity of mildew were frequently higher in the SO_2 treated plots while, in contrast, leaf blotch infection was usually lower. Eyespot levels were variable and although on one date incidence of eyespot was significantly lower in a plot exposed to 38 ppb SO_2 than the control (5 ppb), on all other sampling dates in the three seasons no significant differences were detected and no consistent trend emerged. In the case of black ear moulds, incidence was at times significantly higher in the SO_2 treated plots than the controls; but at other times was significantly lower. None of the other diseases assessed differed significantly between the SO_2 treatments in any season.

Crop growth

SO_2 treatment appeared to affect crop growth significantly through the season but the only significant effect on final yield of grain was in 1987 when this was reduced in the medium SO_2 plot exposed to a seasonal mean SO_2 concentration of 28 ppb. The growth parameters tiller density, total weight, leaf weight and LAI frequently differed significantly between SO_2 treatments and the direction of the response was similar in all parameters but varied between the seasons. It was striking that growth did not differ significantly between the paired control plots on any date in 1985-6 or 1986-7. The seasonal variation in the response of crop growth to SO_2 concentration suggests that the response is dependent on seasonally variable factors which influence growth in the field, such as temeperature, wind speed, background air pollutants (eg. NO_2 and O_3), pests and diseases.

In both 1984-5 and 1986-7 tiller density and total weight were reduced in the high SO_2 plots (48 and 38 ppb SO_2, respectively) during winter but recovered by late spring. Such a winter depression in growth was also seen in the open-air fumigation experiments at Sutton Bonington (Baker et al., 1986) in plots of Igri barley exposed to 84, 113,108 or 196 ppb SO_2. In the Sutton Bonington experiments grain yield was correlated negatively with SO_2 concentration and yield was reduced at SO_2 levels as low 51 ppb (fumigation mean) compared with ambient (15 ppb). This yield effect is in contrast to the effects seen in the present study. The reasons for this difference are unclear, but maybe due to a number of factors which differ between Sutton Bonington and Littlehampton such as site conditions, background NO_2 and O_3 levels, SO_2 treatment (the fumigation period was shorter at Sutton Bonington and the SO_2 concentrations higher), and

background SO_2 levels (higher mean and peak concentrations at Sutton Bonington). Pest and disease levels may also have differed between the two sites, but no assessments were carried out at Sutton Bonington to compare with the Littlehampton records. If, for example, powdery mildew was more severe at Sutton Bonington, and leaf blotch less severe than at Littlehampton (this would be probable in these areas), and if the responses of these diseases to SO_2 was similar to the responses observed in this study, then a greater yield reduction with SO_2 would be expected at Sutton Bonington as mildew levels increased with increasing SO_2.

The component of grain yield reduced most by SO_2 in the Sutton Bonington experiments was the number of grains per ear, whereas in the Littlehampton experiments the greatest effect was on grain size and ear density. Powdery mildew infection on spring barley in field experiments was found to reduce ear density and grain size but not affect grain number/ear (Brooks, 1972; Jenkyn, 1974). This indicates that mildew is probably not the main factor reducing grain yield at Sutton Bonington, although it would be significant at Littlehampton.

The peak SO_2 concentrations, particularly at critical times in grain development may have an important effect on grain yield. The only year in which a significant reduction in grain yield with SO_2 was found at Littlehampton was in 1987 and the peak SO_2 concentrations were higher in the winter of this season than in the other seasons. Peak SO_2 concentrations at Sutton Bonington are higher than normally occur at Littlehampton and the grain yield reductions could be related to these peak concentrations.

Leaf diseases

In each season levels of powdery mildew and leaf blotch differed significantly between plots.

The design of the open-air fumigation system precluded the use of replicated and randomised field plots in the experiments. Instead, SO_2 treatments were applied to whole plots and individual plants within each fumigation plot were considered as replicates. In order to determine whether differences in disease were attributable to differences in SO_2 treatment, the normal variation in the field should be considered. The experiment was conducted over three seasons with the plots re-randomised each year to examine the consistency of the effects. Despite the position of the plots in the field changing in each season, similar trends in leaf disease levels with SO_2 concentration were seen each summer.

The results show that both the proportion of tillers infected with powdery mildew or leaf blotch and the severity of disease on those leaves which do become infected is influenced by SO_2 concentration. These effects were not always observed at each concentration. Indeed, mildew was often more affected by the low SO_2 treatment than the medium SO_2 treatment and this shaped response was also observed for leaf blotch in 1986. However, whenever significant differences can be discerned by analysis they all point to a reduction in leaf blotch and from stem extension onwards, an increase in mildew in SO_2 treated plots, even at concentrations as low as 14 ppb SO_2. The effect of SO_2 on mildew infection before stem extension is less clear since levels of mildew sufficient to make statistically valid comparisons only occurred during tillering in one season (1987), when mildew appeared to be reduced by SO_2, but mildew levels in the field were variable

and once the disease became more established in the crop these effects disappeared.

The four SO_2 treatments were not identical in each year, mainly due to differences in wind speeds affecting SO_2 deposition and fumigation system shut-down and because background SO_2 concentrations decreased through the three seasons.

SO_2 exposure is characterised not only by seasonal mean concentrations, but also by the fluctuations in SO_2 concentration. The SO_2 concentrations fluctuated diurnally and seasonally: the highest concentrations were recorded during the day and in winter and the lowest concentrations in June and July. This could be why crop growth is more affected in winter (Baker et al., 1986; Mcleod et al., 1988) and pathogen response may also be different if infection occurs early in the season.

The peak concentrations of SO_2 may be important in determining the response of a disease as well as seasonal mean concentrations, particularly if these coincide with stages in the disease cycle at which the pathogen is more exposed to SO_2, for example during spore germination or production, or with certain crop growth stages. In 1985, the maximum SO_2 concentration recorded in the low SO_2 plot was higher than in the medium or high SO_2 plots, which could explain why mildew was more affected by the low than the medium SO_2 treatment.

Analysis of the progress of the powdery mildew epidemic in 1987 provides clear evidence of how SO_2 can influence the epidemiology of a disease. Mildew levels were lower in this season probably as a result of unfavourable weather conditions for disease development with particularly high rainfall in April and June. The main effect of SO_2 was on the incidence of disease in the crop but not on the percentage leaf area affected by disease on infected tillers, which

remained low. In plotting the cumulative total number of leaves infected against time, it could be seen that mildew was spreading to new leaves more rapidly in the SO_2 treated plots than the control plots. There also appeared to be a trend across the field but comparison of SO_2 treated plots with the adjacent control plots revealed significant increases in apparent infection rate (Van der Plank, 1963) in the low and high SO_2 plots.

Surprisingly, the apparent infection rate for leaf blotch in the low SO_2 plot in 1986 was significantly higher than in the control plot. This analysis is slightly misleading in this case, however, because the number of infected leaves in the control plot was much higher to start off with so two different phases of the epidemic are being compared - the exponential phase in the control plot and the plateau phase in the low SO_2 plot.

Disease assessments in the additional control plots in the last two seasons allowed the variation across the field to be monitored. The results show that there was some variation in leaf diseases between control plots which at times was significant but even so comparisons between SO_2 treated plots and their adjacent controls through the three seasons showed recurring patterns of foliar disease development.

The effects on both diseases were slightly erratic in that differences in disease levels between SO_2 treated plots and control plots were not always significant. This is partly a reflection of the small scale of the experiment, without plot replication, making it difficult to detect slight effects against the background variation.

By analysing the incidence and the maximum severity of disease on individual leaves during the main epidemic after stem extension,

some of the variation is removed since comparisons are between leaves of similar physiological age and between disease levels at the peak of the epidemic (thereby minimising variation due to time of onset of epidemic in different plots). In consequence, fewer conflicting results are obtained. Significant differences in infection of mildew were observed on each of the four upper leaves and of leaf blotch on each of the six upper leaves, over the three seasons. The exposure of each leaf to SO_2 would differ depending on it's position in the canopy when infection occurred and leaf area duration. SO_2 concentration decreases exponentially down the canopy and at soil level is generally only 10% of the concentration above the canopy (Mcleod, 1988). Disease levels on different leaves were affected by the SO_2 treatments in each season and this seemed to be mainly related to differences in disease levels between seasons.

Despite the limitations of the experimental design, this study has provided evidence that SO_2 does have a real impact on the development of powdery mildew and leaf blotch diseases of barley.

Of the other leaf diseases which infected the crops (brown rust, net blotch and Septoria), none differed significantly between treatments. This was largely because the diseases did not develop to the extent at which effects of SO_2 could be detected, therefore it cannot be assumed that they were insensitive. Mcleod (1988) reported that brown rust infestation was reduced in winter barley cv. Sonja exposed to 23-58 ppb SO_2, compared with 10 ppb SO_2, in the open-air fumigation system in 1982-3. Further investigation is required to establish the response of these leaf diseases to SO_2.

In reviewing the evidence for air pollutant effects on plant pathogens, Heagle (1973) suggested that reports so far indicated that

obligate parasitism is generally decreased and facultative parasitism generally increased by pollutant stress. The response of mildew and leaf blotch to SO_2 found at Littlehampton does not follow this trend but all the experiments reviewed by Heagle used comparatively high SO_2 concentrations. Fumigation chamber experiments on the effects of SO_2 on powdery mildews have demonstrated reductions in disease at concentrations exceeding 180 ppb SO_2, for example post-inoculation fumigation of barley powdery mildew with 187-936 ppb reduced disease severity (Krieg & Knösel, 1983) and infection of lilac powdery mildew (Microsphaera alni) decreased at 300-400 ppb SO_2 (Hibben & Taylor 1975). While SO_2 at such high concentrations may be toxic to powdery mildews, within the range of concentrations tested at Littlehampton (less than 50 ppb), which are more representative of rural levels in Britain, SO_2 may have very different effects on host or pathogen leading to stimulation of disease. Such a dose-response pattern has recently been reported for another obligate parasite Uromyces viciae-fabae when infection of beans was increased at 70 ppb SO_2 but decreased at 137 ppb (Lorenzini & Panattoni, 1986). It is also possible that the response of powdery mildews to SO_2 may be different in fumigation chambers from in the field where many more factors could interact with SO_2 to influence disease development. The only other report of the effect of SO_2 on powdery mildew in the field at comparable concentrations is on wheat grown in the CERL open-air fumigation system in 1984 when the proportion of leaves infected with mildew was higher in the SO_2 fumigated plots (24-57 ppb) than the control plots (11 ppb) (Mcleod, 1988) suggesting that similar mechanisms control the response of wheat and barley powdery mildews to SO_2.

The effects of SO_2 on leaf blotch observed in this study on barley cv. Igri confirm the observations on barley cv. Sonja in the

CERL open-air fumigation system in 1983 when incidence of leaf blotch during tillering was significantly lower in the SO_2 treated plots (24, 38 and 58 ppb) than in control plots (10 ppb) (Mcleod, 1988). Experiments carried out after 1972 have demonstrated that several other facultative pathogens respond negatively to high SO_2 concentrations (Heagle, 1982). For example pre-inoculation fumigation of maize with 150 ppb SO_2 decreased lesion numbers of southern corn leaf blight caused by Helminthosporium maydis (Laurence et al., 1979). It seems that the sensitivity of plant diseases to sulphur dioxide is not simply related to the type of fungal parasitism since cases of SO_2 stimulating, inhibiting or not affecting have been demonstrated in both biotrophic and necrotrophic pathogens.

There are several likely mechanisms by which SO_2 could affect fungal diseases: direct effects of gaseous SO_2 or SO_2 dissolved in rain water on the pathogen at different stages in the disease cycle; changes in host biochemistry or structure affecting host susceptibility to disease, pathogen dispersal and microclimate; SO_2 effects on populations of phyllophane microorganisms or insect pests which influence disease development. The response of a plant disease to SO_2 may thus be the net result of several mechanisms interacting to affect disease development. The significance of the different mechanisms will differ between plant pathogens and with SO_2 concentrations, environmental conditions or crop development. The contrasting responses of mildew and leaf blotch could be a reflection of the differences in the nature of the pathogens influencing their tolerance to SO_2: Erysiphe graminis is biotrophic ectophytic, dry dispersed and favoured by sunny weather; Rhynchosporium secalis is nectrotrophic, endophytic, splash dispersed and favoured by wet weather.

As a fungus with an ectophytic growth habit, Erysiphe graminis would be directly exposed to atmospheric SO_2 during spore germination and germ tube growth, pustule formation and dispersal. Investigations into the direct effects of SO_2 on powdery mildew fungi have shown that germination of E. graminis is inhibited by very high concentrations (936 ppb SO_2) but unaffected by lower concentrations of 187 ppb or 562 ppb SO_2 (Krieg & Knösel, 1983). The maximum SO_2 concentration recorded in the field plots during the open air fumigation experiments was 250 ppb and the peak concentrations occurred in winter when mildew levels were low, and consequently such inhibitory effects are unlikely.

In an open-air fumigation system other pollutants such as O_3 and NO_x are inevitably not excluded and it is possible that the effects observed are due to interactions between SO_2 and these pollutants. The results of Fehmann et al., (1986) indicate SO_2 and O_3 can act synergistically in increasing the susceptibility of barely to Drechslera teres. Infection by Erysiphe graminis has been shown to be inhibited by O_3 and the spores are most sensitive during sporulation (Heagle & Strickland, 1972). Peak O_3 levels occur in summer when powdery mildew is most active. Mean seasonal concentrations of O_3 in the experimental field at Littlehampton were similar in each season (17-20 ppb) but the highest peak O_3 concentrations were reached in the summer of 1986 (112 ppb). The response of mildew to SO_2 treatment in this year was not markedly different than in the other seasons. Background NO_2 and NO concentrations were highest in 1985. It is not possible to draw conclusions about the significance of any pollutant interactions from these field experiments since other environmental factors would have also varied between seasons.

Conidia of Rhynchosporium secalis are spread by rain splash and germinate in free water on the leaf surface. During germination and

germ tube growth the fungus may be sensitive to SO_2 dissolved in the water but once penetration has taken place, any effects are likely to be plant mediated.

Disease development is closely related to host growth. However, despite differences in the response of crop growth to SO_2 in different seasons, the responses of leaf blotch and mildew were relatively consistent, indicating that the former is not the most important mechanism by which disease is affected by SO_2. Nevertheless, the significant changes in tiller density, dry weight, leaf weight and leaf area in the SO_2 plots are likely to have influenced disease development and conversely the disease levels may have altered crop growth.

Development of mildew in a crop has a marked relationship with crop growth, with an increase in disease being associated with periods of rapid growth when large amounts of susceptible host material become available. For example, Sreeramulu (1964) found a sudden increase in the number of mildew spores above a crop during stem extension. The high mildew severity in control plots between February and April 1987 following a period when leaf area was higher in control plots may be explained by such an effect. The increase in leaf area in SO_2 treated plots between March and June 1985 may similarly have lead to the high incidence of mildew in the low and high SO_2 plots, although a high incidence in the medium SO_2 plot would also have been expected. Differences in mildew levels were observed in different plots in 1986 even though leaf area did not differ significantly.

Tiller density can affect disease progress by alteration of the microclimate (a high tiller density leads to a higher relative humidity) and by changing spore dispersal patterns. Conidial germination of both R. secalis and E. graminis is promoted by high humidity (Caldwell, 1937; Manners & Hossain 1963). Leaf blotch incidence and severity did not appear to be correlated with tiller

density. High mildew severities were sometimes preceded by high tiller densities (in 1986 in the low SO_2 plot and in 1987 in the control plots during tillering) but the effects were not consistent. Mildew has been shown to reduce leaf area, dry weight, tiller density, ear number, ear weight and grain size (Last, 1962; Wolfe, 1969; Jenkyn, 1974), but there was no correlation between increased mildew severity and a decrease in any of these parameters in any year.

The nutrient status of a plant can have significant effects on fungal disease. Cultivars most susceptible to leaf blotch have lower levels of water soluble carbohydrate and higher levels of nitrogen (Jenkyn & Griffiths, 1978). Similarly an increase in powdery mildew of brussels sprouts has been shown to be associated with a decrease in total soluble amino acid and an increase in carbohydrate content of the leaves (Rudgard, 1982). An analysis of the nutrient contents of plants in the open-air fumigation system was carried out by Aminu-Kano (1987) on samples taken in the 1984-5 season. These showed that soluble nitrogen content increased with increasing SO_2 concentration in the plots and that reducing sugar content tended to decrease with increasing SO_2. Cultivars most susceptible to leaf blotch have higher levels of nitrogen and lower levels of water soluble carbohydrate (Jenkyn & Griffiths, 1978). It would therefore be expected that leaf blotch would increase with SO_2 concentration, the opposite effect to the one actually observed.

Measurements of the occurrence of phyllophane micro-organisms on leaves in the field plots by Magan (1989) showed that populations of *Sporobolomyces roseus* were reduced and those of *Aureobasidium pullulans* were increased in the medium and high SO_2 plots in 1986 and 1987. These changes could be related to changes in powdery mildew and leaf blotch. In inoculation experiments, Fokkema and Van der Meulen (1976)

found that as colonisation of A. pullulans increased and S. roseus decreased the severity of the pathogen Septoria nodorum was reduced. Since the dispersal, germination, infection processes and conidia morphology of R. secalis is similar to that of S. nodorum interactions between R. secalis and the leaf saprophytes may also occur similarly and could be the cause of the reduction in leaf blotch in SO_2 treated plots.

Other factors which could influence disease development and have also been show to be affected by SO_2 include host biochemistry (Hughes & Laurence, 1983); stomatal opening (Unsworth et al., 1972), structure of wax layer (Koziol & Cowling, 1981) and nutrient content (Prasad & Rao, 1981). These interactive effects may be occurring in the field plots, but without measurements it is impossible to say what role they play in determining the responses of disease to SO_2.

Stem base diseases

The stem base diseases eyespot, sharp eyespot and Fusarium were apparently less sensitive to SO_2 concentration than the leaf diseases in the field. This could be because pathogens are less exposed to SO_2 at the base of the canopy - measurements of SO_2 concentration at the base of a fumigated mature cereal canopy are frequently less than 10% of the concentration above the canopy (Mcleod, 1988).

There was no consistent relationship between eyespot infection and SO_2 concentration. In 1984-5 there appeared to be a trend of decreasing disease with increasing SO_2 concentration but the differences were not significant. Only on one sample date, in 1987 was there a significant difference in incidence of eyespot when fewer tillers in the high SO_2 plot were infected than the adjacent control plot. This was preceeded by a period when the tiller density in the high SO_2 plot was significantly less than in the control plot which could explain the difference in eyespot incidence since occurrence of severe lesions is favoured by dense crops (Sprague, 1937). However, the effect was transitory and many differences in tiller density occurred in all three seasons without a concomitant change in incidence or severity. Although eyespot spores would be exposed to direct effects of SO_2 in the air or dissolved in rain and surface water, during the initial infection stages, once the outer leaf sheath has been penetrated the fungus would be protected from direct contact with SO_2 and any effect would most likely be related to crop growth affecting the rate of penetration through the leaf sheaths to the stem (Fitt, 1985).

The responses of eyespot to SO_2 reported by Mcleod (1988) in previous seasons also varied: a significant increase in eyespot incidence at 38 ppb SO_2 was recorded on barley cv. Sonja but a significant decrease at 46 and 57 ppb SO_2 on wheat cv. Rapier.

Mcleod (1988) also reported a significant decrease in incidence of Fusarium on wheat.

Infection of the stem base by P. herpotrichoides, R. cerealis or Fusarium spp. is related to previous cropping since the main source of inoculum is infected straw (Macer 1961). Any effects of SO_2 treatment may therefore be confounded by the level of disease in a plot in the preceding seasons, which cannot be accounted for with unreplicated plots.

Two main pathotypes of P. herpotrichoides have been distinguished, the W-type (wheat-type) which is generally more pathogenic to wheat than barley and the R-type (rye-type) which is about equally pathogenic to wheat and barley (Hollins et al., 1985). There has been a shift in the incidence of the different pathotypes on barley crops over the past decade and the R-type has now replaced the W-type as the dominant pathotype in UK populations. It is possible that the pathotypes differ in their sensitivity to SO_2 which could account for some of the variability in the responses of eyespot to SO_2 observed.

Further investigations are required before any definite conclusions are drawn on the effect of SO_2 on stem base diseases.

Ear Diseases

Incidence of black ear moulds differ significantly between plots on several occasions in the 1984-5 and 1986-7 seasons but the response to SO_2 was variable: at GS83 in 1985, incidence of black ear moulds was significantly lower in the high SO_2 treatment (43 ppb) compared with the control (9 ppb) but by GS87 incidence was significantly higher in the medium SO_2 treatment (32 ppb) than the control while in 1987 at GS87 incidence was significantly lower in both the medium (28 ppb) and high (38 ppb) SO_2 treatments compared with the control (5 ppb).

The causal organisms Cladosporium and Alternaria spp. are weakly pathogenic and are common colonisers of the ear surface where they would be directly exposed to atmospheric SO_2. Germination of Alternaria spores has been shown to be reduced by direct exposure to SO_2 concentrations above 50 ppb with an increasing sensitivity with increasing relative humidity (Couey, 1965). Conidial germination of Cladosporium herbarum was significantly reduced by fumigation with 100 and 200 ppb SO_2, but A. Alternata germination was unaffected at these concentrations, although germ tube growth was significantly reduced (Magan & Mcleod, 1988). It is probable, therefore, that the significant decreases in black ear moulds in the medium and high SO_2 treatments were caused by direct inhibition of the fungi by SO_2, although inhibitory effects have not yet been demonstrated within the range of SO_2 concentrations under investigation.

Assessments of the microbial populations on the ripening ears were carried out on the experimental plots at Littlehampton by Naresh Magan (Magan & Mcleod 1987; Magan & Mcleod, 1989). Populations of Cladosporium herbarum were not significantly affected by the SO_2 treatments in 1986 or 1987 although in both years there was a slight

reduction in populations in the medium and high SO_2 plots compared with the control plots. In 1985 Cladosporium spp. populations were markedly decreased in the SO_2 treated plots during grain filling (Magan & Mcleod, unpublished data).

Colonisation by black ear moulds is likely to be affected by shifts in populations of saprophytes on the ear surface (Magan & Mcleod; 1989). The assessments by Magan showed that populations of Sporabolomyces roseus on the ripening ears were reduced and those of Aureobasidium pullulans was increased in the medium and high SO_2 plots in 1986 and 1987, reflecting the changes in phylloplane microflora. A. pullulans has been shown to be antagonistic to Alternaria solani (Brame & Flood, 1983). It may well also be antagonistic to the Alternaria spp. involved in the sooty mould complex. If this were so the increase in A. pullulans population may have contributed to the decline in the sooty mould symptoms observed on the ears in 1987.

In 1985, the crop was infested with the aphids Sitobion avenae and Metapolophium dirhodum late in the season. At growth stage 85 the number of aphids was higher in the SO_2 treated plots (Aminu-Kano, 1987). Sugar exuded by the aphids during feeding provides a source of energy for germinating spores on the surface of the ear. An accumulation of this could have led to the increase in black ear moulds observed after growth stage 87 in 1985. Aphid populations did not differ significantly between SO_2 treatments in 1986 and in this year black ear moulds did not differ between treatments either. In 1987 aphid populations were not assessed. This interaction would be worth investigating further.

Yield losses

The levels of disease recorded in the experiments could reduce grain yield.

Grain yield is closely correlated with disease at growth stage 75 and several attempts have been made to quantify the relationship between disease at GS75 and yield loss. Table 31 . shows the estimated yield losses due to disease calculated from measurements of the main diseases in each season: mildew, leaf blotch and eyespot at GS75 (Table 30 .) using the formulae of Large & Doling (1962), Khan & D'Antuono (1985) and Scott & Hollins (1974) respectively.

It can be seen that the estimated percentage yield loss due to mildew was always greater in the SO_2 treated plots than the control plots, with up to 4% greater yield loss. In contrast, estimated yield losses due to leaf blotch were lower than for mildew and were always less in the SO_2 treated plots than control plots, with up to 3% difference. Estimated losses due to eyespot were variable but in 1985 were likely to have been considerably less in the SO_2 treated plots.

These results show that under normal agricultural conditions atmospheric sulphur dioxide concentration could influence the development of fungal disease in a crop of winter barley to an extent at which grain yield is affected. However, the changes in crop growth and yield measured in the experiments cannot be entirely accounted for by changes in disease levels. Since different diseases appear to have contrasting responses to SO_2, the overall effect of changes in disease development induced by SO_2 on grain yield will depend on which diseases are dominant in the crop, the severity of those diseases which do occur in the crop and the environmental conditions during the season. These experiments have provided evidence of SO_2

significantly affecting the epidemiology of two important diseases of winter barley: powdery mildew and leaf blotch. These are many different ways by which SO_2 may influence disease development. These are difficult to identify in a field situation when there are many interacting variables. Further investigation is warranted to determine the critical mechanisms by which each disease is affected by SO_2, in order to understand the effects observed and to predict the response of disease to SO_2. The laboratory experiments described in Section II therefore aimed to examine the interactions observed in the field in more detail.

Table 30. Severity of main diseases at GS75 in each season

Year	SO_2 treatment	% leaf area affected by		% tillers with eyespot	
		mildew	leaf blotch	moderate	severe
1985	Control	2.9	1.8	30	10
	Low	11.3	1.1	10	0
	Medium	5.5	0.3	20	0
	High	11.5	1.1	0	0
1986	Control [a]	0.6	5.9	18	3
	Low	3.7	2.3	10	5
	Medium	1.6	2.3	10	0
	High	6.0	2.4	10	0
1987	Control [a]	0.6	10.4	14	7
	Low	1.1	6.2	10	5
	Medium	3.3	2.1	5	0
	High	3.2	3.9	5	15

[a] Mean of control plot P4 and companion control plots C1, C2, C3 and C4.

Table 31. Grain yield and estimated percentage yield losses due to disease

Year	SO_2 treatment	Yield (t/ha)	Estimated % yield loss due to mildew [a]	leaf blotch [b]	eyespot [c]	Total
1985	Control	8.0	4.3	0.6	6.6	11.5
	Low	9.1	8.4	0.4	1.0	9.8
	Medium	8.3	5.9	0.1	2.0	8.0
	High	7.8	8.5	0.4	0	8.9
1986	Control	8.7	1.9	2.0	2.9	6.8
	Low	7.7	4.8	0.8	2.8	8.4
	Medium	9.1	3.2	0.8	1.0	5.0
	High	8.2	6.1	0.8	1.0	7.9
1987	Control	7.8	1.9	3.5	3.9	9.2
	Low	7.9	2.6	2.1	2.8	8.6
	Medium	6.8	4.5	0.7	0.5	6.2
	High	7.1	4.5	1.3	5.9	9.5

[a] % yield loss = 2.5 . (mean % leaf area affected by mildew at GS75) (Large & Doling, 1962).

[b] % yield loss = 0.33 .(mean % leaf area affected by leaf blotch at GS75) (Khan & D'Antuono, 1985).

[c] % yield loss = $(0.1n_2 + 0.36n_3)/n_t \cdot 100$ (Clarkson, 1981) where n_2 = number of tillers with moderate eyespot lesions, n_3 = no. tillers with severe eyespot lesions; n_t = total no. tillers (Scott & Hollins, 1974).

SECTION II

EXPERIMENTS IN FUMIGATION CHAMBERS

INTRODUCTION

The results of the field experiments described in Section I indicated that infection of Igri barley with fungal pathogens was influenced by atmospheric SO_2 concentration. It is difficult in field experiments to identify that mechanisms by which SO_2 affects fungal disease because disease development in field crops is determined by many interacting factors. The use of fumigation chambers to expose fungal pathogens and plants to controlled amounts of SO_2 enable the effects of SO_2 on different components of disease to be examined in more detail. Thus it may be possible to explain how the effects witnessed in the field may have occurred.

Many workers have used fumigation chambers in their investigations into the effects of SO_2 on plants and pathogens. Chamber designs have been adapted in attempts to make exposure conditions more realistic. Apparent differences in sensitivity of ryegrass to similar SO_2 concentrations in different fumigation systems were shown to be due to differences in air movement affecting SO_2 uptake by the leaves (Ashenden & Mansfield, 1977), and to interactions with climatic conditions in environmentally uncontrolled systems (Bell, 1985). It is therefore important in using chambers to simulate air conditions in the field as far as possible.

The main pathways through which SO_2 could affect the development of a disease are by directly affecting the pathogen, by altering the host susceptibility and by influencing the host/pathogen interaction.

Gaseous SO_2 or SO_2 dissolved in water may be absorbed directly by a fungal pathogen and could thus alter pathogen development. The direct effects of SO_2 on *Alternaria* spp. were studied by Couey (1965) by fumigating conidia on filter paper. Germination was reduced after

exposure to SO_2, in proportion to the concentration and exposure time, wet conidia being more sensitive than dry conidia.

Secondly, SO_2 could affect the metabolism and structure of the host plant in such a way that its susceptibility to a particular pathogen is altered. Changes that could affect host penetration (e.g. stomatal opening and epicuticular wax structure) and subsequent pathogen development (e.g. nutrient availability in host tissues and host defence mechanisms) have been observed in plants subjected to air pollution (Biscoe et al., 1973; Shelvey & Koziol, 1985; Prasad & Rao, 1981; Curtis et al., 1976). Fumigation of field beans with 130 ppb SO_2 before inoculation with Uromyces phaseoli was shown to reduce the severity of bean rust (Weinstein et al., 1975).

Similarly, changes in host or pathogen biochemistry induced by SO_2 could influence disease by affecting the colonization of the host by a fungus, lesion or pustule development and sporulation (Hughes & Laurence, 1981). Exposure of wheat cv. Thatcher to SO_2 from 2 days after inoculation with Puccinia graminis f. sp. tritici decreased the number of lesions which developed (Laurence et al., 1979).

The objective of the experiments described in this section was to investigate the role of these pathways in determining the effect of SO_2 concentration on three fungal pathogens causing diseases observed in the field experiments - E. graminis (powdery mildew) P. herpotrichoides (eyespot) and R. secalis (leaf blotch). The sensitivity of the pathogens at different stages in the disease cycle was examined.

GENERAL MATERIALS AND METHODS

A. Fumigation systems

Two different "closed chamber" fumigation systems were used to expose potted plants and agar plates to controlled concentrations of sulphur dioxide between 0 and 700ppb.

The fumigation systems were located at Imperial College, Silwood Park and at the Central Electricity Research Laboratories (CERL), Leatherhead.

(i) Silwood Fumigation System

The system consisted of two Perspex chambers (90cm x 90cm x 90cm), each with a hardboard base (Usher, 1984) one of which was ventilated with charcoal-filtered air with added SO_2 gas (fumigation chamber). The system was housed in a heated and ventilated glasshouse. Conditions in the chambers were 40-60% relative humidity and $20^{o}c \pm 5^{o}c$ during a 16 hour photoperiod, with normal daylight supplemented with a 400W mercury discharge lamp, and $10^{o}c \pm 3^{o}c$ during the remaining 8 hour period of the day.

Ambient air was pumped (centrifugal pump model VBL8, Air Control Installations Ltd) through a filter containing activated carbon (Sutcliffe Speakman Ltd) to remove sulphur dioxide, and then through two stainless steel ducts, 10cm diameter, into the chambers (Fig 14). The rate of flow of air into each chamber was measured with an electronic vane anemometer at the inlet and adjusted to $8ms^{-1}$ into each chamber using the butterfly valves in the ducts. This gave five air changes per minute in each chamber. Both chambers had three outlets, 6cm diameter, fitted with plastic tubing through which air was expelled outside the glasshouse.

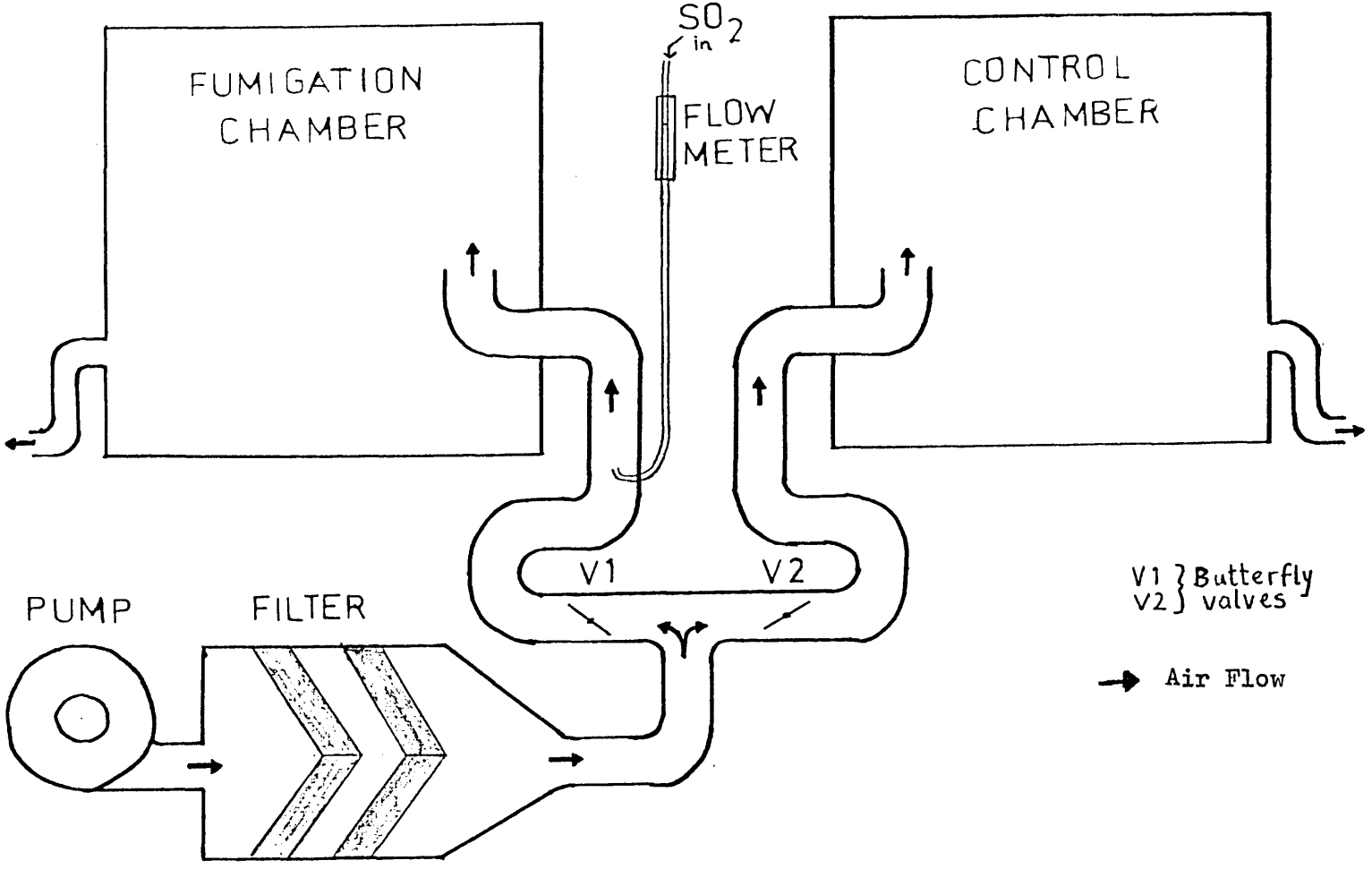


Fig 14. Cross section of the Silwood Fumigation System

Sulphur dioxide gas (1% in Nitrogen) from a cylinder (BO.C. Special Gases) was injected through teflon tubing into the duct at 1.5 meters from the inlet of the fumigation chamber. The rate of flow of SO_2 into the duct was controlled by a flow meter (Platon Ltd; flow range 10-250 $cm^3 min^{-1}$) to give the desired SO_2 concentration in the chamber. The Control chamber received no SO_2 gas.

The concentration of SO_2 in the chambers was measured using a modification of the technique employed by the National Survey of Smoke and SO_2 (Anon, 1966). The sampling system consisted of a diaphragm pump (Charles Austin Ltd) which drew air at a rate of 1 litre min^{-1} from the centre of the chamber through teflon tubing into a Drechsel bottle containing 1 volume hydrogen peroxide (approx. 150 cm^3) adjusted to pH 4.5, then through a gas meter, giving a measure of the volume of air sampled.

After a 5h sampling period 25 cm^3 aliquots of the remaining H_2O_2 solution were titrated against N/250 sodium tetroborate using BDH "4-5" indicator. The mean concentration of SO_2 for the sample period was calculated as follows:

$$SO_2 \text{ (ppb)} = \frac{1582 \times \text{vol.borate}(cm^3) \times \text{vol.}H_2O_2\text{remaining}(cm^3)}{\text{vol.air sampled}(ft^3) \times \text{vol.}H_2O_2 \text{ aliquot}(cm^3)}$$

(ii) CERL Fumigation System

The fumigation system at CERL consisted of four Perspex chambers (61cm x 61cm x 61cm), each with an aluminium base (Fig 15.) and similar in design to those of Lockyer et al. (1976). When fumigating agar plates a perforated Perspex false floor was fitted 13cm above the base of the chamber on which agar plates could be placed between the perforations. The false floor was removed during fumigation of potted plates and the pots were

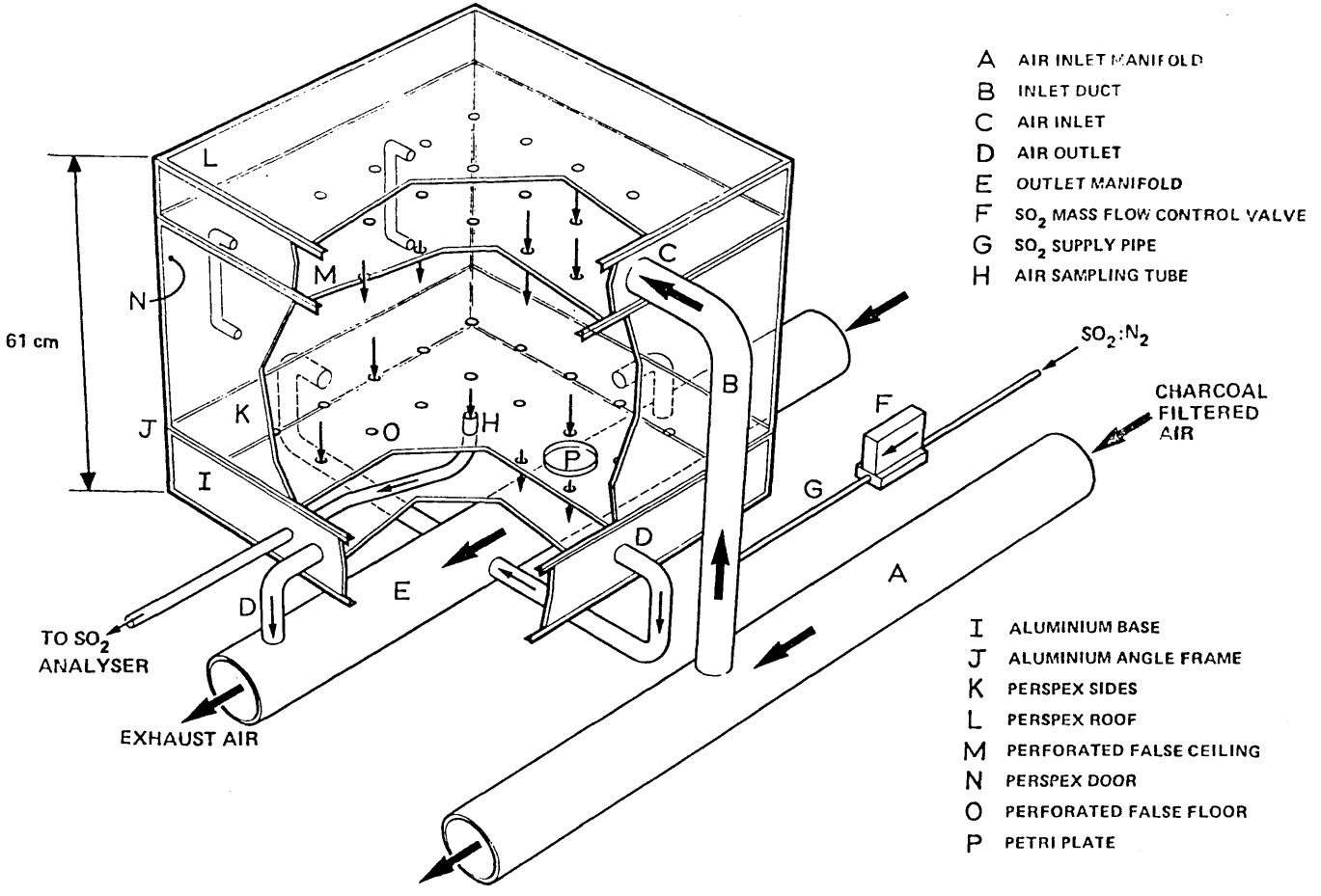


Fig 15. Three dimensional view of a single chamber and associated piping of the fumigation system at CERL. (One corner of the chamber cut away)

placed in individual plastic trays on the base of the chamber. The system was housed in a large glasshouse equipped with heaters and a ventilation fan. Conditions in the chambers were $20^{o}c \pm 10^{o}c$ and 40-60% relative humidity. Plants were exposed to normal daylight during fumigation but polystyrene covers were placed over the chambers to exclude sunlight when plates were fumigated.

Ambient air, drawn through activated charcoal by a centrifugal pump (Air Control Installations Ltd., Model VBL8) passed through a plastic manifold (10cm diameter piping) and then through plastic inlet ducts (5cm diameter) into the top of each chamber (Fig 15). The air flow into each chamber was $4.5ms^{-1}$ giving five air changes per minute.

Air from each chamber passed through four 2.5cm diameter PVC outlet pipes into a plastic manifold (10cm diameter) and was expelled outside the glasshouse. Sulphur dioxide gas (1% in nitrogen) from a cylinder (BOC Special Gases) is injected through stainless steel tubing into the inlet ducts of three of the chambers, at 60cm before the inlet. The SO_2 injection rate is controlled using electronic mass flow control valves, (flow range $0-100cm^3/min$) set using the potentiometers and digital flow display of a valve control unit. The space between the roof of the chamber and the perforated false roof allowed further mixing of the SO_2 and air (Lockyer et al., 1976) before passing over the plants or agar plates.

The concentration of SO_2 in each chamber was measured by a flame photometric detection SO_2 analyser (Meloy Labs. Inc. Model SA285F) calibrated using a permeation tube calibration

source (Meloy Labs Inc. Ltd). The sampling system consisted of air oil-free diaphragm pump (Charles Austin Ltd) and a gas handling unit (The Analytical Development Co. Ltd type WA337-1) through which air was drawn sequentially from each chamber for a 12 minute sample period and passed into the analyser. The reading was recorded on a flat-bed chart recorder (Servoscribe Model 25).

B. Growing of plants

Seeds of winter barley cv. Igri were surface sterilised in sodium hypochlorite solution (1-1.4% available chlorine) for approximately one minute and then sown 2cm deep in 3 in (7.6cm) square pots (10 seeds/pot), containing a peat/sand/fertiliser mixture adapted from University of California Soil Mix I (D) (Matkin & Chandler, 1957). Seedlings were thinned to five seedlings of similar size in each pot 7 days after sowing. Plants were kept in spore-free cabinets (Finney, 1973) within a glasshouse maintained at $20^{\circ} \pm 5^{\circ}$c during a 16h photoperiod provided by a 400w mercury vapour discharge lamp (Phillips TYPS71359) above each cabinet and at $10^{\circ} \pm 3^{\circ}$c during the remaining 8h of the day.

EXPERIMENTS ON POWDERY MILDEW

Methods

A. Fungal Isolates

The isolate of *Erysiphe graminis* f.sp. *hordei* used in all experiments was obtained from ICI Experimental Station, Jealott's Hill, Bracknell.

A stock culture was maintained on barley seedlings (cv.Igri) by shaking plants with sporulating pustules (7d after inoculation) over fresh seedlings at the first leaf stage.

B. Inoculation of plant material

Mature conidia were removed by shaking infector plants (from stock culture) 24h before using them as sources of inoculum. This ensured an inoculum of uniform age and viability. Experimental plants grown to the first leaf stage in filtered air cabinets were transferred to a second cabinet for inoculation using the "cotton wool bud techique". A cotton wool bud was rolled lightly over the first leaf of a sporulating infector plant then rolled once over the central portion (1cm long) of the adaxial surface of the first leaf of the experimental plant. The inoculated plants remained in the filtered air cabinet for 24h following inoculation.

The number and distribution of conidia deposited on the leaf was assessed by taking leaf imprints on sellotape which were mounted in lactophenol and examined. The number of conidia deposited on the leaf sections was fairly uniform (2-3 x 10^3 spores per leaf section) within and between experiments and conidia were evenly distributed with relatively few (4%) in clumps or chains compared with other inoculation methods tested (Appendix 9).

Germination on the inoculated leaf sections was assessed on sellotape imprints taken 24h after inoculation. On plants kept in the filtered air cabinets 75-85% of conidia germinated, whereas on plants placed in the fumigation chambers less than 20% germinated.

C. Inoculation of agar plates

Plates of distilled water agar were inoculated for use in experiments on germination of conidia as follows. Ten agar plates were placed in each fumigation chamber at CERL, and an infector plant (prepared as described above) was shaken over them. The air flow in the cabinets ensured a reasonably even deposition of spores on the surface of the agar ($2\text{-}5 \times 10^2$ spores per cm^2).

D. Disease severity assessment

Disease severity was assessed 8 days after inoculation. The inoculated portion of the leaf was first cut out using a pair of razor blades fixed 11mm apart on a Perspex block. The number of pustules on the leaf section was recorded and the percentage area of leaf section covered by pustules was assessed using a key (Fig 16).

E. Sporulation Assessment

Sporulation was assessed 8 days after inoculation. The inoculated leaf section was removed and placed directly into a McCartney bottle containing 10ml 0.01% Tween 80. Ten leaf sections from one treatment were placed in each bottle and shaken vigorously on a mechanical shaker for 15 min. The concentration of conidia in the suspension was then determined using a haemocytometer (Weber Scientific International Ltd, Improved Neubauer counting chamber).

Ten counts of each suspension were made, taking samples from the suspension at random using a Pasteur pipette.

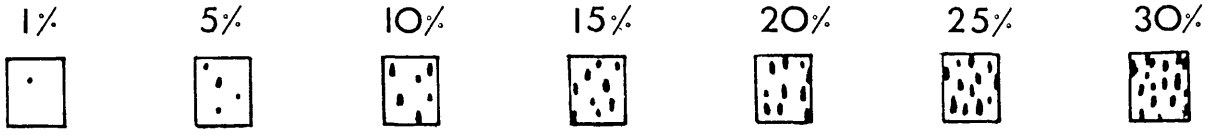


Fig 16. Key used for assessing powdery mildew severity on leaf sections of Igri barley, showing the percentage area of leaf covered by mildew pustules.

Experimental

(i) Effect of SO_2 on germination of conidia

Plates of distilled water agar inoculated with conidia were exposed to 0, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 2 ppb) respectively for 24h in the chambers at CERL. The temperature in the chambers was $25^{\circ} \pm 2^{\circ}c$ for 16h and $15^{\circ}c \pm 5^{\circ}c$ for 8 h of the fumigation period.

After fumigation the plates were removed, flooded with 2-3ml acid fuchsin and examined under the microscope. Germination was assessed on 100 conidia chosen at random across the diameter of the plate. A conidium was considered as germinated when the germ tube length was equal to or greater than the diameter of the conidium.

Germination was similar in all treatments (Table 32.).

(ii) Effect of SO_2 on viability of conidia

Infector plants (four pots of 10 plants) were blown with air to remove mature conidia, then fumigated in the CERL chambers at 2, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 2 ppb) for 24h during conidial formation. The temperature in the chambers was $20^{\circ} \pm 5^{\circ}c$ for 16h and $10^{\circ}c \pm 5^{\circ}c$ for 8h of the fumigation period.

After fumigation the infector plants from each treatment were used to inoculate 10 plates of distilled water agar. The plates were incubated at $25^{\circ}c$ for 24h, then the conidial germination was assessed as described in E (i).

Significantly ($P < 0.01$) more conidia had germinated from infector plants exposed to 20, 40 or 60 ppb SO_2 than from plants exposed to filtered air only (2 ppb SO_2), but otherwise SO_2 concentration had no significant effect on germination (Fig. 17 and Appendix 10.).

Table 32. Percentage germination of Erysiphe graminis conidia on water agar after 24h exposure to different concentrations of sulphur dioxide

	Concentration of SO_2 (ppb)			
Replicate *	0	20	40	60
1	68	74	69	73
2	66	67	76	63
3	69	75	67	62
4	57	68	74	80
5	63	61	71	67
6	64	60	74	69
7	60	58	54	61
8	62	55	58	62
9	63	69	60	56
10	61	67	59	66
Mean	63.3	65.4	66.2	65.9

* 100 conidia were examined on each agar plate

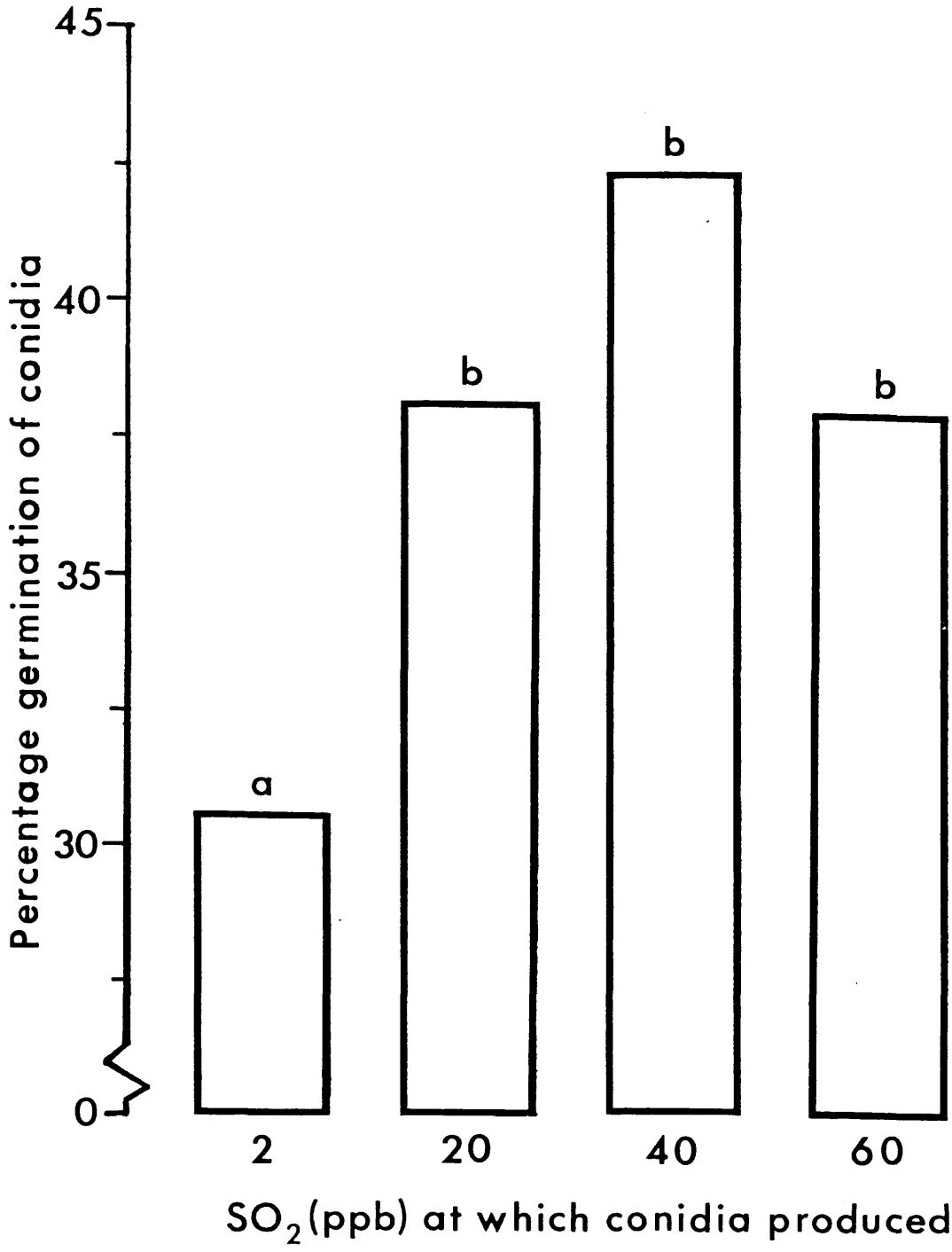


Fig 17: Germination of Erysiphe graminis conidia produced on plants of barley cv. Igri during exposure to different concentrations of SO_2 for 24h.

Germination was assessed on 10 samples of 100 conidia after incubation on water agar for 24h at 25°c.

Values with no common letter are significantly different at $P < 0.01$ (anovar on arcsin transforms of percentage data).

(iii) Effect of SO_2 on the pathogenicity of *Erysiphe graminis*

Infector plants (four pots of 10 plants) were blown with air to remove mature conidia then fumigated in the CERL chambers at 0, 20, 40 and 60 ppb SO_2 (mean concentration $\pm$ 1 ppb) for 24h during conidial formation. The temperature in the chambers was 25°c $\pm$ 5°c for 16h and 16°c $\pm$ 3°c for 8h of the fumigation period.

After fumigation infector plants from each treatment (five plants from each of the four pots) were used to inoculate leaves by the cotton wool bud technique. Disease severity and sporulation were assessed 8 days after inoculation.

The number of pustules which developed on the inoculated leaf sections was similar for all sources of inoculum (Table 33. and Appendix table 10b). However the percentage leaf area covered by pustules was significantly ($P < 0.05$) greater on leaves inoculated with conidia from plants exposed to 40 or 60 ppb than from plants exposed to 0 or 20 ppb SO_2 (Fig 18 , Appendix 11). Correspondingly, the number of conidia produced on the leaf sections differed significantly ($P < 0.001$) in a manner which appeared to be related to the SO_2 concentration in which the original inoculum was produced (Fig 19 , Appendix 12).

(iv) Effect of SO_2 on susceptibility of barley to powdery mildew

Seedlings of barley cv. Igri. (six pots of five seedlings) were fumigated at CERL for 7 days from coleoptile emergence (7 days after sowing) until the first leaf stage with mean SO_2 concentrations ($\pm$ 2 ppb) of 5, 21, 41 or 62 ppb.

After fumigation the plants were inoculated in the glasshouse with conidia, and disease severity and sporulation on the inoculated leaves were assessed 8 days later.

Table 33: No. powdery mildew pustules on leaf sections 8 days after inoculation with Erysiphe graminis conidia produced on plants during exposure to different concentrations of SO_2 for 24h

Inoculated material		Infector plants exposed to SO_2 at			
Pot	Seedling *	0	20	40	60 ppb
1	1	6	10	10	7
	2	12	2	12	5
	3	1	8	9	4
	4	9	5	6	8
	5	8	10	7	5
2	1	8	4	3	5
	2	4	2	10	10
	3	9	6	11	10
	4	3	9	12	11
	5	8	5	8	14
3	1	4	13	10	10
	2	11	7	6	11
	3	7	7	4	8
	4	8	9	7	12
	5	10	4	10	9
4	1	10	8	5	8
	2	10	6	4	10
	3	7	7	5	9
	4	4	6	8	6
	5	2	5	6	6
Mean		7.05	6.65	7.65	8.4

* Pustule number assessed on leaf section of the first leaf of each seedling.

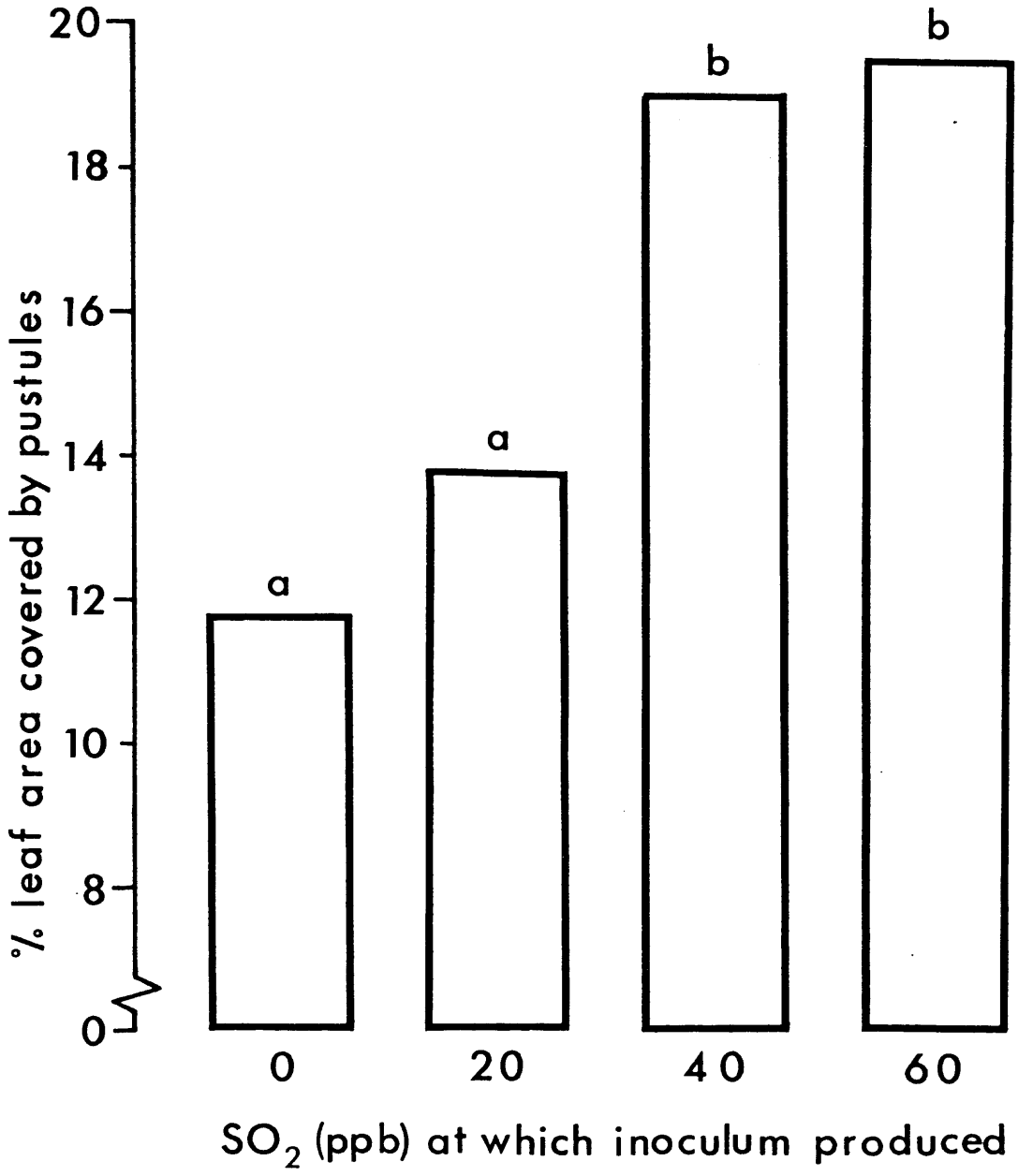


Fig 18: Severity of powdery mildew on leaf sections 8 days after inoculation with Erysiphe graminis conidia produced on plants during exposure to different concentrations of SO_2 for 24h.

Values with no common letter are significantly different at $P < 0.05$ (anovar on arcsin transforms of percentage data).

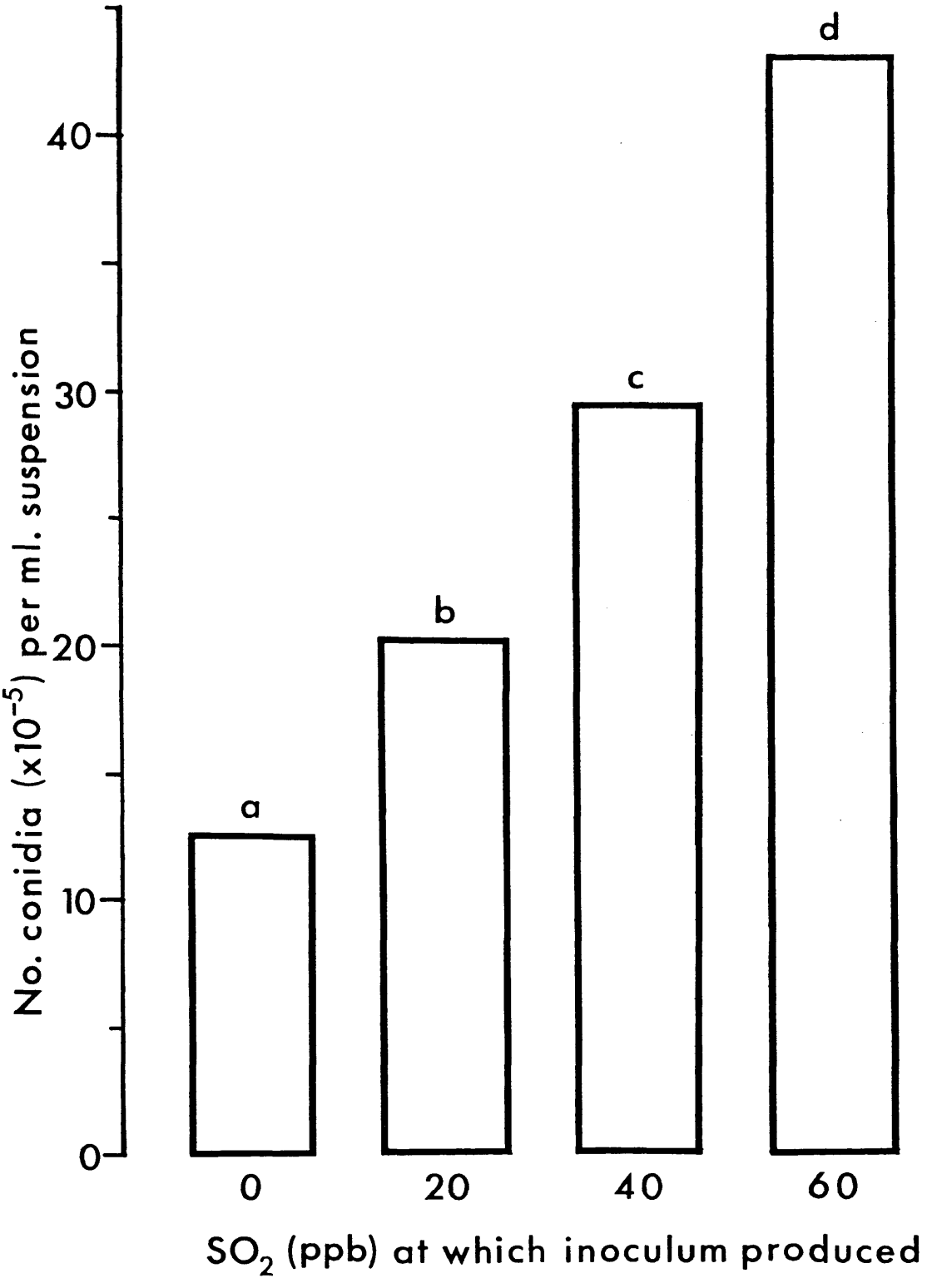


Fig 19: Sporulation of powdery mildew on leaf sections 8 days after inoculation with Erysiphe graminis conidia produced on plants during exposure to different concentrations of SO_2 for 24h.

Sporulation was assessed from haemocytometer counts on a suspension obtained by shaking 10 leaf sections in 10ml 0.01% Tween 80.

Values with no common letter are significantly different at $P < 0.001$.

There was no significant difference ($P < 0.05$) in the number of pustules which developed on leaf sections from plants previously exposed to different amounts of SO_2 nor did the percentage area covered by pustules differ significantly (Table 34 and Appendix Table 13), although there was an apparent tendency for a greater mildew severity on plants previously exposed to 21 and 41 ppb SO_2.

However, significantly more ($P < 0.05$) conidia were produced on leaves from plants previously exposed to 21 or 41 ppb SO_2 than from those exposed to 5 or 62 ppb SO_2 and the number of conidia in the latter two treatments also differed significantly (Fig 20 , Appendix 14).

(v) Development of powdery mildew on plants exposed to SO_2 after inoculation

Seedlings of barley cv. Igri were inoculated at the first leaf stage and 24h later were placed in the fumigation chambers at CERL and exposed to 2, 20, 40 and 60 ppb SO_2 (mean concentration $\pm$ 3 ppb) for 7 days. There were six pots in each chamber, all with five seedlings. After fumigation the inoculated leaf sections were assessed for disease severity and sporulation.

The number of pustules which developed, and the leaf area covered by the pustules did not vary significantly ($P < 0.05$) between the SO_2 treatments (Appendix 15).

Significantly more ($P < 0.05$) conidia were produced on leaves exposed to 60ppb SO_2 during disease development than at the lower SO_2 concentrations, and significantly more conidia were produced at 40 ppb SO_2 than at 2 ppb SO_2 (Fig 21 , Appendix 16.).

Table 34 : Development of powdery mildew on seedlings exposed to different concentrations of SO_2 for 7 d before inoculation

Powdery mildew *	Seedlings exposed to SO_2 at			
	5	21	41	62 ppb
No. pustules	11.3	12.1	13.7	10.5
% Area with pustules	14.3	17.3	19.3	14.8

* Mean values based on sections taken from the first leaf of 20 seedlings, 8 d after inoculation.

Table : Development of powdery mildew on seedlings during exposure for 7 days to different concentrations of SO_2 *

Powdery mildew **	SO_2 (ppb)			
	2	20	40	60
No. pustules	6.2	7.2	5.1	6.4
% Area with pustules	10.9	11.6	10.8	13.5

* Exposure to SO_2 started 24 h after inoculation

** Mean values based on sections taken from the first leaf of 20 seedlings, 8 days after inoculation.

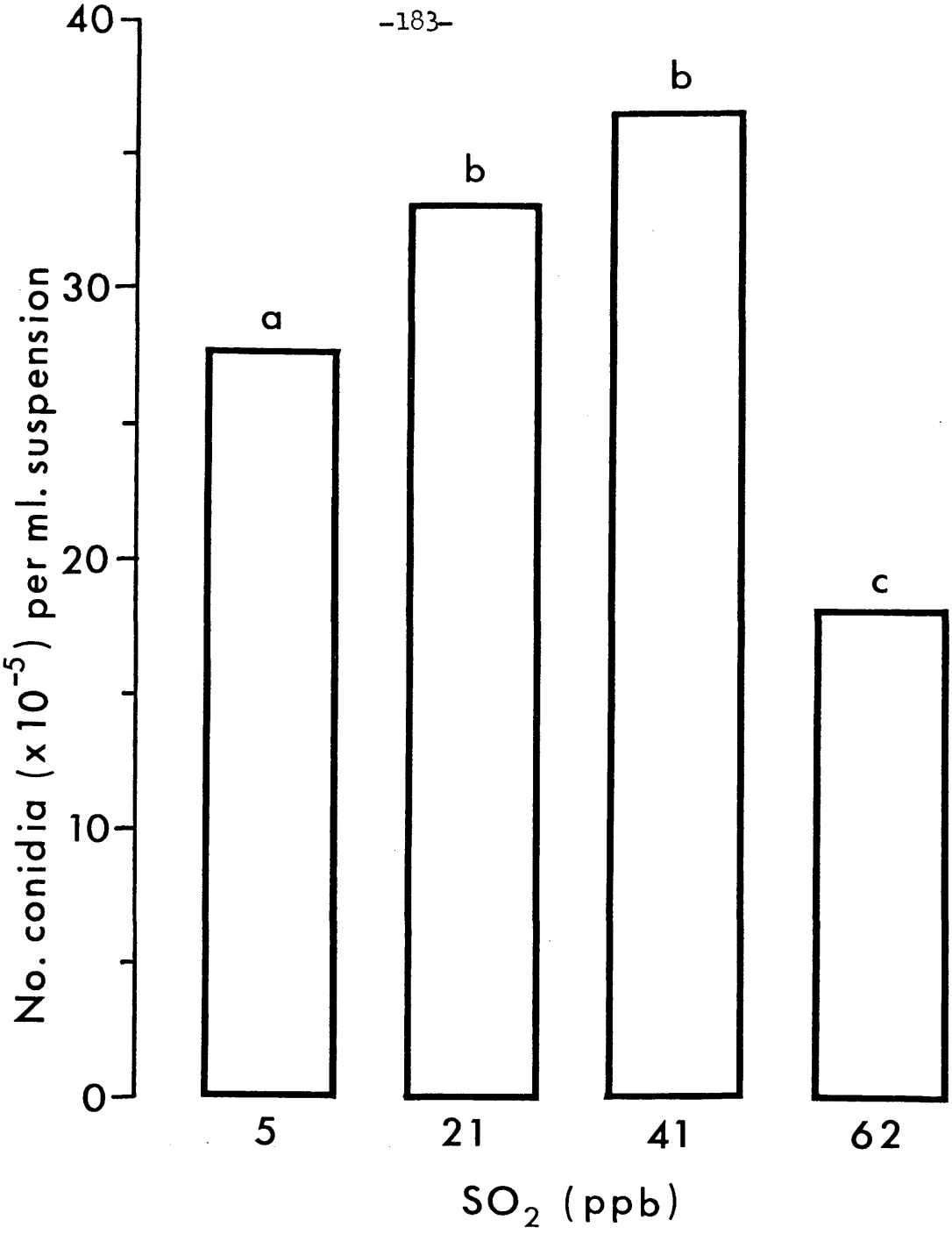


Fig 20: Sporulation of powdery mildew on seedlings exposed to different concentrations of SO_2 for 7 days before inoculation.

Sporulation was assessed from haemocytometer counts on a suspension obtained by shaking 10 leaf sections in 10ml 0.01% Tween 80, 8 days after inoculation.

Treatments with no common letter are significantly different at $\underline{P} < 0.05$.

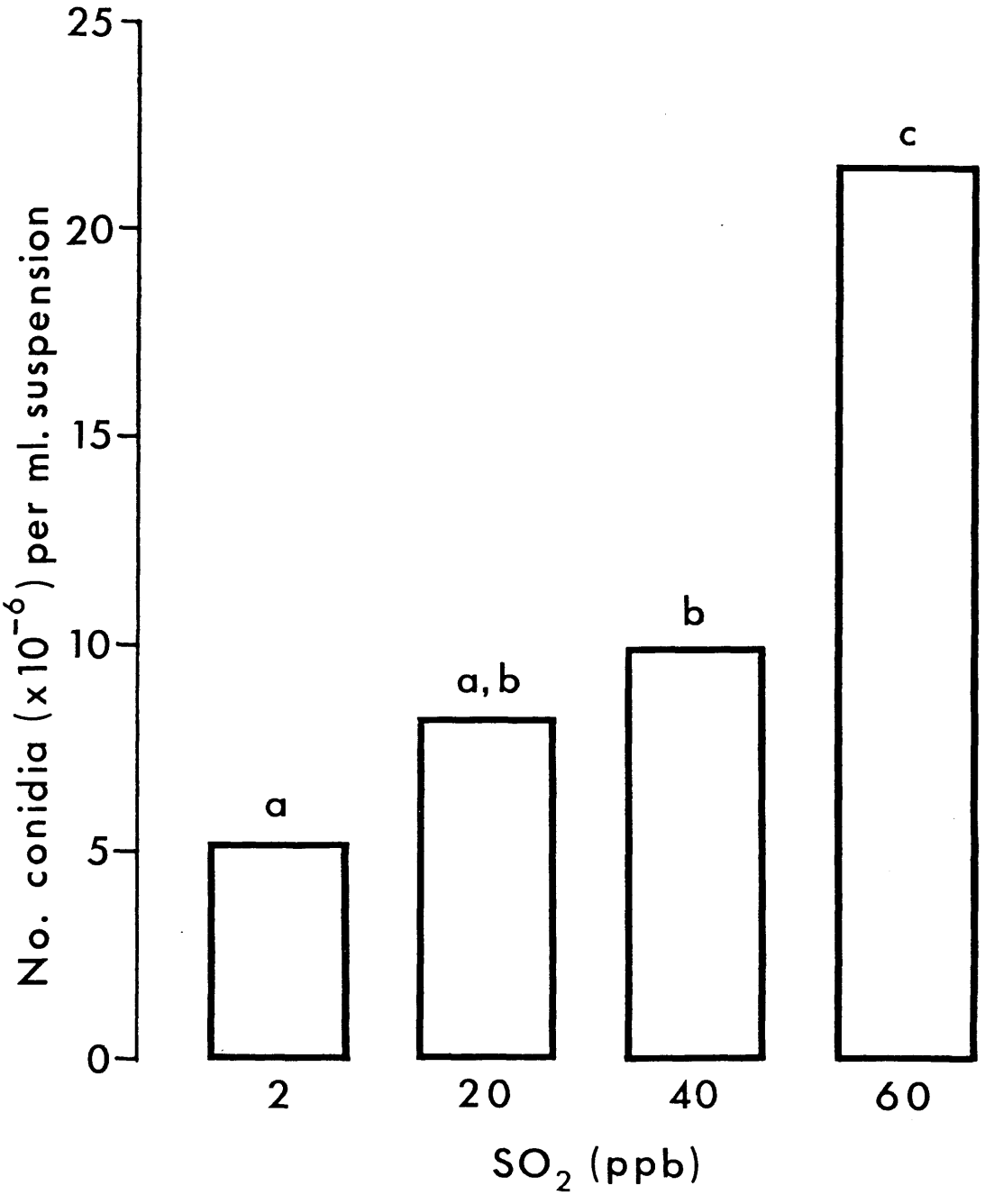


Fig 21: Sporulation of powdery mildew on seedlings following exposure for 7 days to different concentrations of SO_2.

Exposure to SO_2 started 24h after inoculation. Sporulation was assessed from haemocytometer counts on a suspension obtained by shaking 10 leaf sections in 10ml 0.01% Tween 80, 8 days after inoculation.

Treatments with no common letter are significantly different at $P < 0.05$.

(vi) Development of powdery mildew on plants exposed to SO_2 before and after inoculation

Six pots all with five seedlings of barley cv. Igri at coleoptile emergence (7 days after sowing) were placed in each of the chambers at CERL and fumigated at 2, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 2 ppb) continuously for 7 days.

The seedlings were then inoculated and kept in the glasshouse for 24 h.

Subsequently the seedlings were replaced in the same chambers and fumigated for a further 7 days at the original SO_2 concentrations.

After the final fumigation the leaves were assessed for mildew severity and sporulation.

Neither the number of pustules which developed, nor the percentage area covered by the pustules varied significantly ($\underline{P} < 0.05$) between SO_2 treatments (Table 35 , Appendix 17).

Significantly more ($\underline{P} < 0.05$) conidia were produced on leaves exposed to 40 ppb SO_2 before and after inoculation than on those exposed to 2, 20 or 60 ppb SO_2. Also, more conidia were produced at 20 ppb SO_2 than at 2 or 60 ppb SO_2 (Fig 22 , Appendix 18).

Table 35 : Development of powdery mildew on seedlings exposed to different concentrations of SO_2 for 7 days before and 7 days after inoculation *

Powdery mildew **	SO_2 (ppb)			
	2	20	40	60
No. pustules	4.0	3.6	3.7	2.9
% Area with pustules	5.7	6.0	7.5	4.7

* Exposure to SO_2 re-commenced 24 h after inoculation.

** Mean values based on sections taken from the first leaf of 20 seedlings, 8 days after inoculation.

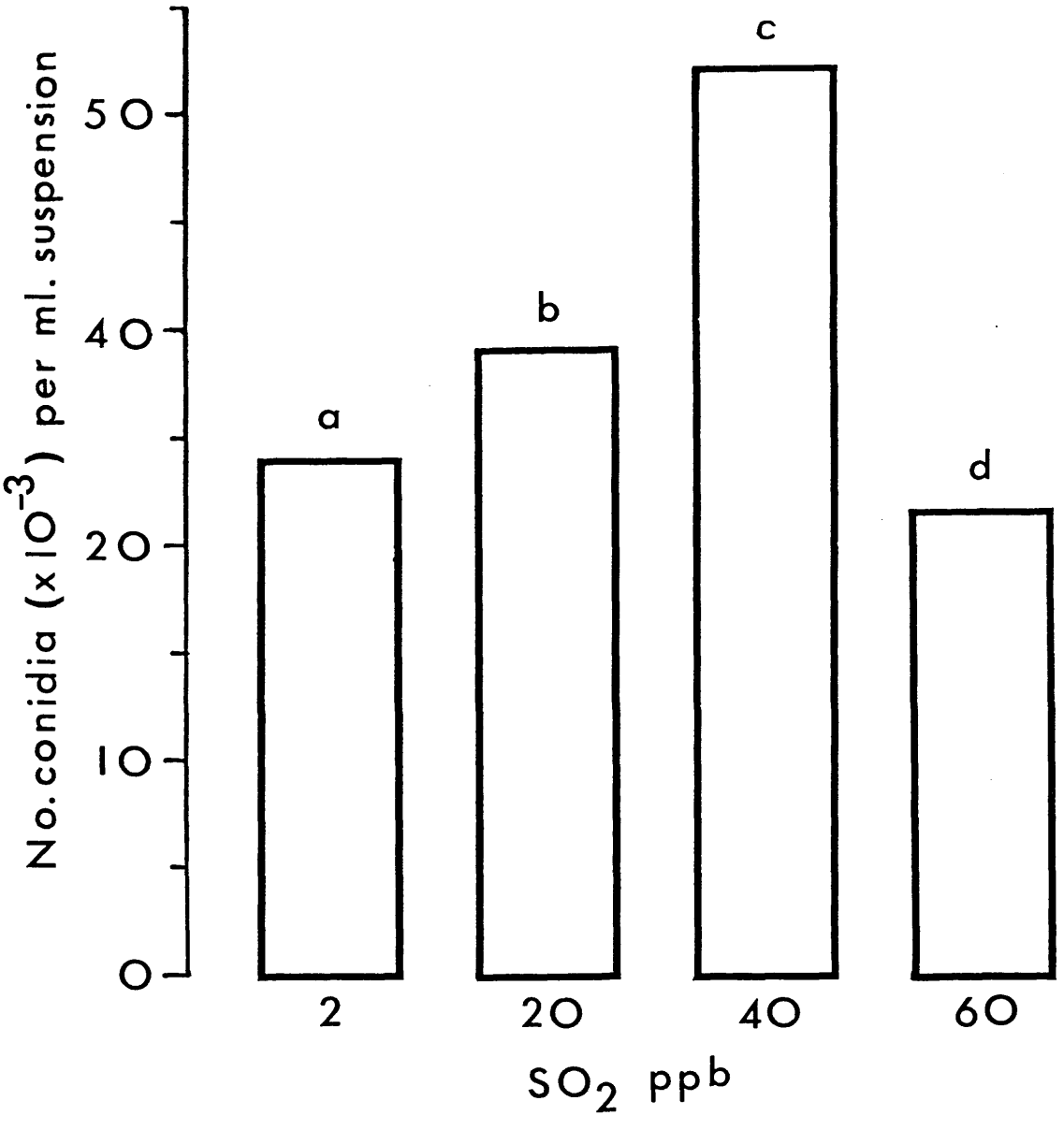


Fig 22: Sporulation of powdery mildew on seedlings exposed to different concentrations of SO_2 for 7 days before and 7 days after inoculation.

Exposure to SO_2 recommenced 24h after inoculation. Sporulation was assessed from haemocytometer counts on a suspension obtained by shaking 10 leaf sections in 0.01% Tween 80, 8 days after inoculation.

Treatments with no common letter are significantly different at $\underline{P} < 0.05$.

EXPERIMENTS ON EYESPOT

Methods

A. Fungal Isolates

The isolate of *Pseudocercosprorella herpotrichoides* (Fron.) Deighton used in all the experiments was obtained from Rothamsted Experimental Station and had been isolated from winter wheat (cv. Armada) by Dr Sally Higgins in 1980. It had been shown to be sensitive to benzimidazole.

Stock cultures were maintained under glycerol on potato dextrose agar (PDA) slopes in McCartney bottles stored at 4°c. When required these were subcultured onto PDA plates which were then incubated at 15°c for 3 weeks. Sporulation was induced by growing sub-cultures on distilled water agar (DWA) plates under near ultraviolet light (Phillips 40W, BL08) during a 16 h photoperiod at 10°c for 4 weeks.

B. Inoculation of plant material

A suspension of conidia was obtained by flooding a sporulating culture (4 weeks old) with 10 ml sterile distilled water and dislodging the conidia with a sterile glass spreader. The concentration of conidia was assessed using a haemocytometer, adjusted to 1×10^3 conidia/ml sterile distilled water, and 10 ml of suspension were placed in a sterile McCartney bottle.

Eleven sterile filter paper strips (20 x 10mm) were placed in each bottle and shaken for 10 min on a mechanical shaker. Each strip was then wrapped round the stem base of a barley seedling, inside the coleoptile, and a strip of parafilm wrapped round the filter paper to hold it in place and to maintain high humidity. The strips were removed 48 h later. The number of conidia on the

strip was estimated by staining one strip per bottle with lactophenol cotton-blue and counting the number of conidia present.

This method was adapted from the "filter paper disc" method used at Rothamsted Experimental Station (Higgins, 1984). The method was chosen because it produced uniform infection.

C. Inoculation of agar plates

Plates of distilled water agar were inoculated with conidia for use in experiments on germination and initial mycelial growth. A drop (0.5ml) of conidial suspension, prepared as described above, was placed on each plate, using a digital micropipetter (Volac, 50-500µl) and spread evenly over the agar surface with a sterile glass angle.

D. Assessment of germination of conidia and initial mycelial growth

Germination of conidia was assessed by examining 100 conidia chosen at random across each replicate slide/plate, and recording the first appearance of germ tube.

Initial mycelial growth was assessed 42 h after inoculation by examining 20 conidia chosen at random across each replicate slide/plate, and recording the total number of hyphae per conidium, the presence of secondary hyphal branches and length of the hyphae. Hyphal length was measured using an eyepiece graticule calibrated using a slide micrometer.

E. Disease severity assessment

The severity of the lesion(s) which developed on the outer leaf sheath was assessed 28 days after inoculation using the scale devised by Scott and Hollins (1974) adapted for use with young plants:

0 - Uninfected

1 - Slight eyespot (one or more lesions occupying in total less than half the circumference of the outer green leaf sheath).

2 - Moderate eyespot (one or more leions occupying at least half the circumference of the outer green leaf sheath).

3 - Severe eyespot (one or more lesions occupying at least half the circumference of the outer green leaf sheath and tissues softened so that lodging could readily occur).

The number of leaf sheaths penetrated by the lesion was assessed by peeling back successive sheaths until no sign of necrosis was visible.

A disease severity score (Higgins, 1984) was calculated using the formula:

$$\text{Disease severity} = \left[\frac{\sum_{1}^{3} n_1 x_1}{3n_t} + \frac{\sum_{1}^{9} n_1 y_1}{9n_t} \right] . 100$$

Where x_1 = lesion severity (0-3 on the Scott & Hollins scale).

y_1 = no. leaf sheaths penetrated (1-9).

n_1 = no. mainstems with a given score.

n_t = total no. mainstems in each sample.

EXPERIMENTAL

Effects of bisulphite at different pH levels on the germination of conidia and initial mycelial growth

Suspensions of conidia of Pseudocercosporella herpotrichoides were prepared, as described below, in the following solutions:-

pH 4: 0.5M citrate buffer (citric acid + sodium citrate)
pH 5.6: Distilled water
pH 7.0 0.5M phosphate buffer (KH_2PO_4 + Na_2HPO_4)

Using a graduated syringe fitted with a microfilter ("Acrodisc", 0.2 um, Gelman UK Ltd.), 10 ml of each solution were placed on separate sporulating cultures of P. herpotrichoides, the surface of agar was agitated with a glass spreader to dislodge the conidia and the resulting suspension transferred to a sterile test tube. The concentration of conidia in the suspensions was adjusted to 10^5conidia/ml with the appropriate solution and then 9 ml of each suspension were transferred to two sterile McCartney bottles. Next, solutions of 1mM sodium bisulphite were prepared in each buffer and in distilled water. To one bottle of each suspension, 1 ml of 1mM sodium bisulphite (made up in the corresponding solution) was added and to the other bottle 1 ml of buffer/water was added, as appropriate. The bottles were shaken on a Whirlimixer (Fisons Scientific Apparatus Ltd) for 30 sec, then left to stand for 30 min.

Using a digital micropipette, 50 µl drops of each suspension were placed on glass slides (five drops per slide) which were mounted on glass rods (6 mm diameter) lying on a piece of damp tissue (Kim-wipe) inside a plastic box (17.5cm x 11.5cm x 6cm). The boxes were sealed with cling film to maintain a high humidity and incubated at 15°c. Five slides per treatment were removed after 18 h and five more after 42 h, mounted with clear lactophenol

and examined under the microscope for assessment of conidial germination and initial mycelial growth respectively.

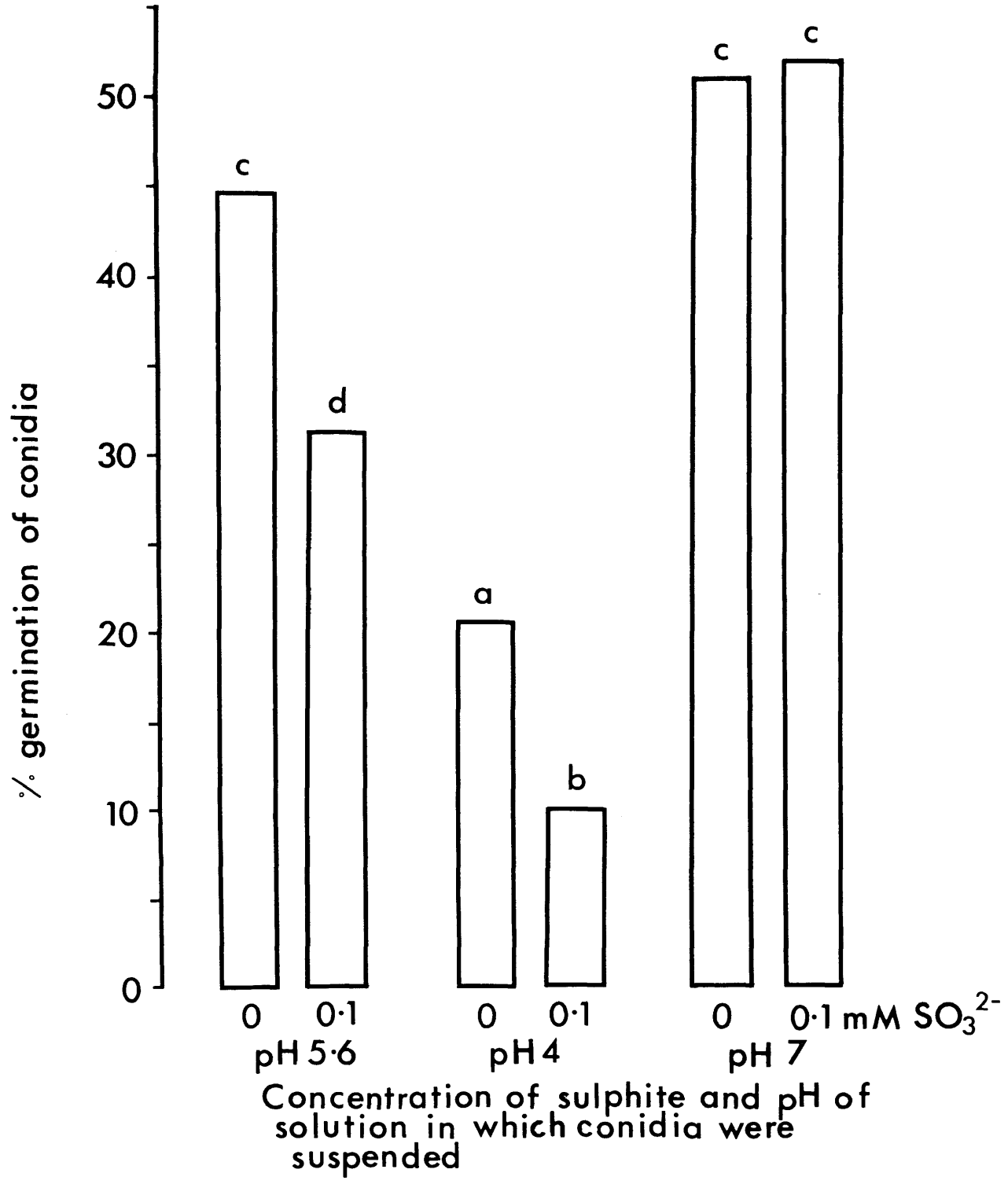
Both conidial germination and the development of hyphae was significantly affected ($P<0.05$) by sulphite and the manner and extent of the response depended on the solution in which the conidia were suspended (Figs 23 & 24 , Appendix 19).

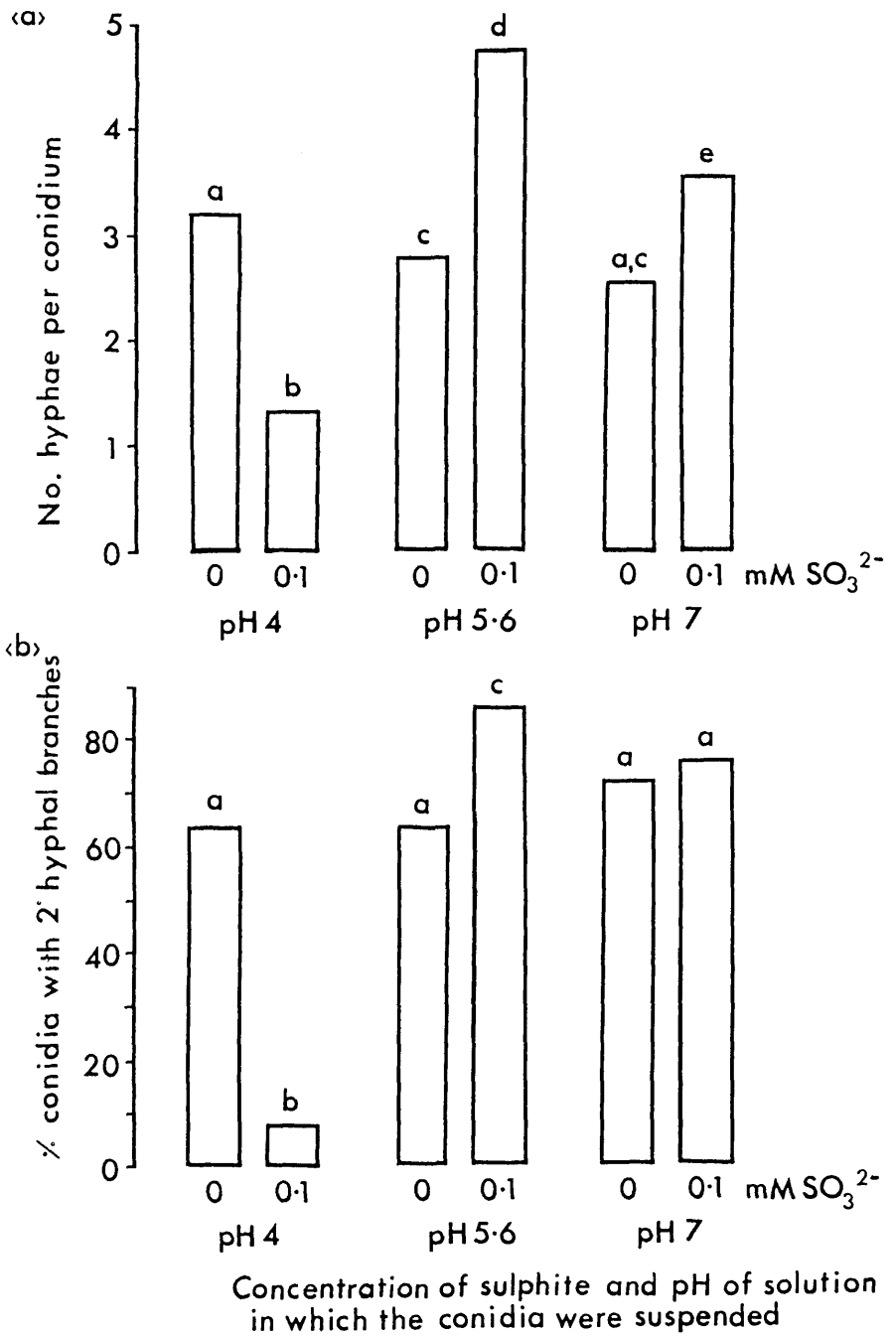
Significantly fewer ($P < 0.01$) conidia germinated in 0.1mM bisulphite in distilled water (pH 5.6) than in distilled water alone. However, the germinated conidia had significantly more ($\underline{P} < 0.01$) hyphae, more secondary hypae and the total hyphal length per conidium was significantly greater ($P < 0.001$) in the bisulphate treatment.

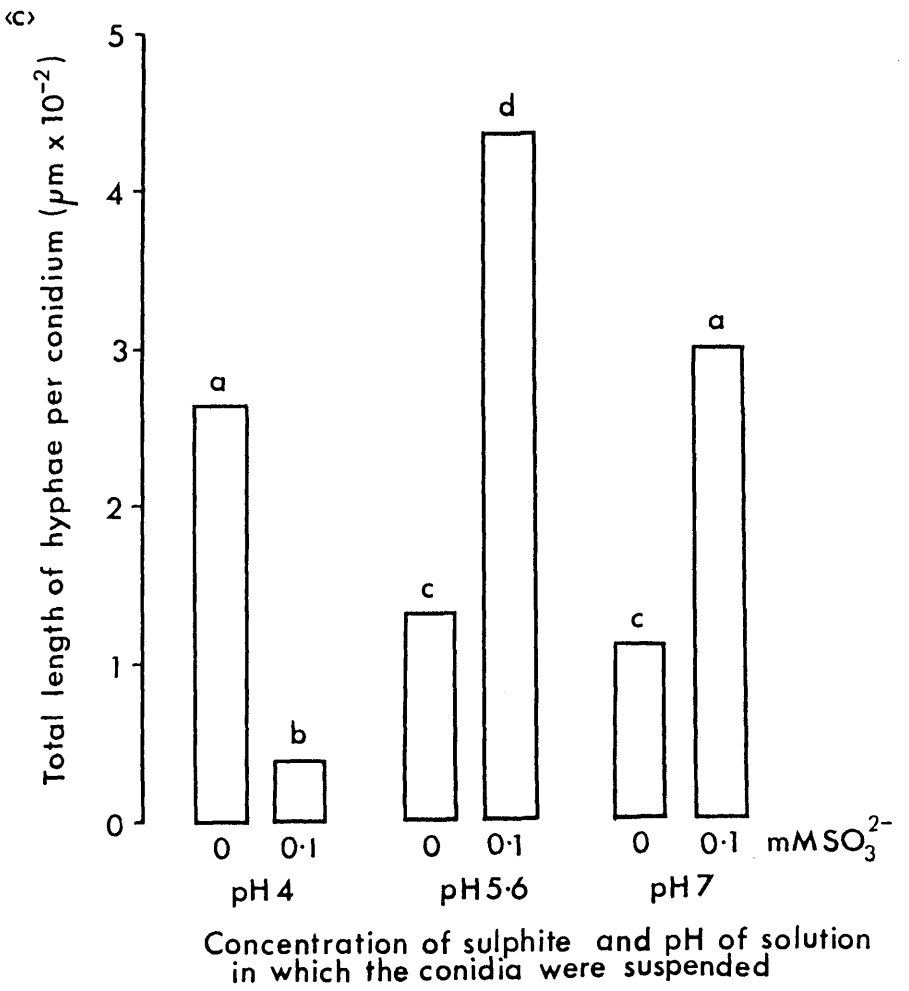
Similarly, significantly fewer ($P < 0.001$) conidia germinated 0.1mM bisulphite in citrate buffer (pH4) than in buffer alone. In this case, the germinated conidia in sulphite had significantly fewer ($\underline{P} < 0.001$) hyphae, fewer ($\underline{P} < 0.001$) secondary hyphae and the total hyphal length was significantly smaller ($\underline{P} < 0.001$) than conidia in buffer.

There was no significant ($\underline{P} < 0.05$) effect of bisulphite on conidial germination or the number of germinated conidia with secondary hyphae in phosphate buffer (pH7), but the number of hyphae and the total hyphal length per conidium were significantly greater ($\underline{P} < 0.01$) in 0.1mM bisulphite than in buffer alone.

There were also significant differences in germination and mycelial growth in the different solutions, regardless of bisulphite.


% germination of conidia
0
10
20
30
40
50
c
d
a
b
c
c
0
0·1
0
0·1
0
0·1 mM SO_3^{2-}
pH 5·6
pH 4
pH 7
Concentration of sulphite and pH of solution in which conidia were suspended


(a)
No. hyphae per conidium
0
1
2
3
4
5
a
b
c
d
a,c
e
0
0·1
0
0·1
0
0·1
mM SO_3^{2-}
pH 4
pH 5·6
pH 7
(b)
% conidia with 2° hyphal branches
0
20
40
60
80
a
b
a
c
a
a
0
0·1
0
0·1
0
0·1
mM SO_3^{2-}
pH 4
pH 5·6
pH 7
Concentration of sulphite and pH of solution in which the conidia were suspended


(c)
Total length of hyphae per conidium ($\mu m \times 10^{-2}$)
0
1
2
3
4
5
a
b
c
d
c
a
0
0·1
0
0·1
0
0·1
mM SO_3^{2-}
pH 4
pH 5·6
pH 7
Concentration of sulphite and pH of solution in which the conidia were suspended

(i) Effect of SO_2 on germination of conidia and initial mycelial growth of *P. herpotrichoides*

Plates of distilled water agar were inoculated with conidia and exposed to 5, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 2 ppb) at 15°c $\pm$ 5°c for 24 h in the chambers at CERL. Conidial germination was assessed on six plates per treatment directly after fumigation and initial mycelial growth was assessed on six more plates after 18 h incubation at 15°c. The plates were flooded with 2-3 ml acid fuchsin before assessments.

Significantly fewer ($P < 0.001$) conidia germinated at 40 or 60 ppb SO_2, and fewer ($P < 0.001$) conidia germinated at 60 than 40 ppb but there was no difference in germination at 5 or 20 ppb SO_2 (Fig 25 Appendix 20). The germinated conidia exposed to 40 or 60 ppb had fewer hyphae, fewer secondary hyphae and less hyphal growth than those exposed to 5 or 20 ppb. There were smaller (but significant) differences between number of hyphae at 40 and 60 ppb, and the percentage of conidia with secondary hyphae at 5 and 20 ppb (Fig 27 Appendix 20).

A parallel experiment investigated the effect of SO_2 absorbed onto agar on conidial germination and initial mycelial growth. Uninoculated plates of distilled water agar were exposed in the chambers at the same time as the inoculated plates above. After exposure the plates were removed and two of them were flooded with universal indicator solution. The remaining plates were inoculated, incubated at 15°c and the conidial germination was assessed after 24 h and initial mycelial growth after 42 h as described above.

A similar pattern of response to SO_2 was observed for conidia germinating on exposed agar plates as for conidia directly exposed

to SO_2 in air (Figs 26 and 28 and Appendix 21), except that the percentage of conidia having secondary hyphae and hyphal growth were both significantly ($P < 0.05$) less on plates previously exposed to 60 ppb than to 40 ppb and there was no significant ($P < 0.05$) difference in percentage of conidia with secondary hyphae on plates exposed to 5 and 20 ppb SO_2.

No differences in pH of agar plates after exposure to different SO_2 concentrations was detected, although the indicator was only accurate to $\pm$ 1 pH unit.

(ii) Effect of SO_2 on the growth of vegetative mycelium

A series of experiments in the chambers at Silwood Park investigated the effects on subsequent growth of exposing mycelial disks of *P. herpotrichoides* to SO_2. There were eight experiments in each of which disks were exposed for 3 h either to SO_2 or air filtered through charcoal. The concentrations of SO_2 were high and generally above those normally encountered in rural areas, viz:

Experiment	SO_2 (ppb)
1	53
2	100
3	130
4	150
5	154
6	425
7	700
8	720

Disks were then transferred to plates of PDA containing streptomycin (5.4mg ml^{-1}) which were incubated at 15°c. Colony diameters were measured at intervals to 35 days after fumigation.

Only transitorily in one experiment (Experiment 5) was any

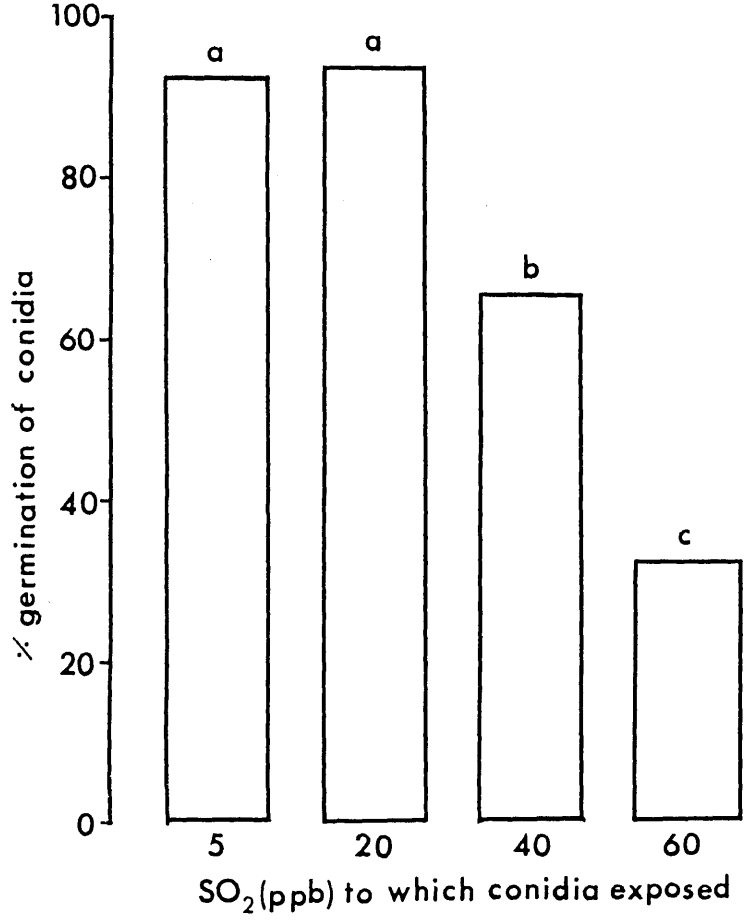


Fig 25: Germination of P. herpotrichoides conidia on plates of distilled water agar after 24h exposure to different concentrations of sulphur dioxide.

Germination assessed on 100 conidia chosen at random across each plate.

Values with no common letter are significantly different at $P < 0.001$.

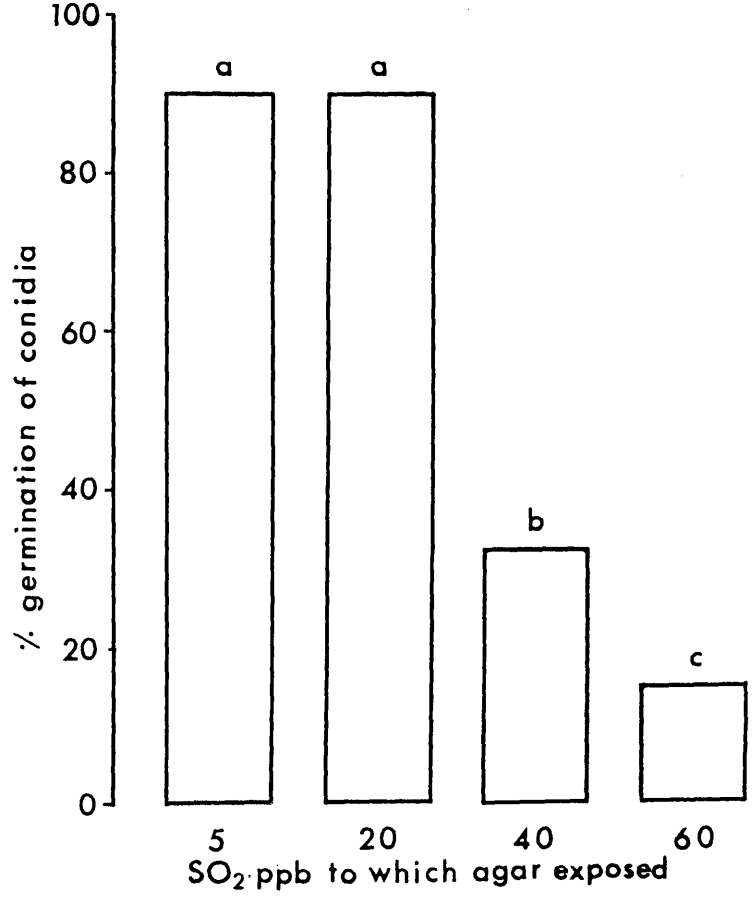
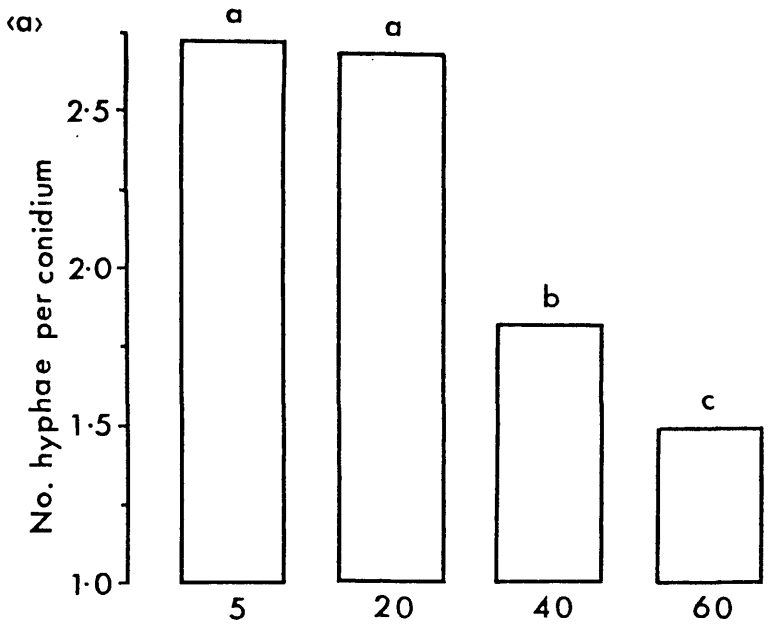
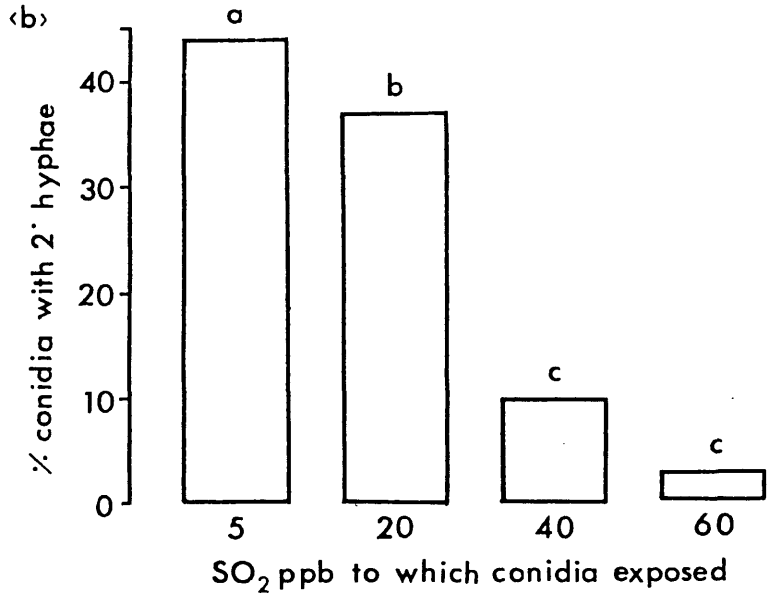
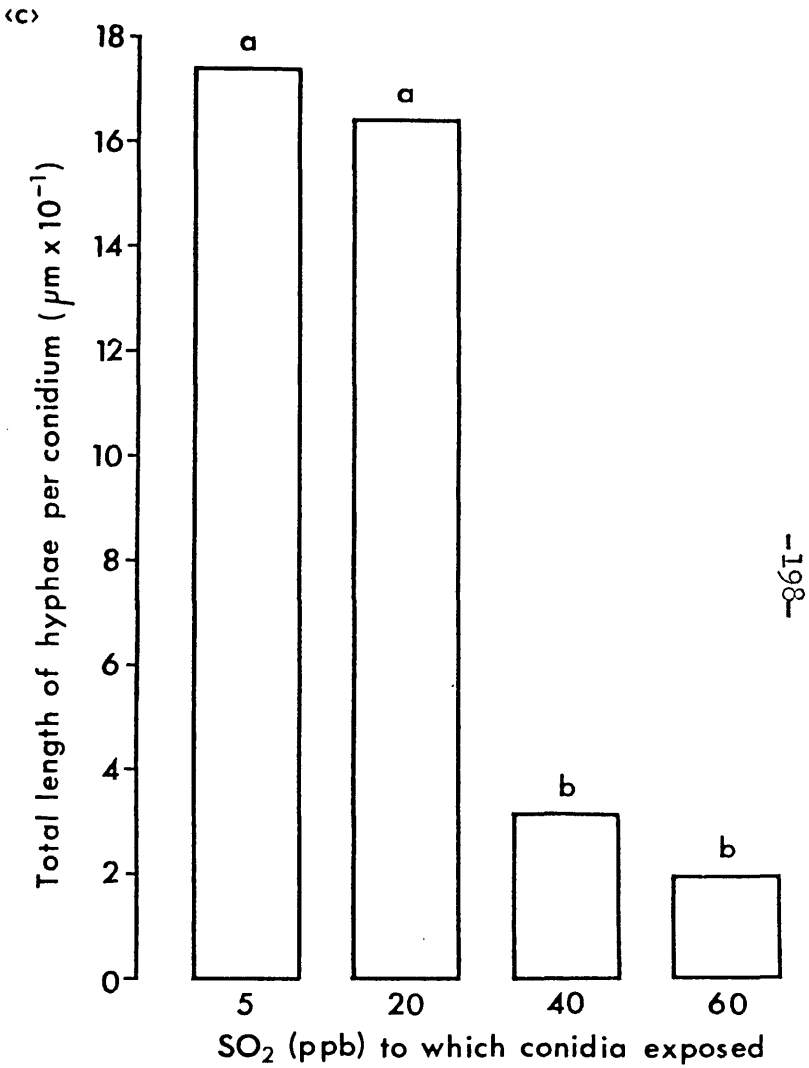


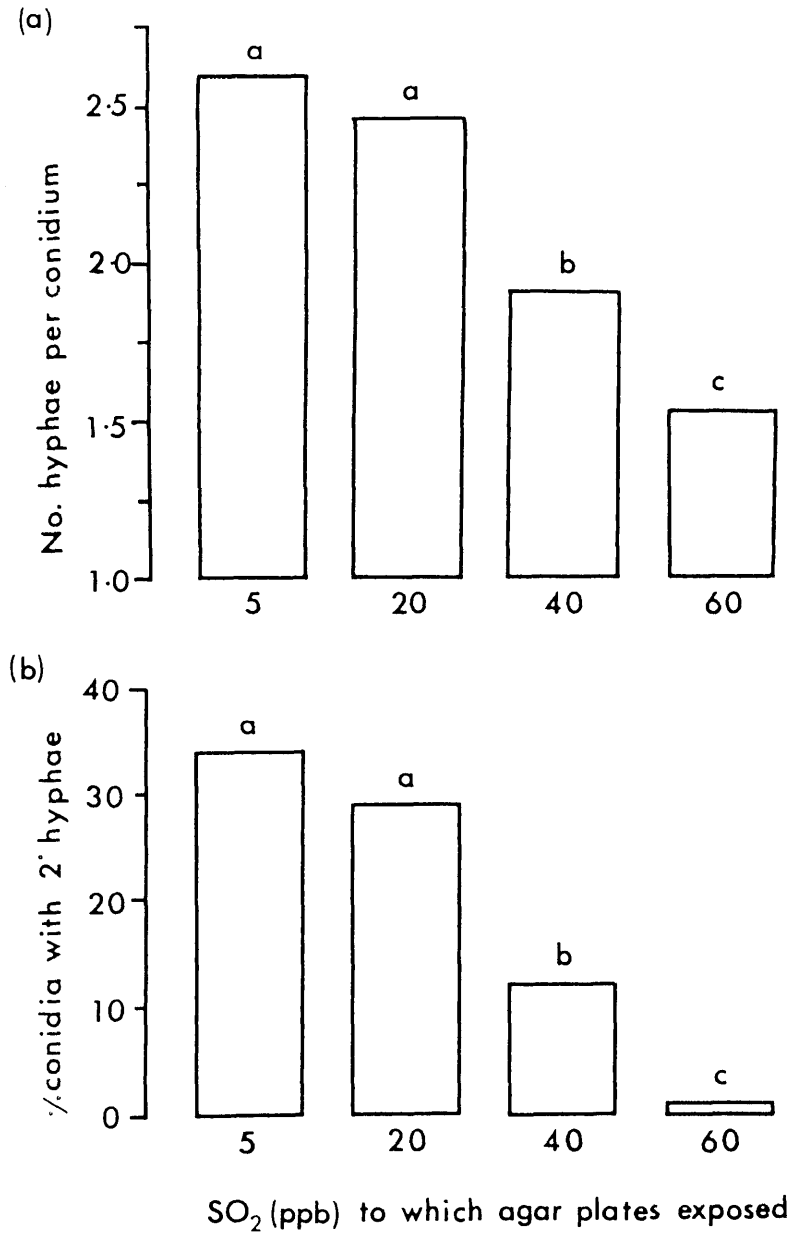
Fig 26: Germination of P. herpotrichoides conidia on plates of distilled water agar previously exposed to different concentrations of sulphur dioxide for 24h before inoculation.

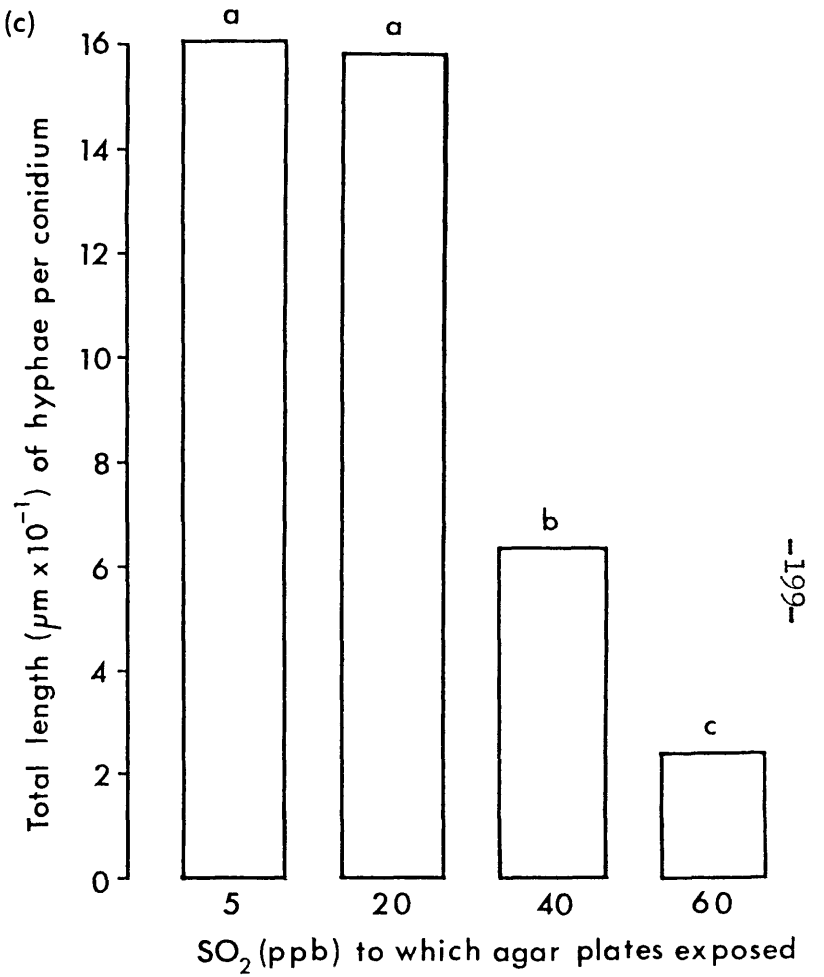
Germination was assessed on 100 conidia after incubation for 24h following exposure.


(a)
No. hyphae per conidium
2·5
2·0
1·5
1·0
a
a
b
c
5
20
40
60


(b)
% conidia with 2° hyphae
40
30
20
10
0
a
b
c
c
5
20
40
60
SO2 ppb to which conidia exposed


(c)
Total length of hyphae per conidium (μm x 10⁻¹)
18
16
14
12
10
8
6
4
2
0
a
a
b
b
5
20
40
60
SO2 (ppb) to which conidia exposed


(a)
No. hyphae per conidium
2·5
2·0
1·5
1·0
a
a
b
c
5
20
40
60
(b)
% conidia with 2° hyphae
40
30
20
10
0
a
a
b
c
5
20
40
60
SO2 (ppb) to which agar plates exposed


(c)
Total length (μm x10⁻¹) of hyphae per conidium
16
14
12
10
8
6
4
2
0
a
a
b
c
5
20
40
60
SO2 (ppb) to which agar plates exposed

significant difference noted in growth from disks exposed to SO_2 and the corresponding control.

In one other experiment the growth of P. herpotrichoides was examined on PDA plates previously exposed for 3 h to 425 ppb SO_2 and compared to growth on plates exposed to filtered air. No significant difference in growth was observed.

The data from these experiments is summarised in Appendix 22 .

(iii) Effects of SO_2 on the susceptibility of barley to eyespot

Seedlings of barley cv. Igri (six pots of 10 seedlings) were exposed to 5, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 3 ppb) in the CERL chambers for 14 d from celeoptile emergence. (7 days after sowing). The mean temperature in the chambers was $12^{o}c \pm 5^{o}c$. After fumigation the plants were removed to the glasshouse and inoculated with conidia of P. herpotrichoides. Disease incidence and severity were assessed 28 days later.

The incidence of disease was similar in all treatments and a minimum of eight plants per pot developed obvious symptoms.

There were no significant differences in disease severity at different SO_2 levels (Table 36 , Appendix 23).

(iv) Effect of SO_2 on the development of eyespot lesions on barley exposed after inoculation

Seedlings of barley cv. Igri were inoculated at the second leaf stage (21 days after sowing) and 48 h later were placed in the chambers at CERL and exposed to 5, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 5 ppb) for 26 days. There were five pots in each chamber, each with 10 seedlings. After fumigation the incidence and severity of eyespot lesions and also the extent of leaf

yellowing were assessed.

There were no significant differences in the incidence or severity of eyespot between the different SO_2 treatments (Table 37 , Appendix 24).

Leaf chlorosis was significantly affected by SO_2 concentration (Table 37 Appendix 24). The incidence of chlorosis on the first leaves was significantly ($\underline{P} < 0.05$) higher on seedlings growing in the SO_2 fumigated chambers than in the control chamber. Incidence of chlorosis on the second leaves was significantly ($\underline{P} < 0.05$) higher at 40 and 60 ppb than at 5 or 20 ppb SO_2. The incidence of leaf chlorosis on the third leaves was low and did not differ significantly with SO_2.

The percentage leaf area chlorotic on the first and second leaves was significantly ($\underline{P} < 0.05$) greater at 60 ppb than at 5, 20 or 40 ppb and also significantly ($\underline{P} < 0.05$) greater at 40 than at 5 or 20 ppb SO_2. On the third leaf the percentage chlorotic leaf area was significantly ($\underline{P} < 0.05$) greater at 60 than at 20 ppb SO_2, with no other significant differences between treatments.

Table 36 : Development of eyespot lesions on seedlings exposed to different concentrations of SO_2 for 14 d before inoculation

Eyespot	Seedlings exposed to SO_2 at			
	5	20	40	60 ppb
Incidence *	9.3	9.5	9.2	9.0
Severity **	76.7	73.1	75.1	75.4

* Mean no. seedings infected with eyespot per pot of 10 seedlings 28 d after inoculation.

** Mean disease severity score, calculated as described in part E, 28 d after inoculation

Table 37 : Development of eyespot lesions and leaf chlorosis on barley during exposure to different concentrations of SO_2 for 26 days.

Parameter assessed	SO_2 (ppb) at which seedlings exposed			
	5	20	40	60
Eyespot				
Incidence *	60	64	62	64
Severity **	51.5	50.7	44.2	50.2
Leaf chlorosis ***				
Incidence				
1st leaf	$5^{(a)}$	$10^{(b)}$	$12^{(b)}$	$12^{(b)}$
2nd leaf	$5^{(a)}$	$8^{(a)}$	$11^{(b)}$	$12^{(b)}$
3rd leaf	$3^{(a)}$	$1^{(a)}$	$2^{(a)}$	$5^{(a)}$
% chlorosis				
1st leaf	$27.5^{(a)}$	$26.7^{(a)}$	$47.5^{(b)}$	$80.8^{(c)}$
2nd leaf	$9.2^{(a)}$	$8.3^{(a)}$	$18.3^{(b)}$	$37.5^{(c)}$
3rd leaf	$2.5^{(a,b)}$	$0.8^{(a)}$	$0.2^{(a,b)}$	$8.3^{(b)}$

* Mean no. seedlings infected with eyespot per pot of 10 seedlings, 28 days after inoculation.

** Mean disease severity score, calculated as described in part E, 28 days after inoculation.

*** Number of seedlings with leaf chlorosis and percentage leaf area chlorotic, assessed on 12 seedlings per treatment chosen at random, 21 days after inoculation. Treatments with no common letter are significantly ($\underline{P} < 0.05$) different.

EXPERIMENTS ON LEAF BLOTCH

Methods

A. Fungal isolates

The isolate of Rhynchosporium secalis (Oud.) Davis used in all experiments was isolated from barley cv. Igri growing in a non-fumigated area of the field at Littlehampton in 1987.

Stock cultures were maintained under glycerol on potato dextrose agar slopes in McCartney bottles stored at 4°c. When required, these were sub-cultured onto lima bean agar in 3cm diameter petri dishes and incubated at 15°c for 14 days.

B. Inoculation of plant material

A suspension of conidia was obtained by flooding a sporulating culture (14 days old) with 5ml sterile distilled water, mashing up the agar with a blunt glass rod then straining through cheese-cloth to remove fragments of mycelium and agar (Caldwell, 1937). The concentration of conidia in the resulting suspension was estimated using a haemocytometer and was adjusted to 10^8 conidia/ml. Then 20ml suspension was transferred to a Universal bottle and a drop of Tween 80 was added. An atomiser (S. Murray & Co Ltd) was screwed onto the bottle and the suspension was sprayed onto 40 seedlings of Igri barley at the third leaf stage (21 days after sowing). The pots containing the plants were stood in water, 1cm deep, in a plastic tray and covered with a polythene frame (50cm x 50cm x 60cm), sprayed with water on the inside to maintain a high relative humidity (80-100%). The polythene frame was removed 48 h after inoculation.

C. Inoculation of agar plates

A drop (0.5ml) of suspension, containing 10^6 conidia/ml was placed on each of several plates of distilled water agar, using a digital micropipette. The drop was spread evenly over the surface of the agar using a glass angle.

D. Disease Assessment

Inoculated plants did not develop scald-like lesions characteristic of field infections of leaf blotch of barley (Melville & Jenkins, 1979). However, small (c.2mm), ovoid necrotic lesions appeared on the top half of the leaves from 10 days after inoculation and were well defined by 14 days after inoculation. The lesions developed consistently on repeated inoculations, when several variations in inoculation technique or incubation conditions were attempted. In order to verify that these lesions were caused by R. secalis, re-isolation of the pathogen was attempted, as follows.

Small pieces of lesions were surface sterilised by dipping in 1% sodium hypochlorite for 30 secs, then washed in sterile distilled water and placed on lima bean agar containing streptomycin (5.4mg/ml). After 7 days at 15°c the culture was examined microscopically. Successful isolations were made from all leaf sections with lesions (except for contaminated plates).

The key used for assessment of leaf blotch in the field (James, 1967) was not appropriate for the small ovoid lesions which developed, but their size, shape and distribution fitted the key for barley brown rust (ADAS, 1976). Lesion severity was assessed using this key, on the first, second and third leaf 14 days after inoculation.

E. Assessment of germination of conidia and germ tube length

Germination of conidia was assessed by examining 100 conidia chosen at random across each replicate plate/slide, and recording the first appearance of a germ tube.

Germ tube length was measured 18 h after inoculation on 10 germinated conidia, chosen at random across each plate/slide, using an eyepiece graticule calibrated against a slide micrometer.

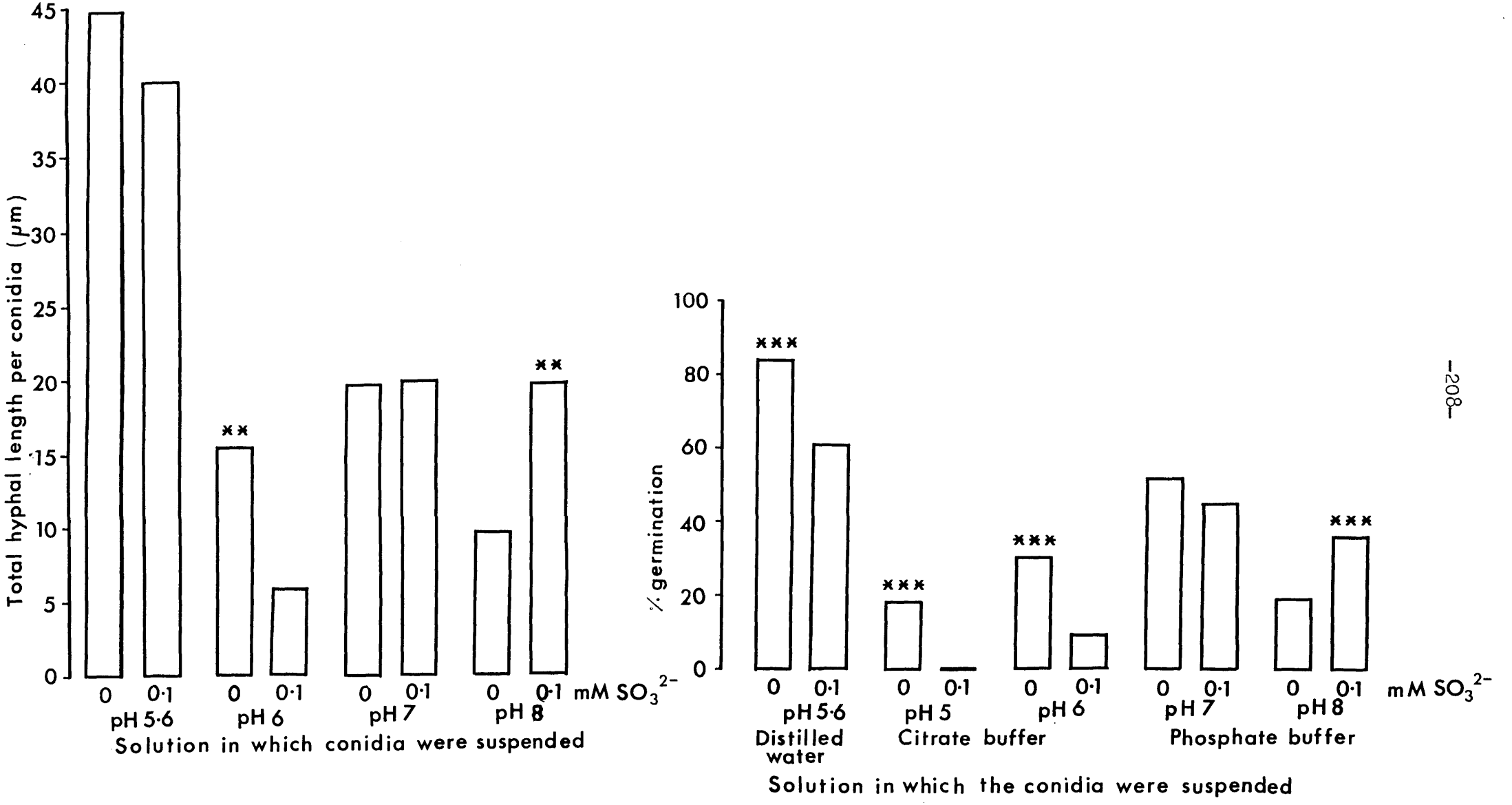
EXPERIMENTAL

A. Effects of bisulphite at different pH levels on germination of conidia and germ tube growth of R. secalis

Suspensions of conidia of Rhynchosporium secalis were prepared in 0.5M citrate buffer adjusted to pH 4, 5 and 6 (sodium citrate + citric acid); 0.5M phosphate buffer adjusted to pH 7 and 8 (KH_2PO_4 + Na_2HPO_4) and in distilled water (pH5.6). Tests for germination and germ tube length in 0 and 0.1m M bisulphite in these solutions were carried out by the method described in Experiments on Eyespot except that 10 slides per treatment were incubated at 20°c for 18 h before assessments.

Conidial germination and germ tube growth was significantly affected ($P < 0.001$) by bisulphite concentration although the response to bisulphite depended on the solution (pH) in which the conidia were suspended (Fig 29 , Appendix 25). All buffers appear to depress germination and germ tube growth in comparison with the water control. Significantly ($P < 0.001$) fewer conidia germinated in distilled water when bisulphite was added, but there was no significant ($P < 0.05$) effect of bisulphite on germ tube growth. Germination was negligible in citrate buffer at pH4, poor at pH5 and 6 and even worse ($P < 0.001$) when bisulphite was added, with germ tube length also reduced ($P < 0.001$) in bisulphite at pH6. In phosphate buffers germination and germ tube growth was unaffected by bisulphite at pH7 and at pH8 germination and germ tube growth was poor but improved ($P < 0.01$) with bisulphite.

From the results the question arose of whether the addition of bisulphite to the solutions altered the pH of solution, thereby compounding any direct effects of bisulphite on conidial germination


Total hyphal length per conidia (μm)
45
40
35
30
25
20
15
10
5
0
**
**
0 0·1 0 0·1 0 0·1 0 0·1 mM SO_3^{2-}
pH 5·6 pH 6 pH 7 pH 8
Solution in which conidia were suspended
% germination
100
80
60
40
20
0
***
***
***
***
0 0·1 0 0·1 0 0·1 0 0·1 0 0·1 mM SO_3^{2-}
pH 5·6 pH 5 pH 6 pH 7 pH 8
Distilled water
Citrate buffer
Phosphate buffer
Solution in which the conidia were suspended

or germ tube growth. In order to test this, the solutions, with or without bisulphite, were prepared as before but containing no conidia. The pH of the solutions was tested using a pH meter accurate to ± 0.1 pH unit (Pye, Model 79). The solutions, in McCartney bottles, were incubated at 20°c for 18 h then the pH was re-tested.

There was no change in pH after addition of bisulphite to any of the citrate buffers at pH4, 5 or 6. However, the addition of bisulphite decreased the pH of both phosphate buffers and of distilled water. Phosphate buffer at pH7 and pH8 were reduced to pH6.8 and pH7.7 respectively 18 h after addition of bisulphite. The pH of distilled water was reduced from pH5.6 to pH4.0.

(i) Effect of SO_2 on the germination and germ tube growth of R. secalis conidia

Plates of distilled water agar inoculated with conidia were exposed to 0, 20, 40 or 60 ppb SO_2 (mean concentration ± 1 ppb) for 24 h in the CERL chambers. The temperature in the chambers was 24°c ± 5°c for 16 h and 14°c ± 5°c for 8 h of the fumigation period.

After fumigation the plates were removed, flooded with 2-3ml acid fuchsin and examined under the microscope. Germination was assessed on 100 conidia and germ tube growth on 10 germinated conidia per plates.

There was no apparent effect of SO_2 on either germination or germ tube growth (Table 38 and Appendix 26).

(ii) Effect of SO_2 on the pathogenicity of R. secalis

Sporulating cultures of R. secalis (14 days old) were exposed to 0, 2), 40 or 60 ppb SO_2 (mean concentration ± 2 ppb) for 24 h in the chambers at CERL (four plates per chamber) at 20°c ± 5°c.

Table 38 : Germination and germ tube growth of Rhynchosporium secalis conidia on water agar after 24 h exposure to different concentrations of sulphur dioxide

(a) Percentage germination of conidia

Replicate *	Concentration of SO_2 (ppb)			
	0	20	40	60
1	73	82	78	88
2	80	87	75	85
3	89	74	82	75
4	67	71	84	81
5	84	80	91	86
6	90	89	77	82
7	72	75	83	89
8	76	70	79	77
9	83	86	83	76
10	85	90	87	79
Mean	79.9	80.4	81.9	89.4

* Germination assessed on 100 conidia per plate.

(b) Germ tube length

	Concentration of SO_2 (ppb)			
	0	20	40	60
Mean *	26.68	32.43	30.53	28.38

* Mean value (Standard Error $\pm$ 2.26) based on 10 germinated conidia per plate.

After fumigation, suspensions of conidia were made from the plates and used to inoculate seedlings of barley cv. Igri at the third leaf stage, in the greenhouse. Disease severity was assessed on the first, second and third leaves 14 days after inoculation.

Disease severity was similar in all SO_2 treatments on all leaves. (Table 39, Appendix 27).

(iii) Effect of SO_2 on the susceptibility of barley to leaf blotch

Seedlings of barley cv. Igri were exposed to 2, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 4 ppb) for 14 days in the CERL chambers from coleoptile emergence until third leaf stage. The temperature in the chambers was $26^{o}c \pm 12^{o}c$.

After fumigation the plants were removed to the glasshouse, inoculated with R. secalis conidia, and disease severity on the first, second and third leaf was assessed 14 days later.

Disease severity on all leaves was not significantly ($P < 0.05$) different between the various SO_2 treatments. (Table 40 , Appendix 28).

(iv) Effect of SO_2 on lesion development in plants exposed after inoculation with R. secalis

The seedlings of barley cv. Igri at third leaf stage were inoculated with R. secalis conidia then exposed to 2, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 4 ppb) for 14 days in the chambers at CERL at $26^{o}c \pm 12^{o}c$. Disease severity was assessed on the first, second and third leaves directly after fumigation.

There was no significant ($P < 0.05$) effect of SO_2 on the percentage area of the first leaves affected by lesions (Table 41, Appendix 29). On both the second and third leaves, however, the

Table 39 : Percentage leaf area affected by lesions 14 days after inoculation of Rhynchosporium secalis conidia exposed for 24 h previously to different concentrations of sulphur dioxide.

Leaf position *	SO_2 (ppb) to which conidia exposed			
	0	20	40	60
First	5.00	7.10	5.40	3.95
Second	3.25	4.50	3.30	3.00
Third	3.20	6.35	4.05	3.70

* Mean percentage values based on the first, second and third leaves of 20 seedlings.

Table 40 : Percentage leaf area affected by leaf blotch on seedlings exposed to different concentrations of sulphur dioxide for 14 days before inoculation

Leaf position *	SO_2 (ppb) to which seedlings exposed			
	2	20	40	60
First	6.55	4.20	5.55	5.20
Second	7.05	6.00	5.75	5.10
Third	9.70	8.55	8.85	12.20

* Mean percentage values based on the first, second and third leaves of 20 seedlings assessed 14 days after inoculation.

Table 41: Percentage leaf area of first leaf affected by leaf blotch on seedlings exposed to different concentrations of sulphur dioxide for 14 days after inoculation

Pot	Seedling *	SO_2 (ppb) to which seedlings exposed			
		2	20	40	60
1	1	5	5	2	5
	2	2	5	10	2
	3	5	10	5	1
	4	1	10	10	2
	5	5	5	2	2
2	1	10	20	2	2
	2	15	2	2	10
	3	10	10	2	15
	4	10	10	2	10
	5	10	10	2	10
3	1	5	10	1	10
	2	25	20	10	5
	3	10	2	5	2
	4	10	10	10	10
	5	5	10	5	2
4	1	10	2	2	5
	2	10	2	10	2
	3	10	2	5	1
	4	20	5	5	2
	5	2	5	2	2
Mean		8.30	7.75	4.70	5.00

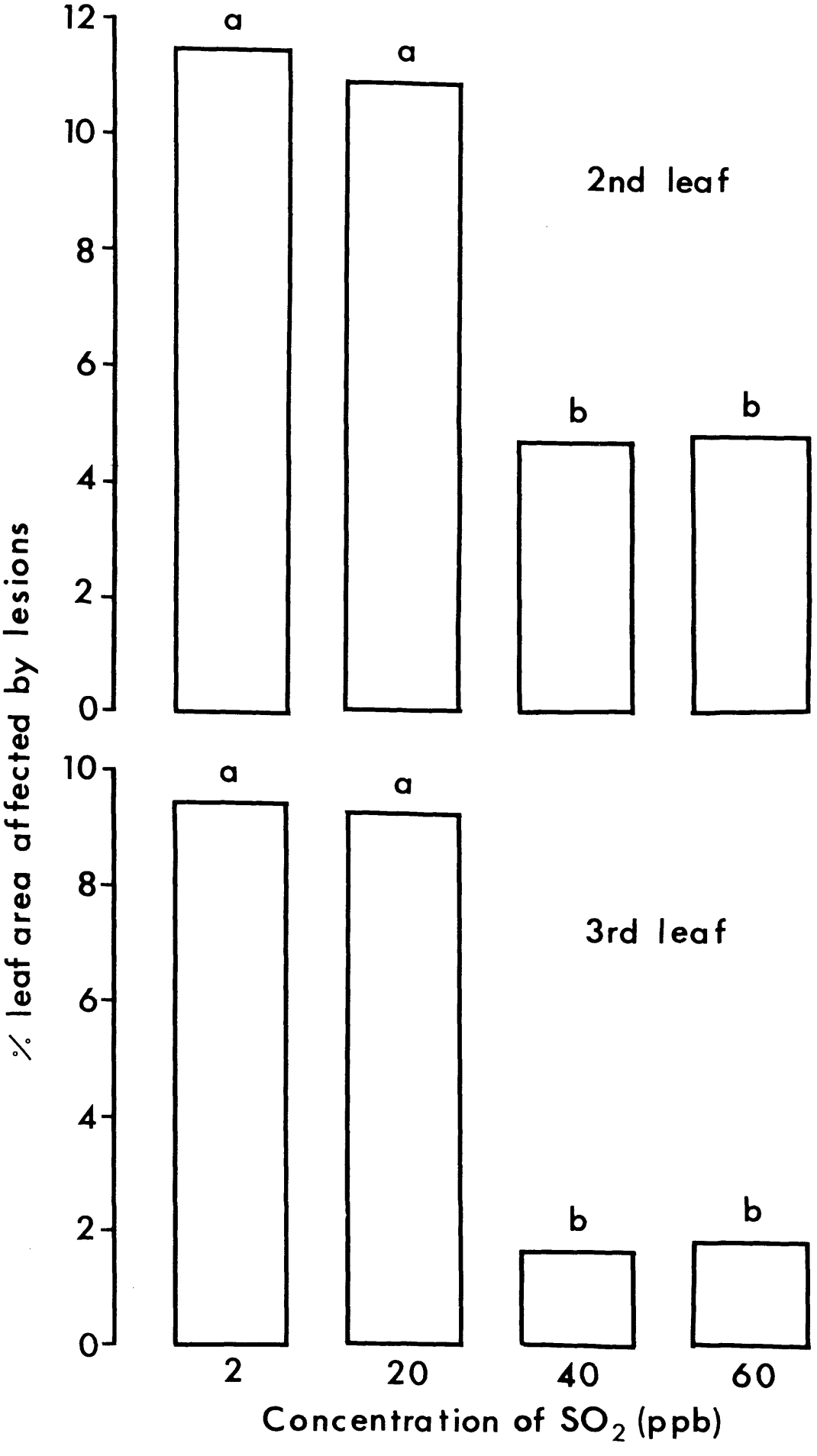
* Assessments 14 days after inoculation.

percentage leaf area affected by lesions was significantly reduced ($\underline{P} < 0.01$) on seedlings previously exposed to 40 or 60 ppb SO_2 compared with those exposed to 2 or 20 ppb SO_2 (Fig 30 , Appendix 29).

(v) Effect of SO_2 on lesion development in plants exposed before and after inoculation with R. secalis

Seedlings of barley cv. Igri were exposed to 2, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 5 ppb) for 14 days in the CERL chambers. The plants were removed to the glasshouse and inoculated with conidia of R. secalis. After 48 h the plants were replaced in the same chambers and exposed to 2, 20, 40 or 60 ppb SO_2 (mean concentration $\pm$ 4 ppb) for a further 14 days. Chamber temperatures were $23^{o}c \pm 11^{o}c$. Disease severity was assessed directly after fumigation.

The percentage area of the first leaf affected by leaf blotch was significantly ($\underline{P} < 0.01$) reduced on seedlings exposed to 20, 40 or 60 ppb compared with those exposed to 2 ppb SO_2, and significantly reduced ($\underline{P} < 0.05$) at 60 ppb compared with 20 or 40 ppb SO_2. (Fig 31 , Appendix 30). There were no significant ($\underline{P} < 0.05$) differences in disease severity on the second leaf (Table 42 , Appendix 30). The percentage area of the third leaf affected by leaf blotch was significantly ($\underline{P} < 0.001$) reduced on seedlings exposed to 20, 40 or 60 ppb compared with those exposed to 2 ppb SO_2 (Fig 31,Appendix 30).


12
10
8
6
4
2
0
a
a
b
b
2nd leaf
% leaf area affected by lesions
10
8
6
4
2
0
a
a
b
b
3rd leaf
2
20
40
60
Concentration of SO_2 (ppb)

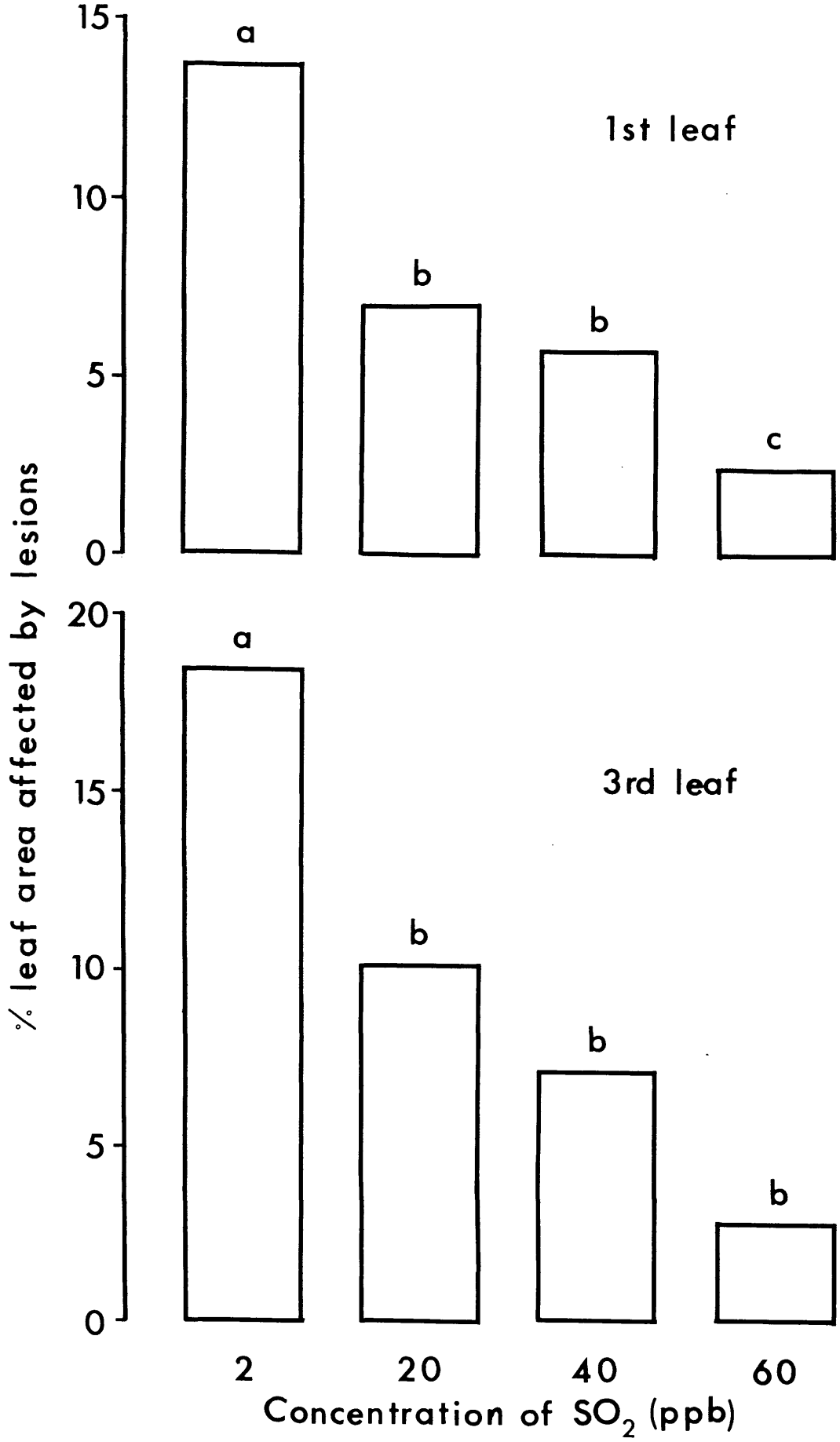

15
10
5
0
a
b
b
c
1st leaf
20
15
10
5
0
a
b
b
b
3rd leaf
% leaf area affected by lesions
2
20
40
60
Concentration of SO_2 (ppb)

Table 42 : Percentage area of second leaf affected by leaf blotch on seedlings exposed to different concentrations of sulphur dioxide for 14 days before and 14 days after inoculation.

Pot	Seedling *	SO_2 (ppb) to which seedlings exposed			
		2	20	40	60
1	1	30	25	10	2
	2	5	5	5	10
	3	5	20	5	10
	4	20	20	10	10
	5	15	30	2	5
2	1	15	20	15	10
	2	30	15	20	5
	3	50	30	20	10
	4	30	10	5	10
	5	10	25	5	15
3	1	10	5	10	15
	2	10	10	25	2
	3	10	10	2	10
	4	50	10	2	5
	5	25	15	15	15
4	1	30	25	25	10
	2	15	10	20	2
	3	10	10	20	10
	4	20	5	30	10
	5	30	2	30	5
Mean		21	15.1	13.8	8.55

* Assessments 14 days after inoculation.

Table 43 SUMMARY OF RESULTS FROM SECTION II EXPERIMENTS IN FUMIGATION CHAMBERS AND IN VITRO

EXPERIMENT	POWDERY MILDEW	EYESPOT	LEAF BLOTCH
A: Effects of 0.1 m M sulphite on*		pH 4 pH 5.6 pH 7	pH: 4 5 5.6 6 7 8
i. Germination of conidia		- - NS	NS - - - NS +
ii. Initial mycelial growth (No. hyphae		- + +	
(No. 2° hyphae		- + NS	
(Hyphal length		- + +	NS - NS +
B: Effects of SO_2 on**	0-5 20 40 60 ppb	0-5 20 40 60 ppb	0-5 20 40 60
i. Germination of conidia***	---------NS---------	3 3 2 1	------NS-------
ii. Initial mycelial growth (No. hyphae		3 3 2 1	
(No. 2° hyphae		3 2 1 1	
(Hyphal length		2 2 1 1	------NS-------
iii. Viability of pre-fumigated conidia****	1 2 2 2		
iv. Pathogenicity of pre-fumigated conidia (No. pustules****	---------NS---------		
(Severity	1 1 2 2		------NS-------
(Sporulation	1 2 3 4		
v. Susceptibility of pre-fumigated plants (No. pustules	---------NS---------		
(Severity	---------NS---------	--------NS---------	------NS-------
(Sporulation	2 3 3 1		
vi. Disease development (post-inoculation fumigation) (No. pustules	---------NS---------		(1st leaf ------NS-------
(Severity	---------NS---------	--------NS---------	(2nd leaf 2 2 1 1
(Sporulation	1 1,2 2 3		(3rd leaf 2 2 1 1
vii. Disease development (pre- and post-inoculation fumigation) (No. pustules	---------NS---------		(1st leaf 3 2 2 1
(Severity	---------NS---------		(2nd leaf ------NS-------
(Sporulation	1 2 3 1		(3rd leaf 2 1 1 1

* Increase in parameter in sulphite treatment represented by +, decrease by -, NS = no significant difference.

** SO_2 treatments with no common number are significantly ($P < 0.05$) different, a higher number indicates a higher value of parameter assessed.

*** Conidia fumigated during germination.

**** " " " formation on conidiophores.

DISCUSSION

The experiments described in this section investigated in more detail the effects of SO_2 (at 20, 40 and 60ppb) on three diseases which had shown sensitivity in the field - powdery mildew (*E. graminis*), eyespot (*P. herpotrichoides*) and leaf blotch (*R. secalis*). In particular they examined, under controlled conditions in fumigation chambers, three ways in which SO_2 might affect disease:

1. By directly affecting the pathogen
2. By altering host susceptibility
3. By influencing the pathogen/host interaction

1. Effects on the pathogen

(a) Germination of conidia

(i) Gaseous SO_2

Neither of the foliar pathogens, *E. graminis* and *R. secalis*, was sensitive to SO_2 concentration (0-60ppb) during conidial germination whereas both germination and initial mycelial growth of *P. herpotrichodes* were inhibited above 40ppb SO_2. When incubated on agar which had already been exposed to SO_2, the inhibition of germination was even more marked. Absorption of SO_2 into agar may lead to the formation of sulphurous acid, sulphate, bisulphite or sulphite ions, making the agar more acid which itself may inhibit germination (McCallan & Weedon,1940, Vas & Ingram, 1949). However, no change in the pH of the agar was detected after fumigation so this cannot explain the greater inhibition observed here. It seems more likely that as sulphite rapidly oxidises to sulphate, that accumulation of sulphate in pre-fumigated plates accounted for the greater inhibition of germination.

However, this does not explain why germination of *P. herpotrichoides* was sensitive to SO_2 concentration and germination of *E. graminis* and

R. secalis apparently was not. One possible explanation is that SO_2 was absorbed to different extents by the three types of conidia. There is, for example, evidence that large quantities of lipids in the cell walls of powdery mildew conidia may limit their permeability to water and various toxicants such as copper sulphate (Yarwood, 1945; Johnson et al., 1976). In particular Gay et al., (1985) showed that the conidia of E. pisi could germinate on a variety of poisons. This they attributed to a non-specific permeation barrier at or outside the plasmalemma of conidia and germ tubes. Such a mechanism could account for the relative insensitivity to SO_2 of E. graminis conidia observed here and that reported to ozone by Schuette (1971) and by Heagle & Strickland (1972).

There may also be differences in the composition of the conidial cell walls of P. herpotrichoides and R. secalis though these are not documented. Alternatively, the larger surface area of the filiform conidia of P. herpotrichoides may have allowed a greater absorption than in the smaller conidia of R. secalis.

Although environmental conditions such as temperature and relative humidity have been shown to affect the sensitivity of some fungal spores to SO_2 (Couey & Uota, 1961; Couey, 1965), it seems unlikely that variation in these factors within the experiments was sufficiently great to explain the marked differences in sensitivity.

(ii) Sulphite

Both P. herpotrichoides and R. secalis are spread primarily by rain splash and germinate readily in free water (Skoropad, 1957; Schrödter & Fehrmann, 1971). Thus the concentration of bisulphite in water on the plant surface, which is correlated with atmospheric SO_2 (Davies, 1979), may be more relevant in considering effects on conidial germination.

There were apparently marked effects on germination and germ tube growth of both fungi when bisulphite (0.1mM HSO_3^-) was added to condial suspensions. However, there are problems in interpreting these results because the response depended on the solution in which the conidia were suspended. For example, germination of R. secalis was inhibited by adding bisulphite to suspensions in citrate buffers at pH5 and 6 and in distilled water (pH 5.6) but stimulated in phosphate buffers at pH8 (Fig 29.). The first problem is to separate the effect of the buffer itself from that of pH. Over 80% of conidia germinated in distilled water (pH 5.6), with no bisulphite added, compared with less that 20% and about 30% in unamended citrate buffer at pH 5 and 6 respectively. It seems highly unlikely that shifts in pH itself of the order of $\pm$ 0.5 would have such dramatic effects but much more likely that germination in citrate buffer is relatively poor. Whether phosphate buffer itself affects germination cannot be determined directly from the results but a percentage germination of about 50 in unamended buffer at pH7 suggests that it might.

The next problem is to consider effects of pH within the context of the buffer used. In citrate buffer at pH 6 addition of sulphite reduced germination by 68.5%. This result is consistent with the observed trend that inhibition by SO_2 in solution is greater as pH decreases (Cruess et al., 1931; Saunders, 1966; Hill, 1971; Turk & Würth, 1975, Marti, 1983), an effect related to the pH dependance of the rate of HSO_3^- oxidation and the differential toxicities of the oxidation states (Vas & Ingram, 1949; Gilbert, 1970; Cape, 1984). However, even this result must be treated with caution because sulphite added to water at pH 5.6 reduced germination only by 26.9% emphasizing the confounding of pH and buffer effects.

The reason for the increase in germination when sulphite was added to phosphate buffer at pH8 remains a matter of speculation. It did, however, shift the pH to 7.5 and this in itself might have favoured germination.

(b) Viability and infectivity of conidia formed during exposure to SO_2

Conidia of E. graminis exposed to SO_2 during their formation germinated better and grew better on leaves than untreated conidia. In the latter instance the effect seemed to be one of increased vigour since the area of leaf covered was affected not the initial number of pustules. Correspondingly, therefore, more conidia were produced from these infections than from those initiated by untreated conidia. The reason for this increased vigour is not known but it could be that the incorporation of sulphur from the SO_2 (before wall formation was complete) either affected amino-acid and protein synthesis or activated enzyme systems associated with the processes of germination.

Certainly these effects require further investigation because other work with powdery mildews, though with high concentrations of SO_2, has indicated either inhibitory effects or lack of response. For example, Hibben & Taylor (1975) found that the germination of Microsphaera alni conidia exposed during formation to 300-400 ppb was unaffected. In contrast to this Krieg & Knösel (1983) obtained a 50% reduction in germination of E. graminis conidia exposed during formation to 936 ppb SO_2. Lower amounts of SO_2 (187 or 562 ppb) had no effect.

Powdery mildew conidia have also been shown to be sensitive to ozone during their formation (Heagle & Strickland, 1977).

In the case of plants inoculated with conidia of R secalis the symptoms which developed were slight and atypical of the disease

symptoms seen in the field. The results should be confirmed on plants developing typical lesions before drawing conclusions on the infectivity of the conidia.

2. Effects on host susceptibility

Seedlings fumigated with SO_2 before inoculation were no more susceptible than the control to infection by R. secalis or P. herpotrichoides. However, there were some indications that E. graminis grew better on leaves pre-fumigated with 20 or 40 ppb SO_2 than the control but worse on plants pre-fumigated with 60 ppb SO_2. More pustules developed after 20 or 40 ppb SO_2, covering more of the leaf surface than in other treatments and producing more conidia. However, only in terms of conidia produced were the differences significant ($P < 0.05$). Nevertheless, this still implies that SO_2 at these concentrations has altered the plant in some way and made it more suitable for mildew growth, at least as measured in terms of sporulation.

It is known that SO_2 can alter the chemical composition of a plant (Wellburn et al., 1976; Hughes & Laurence, 1983). For example, Koziol & Jordan (1978) found that total amounts of free sugars and starch were increased in SO_2 fumigated plants; Prasad & Rao (1981) showed that the carbohydrate content of wheat was increased by low levels of SO_2; Godzik & Linskens (1974) demonstrated an increase in amino acid content and a decrease in protein content of peas exposed to SO_2. Such changes could affect disease development. Indeed, the amount of field infection by Erysiphe cruciferarum appears to be linked with changes in total soluble amino acids and carbohydrates in Brussels sprouts (Rudgard, 1982).

Other mechanisms are possible such as alterations in the epicuticular wax or suppression of other micro-organisms on the leaf surface which

might limit mildew establishment or growth. However, such mechanisms might be expected to be more clearly evidenced by reductions of colonies than was seen in these experiments.

3. Effects on the pathogen/host interaction (disease)

Eyespot lesion development was apparently unaffected by SO_2 concentration, in seedlings exposed from after establishment of infection up to symptom expression. Both mildew and rhynchosporium appeared to be sensitive to SO_2 during disease development but their responses differed. Although there were no significant differences in the number of pustules or the leaf area affected by mildew, the number of conidia produced by the pustules increased significantly ($P < 0.05$) with increasing SO_2 concentration. In contrast, rhynchosporium severity was reduced above 40 ppb SO_2.

Any effect of SO_2 on disease development during fungal colonisation of the host cells would be due to changes in host physiology or biochemistry rather than direct effects of SO_2 on the pathogen. As discussed earlier, SO_2 can alter the nutrient composition of a plant and there is also evidence that SO_2 can induce the production of antimicrobial substances. For example peroxidase activity in pea seedlings was elevated after exposure to SO_2 (Horsman & Wellburn, 1975), which with concominant increases in phenols and isoflavanoids has implications for plant disease resistance (Curtis et al., 1976). This is one possible explanation for the reduction in rhynchosporium severity observed at high SO_2 levels.

The reason for the different responses of the three pathogens to SO_2 during this stage of the disease remains unknown, although there are marked differences in their growth in or on host tissue. In particular, powdery mildew is more ectoparasitic in nature than

either of the other pathogens. *E. graminis* would be most exposed to SO_2 during spore formation and the effect of SO_2 on sporulation, observed in this experiment, may be a direct one on the fungus, as seen in the earlier experiments, leading to an increase in the number of conidia produced.

An additional effect of SO_2 was observed during the experiment on eyespot. During fumigation with SO_2 (post-inoculation with *P. herpotrichoides*) the older leaves began to yellow and the extent of the yellowing increased significantly ($P < 0.05$) with increasing SO_2 concentrations. This could have indirect effects on disease development.

The next experiments on mildew and rhynchosporium combined a pre-inoculation SO_2 treatment with a post-establishment treatment. In the case of rhynchosporium, disease severity was significantly reduced on the first and third leaves of plants exposed to more than 20 ppb SO_2, compared with controls. Since in plants in the previous experiment which had only been exposed pre-inoculation the effect of SO_2 was only significant above 40 ppb, this suggests that there may be a cumulative effect of SO_2 on the host which only becomes apparent after longer fumigations.

The response of mildew was also similar to that observed for seedlings which were fumigated with SO_2 pre-inoculation only: pustule number and severity were unaffected, whilst more conidia were produced in the 20 and 40 ppb treatment. There appear to be different mechanisms by which SO_2 affects disease development in plants exposed before and those exposed after inoculation of the pathogen, since on plants only exposed *after* establishment, sporulation was increased at 60 ppb SO_2. The fact that this effect was not apparent when plants

were exposed both before and after inoculation indicates that the mechanism operating before inoculation has an over-riding effect of disease development.

Conclusions

These experiments show that the three pathogens (E. graminis, R. secalis and P. herpotrichoides) and the diseases induced by them in barley c. Igri, respond differently to SO_2 and are sensitive at different stages in the disease cycle.

Powdery mildew appears to be insensitive during spore germination when exposed directly to SO_2 but sensitive during spore formation leading to an increase in viability and infectivity of spores at higher SO_2 concentrations up to 60 ppb. SO_2 also apparently affected conidial development via changes in host susceptibility. Conidial production consistently increased up to 40 ppb SO_2.

Eyespot responded very differently. Only direct effects of SO_2 and sulphite on the pathogen were significant. Spore germination and germ tube growth were both reduced by SO_2 and by sulphite at pH4 but sulphite in distilled water increased germ tube growth. No significant effects of SO_2 on host susceptibility or on lesion development after infection had been established were observed.

Conidia of Rhynchosporium secalis did not show sensitivity to SO_2 during germination or formation. However, sulphite solutions below pH6 decreased germination and germ tube growth but sulphite at pH8 increased these parameters. No marked effects of SO_2 on host susceptibility to rhynchosporium were demonstrated but high SO_2 concentrations after establishment of infection resulted in reduced disease severity.

GENERAL DISCUSSION

The research described in this thesis has taken two basic approaches in examining the interactions between atmospheric sulphur dioxide and fungal pathogens of winter barley. The experiments described in Section I aimed to assess the effects of a range of SO_2 concentrations on the development of diseases in field crops of winter barley cv. Igri, while those in Section II examined some of the pathways by which SO_2 could affect disease caused by Erysiphe graminis, Pseudocercosporella herpotrichoides and Rhynchosporium secalis, with a view to interpreting the field observations.

Environmental conditions in the field and fumigation chamber differ in many respects. In the field the crop would be exposed to fluctuating concentrations of SO_2 and other pollutants, while in the chambers the SO_2 concentration remained constant and ozone and NOx would have been removed from the air by the carbon filter. The distribution of various other components of the ecosystem such as other pathogens and saprophytes, insects and weeds is restricted in chambers. The wind, light and humidity are likely to differ. In the chamber experiments only young barley plants were tested, whereas in the field the main disease epidemics affected plants after stem extension. Despite these differences, the pattern of the responses of mildew, leaf blotch and eyespot to SO_2 in the chambers was remarkably similar to their responses to SO_2 in the field. As has been discussed previously, changes in factors such as crop growth, leaf saprophyte populations and aphid populations induced by SO_2 may modify disease development. However, the experiments on powdery

mildew and leaf blotch show that the consistent effects of SO_2 on development of these diseases in the field may be accounted for by the direct effect of SO_2 on the host and pathogen.

In order to relate the results of the experiments to the influence of SO_2 on barley crops growing in the UK, the concentrations of SO_2 used in the experiments will first be compared with those measured in rural UK.

The annual mean SO_2 concentration in most cereal growing areas of the UK is 4-8 ppb and the highest levels have been recorded in the Midlands (15-25 ppb) by the Warren Spring Laboratory (1986). The low SO_2 treatments (14-21 ppb) in the field experiments are therefore within the range of annual mean SO_2 concentrations that would occur in cereal growing areas of the UK but the medium SO_2 (28-32 ppb) and high SO_2 (38-48 ppb) treatments exceed this range, except possibly in the vicinity of a limited number of low level pollution sources. The SO_2 concentrations used in the fumigation chamber experiments (0-5, 20, 40 and 60 ppb SO_2) were initially chosen to reflect the SO_2 concentrations expected in the field experiments but the actual SO_2 levels achieved in the field (5-48 ppb) did not always match these. This was because the automatic shut down of gas release in the open-air fumigation system was triggered more frequently than had been anticipated, making the mean seasonal SO_2 concentration lower than expected. The highest SO_2 concentration used in the fumigation chamber experiments (60 ppb) was therefore higher than the mean seasonal SO_2 concentrations measured in any treatment plots in the field experiment, although this concentration was reached in all plots for short periods of time. However, the concentrations used in the fumigation chamber experiments for the control (0-5 ppb), low (20 ppb) and medium (40 ppb) SO_2 treatments lie within the range of the mean

seasonal SO_2 concentrations measured in the field in the control (5-9 ppb), low SO_2 (14-21 ppb) and high SO_2 (38-48 ppb) treatments of the field experiments.

In the experiments on P. herpotrichoides and R. secalis described in Section II, the effect of bisulphite was investigated as well as gaseous SO_2. Dry deposited sulphur dioxide is almost instantly solubilised and oxidised to sulphite on wet plant surfaces, while SO_2 in rain is already oxidised to dissolved sulphite (Marti, 1983). Sulphite in rainwater is readily oxidised to sulphate (Martin & Barber, 1978, Garsed, 1981). Measurements of sulphite in rainwater by Meurrens (1974) found concentrations to vary within the range 0.8-1.4 µg/ml (0.001-0.018mM) SO_3^{2-}, and he predicted that at an atmospheric SO_2 concentration of 50 ppb and rain pH5, sulphite concentration in the rain would be about 1 µg/ml (0.013mM). Davies (1979) measured sulphite and sulphate concentrations in rainfall in rural parts of Norfolk and found mean concentrations to be 0.3 µg/ml (0.004mM) and 2.3 µg/ml (0.03mM), respectively, with an atmospheric SO_2 concentration of 9 ppb and rain pH 4.7. The concentration of bisulphite (0.1mM) used in the experiments on conidial germination of Rhynchosporium secalis and Pseudocercosporella herpotrichoides was therefore at least tenfold higher than would be expected in rain. However, it is possible that sulphite concentration on the leaf surface would be higher than in rainwater since atmospheric SO_2 would be continually solubilised into a small quantity of water until saturation point was reached but this has not been tested.

The results of the field experiments showed that fungal disease development in field crops of winter barley was significantly influenced by the concentration of SO_2 in the air above the crop, within the range 5-48 ppb annual mean, in all three seasons.

Seasonal crop growth was also affected by SO_2 but final grain yield was unaffected in two seasons and in the third season appeared to be reduced by 28 ppb SO_2 compared with 5 ppb SO_2 (annual means). Various responses to elevated SO_2 levels were exhibited by different fungal pathogens ranging from stimulation to inhibition to insensitivity. The more detailed experiments on the diseases described in Section II went some way towards explaining these effects.

McCune et al., (1973) proposed three possible ways in which pollutants could alter pathogen success:

1. A direct effect on the pathogen
2. An indirect effect on the pathogen through modification of the physiology or biochemistry of the host
3. An alteration in the suitability of the hosts organs as habitats of the pathogens (such as fewer infection sites)

The experiments described in Section II showed that the three pathogens examined (E. graminis, R. secalis and P. herpotrichoides) and the diseases induced by them in barley cv. Igri respond differently to SO_2 and were sensitive at different stages in the disease cycle. The responses were generally in accord with the responses observed in the field.

Powdery mildew (*Erysiphe graminis*)

In both the field and chamber experiments there was a general trend for powdery mildew infection to increase with increasing SO_2 levels.

A greater proportion of tillers growing in the field plots exposed to annual mean SO_2 concentrations of 14-21 ppb became infected with powdery mildew than in those exposed to 5-9 ppb during the summers of 1985, 1986 and 1987. This gives strong evidence that crops growing in areas of the United Kingdom with high SO_2 levels, such as the Midlands, would be more likely to have higher levels of powdery mildew than in less polluted areas. In a survey of winter barley diseases carried out in July 1987 (Ministry of Agriculture, Fisheries and Food, 1987) a higher percentage of crops in the Midlands were affected by powdery mildew than any other region in England and Wales, with 92% crops affected. Geographical distribution of disease is determined largely by weather conditions, cropping levels (which will affect inoculum density) and choice of variety. Factors known to be important in influencing mildew development in barley include sowing date (Last, 1957), temperature (Last 1963), relative humidity (Manners & Hossain, 1963) and fertiliser application (Last, 1962a). The results of the field experiments suggest that atmospheric sulphur dioxide levels could also influence the distribution of barley powdery mildew, a factor which has been hitherto overlooked.

Results of the fumigation chamber experiments showed that conidia of *E. graminis* exposed to 20, 40 or 60 ppb SO_2 during formation were more likely to germinate than those exposed to 0-5 ppb SO_2. For obligate parasites such as powdery mildews, conidial germination and primary infection are crucial in determining whether host

colonisation is successful (Masri & Ellingboe, 1966). The effect of SO_2 on germination of E. graminis conidia seen in the chamber experiments could explain the increased incidence of mildew on leaves in the field plots exposed to 14-48 ppb, compared with 5-9 ppb, if conidia are similarly more likely to germinate and cause infection in the field situation.

Experiments in fumigation chambers investigating the effect of SO_2 on host susceptibility to mildew showed that pre-fumigation of barley for 7 days before inoculation with E. graminis did not significantly affect mildew severity but the number of conidia produced by pustules was higher in plants pre-fumigated with 20 or 40 ppb SO_2. This indicates that host susceptibility to mildew can be increased by exposure to these SO_2 levels, an effect which may have contributed to the higher incidence of mildew infection in plants in the field exposed to 14-38 ppb.

The effect of SO_2 concentration on production of conidia demonstrated in the chamber experiments has important implications for mildew development. More conidia were produced on plants exposed to 20 or 40 ppb SO_2 for 7 days before inoculation, 7 days after inoculation, or 7 days before and after inoculation, compared with plants exposed to 0-5 ppb. Increased conidial production was also detected on plants inoculated with pre-fumigated conidia. If conidial production was similarly affected in the field situation a greater amount of inoculum would thus be produced at these SO_2 concentrations (which are similar to those experienced in the SO_2 treated plots) and secondary infection of mildew would be more likely to occur resulting in a higher incidence of mildew. Indeed analysis of the mildew epidemic in the summer of 1987 showed that

the rate of spread of mildew to new plants was significantly higher in field plots exposed to 14 or 38 ppb SO_2 as would be expected if more conidia were being produced during each disease cycle.

The field experiments also showed that on those leaves which became infected with mildew the percentage leaf area affected was frequently greater in the plots with higher SO_2 concentrations, indicating that SO_2 also influences the development of Erysiphe graminis on the leaf once infection has occurred. This was confirmed in a chamber experiment in which plants were fumigated from 24 h after inoculation (i.e. after establishment) until pustule development. Although no significant effect of SO_2 on number of pustules or severity of mildew was detected, since significantly more conidia were produced in plants fumigated with 40 or 60 ppb, SO_2 must have influenced the development of E. graminis in some way after the initial infection stage.

One aspect of mildew development which was not investigated in these experiments, was the effect of SO_2 on haustorial development. According to Masri & Ellingboe (1966), the formation of functional haustoria is the crucial step in the development of a compatible host-parasite relationship for cereal powdery mildew. They found that in barley varieties resistant to mildew, sporulation was suppressed even after haustoria had been formed and that haustoria were smaller and distorted. SO_2 is known to disrupt the membrane structure of chloroplast (Wellburn et al., 1972). Any effect of SO_2 on haustorial membrane could alter the availability of nutrients for production of conidia. In future experiments it would be worthwhile investigating the interaction between SO_2 and haustorial function in E. graminis as this may give insight into why sporulation of E. graminis differed at different SO_2 levels. This would

not be difficult to check by microscopic examination or using histochemical techniques on infected leaves exposed to different SO_2 concentrations.

It is also possible that SO_2 disrupted the plant cell membranes leading to a leakage of nutrients from the plant cells which promoted fungal growth and conidial production. Puckett et al., (1977) found aqueous SO_2 increased electrolyte efflux from lichen thalli and this was assumed to be due to injury to the plasmalemma. This may be the mechanism by which pre-fumigated plants were made more susceptible to mildew.

There have been many reports on the effect of SO_2 on metabolic function of plants (Wellburn, 1982). Changes in enzymatic activity or plant biochemistry induced by SO_2 may well have influenced mildew development in these experiments but until further experiments are carried out comparing host metabolism in diseased and healthy plants at different SO_2 concentrations, the significance of such changes will remain uncertain.

The results of the experiments in fumigation chambers suggest that SO_2 can affect mildew development by increasing the susceptibility of barley plants to infection by E. graminis, increasing the pathogenicity of E. graminis conidia and influencing pustule development and spore production. These effects fit well with the pattern of powdery mildew development seen in the field during three field seasons.

Leaf blotch (Rhynchosporium secalis)

Significant reductions in severity of leaf blotch with increasing SO_2 concentration were found in both the chamber experiments and the field experiments. Incidence of leaf blotch was almost always lower in field plots exposed to SO_2 concentrations (annual mean) within the range of 14-48 ppb than those exposed to 5-9 ppb SO_2 in all three seasons. These results provide evidence that leaf blotch occurrence could be modified by SO_2 levels which typically occur in cereal growing areas of the UK. However, the main factor determining geographical distribution of leaf blotch is probably rainfall as there is a distinct increase in incidence of leaf blotch from East to West across England and Wales, regardless of SO_2 levels (MAFF, 1987).

The effect of SO_2 on leaf blotch incidence in the field cannot be explained by direct effects of gaseous SO_2 on condial germination and initial fungal development as these parameters were not significantly affected by SO_2 concentrations of 0-60 ppb in the chamber experiments. However, conidial germination and germ tube growth of R. secalis were decreased in 0.1mM sulphite solutions at pH5-6. Since conidia of R. secalis germinate in free water on the leaf surface (Skoropad, 1957), the effect of SO_2 dissolved in surface water to form sulphite and sulphate may be more important than gaseous SO_2 in affecting the initial stages of fungal development. It is unclear how the effects seen in vitro relate to the effects in the field as the sulphite concentrations used in the experiment probably exceed those on the leaf surface. However, this does present a possible explanation for why leaf blotch incidence was reduced in the SO_2 treated plots in the field experiments.

SO_2 did not appear to affect susceptibility of barley plants to leaf blotch infection in the chamber experiments when plants fumigated with SO_2 before inoculation with R. secalis.

In the field experiments the maximum percentage leaf area affected by leaf blotch on those leaves which became infected with R. secalis was often lower in field plots exposed to SO_2 levels higher than 14 ppb, indicating that lesion development is affected by SO_2. Similarly, in the chamber experiments plants exposed to 40 or 60 ppb SO_2 for 14 days during the period from establishment of infection to symptom expression had less severe symptoms of leaf blotch than plants exposed to 2 or 20 ppb SO_2. Severity was also reduced in plants exposed to 20, 40 or 60 ppb SO_2 before and after inoculation compared to plants exposed to 2 ppb SO_2 only. This indicates that lesion development of leaf blotch is directly affected by SO_2 concentration and exposure of plants to SO_2 before infection occurs may also influence the sensitivity of the disease to SO_2. This may be due to a build up of toxic sulphite in the leaf. Measurements of conidial production from diseased leaves were not carried out but it is possible that plants showing less severe symptoms of leaf blotch would produce fewer conidia, thereby reducing the inoculum available for secondary infection. However, measurements of apparent infection rate were actually higher in SO_2 treated field plots with less severe leaf blotch infection. On closer examination of the data it can be seen that leaf blotch severity was already much higher in these plots at the start of the period analysed so this anomaly may be simply because disease in the SO_2 plots had reached the peak, while disease in the control plots was still in the exponential period of growth.

On the whole the most important effect of SO_2 on leaf blotch appears to be during lesion development and the inhibitory effects of sulphite in leaf surface water on the initial infection stages of R. secalis could also be a major factor influencing disease development.

The toxic effects of sulphite could be due to ultrastructural changes in R. secalis, indeed mitochondrial structure in the fungal cells of lichen was affected by aqueous SO_2 (Holopainen & Karenlampi, 1984). Alternatively, SO_2 may inhibit metabolic processes in the fungus (Wellburn, 1982). The toxicity of gaseous SO_2 is likely to depend on leaf wetness, which will affect solubilisation of the SO_2 to sulphite. Leaves in the fumigation chambers were probably drier than leaves in a field crop would normally be so the inhibitory effects of SO_2 on R. secalis may have been underestimated.

In addition to the effects of SO_2 on leaf blotch demonstrated in the fumigation experiments, the disease may also be indirectly affected by SO_2 due to changes in populations of leaf surface microorganisms which can promote, antagonise or compete with the pathogens (as discussed previously). The contrasting effects of SO_2 on powdery mildew and leaf blotch in the field experiments may have been partly because the pathogens were competing for infection sites or nutrients so that as R. secalis was inhibited by SO_2, E. graminis was able to colonise more of the leaf, or vice versa. Interactions between the pathogens and the saprophytic leaf microorganisms, which are also sensitive to SO_2, could also modify the response of diseases to SO_2 in the field but would be unlikely to do so in the chambers because the populations would be greatly reduced. Such interactions need to be examined more closely in fumigation experiments to determine their importance in influencing disease development.

Eyespot (*Pseudocercosporella herpotrichoides*)

The apparent effects of SO_2 on eyespot development in the field experiments were rather contradictory. There was some indication that eyespot infection was reduced at 38 ppb SO_2 in 1987 but the effects were transitory and inconsistent. There was also a trend for decreasing eyespot levels with increasing SO_2 concentration in 1985, but the differences were not significant. No effects of SO_2 on host susceptibility to eyespot or lesion development were apparent in the chamber experiments. However, conidial germination and initial mycelial growth were significantly affected by gaseous SO_2 and sulphite concentrations: conidial germination and germ tube growth were reduced above 40 ppb and hyphal branching was reduced above 20 ppb. Sulphite solution significantly reduced conidial germination of *P. herpotrichoides* at pH4 and pH5.6 and also decreased initial mycelial growth at pH4 but at pH5.6 and pH7 initial mycelial growth was increased by sulphite. As *P. herpotrichoides* germinates in water films or droplets at the stem base the effects of sulphite are probably more relevant than those of SO_2. Such effects on initial fungal development may affect early establishment of eyespot on the stem bases of a barley crop. Subsequent eyespot development is more closely related to fungal colonisation of successive leaf sheaths and the stem and to leaf sheath senescence (Fitt, 1985), but by this stage the fungus would be protected from direct exposure to SO_2 by the leaf sheaths remaining around the culm. The concentrations of SO_2 and sulphite used in the laboratory experiments are probably much higher than those occurring in the field at the stem base, but the results do indicate that the pathogen is sensitive to SO_2 during early stages of fungal development, rather than during subsequent lesion

development. The influence of SO_2 levels on eyespot severity in field crops is still uncertain but SO_2 concentration is probably not a very significant factor determining eyespot development.

Implications of the reported effects of SO_2 on barley diseases

Neither of the stem base diseases Fusarium foot rot or sharp eyespot were found to be sensitive to SO_2 concentration. The effect of SO_2 on the leaf diseases brown rust, net blotch and Septoria was inconclusive as only slight infection occurred but there were no apparent differences in development of these diseases in different field plots.

Incidence of black ear moulds seems to be related to SO_2 levels in the field experiments but the responses were variable. In one season black ear moulds were markedly reduced by 28 and 38 ppb SO_2 while in another infection was increased by similar SO_2 concentrations. This variation may relate to changes in aphid population as discussed. Black ear moulds do not generally affect grain yield but can reduce grain quality due to discoloration of the grain. This would be more significant in a wheat crop affected by black ear moulds as infection can lead to flour discoloration and the grain could be rejected by millers. SO_2 concentration could, therefore, influence the value of the grain by affecting black ear moulds but such an effect is likely to vary between seasons and is probably not very important at SO_2 levels in the UK.

From the field results it was estimated that the increase in powdery mildew in the low SO_2 treatments (14-21 ppb), medium SO_2 treatment (28-32 ppb) and high SO_2 treatment (38-48 ppb) against the control (5-9 ppb) resulted in an average yield loss over three seasons of 3%, 2% and 4% in the respective SO_2 treatments. The decrease in severity of leaf blotch on the other hand was estimated

to increase yield by 1%, 1.5% and 1.2% in the low, medium and high SO_2 treatments, compared with the control. Assuming a grain price of £100 per tonne, the overall yield loss due to changes in these diseases would be equivalent to £16/ha, £4/ha and £23/ha in the low (14-21 ppb), medium (28-32 ppb) and high (38-48 ppb) SO_2 plots. The effect of SO_2 on grain yield is likely to be modified by the relative incidence and severity of different fungal diseases in the crop. Varietal susceptibility, weather conditions, previous cropping and other factors which influence which disease develops in a crop will therefore have an important bearing on the effects of SO_2 on cereals. Mildew was more common on winter barley in England and Wales than leaf blotch in 1986, 1987 and 1988 (MAFF 1987, 1988, 1989).

Since the effect of SO_2 on eyespot severity in the field experiments was variable, it seems unlikely that SO_2 would have a consistent effect on yield loss caused by eyespot but this subject needs further investigation before such a possibility can be excluded. Overall, the disease assessments in the field experiments indicate that the influence of SO_2 on the development of barley diseases could result in up to 5% change in final grain yield. The results do illustrate the importance of considering the indirect effects of SO_2 on crop yield due to modification of the biotic environment of the crop, induced by SO_2. As different fungal diseases respond differently to SO_2 concentration, the overall effect of SO_2 on yield will depend on which particular diseases are dominant in the crop.

The changes in disease levels at different SO_2 concentrations could also influence the use of fungicides on the crop to control disease. The threshold for applying a fungicide to control barley mildew given by the Agricultural Development and Advisory Service is 10% leaf area affected by mildew on the lower leaves, was exceeded

in the low and high SO_2 plots but not in the control plots. Thus at these elevated SO_2 concentrations the use of a fungicide to control mildew may be more common. This would cost approximately £13.60/ha (for Corbel or Mistral at full rate of application).

Since the results reported in this thesis show that SO_2 can significantly influence disease development, the concentration of SO_2 in the air above a crop should be considered when formulating models to predict disease development for use in a forecasting scheme. No forecasting models devised up to now have included SO_2 concentration as a variable, but it could be worthwhile doing so to make accurate predictions of disease incidence and severity. The use of forecasting schemes to optimise fungicide timing is likely to become more important in future if restrictions on fungicide use are imposed. In areas with high SO_2 levels it could also be worthwhile growing a variety with good resistance to powdery mildew as the results indicate that these crops would be more likely to develop severe mildew. Unfortunately, there are still relatively few SO_2 monitoring points in rural areas of the UK so it would be difficult to predict accurately SO_2 concentrations in many cereal crops.

Future work

Since the field experiments relied on natural infection of fungal diseases occurring in the field plots, assessment of the effects of SO_2 on winter barley diseases was inevitably confined to those diseases which happened to occur in the crop. It is probable that other important cereal diseases which were not studied here are also influenced by SO_2 concentration, and these may be of additional significance in determining the response of a crop to the pollutant. One way in which this problem could be overcome in future experiments would be to inoculate the crop artificially. The main limitation in the field experiments was the lack of plot replication making it difficult to determine whether changes in disease levels were due to the treatments or natural variation. The use of artificial inoculation of the plot would reduce variation in initial inoculum level at a given time in the season between plots and in different seasons, thereby enabling parallel epidemics to be compared. Seasonal differences in meteorological conditions would, however, still have a major influence on the course of an epidemic.

Another useful approach would be to use fungicides to control disease in one half of each treated plot while allowing natural development of disease in the other half. It would then be possible to evaluate the overall effect of fungal pathogens (and any saprophytic fungi controlled by the fungicides) on the response of the crop to different SO_2 levels.

It is apparent from the experiments described in this thesis that SO_2 can affect crop yield not only by directly affecting crop growth but by indirect effects on plant pathogens. This principle may also apply to other crops and diseases. For example, there is widespread concern at present over the influence of air pollutants on forests.

Many tree pathogens have been shown to be sensitive to SO_2 (Gryzwacz, 1973) and it is possible that changes in pathogen populations could be involved in forest decline.

In order to determine the dose response relationship between a crop and SO_2 concentration, it is essential to study the crop under normal agricultural conditions so that the influence of interacting biotic and abiotic factors (including pathogen and pest populations and pollutant mixtures) is not ignored. However, in order to understand the mechanisms behind the effects seen in the field and to explain why variations may occur, the field experiments need to be complemented with more controlled experiments. The chamber experiments described here demonstrate that SO_2 can affect pathogen development directly or via the plant and shows how pathogens differ in their sensitivity to SO_2 at different stages in their life cycles. However, the experiments do not establish fully the mechanisms by which changes in conidial germination, lesion development or conidial production are brought about by changes in SO_2 concentration. In order to understand this, measurements of enzyme activity and biochemistry of the fungal pathogens subjected to different SO_2 concentrations could be useful. More detailed examination of fungal development on the host, in particular during the penetration and establishment phases, could also provide some more clues as to why SO_2 is affecting these fungi.

Although the chamber experiments showed that SO_2 could affect host susceptibility, it is still unclear whether this was due to changes in host biochemistry, stomatal opening, ultrastructural changes, erosion of wax layer of any other factor. These interactions could easily be evaluated in chamber experiments. It would also be worthwhile clarifying the role of leaf surface micro-organisms in

modifying the response of a disease to SO_2. This could be done using fumigation chambers with simultaneous inoculations of saprophytic fungi and pathogens during exposure to SO_2 to examine their interactions. Interactions between different pathogens may also be affected by SO_2 and this could be investigated in a similar way. Such interactions could explain the contrasting response of mildew and leaf blotch to SO_2.

There is an obvious need to conduct a detailed survey to determine the relationship between SO_2 concentration and cereal diseases in farmers crops. The relationship would be complicated by the influence of other factors including meteorological conditions, crop density and variety which also vary between different areas and could have an overriding influence on disease. Such a survey for the UK has been hampered until recently by inadequate monitoring of SO_2 in rural areas, but more rural monitoring sites have been established in 1989 by the Warren Spring Laboratory.

In December 1984, the British Government announced its aim of achieving a 30% reduction, from 1980 levels, of SO_2 by the end of the 1990s, to be brought about mainly by fitting flue gas de-sulphurisation (FGD) equipment to three CEGB coal-fired power stations at a cost of £600 million at 1986 prices. The Department of Energy have projected that by the year 2000 SO_2 emissions will be reduced to 2.6-3.5 million tonnes per annum (Environment Committee, 1988). However, recently SO_2 emissions have shown a tendency to rise as a result on increasing demand for energy, from 3.56 mt in 1985 to 3.74 mt in 1986. The DOE's 30% reduction target is only likely to be met if there is low economic growth and high world

energy prices in the next decade or if further power stations are fitted with FGD equipment (Mellanby, 1988).

SO_2 concentrations in rural areas have fallen by about a factor of 2 in the last 20 years, whereas urban concentrations have fallen by about a factor of 5 (Environment Committee 1988). Rural SO_2 concentrations are likely to continue to fall gradually. The influence of SO_2 on cereal pathogens could become less important to UK agriculture in future as SO_2 levels fall, and may need to be re-assessed using lower SO_2 levels.

The effects of SO_2 on cereal diseases reported here also has implications for cereal growers outside Britain. Annual average concentrations of SO_2 in rural areas of Europe range from 8-12 ppb close to industrial regions of West and East Germany, Czechoslovakia, Northern France and the midlands of England, to less than 2 ppb along much of the Atlantic seaboard (D. Fowler, personal communication). Other rural districts experiencing large concentrations of SO_2 are found close to isolated cities or industrial installations, although these only constitute a small proportion of the $2.8 \times 10^5 Km^2$ area of non-Nordic Europe which experience SO_2 concentrations in excess of 8 ppb. Unless emission controls are employed, SO_2 concentrations are likely to remain high in some areas of Europe.

Conclusions

SO_2 concentration within the range currently experienced in rural areas of the UK, influences disease development in winter barley to an extent at which yield would be affected. Different fungal pathogens are affected by SO_2 in different ways. Probably the most important results obtained, in terms of significance to UK agriculture, were the effects of SO_2 at concentrations as low as 14 ppb on powdery mildew and leaf blotch.

Concentrations of SO_2 above 14 ppb increased the incidence and severity of powdery mildew in field crops. Further experiments indicated these effects could be due to the influence of SO_2 on host susceptibility to mildew, pathogen development within the host and conidial production.

In contrast, leaf blotch incidence and severity in field crops was reduced by SO_2 concentrations above 14 ppb and this appears to be mainly due to the influence of SO_2 during lesion development and may also be caused by the inhibitory action of sulphite on the pathogen during conidial germination and germ tube growth.

Both SO_2 and sulphite inhibit conidial germination and initial hyphal growth of P. herpotrichoides but the effects of SO_2 on eyespot in the field were inconsistent.

Sulphur dioxide levels in the UK are likely to influence the distribution of winter barley diseases. The effect of SO_2 on grain yield will depend partly on which disease the crop becomes infected with.

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Appendix 1 . Apparent infection rates of powdery mildew in field plots of Igri barley subjected to different levels of sulphur dioxide (Table 15).

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Apparent infection rate June 11-27	Jun-Jul 27-5	July 5-12	Relative advantage[a]	S[b]	t
P1	9 (Control)	0.066	0.030	0			
P2	21 (Low)	0.039	0.153	0	0.032	0.08	0.7
P4	32 (Medium)	0.018	0.125	0	0.016	0.073	0.37
P3	48 (High)	0.039	0.076	0.17	0.063	0.1	1.09

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Apparent infection rate May 8-22	May 22-29	June 29-5	June 5-12	June 12-19	Relative advantage[a]	S[b]	t
P4	7 (Control)	0.134	0.121	0.176	0.007	0.046	0.025	0.034	1.67
C4	(Control)	0.134	0.043	0.134	0.01	0.036			
P1	14 (Low)	0.185	0.107	0.045	0.031	0.052	0.013	0.059	0.53
C1	(Control)	0.069	0.136	0.069	0.027	0.050			
P3	29 (Medium)	-	0.071	0.169	0.036	0.066	0.026	0.052	0.99
C3	(Control)	-	0.061	0.073	0.063	0.043			
P2	48 (High)	-	0.427	0.101	0.044	0.103	0.116	0.149	1.56
C2	(Control)	-	0.093	0.083	0.021	0.014			

Appendix 1. Cont.

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Apparent infection rate May 14-21	May 21-28	June 28-4	June 4-11	June 11-18	Relative advantage[a]	S^b	t
P4	5 (Control)	-	0	0	0	0	0.026	0.243	0.19
C4	(Control)	-	-	0.044	0.034	0			
P3	14 (Low)	0	0.126	0.043	0.024	0.011	0.06	0.045	2.67*
C3	(Control)	-	0	0	0.061	0.043			
P1	28 (Medium)	0.151	0.18	0.02	0.037	0.019	0.034	0.038	1.98
C1	(Control)	0.11	0.086	0	0.014	0.027			
P2	38 (High)	0.108	0.197	0.049	0.028	0.056	0.043	0.044	2.18*
C2	(Control)	0	0.153	0.039	0.032	0			

* Infection rate differs significantly from that in corresponding control plot at $P < 0.05$.

[a] Relative advantage (increase in r) of each SO_2 treated plot relative to appropriate control.

[b] Comparison of r in SO_2 treated plot and control plot,

where $S^2 = \frac{1}{n-1}.\left[\Sigma x^2 + \frac{1}{n}(\Sigma x)^2\right]$ and $t = \frac{s}{\sqrt{n}}$

Appendix 2 : Maximum % mildew recorded on individual leaves of each tiller in plots at Littlehampton.

(a) 1985 (11/6 - 12/7)

Plot and treatment	Tiller	Leaf number 1	2	3	4	5
	1	0	1	25	2	-
	2	1	25	0	0	-
	3	1	10	2	0	-
	4	10	15	10	0	-
	5	5	10	25	0	0
P1	6	2	0	2	0	0
Control	7	2	10	25	10	0
	8	0	1	0	0	-
	9	2	25	10	0	-
	10	0	2	10	20	-
	11	5	25	0	0	0
	12	1	25	10	-	-
	1	25	50	10	50	-
	2	25	1	10	0	5
	3	10	10	10	5	-
	4	50	10	15	50	-
	5	25	10	25	5	-
P2	6	20	10	20	15	50
Low SO_2	7	25	25	10	50	-
	8	1	5	10	20	-
	9	2	5	20	25	0
	10	1	50	15	25	50
	11	1	10	25	10	-
	12	0	10	50	75	-

Appendix 2 : Cont. (a) 1985

Plot and treatment	Tiller	Leaf number 1	2	3	4	5
	1	1	2	10	10	0
	2	1	5	10	25	30
	3	0	5	5	5	0
	4	25	50	5	0	0
	5	1	2	0	50	0
P4	6	2	5	5	10	0
Medium	7	0	10	10	5	-
SO_2	8	2	1	5	0	0
	9	2	2	10	10	0
	10	1	5	25	15	-
	11	5	50	25	20	-
	12	2	25	25	30	-
	1	0	50	25	50	0
	2	10	1	25	10	25
	3	0	2	5	10	15
	4	25	50	25	50	-
	5	1	25	25	0	-
P3	6	5	30	25	50	-
High SO_2	7	0	50	15	50	-
	8	10	25	10	25	-
	9	10	50	25	5	-
	10	2	25	15	50	-
	11	10	25	25	15	-
	12	5	50	50	15	75

Appendix 2 : Cont.

(b) 1986 (May 8 - Jun 19)

Plot and treatment	Tiller	Leaf number					
		1	2	3	4	5	6
	1	0	0	2	0	0	0
	2	2	1	1	1	2	0
	3	0	2	2	5	5	0
	4	0	0	0	0	0	0
	5	0	2	0	1	2	2
	6	0	0	5	5	0	0
P4	7	0	2	2	1	5	0
Control	8	5	2	2	0	0	0
	9	0	1	5	1	0	0
	10	0	0	2	10	5	0
	11	0	0	2	0	0	0
	12	0	0	1	2	0	0
	13	0	1	2	5	0	0
	14	5	0	2	10	0	0
	15	0	1	5	5	2	0
	1	0	5	0	2	0	0
	2	0	0	2	1	0	1
	3	0	0	0	0	0	0
	4	0	0	1	0	0	0
	5	0	0	1	0	0	0
	6	0	0	2	0	1	0
C4	7	0	0	0	1	1	0
Control	8	0	0	1	2	1	0
	9	0	2	5	2	0	0
	10	0	0	2	1	0	0
	11	1	0	0	0	0	0
	12	0	2	0	0	0	0
	13	0	1	5	0	0	0
	14	0	0	1	0	0	0
	15	0	0	0	0	0	0

Appendix 2 : Cont. (b) 1986

Plot and treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	5	5	2	5	0
	2	1	2	5	2	5	0
	3	0	5	10	10	0	0
	4	0	10	5	10	0	0
	5	0	0	5	1	5	0
	6	1	0	5	5	1	0
P1	7	1	5	10	10	0	0
Low SO_2	8	2	2	2	5	10	2
	9	0	5	10	5	1	0
	10	1	0	0	5	0	0
	11	1	1	5	10	10	0
	12	2	25	2	0	0	0
	13	0	2	5	10	0	0
	14	0	1	5	10	1	0
	15	0	25	0	2	0	0
	1	0	0	0	0	0	0
	2	0	0	1	2	1	2
	3	0	0	0	0	0	0
	4	0	0	2	0	0	0
	5	0	2	0	0	0	0
	6	0	0	2	5	0	0
C1	7	1	1	0	0	0	0
Control	8	0	2	2	0	0	0
	9	2	1	1	0	0	0
	10	0	0	0	0	0	0
	11	2	0	5	5	0	0
	12	0	0	0	0	0	0
	13	0	2	0	0	0	0
	14	2	0	2	5	2	1
	15	0	0	1	2	0	0

Appendix 2 : Cont (b) 1986

Plot and treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	0	5	5	0	0
	2	0	0	5	5	0	-
	3	0	1	5	2	0	0
	4	2	2	2	0	0	0
	5	2	2	5	0	0	0
	6	2	2	2	0	0	0
P3	7	0	5	5	10	0	0
Medium	8	0	2	5	1	0	0
SO_2	9	0	5	5	5	5	0
	10	0	5	0	10	0	0
	11	0	1	2	2	1	0
	12	2	2	5	0	0	0
	13	0	2	5	0	0	0
	14	0	0	2	2	0	0
	15	0	2	5	2	5	0
	1	0	2	5	0	0	0
	2	0	0	2	0	2	0
	3	0	0	0	1	0	0
	4	0	5	1	0	0	0
	5	0	0	0	2	0	0
C3	6	0	0	1	0	0	0
Control	7	0	0	0	0	0	0
	8	0	1	1	0	0	0
	9	0	1	0	0	0	0
	10	0	0	2	5	5	0
	11	0	0	5	0	0	0
	12	0	0	0	0	0	0
	13	0	0	2	0	0	0
	14	0	0	2	0	1	0
	15	0	0	0	0	0	0

Appendix 2: Cont. (b) 1986

Plot and treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	1	25	5	0	0
	2	0	5	5	25	1	0
	3	1	2	2	10	1	0
	4	0	2	25	5	0	0
	5	0	5	0	1	0	-
	6	0	5	25	25	0	0
P2	7	0	1	10	50	1	0
High SO_2	8	0	2	5	2	2	0
	9	0	2	2	10	2	0
	10	0	2	5	25	2	0
	11	0	2	10	0	0	0
	12	5	0	10	0	0	0
	13	2	2	0	0	0	0
	14	0	2	0	5	0	0
	15	0	0	5	10	5	0
	1	0	5	10	5	1	0
	2	0	2	5	0	0	-
	3	0	0	2	0	0	0
	4	0	2	2	0	0	0
	5	0	0	5	0	0	0
	6	0	0	10	5	2	0
C 2	7	0	1	5	2	2	0
Control	8	0	5	5	2	0	0
	9	0	0	5	1	0	0
	10	0	0	5	2	5	0
	11	0	0	2	0	0	0
	12	0	0	0	1	0	0
	13	0	0	1	1	0	0
	14	0	2	2	0	0	0
	15	0	2	5	5	0	0

Appendix 2 : Cont.

(c) 1987 (14 May - 18 June)

Plot and treatment	Tiller	Leaf number					
		1	2	3	4	5	6
	1	0	0	0	0	-	-
	2	0	5	0	0	-	-
	3	0	0	0	0	0	-
	4	0	0	0	0	-	-
	5	0	0	0	0	0	-
	6	0	0	0	0	-	-
P4	7	0	0	0	0	-	-
Control	8	0	0	0	0	-	-
	9	0	0	0	0	-	-
	10	0	1	0	0	-	-
	11	0	0	0	-	-	-
	12	0	0	0	0	0	-
	13	0	0	0	0	-	-
	14	0	0	0	0	-	-
	15	0	0	0	0	-	-
	1	0	0	0	1	-	-
	2	0	1	0	0	-	-
	3	0	0	0	0	0	-
	4	0	0	0	0	-	-
	5	0	0	0	0	-	-
	6	1	0	0	0	0	-
C4	7	0	0	5	5	0	0
Control	8	0	0	0	0	0	-
	9	0	0	0	0	-	-
	10	0	0	0	0	0	-
	11	0	0	0	0	-	-
	12	0	0	0	0	-	-
	13	0	0	0	0	0	-
	14	0	0	0	0	0	-
	15	0	0	0	0	-	-

Appendix 2 : Cont. (c) 1987

Plot and treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	0	0	-	-	-
	2	0	0	0	0	-	-
	3	0	0	0	0	-	-
	4	0	0	0	0	0	-
	5	1	0	0	5	0	-
	6	0	2	5	5	-	-
P3	7	0	0	0	0	-	-
Low SO_2	8	0	0	5	5	-	-
	9	0	1	0	0	-	-
	10	0	5	5	0	0	-
	11	0	0	2	0	0	-
	12	0	0	15	0	-	-
	13	0	2	5	10	5	0
	14	0	0	0	0	0	-
	15	0	0	2	0	0	-
	1	10	10	0	-	-	-
	2	0	5	20	0	-	-
	3	0	0	5	0	-	-
	4	0	0	5	0	-	-
	5	0	15	25	15	-	-
	6	0	0	0	-	-	-
C1	7	0	0	0	-	-	-
Control	8	5	5	5	0	-	-
	9	0	0	30	-	-	-
	10	0	0	10	-	-	-
	11	0	0	0	-	-	-
	12	0	0	0	-	-	-
	13	0	0	0	0	-	-
	14	0	2	0	2	-	-
	15	1	0	0	0	-	-

Appendix 2 : Cont (c) 1987

Plot and treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	5	5	20	5	0	-
	2	0	2	5	10	0	-
	3	1	0	0	0	-	-
	4	0	10	10	5	0	-
	5	2	10	0	0	-	-
	6	1	1	10	5	5	-
P2	7	0	0	0	-	-	-
High SO_2	8	0	2	15	0	0	-
	9	0	5	1	0	-	-
	10	1	10	25	0	-	-
	11	5	2	1	0	-	-
	12	2	10	10	5	-	-
	13	1	0	0	-	-	-
	14	2	10	1	0	-	-
	15	0	10	0	-	-	-
	1	0	0	0	0	-	-
	2	0	0	0	0	-	-
	3	0	0	0	0	-	-
	4	0	0	0	0	-	-
	5	0	0	0	0	-	-
	6	0	5	0	-	-	-
C3	7	0	0	0	-	-	-
Control	8	0	0	0	-	-	-
	9	2	5	0	-	-	-
	10	0	0	0	-	-	-
	11	0	0	0	0	-	-
	12	0	0	0	-	-	-
	13	0	0	0	-	-	-
	14	0	0	0	-	-	-
	15	0	0	0	-	-	-

Appendix 2 : Cont. (c) 1987

Plot and treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	0	0	0	-	-
	2	0	5	10	5	-	-
	3	0	5	0	0	-	-
	4	5	2	5	0	0	-
	5	0	0	10	2	-	-
	6	0	2	5	0	-	-
P1	7	2	20	5	0	-	-
Medium	8	1	0	15	0	-	-
SO_2	9	0	0	0	0	-	-
	10	0	0	2	0	-	-
	11	10	10	25	0	0	-
	12	0	5	5	0	-	-
	13	0	10	0	0	-	-
	14	0	10	5	0	-	-
	15	2	10	25	0	2	-
	1	0	0	0	0	-	-
	2	0	0	0	-	-	-
	3	0	0	0	0	-	-
	4	0	0	1	-	-	-
	5	0	2	0	0	-	-
	6	0	0	2	0	-	-
C2	7	0	0	0	0	-	-
Control	8	0	0	2	0	-	-
	9	0	10	0	0	-	-
	10	0	0	15	0	-	-
	11	0	2	1	-	-	-
	12	0	0	0	-	-	-
	13	0	0	5	-	-	-
	14	0	0	1	-	-	-
	15	0	0	5	0	-	-

Appendix 3 . Apparent infection rates of Rhynchosporium secalis in field plots of Igri barley subjected to different levels of sulphur dioxide (Table 21)

(a) 1985 (11 June - 12 July)

Plot No.	Mean annual ppb SO_2 (Treatment)	Apparent infection rate June 11-27	June 27-5	July 5-12	Relative Advantage[a]	s^b	t
P1	9 (Control)	0.083	0	0			
P2	21 (Low)	0.045	0.119	0.011	0.030	0.081	0.85
P4	32 (Medium)	0.079	0.198	0.011	0.068	0.113	1.35
P3	48 (High)	0.042	0.089	0.022	0.023	0.065	0.81

(b) 1986 (8 May - 19 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Apparent infection rate May 8-22	May 22-29	June 29-5	June 5-12	June 12-19	Relative Advantage[a]	s^b	t
P4	7 (Control)	0.056	0.071	0.119	0.058	0.072	0.004	0.021	0.46
C4	(Control)	0.065	0.085	0.079	0.060	0.064			
P1	14 (Low)	0.080	0.075	0.106	0.118	0.051	0.038	0.014	6.09**
	(Control)	0.053	0.054	0.051	0.072	0.012			
P3	29 (Medium)	0.064	0.072	0.153	0.091	0.045	0.020	0.044	1.01
	(Control)	0.087	0.026	0.068	0.097	0.045			
P2	48 (High)	0	0.203	0.199	0.053	0.075	0.045	0.089	1.13
	(Control)	0.08	0.083	0.058	0.046	0.035			

(c) 1987 (14 May - 18 June)

Plot No.	Mean annual ppb SO_2 (Treatment)	Apparent infection rate May 14-21	May 21-28	June 28-4	June 4-11	June 11-18	Relative Advantage[a]	s[b]	t
P4	5 (Control)	0.122	0.07	0.086	0.05	0	-0.006	0.061	0.2
C4	(Control)	0.071	0.068	0.035	0.138	0.045			
P3	14 (Low)	0.113	0.089	0.069	0.098	0	0.007	0.028	0.56
C3	(Control)	0.099	0.079	0.047	0.09	0.039			
P1	28 (Medium)	0.037	0.128	0.021	0.039	0.037	-0.02	0.042	1.23
C1	(Control)	0.132	0.112	0.030	0.056	0.048			
P2	38 (High)	0.143	0.034	0.07	0.093	0.019	-0.011	0.018	1.30
C2	(Control)	0.161	0.062	0.089	0.1	0			

** Infection rate differs significantly ($P<0.01$) from that in corresponding control plot.

a Relative advantage (increase in r) of each SO_2 treated plot relative to appropriate control.

b Comparison of r in SO_2 treated plot and control plot, where

$$s^2 = \frac{1}{n-1} \cdot \left[\Sigma x^2 + \frac{1}{n} (\Sigma x)^2\right]$$

and $$t = \frac{s}{\sqrt{n}}$$

Appendix 4 : Maximum % leaf blotch recorded on individual leaves of each tiller in plots at Littlehampton

(a) 1985 (11 June - 12 July)

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5
	1	0	5	1	0	-
	2	10	10	10	0	-
	3	5	10	0	0	-
	4	2	5	2	5	-
P1	5	5	10	0	0	0
Control	6	0	2	2	0	0
	7	0	2	5	0	0
	8	1	0	0	0	-
	9	0	2	2	0	-
	10	0	5	0	2	-
	11	10	10	5	10	50
	12	5	5	0	10	-
	1	10	15	2	0	0
	2	0	5	0	0	-
	3	1	0	0	2	-
	4	10	5	0.5	0	-
P2	5	20	5	0	5	-
Low	6	10	5	2	0	0
SO_2	7	15	10	1	0	-
	8	5	10	15	15	-
	9	1	2	0	0	0
	10	5	0	0	0	0
	11	0	0	0	0	-
	12	1	2	1	10	-
	1	1	5	2	0	0
	2	1	10	2	0	5
P4	3	1	2	2	0	0
Medium	4	2	5	2	0	0
SO_2						

Appendix 4. cont

(a) 1985

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5
	4	2	5	2	0	0
P4	5	1	1	0	0	0
Medium	6	5	2	5	0	0
SO_2	7	1	10	2	0	-
	8	1	2	0	0	0
	9	2	2	0	0	0
	10	1	0	5	5	-
	11	2	2	0	0	-
	12	1	1	5	0	-
	1	5	10	0	0	0
	2	10	5	0	5	20
	3	5	5	0	0	0
	4	5	0	2	5	-
P3	5	1	2	5	0	-
High	6	5	10	0	0	-
SO_2	7	10	0	0	0	-
	8	0	0	0	5	-
	9	5	5	0	0	-
	10	1	5	0	2	-
	11	2	1	1	10	-
	12	5	5	10	0	0

(b) 1986 (8 May - 19 June)

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	0	5	50	0	0
P4	2	2	20	50	10	5	1
Control	3	0	0	5	5	20	2
	4	0	2	2	1	15	2

Appendix 4 . cont

(b) 1986

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5	6
	5	0	0	10	5	5	0
	6	0	0	10	20	20	0
P4	7	0	0	10	50	10	0
Control	8	0	2	50	25	5	10
(cont)	9	0	10	10	20	2	10
	10	0	0	0	15	2	1
	11	0	0	2	5	10	0
	12	0	0	5	5	25	0
	13	5	20	10	25	10	1
	14	0	0	2	2	0	0
	15	0	0	2	5	2	5
	1	0	2	1	5	5	0
	2	0	0	0	2	0	0
	3	0	0	2	5	5	0
	4	0	0	0	2	0	0
C4	5	0	0	0	0	10	0
Control	6	0	0	0	0	10	2
	7	0	0	0	0	5	0
	8	0	0	0	2	0	0
	9	0	0	0	5	2	0
	10	0	0	0	50	0	0
	11	0	0	2	50	0	0
	12	0	1	0	10	0	1
	13	0	0	5	10	0	1
	14	0	10	2	2	5	2
	15	0	5	2	25	5	2

Appendix 4 . cont

(b) 1986

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	0	5	5	0	0
	2	10	5	2	10	0	0
	3	0	0	2	0	0	0
	4	0	0	2	0	0	0
	5	0	0	2	10	0	0
	6	0	0	2	25	0	0
P1	7	0	0	5	10	0	0
Low	8	0	0	0	2	0	0
SO_2	9	0	0	0	25	0	2
	10	0	0	10	0	0	0
	11	0	0	10	5	0	0
	12	0	10	0	0	0	0
	13	0	2	2	25	0	0
	14	0	0	10	10	0	5
	15	0	10	0	0	0	0
	1	0	5	50	10	10	20
	2	1	10	10	50	10	10
	3	0	2	2	10	2	10
	4	0	0	5	10	2	5
	5	0	5	75	0	0	20
	6	0	0	5	10	1	10
C1	7	0	2	20	10	2	5
Control	8	0	0	10	25	10	0
	9	0	25	50	0	5	20
	10	0	0	10	5	20	0
	11	0	0	5	50	5	5
	12	2	20	25	2	0	0
	13	2	5	10	10	5	0
	14	0	0	2	5	30	10

Appendix 4 . cont

(c) 1986

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	5	10	5	0	0
	2	1	10	10	2	0	1
	3	0	0	1	15	0	0
	4	0	0	10	10	2	0
	5	0	0	2	0	0	0
	6	0	0	2	10	0	0
P3	7	0	2	5	50	0	0
Medium	8	0	5	15	25	0	0
SO_2	9	0	0	5	5	2	2
	10	0	2	25	10	10	0
	11	0	10	5	10	0	0
	12	0	1	5	5	10	0
	13	0	0	0	1	5	0
	14	0	5	20	25	0	0
	15	0	0	5	10	20	5
	1	0	2	5	10	2	2
	2	0	0	20	10	20	0
	3	0	0	10	0	10	2
	4	0	0	10	5	2	2
	5	0	0	10	50	20	1
	6	0	0	1	20	5	1
C3	7	0	10	5	5	5	0
Control	8	0	0	5	10	2	1
	9	0	0	2	10	0	0
	10	0	1	5	10	10	0
	11	2	10	10	25	10	1
	12	0	2	2	10	20	0
	13	0	0	2	2	0	0
	14	0	0	10	10	2	0
	15	0	2	25	0	0	0

Appendix 4. cont

(c) 1986

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	0	0	0	5	0	0
	2	0	0	0	0	0	0
	3	0	0	1	2	0	0
	4	0	2	2	2	0	0
	5	0	0	5	20	0	0
P2	6	0	2	5	0	0	0
High	7	0	0	0	0	0	0
SO_2	8	0	0	0	10	5	0
	9	0	1	5	0	2	0
	10	0	0	15	10	0	0
	11	0	0	2	0	2	0
	12	0	0	0	0	0	0
	13	0	0	5	10	0	5
	14	0	0	20	10	5	0
	15	0	0	2	30	5	0
	1	0	0	10	2	5	0
	2	0	0	5	10	2	-
	3	0	5	50	10	0	5
	4	5	5	10	5	0	0
	5	0	0	5	20	0	10
	6	0	20	30	2	2	10
C2	7	0	5	10	20	2	0
Control	8	0	10	5	10	5	5
	9	0	10	5	25	10	0
	10	0	0	10	5	0	15
	11	10	5	5	20	5	10
	12	5	20	75	5	0	10
	13	0	0	25	2	5	0
	14	0	15	30	10	0	0
	15	2	20	5	25	5	5

Appendix 4 . cont

(c) 1987 (14 May - 18 June)

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	40	50	20	10	-	-
	2	20	20	5	50	-	-
	3	0	5	10	0	20	-
	4	20	50	20	20	-	-
	5	0	0	2	2	50	-
	6	0	40	5	5	-	-
P4	7	20	30	40	20	-	-
Control	8	50	50	30	50	-	-
	9	75	60	50	20	-	-
	10	30	20	75	50	-	-
	11	30	50	10	-	-	-
	12	10	0	0	-	-	-
	13	20	10	40	5	-	-
	14	20	50	10	50	-	-
	15	20	20	40	20	-	-
	1	10	30	50	30	-	-
	2	10	30	10	10	-	-
	3	0	20	20	30	30	-
	4	20	10	0	10	-	-
	5	30	30	20	10	-	-
	6	0	20	50	0	0	-
C4	7	0	20	2	20	10	50
Control	8	0	10	50	10	10	-
	9	5	30	30	50	-	-
	10	20	20	10	10	20	-
	11	30	20	1	-	-	-
	12	40	50	75	0	-	-
	13	20	5	50	0	20	-
	14	30	30	10	20	75	-
	15	2	30	2	20	-	-

Appendix 4. cont

(c) 1987

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5	6
	1	10	30	0	-	-	-
	2	20	50	0	50	-	-
	3	30	0	0	0	-	-
	4	0	2	2	2	10	-
	5	0	5	2	2	20	-
	6	20	30	15	10	-	-
P3	7	40	30	10	50	-	-
Low	8	20	30	5	50	-	-
SO_2	9	5	40	0	0	20	-
	10	20	20	15	0	20	-
	11	0	5	10	0	15	-
	12	0	20	0	15	-	-
	13	10	0	20	0	5	-
	14	0	5	10	0	0	-
	15	0	5	30	0	0	-
	1	5	10	5	15	-	-
	2	10	20	0	20	-	-
	3	15	10	10	50	-	-
	4	75	75	20	10	-	-
	5	40	10	0	30	-	-
	6	0	5	15	-	-	-
C3	7	10	5	20	-	-	-
Control	8	10	20	20	-	-	-
	9	10	5	10	-	-	-
	10	5	5	10	-	-	-
	11	0	0	10	50	-	-
	12	10	20	25	-	-	-
	13	30	0	20	-	-	-
	14	30	5	10	-	-	-
	15	2	0	10	-	-	-

Appendix 4. cont

(c) 1987

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5
	1	2	0	0	10	-
	2	10	0	5	0	-
	3	0	10	10	0	-
	4	0	0	5	10	0
	5	20	20	0	2	-
	6	30	0	2	0	-
P1	7	10	20	0	10	-
Medium	8	0	0	0	0	-
SO_2	9	0	5	0	0	-
	10	30	0	0	0	-
	11	0	2	5	20	0
	12	0	5	5	10	-
	13	0	15	0	0	-
	14	5	0	0	0	-
	15	10	10	20	0	2
	1	10	5	30	-	-
	2	5	5	5	50	-
	3	50	20	5	50	-
	4	30	30	30	50	-
	5	30	10	10	50	-
	6	10	10	50	-	-
C1	7	20	50	15	-	-
Control	8	50	10	10	50	-
	9	20	0	20	-	-
	10	20	5	10	-	-
	11	40	5	50	-	-
	12	40	10	10	-	-
	13	30	20	5	20	-
	14	0	5	5	20	-
	15	20	30	5	50	-

Appendix 4 . cont

(c) 1987

Plot and Treatment	Tiller	Leaf number 1	2	3	4	5
	1	0	20	0	0	50
	2	0	5	0	0	0
	3	0	5	2	10	-
	4	0	20	5	0	0
	5	20	20	0	50	-
	6	0	2	20	0	0
P2	7	50	20	5	-	-
High	8	30	10	5	0	5
	9	0	20	0	0	-
	10	20	20	5	10	-
	11	0	5	0	5	-
	12	10	2	0	5	-
	13	5	0	10	-	-
	14	5	10	0	30	-
	15	50	20	5	-	-
	1	2	40	0	50	-
	2	30	40	5	-	-
	3	20	10	5	50	-
	4	50	30	0	-	-
	5	50	5	0	20	-
	6	20	30	20	0	-
C2	7	60	30	5	10	-
Control	8	30	10	0	0	-
	9	30	20	10	20	-
	10	10	20	20	20	-
	11	30	0	0	-	-
	12	10	20	10	-	-
	13	30	10	10	50	-
	14	0	2	30	-	-
	15	5	5	2	20	-

Appendix 5 . Lodging of barley tillers in July 1986 (at GS 91) in field plots of Igri barley subjected to different levels of SO_2

Plot No.	Mean annual ppb SO_2 (Treatment)	% area of plot lodged
P4	7 (Control)	30
C4	(Control)	10
P1	14 (Low)	0
C1	(Control)	10
P3	29 (Medium)	0
C3	(Control)	0
P2	48 (High)	20
C2	(Control)	0

Appendix 6. Incidence of sharp eyespot in field plots of barley subjected to different levels of SO_2

(a) 1984-5

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on July 5	12	19	31	
P1	9 (Control)	30	30	20	10	(10)[c]
P2	21 (Low)	10	0	10	30	(30)
P4	32 (Medium)	20	10	10	20	(10)
P3	48 (High)	30	0	10	10	(10)
Growth Stage [b]		85	87	91	92	

(b) 1985-6

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on May 22	June 5	June 19	July 2	July 17
P4	7 (Control)	5	15	20	20	20
C4	(Control)	5	15	10	15	25
P1	14 (Low)	20	20	25	10	15
C1	(Control)	5	10	20	25	25
P3	29 (Medium)	15	15	20	15	15
C3	(Control)	0	15	15	15	15
P2	48 (High)	5	20	15	15	15
C2	(Control)	10	10	5	10	10
Growth Stage [b]		47	61	83	87	91

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on							
		Dec	Feb	Apr	May		June		July
		11	11	16	14	28	11	25	9
P4	5 (Control)	5	5	5	5	10	30	50	60
C4	(Control)	15	5	5	0	5	25	50	60
P3	14 (Low)	0	0	0	0	5	30	40	60
C3	(Control)	0	10	0	5	25	35	45	65
P1	28 (Medium)	0	5	15	0	30	45	75	80
C1	(Control)	0	10	5	0	25	55	45	60
P2	38 (High)	10	15	5	0	10	55	35	45
C2	(Control)	5	0	0	0	40	30	45	75
Growth Stage [b]		24	27	30	47	65	83	87	93

[a] Incidence based in 1985 on 10 tillers; in 1986 on 20 tillers; in 1987 on 20 plants to February, thereafter on 20 tillers per plot.

[b] Approximate growth stage (Zadoks *et al.*, 1974) of all plants sampled.

[c] Values in brackets are incidences in companion control plots on 31/7/85.

Appendix 7 . Incidence of brown foot rot (Fusarium spp.) on barley tillers growing in field plots subjected to different levels of SO_2 throughout three seasons.

(a) 1985

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 11	June 27	July 5	July 12	July 19	July 31	
P1	9 (Control)	20	20	50	40	40	40	(40)[c]
P2	21 (Low)	30	20	50	50	40	30	(40)
P4	32 (Medium)	20	20	20	40	20	30	(50)
P3	43 (High)	10	10	30	40	40	30	(40)
Growth Stage [b]		15	83	85	87	91	92	

(b) 1986

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on May 22	June 5	June 19	July 2	July 17
P4	7 (Control)	15	20	15	30	40
C4	(Control)	20	10	35	50	20
P1	14 (Low)	5	5	20	25	20
C1	(Control)	5	20	20	25	25
P3	29 (Medium)	25	20	25	15	25
C3	(Control)	0	20	30	25	30
P2	48 (High)	5	10	5	15	15
C2	(Control)	10	15	15	20	20
Growth Stage [b]		47	61	83	87	91

(c) 1986-7

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on							
		Dec	Feb	Apr	May		June		July
		11	11	16	14	28	11	25	9
P4	5 (Control)	0	10	5	20	15	25	30	35
C4	(Control)	0	10	20	15	30	30	20	25
P3	14 (Low)	0	35	5	0	10	10	30	45
C3	(Control)	0	25	10	15	10	25	30	60
P1	28 (Medium)	0	5	25	0	5	15	15	40
C1	(Control)	0	10	10	10	10	25	10	50
P2	38 (High)	0	5	0	20	10	10	25	30
C2	(Control)	0	5	0	20	0	25	25	50
Growth Stage [b]		24	27	30	47	65	83	87	93

[a] Incidence based in 1985 on 10 tillers; in 1986 on 20 tillers; in 1987 on 20 plants to February, thereafter on 20 tillers per plot.

[b] Approximate Growth Stage (Zadoks et al., 1974) of all plants sampled.

[c] Values in brackets are incidences in companion control plots on 31/7/85.

Appendix 8 . Incidence of Ear Botrytis in field plots of Igri barley exposed to different SO_2 levels in three seasons.

(a) 1985

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 27	July 5	July 12	July 19
P1	9 (Control)	17	50	67	58
P2	21 (Low)	25	50	50	42
P4	32 (Medium)	0	50	33	33
P3	43 (High)	25	33	58	50
Growth Stage [b]		83	85	87	91

(b) 1986

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on July 2	July 10	July 17	July 23
P4	7 (Control)	0	33	40	53
C4	(Control)	0	27	40	40
P1	14 (Low)	13	13	33	40
C1	(Control)	13	27	33	40
P3	29 (Medium)	0	20	20	20
C3	(Control)	7	20	40	47
P2	48 (High)	27	33	33	40
C2	(Control)	0	20	47	47
Growth Stage [b]		87	87	91	93

(c) 1987

Plot No.	Mean annual ppb SO_2 (Treatment)	% Incidence [a] on June 11	June 18	June 25	July 2	July 9
P4	5 (Control)	0	0	0	7	7
C4	(Control)	0	0	0	7	7
P3	14 (Low)	0	7	13	13	20
C3	(Control)	7	7	7	13	13
P1	28 (Medium)	0	0	0	7	7
C1	(Control)	7	7	7	7	7
P2	38 (High)	7	7	7	13	13
C2	(Control)	0	13	13	20	20
Growth Stage [b]		83	83	87	91	93

[a] Incidence based on 12 tillers per plot in 1985 and 15 tillers per plot in 1986 and 1987.

[b] Approximate growth stage (Zadoks et al., 1974) of all plants sampled on each date.

Apendix 9:Inoculation methods tested for use in chamber experiments on Erysiphe graminis.

Five methods were tested for inoculating conidia of E. graminis on to barley leaves of potted seedlings. Inoculum was obtained from stock infector plants which had been shaken to remove old conidia 24h before inoculation.

1. Cotton wool bud method

See Section II, Experiments on Powdery Mildew, Part B.

2. Paint brush method

A fine camel hair paint brush was gently stroked along about 1cm of a leaf of an infector plant, then stroked once along a central portion (c. 1cm) of the adaxial surface of the first leaf of an experimental plant.

3. Leaf impression method

A leaf of an infector plant with sporulating pustules was pressed lightly on to the adaxial surface of the first leaf of an experimental plant.

4. Cotton sieve method (Schuette, 1971)

Conidia from sporulating pustules were tapped into a plastic cuvette (4.4cm x 1.2cm). A piece of sterile absorbent cotton was fixed tightly over the open end of the cuvette, secured with an elastic band, then the cuvette was inverted over the central portion of an infector plant and tapped several times so that the conidia filtered through the cotton on to the leaf.

5. Aerial deposition method (Simkin, 1973)

Experimental plants were placed around a cardboard cylinder

(13cm diam., 58cm high) positioned in the centre of a glasshouse cabinet, mounted 2.6cm above the sand base. Infector plants were shaken over the top of the cylinder and the fan was switched on and run for 5 mins. The conidia were then allowed to settle on to the experimental plants for the next hour.

Assessment of the number of conidia deposited, the distribution of conidia and conidial germination were made on five leaf sections, as described in the text.

Inoculation method	No. conidia ($x10^{-3}$) per cm^2 leaf	% conidia in chains	% conidia in clumps	% conidia germination *
Cotton wool bud	2.0–3.0	3.5	0.5	75–85
Paint brush	0.5–2.0	6.0	2.0	76–89
Leaf impression	1.0–3.0	10.0	1.0	69–80
Cotton sieve	0.4–0.8	0.5	0.5	87–93
Aerial deposition	0.3–1.5	3.0	1.0	68–90

* Germination assessed on 100 spores after 24h incubation at 25°c

Conidia in clumps or chains have been shown to germinate poorly and irregularly compared with isolated conidia (Brodie, 1945; Hossain & Manners, 1963) and result in non-uniform distribution of germinated conidia in the leaf surface. The cotton wool bud method was ultimately used in the chamber experiments because relatively few conidia occurred in chains or clumps, germination was even, pustule development was fairly uniform and reproducible and the method was easy to use.

Appendix 10 : Percentage germination of Erysiphe graminis conidia produced on plants during exposure to different concentrations of SO_2 for 24 h (see Fig 17).

	SO_2 (ppb) to which infector plants exposed			
Replicate *	2	20	40	60
1	28	37	35	46
2	34	28	40	35
3	33	40	41	34
4	33	47	46	42
5	31	36	38	37
6	32	38	41	43
7	30	44	39	38
8	29	39	48	32
9	26	40	55	41
10	35	39	38	34
Mean	31.1	38.8	42.1	38.2

* Germination assessed on 100 conidia after incubation on water agar for 24 h at 25°c.

Analysis of variance *

Source	DF	MS	F	P
Treatments	3	76.46	9.79	0.001
Error	36	7.81		

*Analysis of arcsin transforms of percentage data

Appendix Table 10a:

Analysis of variance for data of Table 33. *

Source	DF	MS	F
Pots (Blocks)	3	10.78	1.06
SO_2 level (A)	3	11.61	1.14
Error (a)	9	10.19	
Seedlings (B)	4		
Interaction (AB)	12	1.28	0.18
Error (b)	48	12.00	1.70

* Analysis for a split plot design arranged in randomised complete blocks.

Appendix 11 : Percentage leaf area covered by powdery mildew pustules 8 days after inoculation with Erysiphe graminis conidia produced on plants during exposure to different concentrations of SO_2 for 24 h (see Fig 18).

Pot	Seedling *	Infector plants exposed to SO_2 at 0	20	40	60 ppb
1	1	10	20	20	15
	2	15	10	25	15
	3	1	15	20	20
	4	15	5	15	20
	5	15	20	15	15
2	1	20	10	10	15
	2	5	5	25	15
	3	15	15	25	20
	4	5	15	25	20
	5	10	10	20	25
3	1	5	25	20	25
	2	15	10	10	20
	3	10	10	10	25
	4	15	25	10	25
	5	20	10	15	20
4	1	15	15	25	25
	2	15	10	10	20
	3	10	15	15	20
	4	5	10	20	15
	5	5	10	15	15
Mean		11.80	13.75	19.00	19.50

* % area covered by pustules assessed on leaf section from the first leaf of each seedling.

Appendix 11 : Continued

Analysis of variance **

Source	DF	MS	F	P
Pots (Blocks)	3	6.00	0.27	-
SO_2 level (A)	3	210.55	9.46	0.01
Error (a)	9	22.25	0.56	-
Seedlings (B)	4	13.50	0.46	-
Interaction (AB)	12	11.13		
Error (b)	48	23.79		

** Analysis of arcsin $\sqrt{\%/100}$ for a split-plot design arranged in randomised complete blocks

Appendix 12 : No. conidia (x 10^{-3})/ml suspension prepared from leaf sections 8 days after inoculation with Erysiphe graminis conidia produced on plants during exposure to different concentrations of SO_2 for 24 h (see Fig 19).

	Infector plants exposed to SO_2 at			
Replicate *	0	20	40	60 ppb
1	104	152	384	368
2	176	288	264	432
3	136	192	352	432
4	144	144	296	360
5	72	208	224	440
6	184	232	408	544
7	120	176	256	528
8	128	184	240	408
9	112	184	288	344
10	80	272	232	440
Mean	125.6	203.2	294.4	429.6

* Random samples from a suspension of conidia prepared by shaking 10 leaf sections in 0.01% Tween 80.

Analysis of variance

Source	DF	MS	F	P
Treatments	3	170653.87	55.6	0.001
Error	36	3068.80		

Appendix Table 13 : Development of powdery mildew on seedlings previously exposed to different concentrations of SO_2.

(a) Number of pustules

Pot	Seedling *	5	21	41	62 ppb
1	1	12	4	12	14
	2	16	18	13	9
	3	11	13	18	6
	4	11	10	20	7
	5	18	11	17	12
2	1	8	15	10	11
	2	15	10	15	16
	3	13	11	17	11
	4	15	7	15	6
	5	13	8	12	10
3	1	19	16	20	18
	2	10	12	16	13
	3	4	18	13	6
	4	9	13	8	14
	5	14	22	16	13
4	1	7	12	12	10
	2	4	7	14	7
	3	9	5	9	14
	4	14	14	6	4
	5	4	15	11	8
Mean		11.30	12.05	13.7	10.45

* Pustule number assessed on a section from the first leaf of each seedling 8 days after inoculation.

Appendix Table 13 cont.

(b) Percentage area with pustules

Pot	Seedling [a]	Seedlings exposed to SO_2 at 5	21	41	62 ppb
1	1	15	5	20	15
	2	20	30	20	15
	3	15	15	25	10
	4	15	15	30	10
	5	25	15	25	20
2	1	10	20	15	15
	2	20	15	20	20
	3	15	15	20	15
	4	20	10	20	10
	5	15	20	15	20
3	1	25	20	25	25
	2	10	15	20	20
	3	5	20	15	10
	4	10	20	10	15
	5	15	30	25	15
4	1	10	20	15	15
	2	5	10	20	10
	3	10	10	15	20
	4	20	20	10	5
	5	5	20	20	10
Mean		14.25	17.25	19.25	14.75

[a] % area covered by pustules assessed on a leaf section from the first leaf of each seedling 8 days after inoculation.

Appendix Table 13 . cont.

(c) Anovars *

Source	DF	No. Pustules MS	F	% Area ** MS	F
Pots (blocks)	3	69.96	4.26	40.83	1.61
SO_2 level (A)	3	38.15	2.32	75.75	2.99
Error (a)	9	16.42		25.35	1.77
Seedlings (B)	4	11.78	1.29	21.54	3.50
Interaction (AB)	12	40.73	4.47	42.10	
Error (b)	48	9.10		12.01	

* Analysis for a split-plot design arranged in randomised complete blocks.

** Anovar on arcsin transforms of percentage data.

Appendix 14: No. conidia ($x10^{-4}$)/ml suspension prepared from leaf sections of seedlings exposed to different concentrations of SO_2 for 7 days before inoculation with Erysiphe graminis (See Fig 20)

	Seedlings exposed to SO_2 at			
Replicate [a]	5	21	41	62 ppb
1	232	400	320	184
2	336	392	400	120
3	272	264	328	256
4	296	272	360	248
5	248	352	424	208
6	312	368	472	152
7	192	320	288	176
8	288	336	432	160
9	320	360	336	168
10	288	264	296	160
Mean	278.4	332.8	365.6	183.2

[a] Random samples from a suspension of conidia prepared by shaking 10 leaf sections in 0.01% Tween 80, 8 days after inoculation

Analysis of variance

Source	DF	MS	F	P
Treatments	3	63626.67	24.63	0.001
Error	36	2583.11		

Appendix Table 15: Development of powdery mildew on seedlings during exposure for 7 days to different concentrations of SO_2 *

(a) Number of pustules

Pot	Seedling **	Seedlings exposed to SO_2 at 2	20	40	60 ppb
1	1	3	9	4	13
	2	1	6	4	5
	3	10	5	3	1
	4	3	7	5	10
	5	10	6	6	11
2	1	3	2	5	1
	2	8	20	7	7
	3	9	3	6	5
	4	8	11	7	4
	5	6	3	3	7
3	1	1	6	8	1
	2	4	9	4	12
	3	5	5	6	11
	4	9	5	7	6
	5	1	4	5	10
4	1	12	7	6	2
	2	8	1	6	6
	3	10	19	3	5
	4	5	5	4	7
	5	8	11	3	4
Mean		6.2	7.2	5.1	6.4

* Exposure to SO_2 started 24 h after inoculation

** Pustule number assessed on a section from the first leaf of each seedling, 8 days after inoculation.

Apendix Table 15 cont.

(b) Percentage area with pustules

Pot	Seedling *	Seedlings exposed to SO_2 at 2 ppb	20 ppb	40 ppb	60 ppb
1	1	5	15	10	25
	2	1	10	10	10
	3	20	15	5	1
	4	5	10	10	20
	5	20	10	15	25
2	1	5	1	10	1
	2	15	25	15	15
	3	15	5	15	10
	4	10	10	15	5
	5	15	5	5	10
3	1	1	10	15	1
	2	10	25	5	25
	3	10	10	15	25
	4	15	15	15	10
	5	1	5	10	25
4	1	20	10	10	1
	2	15	1	15	20
	3	20	20	5	10
	4	5	15	10	20
	5	10	15	5	10
Mean		10.9	11.6	10.75	13.45

* % leaf area covered by pustules assessed on a leaf section from the first leaf of each seedling, 8 days after inoculation.

Appendix Table 15 : cont.

(c) Anovars *

Source	DF	No. Pustules MS	F	% Area ** MS	F
Pots (blocks)	3	1.55	0.10	10.46	0.25
SO_2 level (A)	3	14.98	0.98	11.14	0.27
Error (a)	9	15.33	0.52	41.95	1.52
Seedlings (B)	4	6.27	1.82	62.98	1.92
Interaction (AB)	12	22.04		79.98	
Error (b)	48	12.10		41.56	

* Analysis for a split - plot design arranged in randomised complete blocks.

** Anovar on arcsin transforms of percentage data.

Appendix 16: No. conidia (x 10^{-6})/ml suspension prepared from leaf sections of seedlings exposed to different concentrations of SO_2 for 7 days after inoculation with Erysiphe graminis (see Fig 21).

	Seedlings exposed to SO_2 at			
Replicate **	2	20	40	60 ppb
1	4	12	16	32
2	4	8	12	16
3	0	12	8	24
4	8	8	12	16
5	8	8	8	20
6	12	8	12	12
7	4	8	4	36
8	0	4	8	12
9	8	0	12	20
10	4	16	8	28
Mean	5.2	8.4	10.0	21.6

* Exposure to SO_2 started 24 h after inoculation.

** Random samples from a suspension of conidia prepared by shaking 10 leaf sections in 0.01% Tween 80, 8 days after inoculation.

Analysis of Variance

Source	DF	MS	F	P
Treatments	3	511.33	18.00	0.001
Error	36	28.4		

Appendix Table 17: Development of powdery mildew on seedlings exposed to different concentrations of SO_2 for 7 days before and 7 days after inoculation *

(a) No. pustules

Pot	Seedlings **	Seedlings exposed to SO_2 at 2 ppb	20 ppb	40 ppb	60 ppb
1	1	1	3	2	3
	2	4	6	5	6
	3	2	11	2	3
	4	2	5	3	2
	5	2	10	1	2
2	1	14	3	1	4
	2	2	1	4	3
	3	8	7	6	1
	4	1	1	1	4
	5	3	3	1	1
3	1	3	1	3	3
	2	1	1	4	5
	3	5	1	1	2
	4	3	4	5	1
	5	1	6	6	2
4	1	8	1	5	4
	2	4	1	3	4
	3	1	4	8	2
	4	2	1	7	4
	5	12	1	6	2
Mean		3.95	3.55	3.7	2.9

* Exposure to SO_2 re-commenced 24 h after inoculation.

** Pustule number assessed on a section from the first leaf of each seedling, 8 days after inoculation.

Appendix Table 17: cont.

(b) Percentage area with pustules

Pot	Seedling *	Seedlings exposed to SO_2 at 2 ppb	20 ppb	40 ppb	60 ppb
1	1	1	5	5	5
	2	5	10	10	10
	3	1	20	5	5
	4	5	10	5	1
	5	1	20	1	5
2	1	20	5	1	10
	2	1	1	5	5
	3	15	10	10	1
	4	1	1	1	5
	5	5	5	1	1
3	1	5	1	5	5
	2	1	1	10	10
	3	10	1	1	1
	4	5	10	10	1
	5	1	10	10	1
4	1	15	1	10	10
	2	5	1	5	10
	3	1	5	20	1
	4	1	1	20	5
	5	15	1	15	1
Mean		5.7	5.95	7.5	4.65

* % leaf area covered by pustules assessed on a leaf section from the first leaf of each seedling, 8 days after inoculation.

Appendix Table 17: cont.

(c) Anovars *

Source	DF	No. pustules MS	F	% Area ** MS	F
Pots (blocks)	3	4.48	0.26	10.46	0.25
SO_2 level (A)	3	4.02	0.23	11.14	0.27
Error (a)	9	17.29	0.49	41.95	1.52
Seedlings (B)	4	2.89	1.56	62.98	1.92
Interaction (AB)	12	9.29		79.98	
Error (b)	48	5.95		41.56	

* Analysis for a split - plot design arranged in randomised complete blocks.

** Anovar on arcsin transforms of percentage data.

Appendix 18: No. conidia (x 10^{-3})/ml suspension prepared from leaf sections of seedlings exposed to different concentrations of SO_2 for 7 days before and 7 days after inoculation (see Fig 22).

	Seedlings exposed to SO_2 at			
Replicate **	2	20	40	60 ppb
1	32	48	72	16
2	32	40	56	24
3	24	40	40	32
4	56	48	64	8
5	16	56	48	8
6	32	32	80	24
7	40	24	56	24
8	8	24	40	40
9	24	32	40	40
10	16	40	48	16
Mean	28.0	38.4	54.4	23.2

* Exposure to SO_2 re-commenced 24 h after inoculation.

** Random samples from a suspension of conidia prepared by shaking 10 leaf sections in 0.01% Tween 80, 8 days after inoculation.

Analysis of Variance

Source	DF	MS	F	P
Treatments	3	1907.20	12.11	0.001
Error	36	157.51		

Appendix 19 : Germination and initial mycelial growth of Pseudocercosporella herpotrichoides conidia in 0 and 0.1 mM sulphite in distilled water and buffers at different pH levels.

(a) Percentage germination of conidia

	Solution in which conidia were suspended *					
	Distilled water (pH5.6)		Citrate buffer (pH4)		Phosphate buffer (pH7)	
Replicate **	-S	+S	-S	+S	-S	+S
1	49	27	12	10	62	56
2	50	30	22	11	51	59
3	45	29	28	8	55	48
4	39	34	24	10	42	53
5	40	37	18	12	45	44
Mean	44.6	31.4	20.8	10.2	51.0	52.0

* Conidia were suspended in 0.5M citrate buffer (ph4), distilled water (pH5.6) or phosphate buffer (pH7), to which either 1ml of sodium bisulphite was added to give a 0.1mM sulphite solution (+S) or 1ml of buffer/water was added as a control (-S).

** Germination assessed on 100 conidia on each glass slide after 18 h at 15°C.

Analysis of variance *

Source		DF	MS	F	P
Treatments	Solution (pH)	2	1379.86	118.70	0.001
	Sulphite	1	203.11	17.47	0.001
	Interaction	2	1278.31	109.96	0.001
Error		24	11.63		

* Analysis of arcsin transforms of percentage data.

Appendix 19 : continued

(b) No hyphae per conidium

	Solution in which conidia were suspended					
	Distilled water (pH5.6)		Citrate buffer (pH4)		Phosphate buffer (pH7)	
Replicate *	-S	+S	-S	+S	-S	+S
1	2.90	4.55	3.00	1.40	2.85	3.50
2	2.95	5.05	3.45	1.30	3.05	3.60
3	2.55	4.85	2.90	1.40	2.85	3.55
4	2.60	5.10	3.10	1.30	3.45	3.75
5	2.90	4.10	3.30	1.35	3.00	3.35
Mean	2.78	4.73	3.15	1.35	3.04	3.55

* No hyphae assessed on 20 germinated conidia on each glass slide after 42h at 15°c.

Analysis of variance

Source		DF	MS	F	P
Treatments	Solution (pH)	2	5.95	105.00	0.001
	Sulphite	1	0.36	6.39	0.05
	Interaction	2	8.95	158.00	0.001
Error		24	0.06		

Appendix 19 : cont.

(c) No. conidia with secondary hyphal branches

	Solution in which conidia were suspended					
	Distilled water (pH5.6)		Citrate buffer (pH4)		Phosphate buffer (pH7)	
Replicate *	-S	+S	-S	+S	-S	+S
1	14	17	12	2	13	14
2	13	16	15	1	14	16
3	12	18	10	2	16	14
4	13	17	13	2	14	16
5	12	18	14	1	15	16

* On each glass slide (replicate), 20 germinated conidia were assessed after 18 h at 15°c.

Contingency tables and X^2 values

	Total no. conidia with primary or secondary hyphal branches *					
	pH4.0		pH5.6		pH7.0	
Sulphite	1°	2°	1°	2°	1°	2°
-	36	64	36	64	28	72
+	92	8	14	86	24	76
X^2	65.65		11.86		0.72	
P	0.001		0.001		0.05	

* Total of 100 germinated conidia assessed (5 slides, 20 conidia per slide).

Appendix 19: cont.

(d) Total length of hyphal per conidium (μm)

	Solution in which conidia were suspended					
	Distilled water (pH5.6)		Citrate buffer (pH4)		Phosphate buffer (pH7)	
Replicate *	-S	+S	-S	+S	-S	+S
1	155	446	250	31	106	361
2	141	441	352	43	127	349
3	125	473	266	38	131	217
4	112	372	228	46	112	335
5	128	456	224	39	120	234
Mean	132.2	437.6	264	39.4	119.2	299.2

* Total hyphal length measured on 20 conidia on each glass slide after 42 h at 15°c.

Analysis of Variance

Source		DF	MS	F	P
Treatments	Solution (pH)	2	44631.65	28.92	0.001
	Sulphite	1	56680.57	36.73	0.001
	Interaction	2	191802.60	124.29	0.001
Error		24	1543.20		

Appendix 20 . Germination and initial mycelial growth of Pseudocercosporella herpotrichoides conidia on water agar after 24 h exposure to different concentrations of sulphur dioxide

(a) Percentage germination of conidia

	Concentration of SO_2 (ppb)			
Replicate *	5	20	40	60
1	94	95	68	24
2	92	96	62	12
3	91	91	71	45
4	96	94	74	52
5	92	92	59	18
6	94	97	65	41
Mean	93.0	94.2	66.5	32

* 100 conidia were examined on each agar plate

Analysis of variance *

Source	DF	MS	F	P
Treatments	3	2405.09	72.88	0.001
Error	20	33.00		

* Analysis of arcsin transorms of percentage data

Appendix 20: continued

b) No. hyphae per conidium

Replicate *	SO_2 (ppb) to which conidia exposed			
	5	20	40	60
1	3.10	2.75	1.65	1.30
2	2.75	2.70	1.70	1.35
3	2.70	2.75	1.85	1.65
4	2.50	2.60	1.75	1.70
5	2.60	2.55	2.05	1.45
6	2.60	2.75	1.90	1.50
Mean	2.71	2.68	1.82	1.49

On each plate 20 germinated conidia were assessed after 18 h incubation at 15°c following exposure.

Analysis of variance

Source	DF	MS	F	P
Treatments	3	2.28	91.77	0.001
Error	20	0.025		

Appendix 20 : continued

(c) No. conidia having secondary hyphal branches

	SO_2 (ppb) to which conidia exposed			
Replicate *	5	20	40	60
1	9	6	1	0
2	7	6	1	0
3	7	7	2	1
4	8	6	2	2
5	7	5	2	0
6	6	7	2	0
Total	44	37	10	3

* On each plate 20 germinated conidia were assessed after 18 h incubation at 15°c following exposure.

Significance test (X^2) *

Treatments compared (ppb SO_2)	^{2} value	P
5 x 20	0.67	NS
5 x 40	26.02	0.001
5 x 60	42.33	0.001
20 x 40	17.89	0.001
20 x 60	27.23	0.001
40 x 60	2.93	0.10 (NS)

* Calculated from total no. conidia with secondary and primary hyphal branches of the 100 conidia assessed per treatment.

Appendix 20 : continued

(d) Total length of hyphae per conidium (μm)

	SO_2 (ppb) to which conidia exposed			
Replicate *	5	20	40	60
1	193	165	26	13
2	187	159	27	17
3	186	164	28	26
4	166	182	40	28
5	156	149	35	15
6	158	167	33	19
Mean	174.3	164.3	31.5	19.7

* On each plate 20 germinated conidia were assessed after 18 h incubation at 15°c following exposure.

Analysis of variance

Source	DF	MS	F	P
Treatments	3	115442.84	1033.74	0.001
Error	20	111.67		

Appendix 21: Germination and initial mycelial growth of *Pseudocercosporella herpotrichoides* conidia on water agar previously exposed to different concentrations of sulphur dioxide (Fig 26).

(a) Percentage germination of conidia

	SO_2 (ppb) to which agar exposed *			
Replicate **	5	20	40	60
1	91	94	43	17
2	88	88	28	8
3	93	89	35	19
4	87	92	39	22
5	90	87	16	9
Mean	89.8	90.0	32.2	14.8

* Uninoculated agar plates were exposed for 24 h before being inoculated with conidia.

** Germination was assessed on 100 conidia per plate after incubation at 15°c for 24 h following exposure.

Analysis of variance

Source	DF	MS	F	P
Treatments	3	3235.33	148	0.001
Error	16	21.82		

* Analysis of arcsin transforms of percentage data.

Appendix 21: continued

(b) No. hyphae per conidium

Replicate *	SO_2 (ppb) to which agar plates exposed			
	5	20	40	60
1	2.60	2.45	2.05	1.95
2	2.50	2.35	2.00	1.35
3	2.60	2.55	1.30	1.55
4	2.65	2.30	1.80	1.45
5	2.55	2.55	2.35	1.35
Mean	2.58	2.44	1.90	1.53

* 20 germinated conidia assessed per plate after 42 h incubation at 15°c following inoculation.

Analysis of variance

Source	DF	MS	F	P
Treatments	3	1.18	20.63	0.001
Error	16	0.06		

Appendix 21: continued.

(c) No. conidia with secondary hyphal branches

Replicate *	SO_2 (ppb) to which agar plates exposed			
	5	20	40	60
1	7	5	3	1
2	7	6	4	0
3	6	6	0	0
4	7	5	1	0
5	7	7	4	0
Total	34	29	12	1

* On each plate 20 germinated conidia were assessed after incubation at 15°c for 42 h following inoculation.

Significance tests (χ^2) *

Treatments compared (ppb SO_2)	χ^2 value	P
5 x 20	0.37	NS
5 x 40	7.23	0.001
5 x 60	35.46	0.001
20 x 40	7.85	0.001
20 x 60	28.59	0.001
40 x 60	8.23	0.001

* Calculated from total number of conidia having secondary or primary hyphal branches of the 100 conidia assessed.

Appendix 21: cont.

(d) Total length of hyphae per conidium (μm)

	SO_2 (ppb) to which agar plates exposed			
Replicate *	5	20	40	60
1	195	132	57	33
2	149	148	93	18
3	149	158	33	28
4	144	177	49	24
5	166	176	87	17
Mean	160.6	158.1	63.8	24

* On each plate 20 germinated conidia were assessed after incubation at 15°c for 42 h following inoculation.

Analysis of Variance

Source	DF	MS	F	P
Treatments	3	23558.58	62.77	0.001
Error	16	375.30		

Appendix 22: Mean colony diameters * (mm) and mean relative growth rates ** of colonies of P. herpotrichoides.

(a) after exposure of mycelial disks for 3h to SO_2/filtered air.

Experiment 1			Experiment 2			Experiment 3		
Days after	SO_2(ppb)		Days after	SO_2(ppb)		Days after	SO_2(ppb)	
fumigation	0	53	fumigation	0	100	fumigation	0	130
0	7	7	0	7	7	0	7	7
10	21.7	21.4	10	35.4	35.7	20	25.6	25.6
13	28.4	28.6	13	64	61	24	28.4	28.7
15	33.4	33.1	16	71	66.9	27	29.6	29.6
17	36.5	36.8	18	74.2	71.3	30	31.1	30.6
19	40.4	41.4	20	76.4	74.4	35	33.4	32.5
21	46.6	46.7	RGR	0.12	0.12	37	34.5	33.8
24	52.5	53.6				RGR	0.04	0.04
26	57.3	56.8						
32	65.4	65.8						
RGR	0.07	0.07						

Experiment 4			Experiment 5		
Days after	SO_2(ppb)		Days after	SO_2(ppb)	
fumigation	0	150	fumigation	0	154
0	7	7	0	5	5
12	16.8	16.2	5	10.3	9.8
14	23.1	22	10	18.8	16.3 *
19	37.9	36.3	14	24	20
22	44.5	43.3	19	27.5	24.6
27	61.9	56	22	31	26.3
34	76.4	76.5	RGR	0.1	0.08
RGR	0.07	0.07			

Appendix 22: cont.

Experiment 6			Experiment 7			Experiment 8		
Days after	SO_2(ppb)		Days after	SO_2(ppb)		Days after	SO_2(ppb)	
fumigation	0	425	fumigation	0	700	fumigation	0	720
0	7	7	0	7	7	0	7	7
2	7	7	2	7	7	2	7	7
4	8.6	8	6	15	14.7	4	8	8
6	10.8	9.8	11	45.3	45.1	6	12.7	13.1
8	18.7	17.5	14	53.9	53.5	8	17.3	17.6
10	22.1	20.6	16	59.9	59.7	10	23.9	23.3
12	27	25.7	18	63.2	62.5	12	29.4	28.9
14	30.9	29.4	20	67.7	67.6	14	33.4	31.8
16	34.4	32.4	22	73	72.4	16	39.6	37.5
18	38.7	36.3	25	78.1	77.8	18	41.9	39.7
20	42.1	39.7	RGR	0.1	0.1	20	46.3	45
22	45	42.8				26	60.8	56.6
24	48.3	45				28	64.2	59.9
26	51.7	48.5				RGR	0.08	0.08
28	54.1	50.9						
30	56.7	52						
RGR	0.07	0.07						

* Mean diameters measured along two right angled axes on each replicate plate, 4-12 replicates per treatment.

** RGR = mean relative growth rate of colony

$$= \frac{\log_e \text{final diameter} - \log_e \text{initial diameter}}{\text{no. days between measurements}}$$

Appendix 22 : cont.

(b) on plates of potato dextrose agar previously exposed to 0 or 425 ppb SO_2 for 3 h before inoculation *

No. days after inoculation	SO_2 (ppb) at which plates exposed	
	0	425
0	7	7
2	7	7
4	7.9	8
6	10.1	9.9
8	16.1	16.3
10	20.6	19.4
12	25.6	25.6
14	28.8	29.2
16	32.2	32.7
18	34.7	36.1
20	37.3	38.9
22	39.6	41.8
24	41.7	44.3
26	43.4	49.8
28	44.8	52.7
RGR	0.07	0.07

* Mean colony diameters based on six replicate plates per treatment, measured along two right angled axes.

Appendix 23. Development of eyespot lesions on seedlings exposed to different concentrations of SO_2 for 14 d before inoculation

(a) No. plants per pot infected with eyespot

	SO_2 (ppb) to which seedlings exposed			
Pot *	5	20	40	60
1	9	10	9	10
2	8	8	9	8
3	10	10	10	9
4	9	10	8	10
5	10	9	9	8
6	10	10	10	9
Mean	9.3	9.5	9.2	9.0

* In each pot 10 plants were assessed 28 d after inoculation.

(b) Disease severity score *

	SO_2 (ppb) to which seedlings exposed			
Pot	5	20	40	60
1	71.1	87.8	72.2	92.2
2	52.2	74.4	75.6	43.3
3	86.7	82.2	85.6	77.8
4	66.7	58.9	55.6	95.6
5	95.6	53.3	73.0	78.9
6	87.8	82.2	88.9	64.4
Mean	76.6	73.1	75.1	75.4

* Mean score per plant (calculated as described in experiments on eyespot, part E), assessed 28 d after inoculation.

Appendix 23: cont.

(c) Anovars

Source	DF	Incidence		Severity	
		MS	F	MS	F
Treatments	3	0.28	0.41	12.76	0.05
Error	20	0.68		241.62	

Appendix 24 : Development of eyespot lesions on barley during exposure for 26 d to different concentrations of SO_2.

(a) No. plants per pot infected with eyespot

	SO_2 (ppb) to which plants exposed			
Pot *	5	20	40	60
1	6	5	7	9
2	4	6	5	6
3	8	8	7	7
4	8	7	8	6
5	4	6	4	4
Mean	6.0	6.4	6.2	6.4

* In each pot 10 plants were assessed, 28 d after inoculation

(b) Disease severity score

	SO_2 (ppb) to which plants exposed			
Pot *	5	20	40	60
1	38.9	35.6	45.6	65.6
2	33.3	56.7	33.3	54.4
3	73.3	52.2	52.2	52.2
4	73.3	56.7	51.1	38.9
5	38.9	52.2	38.9	40.0

* Mean score per plant (calculated as described in Experiments in eyespot, Part E), assessed 28 d after inoculation.

(c) Anovars for Eyespot

		Incidence		Severity	
Source	DF	MS	F	MS	F
Treatments	3	0.18	0.08	56.0	0.30
Error	20	2.26		166.4	

Appendix 24 : cont.

(d) Percentage area of first, second and third leaf yellowing *

	SO_2 (ppb) to which plants exposed											
	5			20			40			60		
Plant	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1	0	0	0	20	10	0	60	20	0	90	50	30
2	0	0	0	40	10	0	40	0	0	80	50	40
3	50	30	10	60	20	0	40	20	0	40	10	0
4	90	30	10	30	10	0	70	40	10	70	30	0
5	0	0	0	0	0	0	40	20	0	60	30	0
6	0	0	0	0	0	0	50	10	0	90	40	0
7	0	0	0	30	10	0	20	20	0	90	60	10
8	0	0	0	10	0	0	50	20	0	100	30	10
9	10	0	0	30	10	0	50	20	0	90	30	0
10	0	10	0	10	0	0	30	10	0	70	20	0
11	20	20	10	50	20	10	80	30	10	100	60	10
12	60	20	o	40	10	0	40	10	0	90	40	0
Mean	28	9	3	27	8	1	48	18	2	81	38	8

* Assessed on 12 seedlings chosen at random from each chamber, 21 days after inoculation

(e) Anovar for leaf yellowing *

		1st leaf		2nd leaf		3rd leaf	
Source	DF	MS	F	MS	F	MS	F
Treatments	3	85.88	20.39	34.27	13.09	7.72	3.64
Error	44	4.21		2.62		2.12	

* Analysis of $\sqrt{x + 0.5}$ transforms of percentage data.

Appendix 25 : Germination and germ tube growth of Rhynchosporium secalis conidia suspended in 0 or 0.1mM sulphite in distilled water and buffers at different pH levels.

(a) Percentage germination of conidia

	Solution in which conidia were suspended *											
	Distilled water		Citrate buffer						Phosphate buffer			
	pH5.6		pH4		pH5		pH6		pH7		pH8	
Replicate **	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S
1	89	54	2	2	21	0	35	9	41	35	28	24
2	67	37	1	0	17	0	24	10	67	58	15	31
3	91	68	0	1	11	1	28	2	39	37	15	44
4	81	81	0	2	11	0	31	7	51	38	16	20
5	74	61	3	1	25	0	22	5	43	59	20	40
6	82	50	0	1	7	0	43	9	59	44	28	50
7	90	77	0	2	17	2	27	11	65	45	17	46
8	88	77	2	1	23	0	42	12	69	39	16	42
9	91	65	1	0	17	1	27	18	48	41	14	45
10	86	43	1	1	30	0	26	13	40	54	19	19
Mean	83.9	61.3	1.1	1.1	17.9	0.4	30.5	9.6	52.2	45	18.8	36.1
t value ***	4.37		0		11.32		7.75		1.55		4.38	

* Conidia were suspended in distilled water (pH5.6), citrate buffer at pH4, 5 or 6 or phosphate buffer at pH7 or 8 to which either 1ml sodium bisulphite was added to give a 0.1mM sulphite solution (+S), or 1ml water/buffer was added as a control (-S).

** Germination assessed on 100 conidia on each glass slide after 18 h at 20°c.

*** Comparison of means of angular transforms of percentage data using a t test for small samples of equal variance.

Appendix 25: cont.

(b) Germ tube length (μm)

Replicate **	Solution in which conidia were suspended							
	Distilled water		Citrate buffer		Phosphate buffer			
	pH5.6		pH6		pH7		pH8	
	-S	+S	-S	+S	-S	+S	-S	+S
1	65.50	40.25	11.75	7.50	15.25	16.25	7.50	11.50
2	52.50	50.00	9.00	5.25	19.75	17.50	8.00	16.75
3	49.50	46.25	7.00	6.25	16.25	16.25	6.00	16.75
4	28.50	42.25	21.00	5.00	17.00	21.75	10.75	13.25
5	60.25	34.75	14.50	4.75	13.00	23.50	9.75	25.75
6	47.25	26.25	21.75	6.00	20.00	21.25	12.75	21.00
7	43.00	45.00	18.75	10.75	26.75	16.75	7.25	16.25
8	25.50	53.00	26.75	8.25	12.50	18.25	8.25	15.00
9	34.50	33.50	20.75	4.25	28.50	23.25	8.25	33.50
10	40.75	31.00	9.50	10.00	25.00	23.25	16.25	24.50
Mean	44.73	40.23	16.08	6.80	19.40	19.80	9.48	19.43
t value **	0.91		4.19		0.196		4.24	

* Germ tube length was measured on 10 germinated conidia per slide after 18 h at 20°c.

** Comparison of means using t test for small samples of equal variance.

Appendix 26 : Germ tube length (µm) from Rhynchosporium secalis conidia on water agar after 24 h exposure to different concentrations of sulphur dioxide

Replicate *	Concentration of SO_2 (ppb)			
	0	20	40	60
1	28.25	34.25	33.50	35.25
2	21.00	19.25	30.75	25.75
3	22.50	38.00	31.25	20.75
4	28.50	30.25	26.75	28.25
5	25.50	39.00	21.50	28.25
6	33.00	30.00	25.00	28.25
7	24.75	33.75	35.50	27.75
8	26.25	31.50	33.25	23.50
9	23.75	33.50	35.25	24.75
10	33.25	34.75	32.50	40.75
Mean	26.68	32.43	30.53	28.38

* Germ tube length assessed on 10 germinated conidia per plate.

Analysis of Variance

Source	DF	MS	F	P
Treatments	3	62.85	2.46	NS
Error	36	25.57		

Appendix 27: Percentage leaf area affected by lesions 14 days after inoculation of Rhynchosporium secalis conidia exposed for 24 h previously to different concentrations of sulphur dioxide.

(a) First leaf

Pot	Seedling	SO_2 (ppb) to which conidia exposed			
		0	20	40	60
1	1	2	5	5	2
	2	5	2	5	2
	3	5	5	5	2
	4	5	10	2	10
	5	5	10	2	10
2	1	5	2	5	5
	2	5	10	2	2
	3	5	10	10	2
	4	10	2	5	5
	5	5	10	5	5
3	1	5	5	5	2
	2	5	2	5	2
	3	2	15	5	2
	4	10	20	10	5
	5	5	5	5	2
4	1	5	10	10	2
	2	2	10	5	2
	3	2	5	5	5
	4	2	2	10	10
	5	10	2	2	2
Mean		5.00	7.10	5.40	3.95

Appendix 27: cont.

(b) Second leaf

Pot	Seedling	SO_2 (ppb) to which conidia exposed			
		0	20	40	60
1	1	1	5	5	2
	2	2	2	10	2
	3	5	2	2	2
	4	5	5	2	2
	5	5	5	5	2
2	1	2	2	5	2
	2	2	2	2	5
	3	5	10	5	10
	4	2	2	2	2
	5	2	5	2	2
3	1	2	5	5	2
	2	2	5	2	5
	3	5	10	2	2
	4	2	2	2	5
	5	2	5	2	2
4	1	2	5	2	2
	2	2	10	5	2
	3	2	2	2	2
	4	5	5	2	2
	5	10	1	2	5
Mean		3.25	4.50	3.30	3.00

Appendix 27 : cont.

(c) Third Leaf

Pot	Seedling	SO_2 (ppb) to which conidia exposed			
		0	20	40	60
1	1	1	5	5	2
	2	2	2	5	5
	3	2	5	2	2
	4	2	5	1	5
	5	2	2	10	2
2	1	2	2	2	2
	2	2	5	2	5
	3	2	10	20	2
	4	5	5	2	2
	5	5	10	5	5
3	1	1	20	10	2
	2	2	5	2	2
	3	5	2	1	2
	4	2	5	2	2
	5	2	5	2	10
4	1	2	2	2	2
	2	5	5	2	5
	3	5	10	2	10
	4	5	2	2	2
	5	10	20	2	5
Mean		3.20	6.35	4.05	3.70

Appendix 28: Percentage leaf area affected by leaf blotch on seedlings exposed to different concentrations of sulphur dioxide for 14 days before inoculation.

(a) First Leaf

		SO_2 (ppb) to which seedlings exposed			
Pot	Seedling	2	20	40	60
	1	10	2	10	15
	2	10	5	5	10
1	3	10	5	2	2
	4	5	5	5	5
	5	2	5	10	5
	1	10	2	5	2
	2	10	2	5	2
2	3	5	5	5	2
	4	5	5	5	5
	5	5	5	10	5
	1	2	5	5	5
	2	5	2	2	5
3	3	5	2	5	2
	4	5	2	5	2
	5	10	5	2	5
	1	2	10	5	5
	2	5	5	10	5
4	3	10	5	5	10
	4	10	2	5	10
	5	5	5	5	2
Mean		6.55	4.20	5.55	5.20

Appendix 28 : cont.

(b) Second leaf

Pot	Seedling	SO_2 (ppb) to which seedlings exposed			
		2	20	40	60
1	1	10	2	5	5
	2	10	5	2	10
	3	5	5	2	10
	4	5	5	5	2
	5	5	10	2	2
2	1	2	10	5	5
	2	5	2	15	5
	3	5	5	10	2
	4	5	2	2	5
	5	2	5	10	5
3	1	5	5	5	5
	2	10	5	5	15
	3	10	2	5	2
	4	5	5	5	5
	5	10	15	5	2
4	1	5	10	10	5
	2	10	10	10	5
	3	2	10	2	5
	4	10	2	5	2
	5	20	5	5	5
Mean		7.05	6.00	5.75	5.10

Appendix 28 : cont.

(c) Third leaf

Pot	Seedling	SO_2 (ppb) to which seedlings exposed			
		2	20	40	60
1	1	5	5	5	10
	2	5	5	10	20
	3	10	10	5	10
	4	2	5	10	2
	5	20	15	10	10
2	1	20	5	5	5
	2	5	10	5	10
	3	5	10	2	5
	4	10	20	5	20
	5	10	2	10	10
3	1	10	10	15	10
	2	10	10	15	2
	3	15	10	10	25
	4	15	2	10	15
	5	10	10	5	10
4	1	5	10	15	15
	2	10	10	10	25
	3	2	2	5	20
	4	10	10	10	10
	5	15	10	15	10
Mean		9.70	8.55	8.85	12.20

Appendix 28 : cont.

(d) Analysis of vairance *

Source	DF	MS	F	P
Blocks	3	35.00	1.80	NS
SO_2 (A)	3	41.59	2.14	NS
Error (a)	9	19.40		
Seedlings (B)	4	5.77	0.25	NS
Interaction (AB)	12	55.93	2.45	NS
Error (b)	48	22.87		

* Analysis of arcsin transforms of percentage leaf area affected on the third leaf, for a split-plot design arranged in randomised blocks.

Appendix 29 : Severity of leaf blotch on seedlings exposed to different concentrations of sulphur dioxide for 14 days after inoculation (Fig 30 , Table 41).

(a) Percentage area of second and third leaves affected by lesions

		SO_2 (ppb) to which seedlings exposed							
		2		20		40		60	
Pot	Seedling *	2nd	3rd	2nd	3rd	2nd	3rd	2nd	3rd
1	1	10	10	2	20	5	5	15	2
	2	10	10	15	5	5	5	5	1
	3	10	5	5	15	5	2	1	5
	4	10	10	20	15	10	2	1	1
	5	10	10	5	2	2	0	10	1
2	1	5	15	10	10	2	0	10	1
	2	10	10	10	2	5	1	2	2
	3	20	5	20	5	2	0	2	2
	4	20	5	15	10	2	1	5	5
	5	15	5	5	5	5	5	5	1
3	1	5	10	15	10	5	0	5	1
	2	20	10	5	2	5	5	10	1
	3	10	5	1	10	5	1	2	1
	4	10	10	10	5	10	0	2	0
	5	10	5	10	5	5	1	5	0
4	1	5	10	15	10	2	2	5	2
	2	15	15	10	15	5	2	2	2
	3	20	15	20	10	5	0	5	2
	4	10	20	15	10	5	0	2	5
	5	5	5	10	20	5	2	2	2
Mean		11.5	9.5	10.9	9.3	4.75	1.7	4.80	1.85

* Assessed 14 days after inoculation.

Appendix 29: cont.

(b) Anovars *

Source	DF	1st leaf		2nd leaf		3rd leaf	
		MS	F	MS	F	MS	F
Blocks	3	11.13	7.52	0.04	0.07	1.47	4.35
SO_2 (A)	3	2.90	1.96	8.22	13.48	18.55	54.97
Error (a)	9	1.48		0.61		0.34	
Seedlings (B)	4	1.01	3.35	0.28	0.46	0.42	1.24
Interaction (AB)	12	1.50	4.98	0.62	1.02	0.83	2.40
Error (b)	48	0.30		0.61		0.34	

* Analysis of $\sqrt{x + 0.5}$ transforms of percentage data for a split-plot design arranged in randomised blocks.

Appendix 30: Severity of leaf blotch on seedlings exposed to different concentrations of sulphur dioxide for 14 days before and 14 days after inoculation.

(a) Percentage area of first and third leaves affected by lesions

		SO_2 (ppb) to which seedlings exposed							
		2		20		40		60	
Pot	Seedling *	1st	3rd	1st	3rd	1st	3rd	1st	3rd
	1	10	10	2	30	15	2	2	5
	2	30	2	5	5	2	2	2	5
1	3	30	10	2	20	10	2	1	2
	4	20	15	10	10	5	2	0	2
	5	20	20	10	15	5	10	5	2
	1	10	20	2	15	15	5	1	2
	2	10	30	10	15	2	5	5	2
2	3	10	30	10	10	2	2	2	2
	4	10	20	10	10	2	2	1	2
	5	10	2	5	20	10	5	2	10
	1	10	30	5	15	5	2	2	2
	2	5	5	2	5	5	2	2	2
3	3	10	30	10	2	5	10	2	2
	4	10	40	5	2	2	10	2	2
	5	20	30	15	5	5	2	1	2
	1	10	30	2	5	2	5	5	5
	2	15	5	20	2	2	20	2	2
4	3	10	2	2	5	2	30	10	2
	4	10	5	5	2	10	5	2	5
	5	15	25	10	10	10	20	1	1
Mean		13.75	18.55	7.10	10.15	5.80	7.15	2.50	2.95

*Assessed 14 days after inoculation

Appendix 30: cont.

(b) Anovars

Source	DF	1st leaf		2nd leaf		3rd leaf	
		MS	F	MS	F	MS	F
Blocks	3	0.57	0.75	2.35	0.90	0.26	0.07
SO_2 (A)	3	14.28	18.79	7.82	3.00	18.78	5.14
Error (a)	9	0.76		2.61		3.66	
Seedlings (B)	4	0.49	0.78	0.81	0.82	2.19	2.48
Interaction (AB)	12	0.53	0.85	2.23	2.28	1.38	1.56
Error (b)	48	0.63		0.98		0.88	

* Analysis of $\sqrt{x + 0.5}$ transforms of percentage data for a split-plot design arranged in randomised blocks.