AERIAL POLLUTANT EFFECTS ON THE GROWTH OF CEREALS AND ON RIBULOSE BISPHOSPHATE CARBOXYLASE IN VITRO

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by

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ABSTRACT

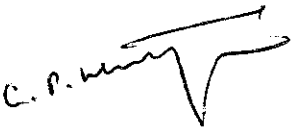
Small areas of barley crop were enclosed within chambers through which air was blown with or without filtration through charcoal. Experiments were conducted in the Marston Valley, Bedfordshire where SO_2 and F are the major gaseous pollutants. The design of the open-topped chambers was based on studies in a wind tunnel, and minimized the influx of ambient air caused by turbulence. A collar and an inner lip were fitted to the top of the chambers. The conditions within the chambers approximated those in the open field although the development of the crops was accelerated by 7-8 days and the yield reduced, resulting from suppressed tillering, even without filtration. Removal of pollutants with charcoal increased straw and grain yields; although the measured pollutant concentrations were similar (~ 50 μgm^{-3}* SO_2 and 0.15 μgm^{-3} F) the magnitude of the effect varied from year to year. The largest increase in yield due to filtration occurred when grain yields from the unenclosed crop were low.

In vitro SO_4^{2-}, SO_3^{2-} and F^- were strong inhibitors of ribulose bisphosphate carboxylase purified from wheat. Inhibition of both carboxylase and oxygenase activities by SO_3^{2-} was biphasic i.e. there was a decrease from the initial velocity over a first phase to a slower and constant rate in the second. SO_3^{2-} may be a stronger inhibitor of ribulose bisphosphate carboxylase than previously reported. SO_4^{2-} was a non-competitive inhibitor with respect to CO_2 and competitive/mixed with respect to ribulose-bisphosphate. F^- was also a competitive inhibitor with respect to CO_2 but an uncompetitive inhibitor with respect to ribulose-bisphosphate.

* $\mu gm^{-3} \longrightarrow \mu g\ m^{-3}$

Additional Statement

All the experimental work was supervised by and the responsibility of M. A. Parry, who was assisted by others in its execution. The work was planned under the supervision of myself and the results discussed by me with M. A. Parry.



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INTRODUCTION

An air pollutant may be defined as any airborne substance that is at a concentration at which it is detrimental to materials, plants or animals. Some pollutants may occur naturally, e.g. HF from volcanoes, but most are man-made. Many substances only become pollutants at high concentrations, and lower concentrations may be essential to life. Thus both CO_2 and O_2 could under certain conditions be regarded as pollutants. If acceptable ground level concentrations are to be identified then the impact of pollutants must be properly assessed.

Pollutants

This thesis concentrates on two important pollutants, SO_2 and F.* A major source of air pollutants is the combustion of fossil fuels. Although the most abundant products of the combustion of coal and oil are CO_2 and water vapour, at the concentrations emitted these are not normally classified as air pollutants. It is the release of smaller quantities of SO_2 and smoke that makes fossil fuels a major source of pollution. Smoke is produced as a result of the incomplete combustion of the fuel and can be reduced by improved combustion technology. Coal and oil contain 1.0-1.6% and 0.75-4.1% sulphur respectively of which 80-100% is liberated on combustion.

Attention in the U.K. has been focussed upon smoke and SO_2 mainly in urban areas and these are the only pollutants monitored by the National Survey.

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*Fluoride(F) is used as a generic term that includes the fluoride ion and combined forms of the element fluorine.

In 1863 Parliament passed an Act setting up the H.M. Alkali and Clean Air Inspectorate in England and Wales and H.M. Industrial Pollution Inspectorate in Scotland (Anon, 1975a). The Act insisted that industry adopt the 'best practicable means' to prevent or minimize pollution. As a result of this action and the introduction of the Clean Air Acts in 1956 and 1968 urban ground level concentrations of smoke and SO_2 have been substantially reduced (Anon, 1979). Although both emissions and ground level concentrations of smoke have fallen, SO_2 emissions doubled between 1940 and 1965 and have remained almost static since then (Anon, 1975b & Anon, 1979). In spite of an increase in emissions from power stations, ground level concentrations have been reduced because most of the increased emission has been discharged at great height.

Whilst the decrease in urban ground level concentrations have been well documented little information is available for rural areas, although one may expect concentrations to have increased as a result of the greater dispersal of pollutants from urban areas.

Although small amounts of F are emitted from domestic and industrial fires burning coal, F has a more local distribution than SO_2 since it arises mainly from specific industrial processes. The main sources are aluminium smelting, glass and glass fibre manufacture, phosphate rock processing, brick-making and ceramics.

Fluctuations and Mixtures

Whilst SO_2 may be the major pollutant in towns, it is rarely if ever present alone. Its measurement is often taken as an index of the

level of other common industrial pollutants although this relationship should not be relied upon as constant mixtures of pollutants rarely exist. Many sources of pollution emit a combination of two or more major pollutants; power stations may be the source of SO_2 and NO_x, domestic coal fires SO_2 and smoke, car exhausts NO_x and hydrocarbons and brickworks SO_2 and F. CO_2 has been recognised to occur in the emissions of most industries.

The concentrations of pollutants fluctuate widely in response to fluctuations in emissions and climatic factors. At sites close to a point source, recurrent episodes of exposure in excess of ten times the annual mean may occur for 2.5% of the time and the maximum hourly peak to annual mean ratio be greater than 100:1. In more remote areas the maximum hourly peak to annual mean ratio is less than 10:1 and episodes of exposure five times in excess of the annual mean occur for 2.5% of the time (I.E.R.E., 1981). In addition several pollutants show a marked diurnal variation which may be particularly important in determining their effects on plants.

Damage may be related to an accumulated dose, i.e. concentration $\times$ time, but it is important to determine whether damage is caused by short-term peaks or a longer term cumulative effect of all exposures. There is no reason to assume that the response to a dose of pollutant is the same for different types of exposure. If short term peaks are important there is no value in expressing results on the basis of average concentration although this is often done.

Accurate description of pollutant concentrations may be prevented by the absence of suitable technology. A number of methods have been developed to analyse the frequency of episodes and can form a valuable means of expression of pollution concentration data (N.E.R.C., 1975).

Biological Effects

Determining acceptable concentrations of pollutants is dependent on assessing their effects. In the past there have been certain disasters due to extreme air pollution which have resulted in the death of people and animals e.g. Meuse Valley in 1930, Donova 1948 and London 1952. Not surprisingly most air pollution control legislation relates to effects on people. There has been no systematic investigation into the effects of pollutants, detrimental or beneficial, on agriculture despite the importance attached to this by the Royal Commission on Environmental Pollution in 1979.

Methodology

Adverse effects caused by industrial emissions have been reported in several areas and a number of methods have been used to investigate these. The available methodology has been assessed in a number of reviews (Mukammal, 1976; Heck et al., 1979; I.E.R.E., 1981; Unsworth, 1982).

Field observations have been successfully used to relate visible injury with pollutant concentrations (Dreisinger & McGovern, 1970; Jones et al., 1979). Visible patterns on leaf surfaces as a result of an increase in subepidermal air space or the death of cells usually occur

about 24 h after exposure and the resultant loss in leaf area can be easily quantified. But even such marked effects can be confounded by gradients in other environmental factors. Although attempts have been made to use the same soil at each site (Cohen & Ruston, 1925; Guderian & Stratmann, 1968; Roberts, personal communication), small variations in climate may prevent effects produced by pollutants, e.g. on growth, being conclusively related to pollutant concentrations.

Most work on the effects of pollutants has been based on fumigation experiments. These experiments usually arrange for a constant concentration and do not simulate the fluctuating concentrations and mixtures observed naturally. Prolonged exposure to a low mean concentration of a single pollutant is not an adequate model for the field situation. A few long-term experiments have begun to use fluctuating concentrations (Garsed et al., 1982) and mixtures (Ashenden & Mansfield, 1978; Ashenden & Williams, 1980; Whitmore et al., 1982). Consequently it is very difficult to relate the results of fumigation experiment to observations in the field (Mukammal, 1976; Black & Unsworth, 1979; Mansfield & Freer-Smith, 1981).

The most direct approach for determining the effects of pollution in the field is to compare plants grown in ambient field air and air that has been cleaned by filtration. Some attempts have been made to do this without using chambers (Jones et al., 1977) but these have had limited success in reducing pollutant concentrations. If chambers are used it is essential that the conditions within them are as close as possible to those in the open field.

The earliest chambers were designed by O. Gara and were modified by numerous other workers (e.g. Thomas & Hill, 1937; Katz & Leedingham, 1939; Brisely & Jones, 1950; Davis, 1972). These chambers had a low air flow and were found to increase temperatures within them by several degrees and reduce light intensities. Consequently the uptake of pollutants was less than half that reported for field crops (IERE, 1981). Other chamber systems have been reported to suffer from similar defects (Leone & Brennan, 1972; Bell & Clough, 1973; Cowling & Koziol, 1978). By increasing the rates of airflow and introducing mixing fans pollutant uptake has been made equal to that in the field (Cowling & Jones, 1978; Ashenden & Mansfield, 1978; Sato et al., 1979). Although Last (1982) suggested that the absence of a moisture film from the leaves for long periods could reduce uptake of pollutants & Unsworth (1982) suggested that plants in chambers may experience an enhanced uptake of pollutants because of a change in concentration gradients around the plants. This may be overcome by improved air distribution systems and the use of crop densities similar to those in the field.

To minimize the effect of enclosure on microclimate open-topped chambers were developed (Mandl et al., 1973; Heagle et al.,1973). In these, temperature, relative humidity and light intensity are close to and fluctuate in the same way as values in the surrounding field. Small but measurable differences in microclimate still exist between open-topped chambers and the field. As with closed chambers the air flow may differ from that of the field, since in most systems air is introduced near to the ground and flows upwards through the plant canopy. This problem has been overcome in a recent design (Roberts et al., 1983) in which air is blown over the surface of the canopy. A further problem

with the open-topped design is that the concentrations of pollutants in the filtered chambers can only be satisfactorily reduced in calm weather and on cool days due to wind-generated and thermal inflows (King & Smith, 1975; Kats et al., 1976; Chamberlain, 1976). This may severely restrict the number of studies in which open-topped chambers can satisfactorily exclude polluted air. Some modifications have been tried (King & Smith, 1975) but such modifications have been ad hoc not the result of aerodynamic studies. Although preferable to closed chambers measurable differences in microclimate exist between the open-topped chambers and the field and any enclosure may affect plant growth and development (Howell et al., 1979). Experiments using chambers have rarely included comparisons with plants grown outside. Whilst (Heagle et al., 1973) found good agreement in the growth and yield of spinach Spinacea oleracea from chambers ventilated with unfiltered air and unenclosed plots, substantial differences in growth and development were found by other workers (Howell et al., 1979; Olszyk, et al., 1980).

Growth Effects

It has been shown that plant productivity may be lowered by pollutants in the absence of visible injury. These effects which may pass unnoticed in the field may be described as hidden injury. Recently Heath (1980) proposed that hidden injury be defined as biochemical or physiological alterations resulting in lowered plant productivity without visible injury.

Often cases of acute injury occur immediately adjacent to a point source and affect a very limited acreage, whereas hidden injury may be found over much larger areas and be of greater economic significance.

Whilst the air filtration method has been used extensively in North America to determine the effects of photochemical oxidants (Heggestad, 1980) only a few such studies have been made in the U.K. (Bleasdale, 1973; Crittenden & Read, 1978 and Bell & Ashmore, personal communication). In all these studies SO_2 was thought to be a major pollutant.

Bleasdale conducted experiments at Fallowfield in Manchester in 1950/51. He compared the growth of ryegrass (Lolium perenne L.) in two small closed greenhouses one of which was ventilated with ambient air and the other with filtered air. The dry weight of plants in the unfiltered air containing 140-180 μgm^{-3} SO_2 was decreased by up to 57%. Crittenden & Read (1978) described experiments conducted in Sheffield in 1973 and 1974 which were similar to those of Bleasdale (1973) but used charcoal filters instead of water scrubbers to clean the air. Growth in ambient air containing 50-90 μgm^{-3} SO_2 was 40% less than in filtered air. Crops differed in their susceptibility to pollutants: Crittenden & Read found no significant decrease in dry matter production in wheat Triticum aestivum cv. Maris Widgeon exposed for 210 days to air containing a mean SO_2 concentration of 55 μgm^{-3} SO_2. Neither Crittenden and Read nor Bleasdale investigated the effect of enclosure on growth or yield. Because of the poor ventilation rates used by Bleasdale and Crittenden and Read, 0.3 and 0.75 air changes min^{-1} respectively, the concentrations of SO_2 in the unfiltered chambers were

much lower than ambient. Bleasdale reported an average depletion of 50% whereas Crittenden & Read found a greater depletion at higher than at low SO_2 concentrations, 55% and 43% respectively. Thus even though the mean and particularly the peak concentrations were reduced in the unfiltered chambers substantial growth reductions were recorded. Mansfield and Freer-Smith (1981) in their investigation of the effects of urban air pollution on plant growth concluded that fumigations with SO_2 at concentrations similar to those found in urban air, in the absence of other pollutants have not usually caused such large decreases. This may be due to the fluctuations of SO_2 concentration in the urban air or to the presence of other pollutants. However, Roberts et al. (1983) and Colvill et al. (1983) reported only small effects on growth in experiments conducted at St. Helens between 1977 and 1980. These authors grew plants in four small open-topped chambers, two of which were ventilated with carbon-filtered air, and in two unenclosed plots. Because the chambers had 6-7 air changes/min the SO_2 concentrations within the unfiltered ones was close to the ambient and fluctuated in the same way. However, because of turbulent inflows the concentration in the filtered chamber was only reduced by 56%, compared to an 80% reduction in the closed chambers of Crittenden and Read and Bleasdale. In an initial experiment conducted in air containing 125 μgm^{-3} SO_2 shoot yields were increased by 16% in the filtered chambers, but in two later experiments done in air containing 73 and 104 μgm^{-3} SO_2 there were no significant differences between the yield of grasses grown in filtered or unfiltered air. In this study the SO_2 concentration in the filtered chambers were high and the ambient concentrations lower than in the earlier work of Bleasdale and Crittenden and Read. The effects described by Roberts et al were ascribed to a reduction in O_3 and NO_2 as well as in SO_2

concentrations; they may be present at concentrations equal to or greater than SO_2. The composition of urban air varies from site to site precluding direct comparisons between the magnitude of results obtained at different sites. Whilst there is good evidence that the low concentrations of pollutants present in ambient air can affect plant productivity it is not possible to identify the role of any individual pollutant. The effects of individual pollutants have been examined in fumigation experiments, but it is difficult to relate the results of these to the field.

In Western Europe most attention has been paid to SO_2 but still the effects of low concentrations in the absence of visible injury are uncertain. In the UK most experiments have been performed with ryegrass Lolium perenne L., an important agricultural crop. Bell et al., (1979) showed that there were significant reductions in growth following exposure to 43 μgm^{-3} SO_2 for 173 days, 106 μgm^{-3} for 194 days, 122 μgm^{-3} for 44 days and 220 μgm^{-3} for 133 days but no effect of 66 μgm^{-3} for 133 days using the same exposure system. Other workers have found no adverse effect on growth at concentrations below 400 μgm^{-3} (Lockyer et al., 1976; Cowling & Lockyer, 1976 and 1978; Cowling & Koziol, 1978). In fact there have been claims that low concentrations of SO_2 may be beneficial (Ross, 1971; Kamprath, 1972) and these suggestions have been supported by the results of Cowling & Jones (1970), Jones et al. (1972) and Cowling & Jones (1978). The beneficial effects of SO_2 have only been observed on plants growing in soils thought to be deficient in sulphur and there are thought to be few agricultural areas of the U.K. where sulphur is in short supply. However, the absence of sulphur from modern fertilizers may lead to sulphur depletion in future.

Godzik & Krupa (1982) reported that some components of yield of barley (Hordeum vulgare L.) can be stimulated by low concentrations of SO_2 but this is not true for grain production. Thus economic yield may decrease even when some other components of plant productivity increase.

Although F pollution has only local distribution it has been intensively studied. Fluoride is more phytotoxic than SO_2 and accumulates in plants (Weinstein, 1977) although a number of reports have observed a loss of F from plants (Knabe, 1970; Davison, Blakemore & Craggs, 1979) and a reduction of F concentrations by growth dilution (Hitchcock, et al., 1971). The rate of F uptake by leaves is more rapid than that of other pollutants, e.g. SO_2, O_3 and NO_x (Bennett & Hill, 1973a). The primary route of entry of gaseous F is via the stomata and once inside the leaf the F moves in the transpiration stream to the tips and the margins. If sufficient F accumulates injury may occur. Only small amounts of F appear to be translocated to other areas (Benedict et al., 1964; Kronberger & Halbwachs, 1978; Garrec & Vavasseur, 1978). As a result of the accumulation of F there is often a gradient of F concentrations in leaves taken from around a point source (Kay, 1974; McClenahon & Weidensaul, 1977). It is however very difficult to relate the fluoride content of leaves to injury but Weinstein (1977) concluded that the threshold for visible injury of susceptible plants was less than 100 $\mu g\ g^{-1}$ and may be lower than 20 $\mu g\ g^{-1}$. However, as a result of recent advances in emission control technology, episodes of visible injury to vegetation are rare in developed countries.

Although F fumigations causing visible injury may have no discernible effect on growth (Hitchcock et al., 1971) many reports e.g. Brewer et al., 1960a, 1960b; MacLean et al., 1977) have demonstrated that growth may be affected in the absence of visible injury. Both harmful and beneficial effects have been reported and the effects on different organs of the plant may not be the same. Thus Brewer et al. (1960a, 1960b, 1967) showed that F increased the linear growth of citrus and roses but decreased the area and weight of individual leaves. A major effect of F appears to be on fruit and seed production. Pack & Sulzbach (1976) found a reduction in both the number and dry weight of pea seeds in plants exposed to between 4.8 and 9.1 μgm^{-3} F. A reduction in reproductive tissue reduced demand for assimilate and therefore vegetative production was stimulated increasing the dry weight of leaves and stems. Effects of F are most pronounced on crops grown for fruit or seed production but these vary in sensitivity. MacLean, Schneider & McCune (1977) exposed tomatoes and beans to 0.6 μgm^{-3} F and found no effect on the yield of tomatoes but bean plants yield was reduced by 25%. This contrasts with the sensitivity of the two species to visible injury, and visible injury caused by F cannot be used to assess the response to lower concentrations. Reduced seed production has been ascribed to the inhibition of pollen germination and pollen tube growth (Sulzbach & Pack, 1972; Facteau et al., 1973). These symptoms are similar to those of calcium deficiency. Although concentrations of 0.6 μgm^{-3} may occur in some areas they would be unlikely to persist for long periods. Growth effects of prolonged exposures to low concentrations of F have yet to be examined.

The interaction between different pollutants has seldom been investigated at realistic concentrations. Tingey et al (1971) studied the effect on leaf injury of SO_2 and NO_2 mixtures at concentrations ranging from 140 to 1400 μgm^{-3}. They concluded that the injury threshold was at much lower concentrations when the two pollutants occurred together rather than in isolation. Bennett et al (1975) confirmed this. A similar synergistic effect of these two pollutants has been found for growth effects. Ashenden and Mansfield (1978) reported that exposure to NO_2 had a small beneficial effect on growth whilst SO_2 caused a yield reduction; however, a much greater reduction was found when the two pollutants occurred together. The interaction between SO_2 and F has received little attention. Those investigators who have considered it used high concentrations to ensure visible damage (Solberg & Adams, 1956; Hitchcock et al., 1962; Matsuhama & Brewer, 1972 and Mandl, et al., 1975). McCune (1980) reviewing this work found no evidence of any non-additive effects on growth but the presence of SO_2 has been found to reduce F accumulation in a number of species e.g. sweetcorn, alfalfa and ryegrass (Mandl et al., 1975).

Physiological and Biochemical Responses to Pollutants

The response of plants to pollutants is complex, nevertheless for growth and yield reductions to occur pollutants must affect cellular metabolism. The evaluation of pollutant effects in the field is highly desirable and can be achieved using appropriate experiments but details of the mechanism are very difficult to elucidate. Most investigations into physiological processes have been conducted in controlled environment facilities rather than in the field. Some investigators have

used parts of plants and unrealistically high pollutant concentrations unrepresentative of the conditions in nature.

Pollutants may react chemically within the water phase of the cell and protoplasm so that the chemical form of the pollutant arriving at the metabolic sites will not be identical with that which is present in the atmosphere outside the plant, e.g. SO_2 will presumably enter the cell as an uncharged molecule and dissociate to form SO_3^{2-} and HSO_3^- and be oxidized to form SO_4^{2-}. Thus it is very important to identify the form which a pollutant has when entering the chloroplast and to determine the potency of various forms in relation to the reactions taking place in the cells.

Photosynthesis

Photosynthesis is the process common to all autotrophic organisms whereby acids produced from the fixation of CO_2 are converted into sugars. Any effects of pollutants on this process will have direct effects on growth. The effects of both SO_2 and F have been examined.

The inhibition of photosynthesis is often regarded as the first sign of SO_2 action on plants (Hill, 1974; Inglis & Hill, 1974; Roberts et al., 1971; Black & Unsworth, 1979). The majority of investigators report a reduction in net photosynthesis although some reports with lichens (Hill, 1974) algae (Hällgren & Huss, 1975; Puckett et al., 1974) chloroplasts (Libera et al., 1973) and higher plants (Bull & Mansfield, 1974; Black & Unsworth, 1979; Winner & Mooney, 1980) have reported on stimulation at low SO_2 concentrations. Generally any such

stimulatory effect is short-lived and has been ascribed to depressed photorespiration or increased stomatal conductance (Black, 1982). The magnitude of the inhibition has, like growth responses, been found to vary with plant species and leaf age. Concentrations of 100 μgm^{-3} have been reported to inhibit photosynthesis in sensitive species (Keller, 1981). The depression of photosynthesis can occur in the absence of visible injury (Black & Unsworth, 1979), their magnitude is dependent on SO_2 concentrations and at low concentrations are readily reversible. Sisson et al. (1981) were able to relate changes in photosynthetic activity directly to sulphur uptake. The effects of higher concentrations of SO_2 do not relate to the SO_2 concentration, and are irreversible due to the breakdown of biochemical systems.

A number of reports have indicated that in the absence of visible injury photosynthesis is unaffected by F (Hill et al., 1958; Hill, 1969; Thompson et al., 1979). Others have reported that photosynthesis is sensitive to F exposure (Thomas & Hendricks, 1956; Thomas, 1958; Thomas & Alther, 1966 and Bennett & Hill, 1973a, 1973b), even at low concentrations (McCune et al., 1976). This is consistent with reports that the chloroplast is the site of highest F accumulation (Chang & Thompson, 1966). As with SO_2 the inhibition of photosynthesis in the absence of visible damage has been shown to be reversible (McCune et al., 1976). This is not compatible with the report of Jacobson et al (1966) that F remains soluble and unbound although some reports suggest the recovery of photosynthesis may be incomplete (Thomas & Alther, 1966).

A number of experiments have demonstrated a modified response to pollutant mixtures. Whilst the combination of some concentrations of SO_2 and NO_2 caused reductions in net photosynthesis which were greater than the summed response of individual pollutants, at other concentrations in the mixture the response may be less than additive (Bull & Mansfield, 1974).

Mechanism of Action

A number of apparently plausible mechanisms for SO_2 effects on photosynthesis have been proposed. However, most are based on *in vitro* experiments conducted on isolated chloroplasts, membranes or enzymes and their relationship with *in vivo* physiological conditions is unclear (Hällgren, 1978). In addition many investigators have used high concentrations of SO_2 which are not consistent with effects on photosynthesis in the absence of visible injury.

Exposure to high concentrations of pollutants can result in the degradation of chlorophyll and other important photosynthetic pigments (Malhotra, 1976; LeBlanc & Rao, 1975; Ricks & Williams, 1974) but lower concentrations, below 100 ppm SO_2,* were found to have no effect on these pigments and 10-50 ppm SO_2 increased chlorophyllase activity. Several investigators have demonstrated that the rate of photosynthesis can be reduced prior to the detection of any changes in chlorophyll content (Showman, 1972; Puckett *et al.*, 1974; Hill, 1974; Hällgren & Huss, 1975 and Bull & Mansfield, 1974).

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*1 ppm $SO_2 \equiv 2860\ \mu gm^{-3}$.

A number of investigators have claimed that photosynthesis can be affected indirectly by changes in the structure and permeability of membranes. Malhotra (1976) related a decrease in O_2 evolution after exposure to 500 ppm SO_2 to ultrastructural changes in the chloroplast, a swelling of the grana thylakoids and granulation of the stroma. Wellburn et al. (1972) found that such changes in ultrastructure were reversible after exposure to low concentrations of SO_2. The leakage of potassium ions and primary photosynthetic products can be explained by a disruption of cellular membranes (Puckett et al., 1974; Nieboer, et al., 1976). The cleavage of disulfide linkages by SO_3^{2-} is one of the proposed mechanisms of damage (Puckett et al., 1974). Grill and Esterbaer (1973) found the S-H content was higher in plants damaged by SO_2 than in those suffering no injury but such evidence is inconclusive.

Reports indicate that SO_2 can affect electron transport and photophosphorylation and thus affect the C_3 cycle (Sij & Swanson, 1974; Asada, et al., 1965; Harvey & Legge, 1979). In vitro studies by Cerovic et al. (1982) have suggested there is competitive inhibition between orthophosphate and SO_2 (or SO_3^{2-}) since both PGA dependent O_2 evolution and photophosphorylation have the same inhibition constant (0.8 mM). However this is not consistent with results from in vivo investigations since Black and Unsworth (1979) and Hällgren and Gezelius (1982) have found no inhibition of photosynthesis at low light intensities when electron flow and photophosphorylation are considered to be rate limiting. This suggests that the primary effect of SO_2 is not on these processes.

The mechanism by which SO_2 interferes with photosynthesis was thought to be the competitive inhibition of Ribulose 1,5-bisphosphate (RuBP) carboxylase by SO_3^{2-} with respect to HCO_3^- (Ziegler, 1972). In 1973 Fischer et al. concluded that the first change in the ultrastructure of chloroplasts exposed to SO_2 was granulation of the stroma. This change preceded the change in grana structure, swelling of the grana compartments and breakdown of internal structure. The granulation was reversible and comparable crystalline assays have been observed in leaves affected by other pollutants, water stress, infection and herbicides (Thompson, Dugger & Palmer, 1966; Dolzmann & Ullrich, 1966) and Steer et al (1968) suggested that the crystalloids observed in several species with C_3 photosynthesis are a crystalline state of RuBP carboxylase. The activity of this enzyme also decreases after SO_2 fumigation (Horsman & Wellburn, 1975; Miszalski & Ziegler, 1980). Hällgren and Gezelius (1982) concluded that the decrease in photosynthesis may be due to lower levels of RuBP carboxylase as a result of decreased protein synthesis but could not explain the reversible inhibition of photosynthesis. But this ignores any reversible inhibition of the enzyme which may occur in vivo and would be lost during extraction procedures. Direct effects of SO_3^{2-} on the catalytic activity of isolated enzymes has been observed. Ziegler (1972) found that SO_3^{2-} inhibited RuBP carboxylase with respect to HCO_3^- in a competitive way the SO_3^{2-} replacing HCO_3^- at the active site on the enzyme. She also reported a non-competitive inhibition by SO_3^{2-} with respect to RuBP and concluded that SO_3^{2-} did not react with the keto group of RuBP. More recently Gezelius and Hällgren (1980) using the same assay conditions as Ziegler (1972) found that SO_3^{2-} was a less potent inhibitor than Ziegler claimed and observed K_i values with

respect to HCO_3^- ranging from 9-13 mM compared to the 3 mM found by Ziegler. In addition the pattern of inhibition that they found was non-competitive. In contrast Khan and Malhotra (1982) found competitive inhibition with respect to HCO_3^- and a K_i of 2.2 mM. The discrepancy in the results may be explained by the conditions under which the enzyme was assayed for it is probable that the enzyme was not fully activated throughout all of the experiments. The activation requirements of RuBP carboxylase have only recently been demonstrated (Lorimer et al., 1976). The results of the in vitro experiments are conflicting and require resolution. Support of Ziegler's conclusions can be found in fumigation experiments where Black (1982) has found that high CO_2 concentrations reduced inhibition of photosynthesis by SO_2, and that this change could not be accounted for by changes in stomatal conductance. Similarly plants with a C_4 type of photosynthesis and an increased concentration of CO_2 in the bundle sheath cells will be less affected by SO_2. Winner and Mooney (1980) compared the influence of SO_2 on two Atriplex species one of which was C_3, the other C_4. They found that the C_3 species was more sensitive to SO_2 than the C_4, but this must also reflect the different morphological configuration of the mesophyll tissues and different response to stomatal opening following exposure to SO_2. Since the C_4 species has a lower conductance it absorbed less SO_2 during the fumigation; furthermore, the C_3 plants were more vulnerable to stimulation of stomatal opening by SO_2. Consequently the rates of SO_2 flux into the C_3 leaf and water flux out of the leaf were both increased whereas C_4 plants are better adapted to SO_2 polluted environments.

Despite attempts to elucidate F induced effects on photosynthesis no clear-cut mechanism of action has been discovered. High concentrations have been shown to affect chloroplast ultrastructure (Horvath et al., 1978; Weinstein & Alscher Herman, 1982) with a dilation of internal membranes and a reduction in pigmented membranes. A reduction in chlorophyll because of decreased pigment synthesis (McNalty & Newman, 1961) could not explain the reversible inhibition of photosynthesis observed in short-term studies (Bennett & Hill, 1973b). In addition Wei & Miller (1972) suggest that any changes in chloroplast ultrastructure will be secondary to changes in other cellular components.

Inhibitory effects of F have been reduced by the addition of Mg^{2+} and it has been suggested that the presence of F may render Mg^{2+} physiologically inactive. Mg^{2+} is essential to several processes including thylakoid function, the Hill reaction and the activation of RuBP carboxylase (Lorimer et al., 1976). Effects of F on RuBP carboxylase have been neglected but Venesland and Turkington (1966) and Ballantyne (1972) have demonstrated an inhibitory effect of F on the Hill reaction of bean chloroplasts.

Present Problem

This study is to define the effects of ambient concentrations of pollutants in a relatively polluted rural area, the Marston Valley, Bedfordshire, on the growth development and yield of barley crops. The valley is the centre of the U.K. brick manufacturing industry from the local clays and there has been considerable local concern about the concentrations of pollution in the area and their effects on health and agriculture (DOE, 1980; Cremer & Warner, 1979). There are a number of

historical reports of pollution damage to plants and animals in the area (Blakemore et al., 1948; Burns & Allcroft, 1964; Thorold, 1968; Chamberlain, Personal communication). Despite the presence of a number of brickworks the area is primarily agricultural.

The clay used to manufacture the bricks is of the lower Oxford series and has a high content (~ 5%) of a lignite-like material. This material acts as a fuel, contributes to the firing of the bricks and represents an important saving in energy. The combustion process gives rise to three major pollutants, SO_2, HF and mercaptans. The mercaptans give the fumes their characteristic odour but there is no evidence that they are harmful to plants. Although brickmaking is scheduled under the Alkali Act of 1906 at the present time and in the absence of suitable technology the alkali inspectorate do not require the removal of pollutants from flue gases. Their principle requirement is for tall chimneys to dispose fumes. However the present kilns have many stacks each 50 m tall; moreover, the emissions have a low efflux velocity which can lead to poor dispersion. Although the ratio of SO_2:HF from the flue gas is 100:1 (DOE, 1980) a higher ratio must be expected at ground level due to the ambient background of SO_2. Under certain adverse conditions the pollutants accumulate and episodes of pollution occur during which sensitive plants are usually damaged. Incidents in which crops are visibly damaged are rare and in the vicinity of the works the normal ambient concentrations of pollution are only slightly increased. The last incident when extensive visible damage was recorded was in 1968 (Chamberlain, personal communication) when the pattern of damage was characteristic of SO_2 injury.

This study determined whether pollution in the Bedfordshire brickfields which did not cause visible injury affected growth and yield of barley. Growth experiments were preceded by a survey of the area. In initial experiments closed and open-topped chambers were used, neither were adequate; conditions in the closed chambers were not a good facsimile of those in the field and pollutant concentrations could not be satisfactorily reduced in the open-topped chambers. Therefore a modified open-topped chamber was developed and used in subsequent experiments. The effects of SO_2 and F on RuBP carboxylase were also studied, because this seemed a probable mechanism of action for yield reductions by these pollutants.

MATERIALS AND METHODS

VEGETATION SURVEY

To assess possible biological effects around the brickworks an initial survey of the vegetation was conducted. Naturally-occurring hawthorn Crataegus monogyna was chosen for this because it was widely distributed throughout the area. Leaf samples of 20 g fresh weight were taken at the end of August from established bushes in hedgerows at various distances from the brickworks. The leaf samples were oven dried at 80°C to a constant weight and then ground coarsely using a mill (Junior Lab) and finely using a ball mill (Glen Creston). Samples were analysed for total sulphur and fluoride content.

Fluoride Determination

Fluoride concentrations were determined using a specific ion electrode after an acid and then alkali extraction (Jacobson & Heller, 1970). The method is simpler and faster than the official method (Anon, 1970) and a collaborative study (Jacobson & Heller, 1975) demonstrated that it gave reproducible results.

A 500 mg sample of the dried vegetation was extracted with 10 ml of 0.025 M H_2SO_4 and five drops of Manoxal (a B.D.H. wetting agent). After shaking for 15 min 10 ml of 0.1 M NaOH was added and the mixture agitated for a further 15 min. At the end of this period the extract was brought to pH 7.0 by the addition of 1 ml of 0.25 M H_2SO_4 and buffered by the addition of 2.5 ml of 2 M sodium acetate (pH 7.0) and

5 ml of 0.5 M trisodium citrate (pH 7.0). Fluoride concentrations in the solution were determined with a specific ion electrode (Orion 901 microprocessor analyser & fluoride and reference electrodes). The electrode response was linear between 10^{-2} and 10^{-5} M F^- concentrations (Fig. 2:1). The microprocessor was programmed to give a direct measurement of sample concentration and was calibrated by adjusting the slope between two standard solutions of the extraction mixture with added fluoride. The blank correction determined for the extraction mixture was automatically removed from sample measurements. The samples were stirred slowly and the concentration measured after 3 min. The analysis was conducted by an assistant.

Sulphur Analysis

The milled powder was made into tablets using ~4 g of sample at 10 t pressure in a 33 mm die. Total sulphur in the tablets was then determined using an x-ray fluorescence spectrometer (Philips 1540 Manual Spectrometer). This method of analysis gives reproducible results fast and these agree with chemical methods of analysis (Brown, Kanaris-Satiriou, 1969).

Field Samples

Samples were taken in 1980 at 30 m intervals along a transect across a field of cereal, spring barley Hordeum vulgare L. var. Ark Royal. The fields were chosen for their uniform soil type and expected gradient of pollution, information supplied by the London Brick Company (personal communication).

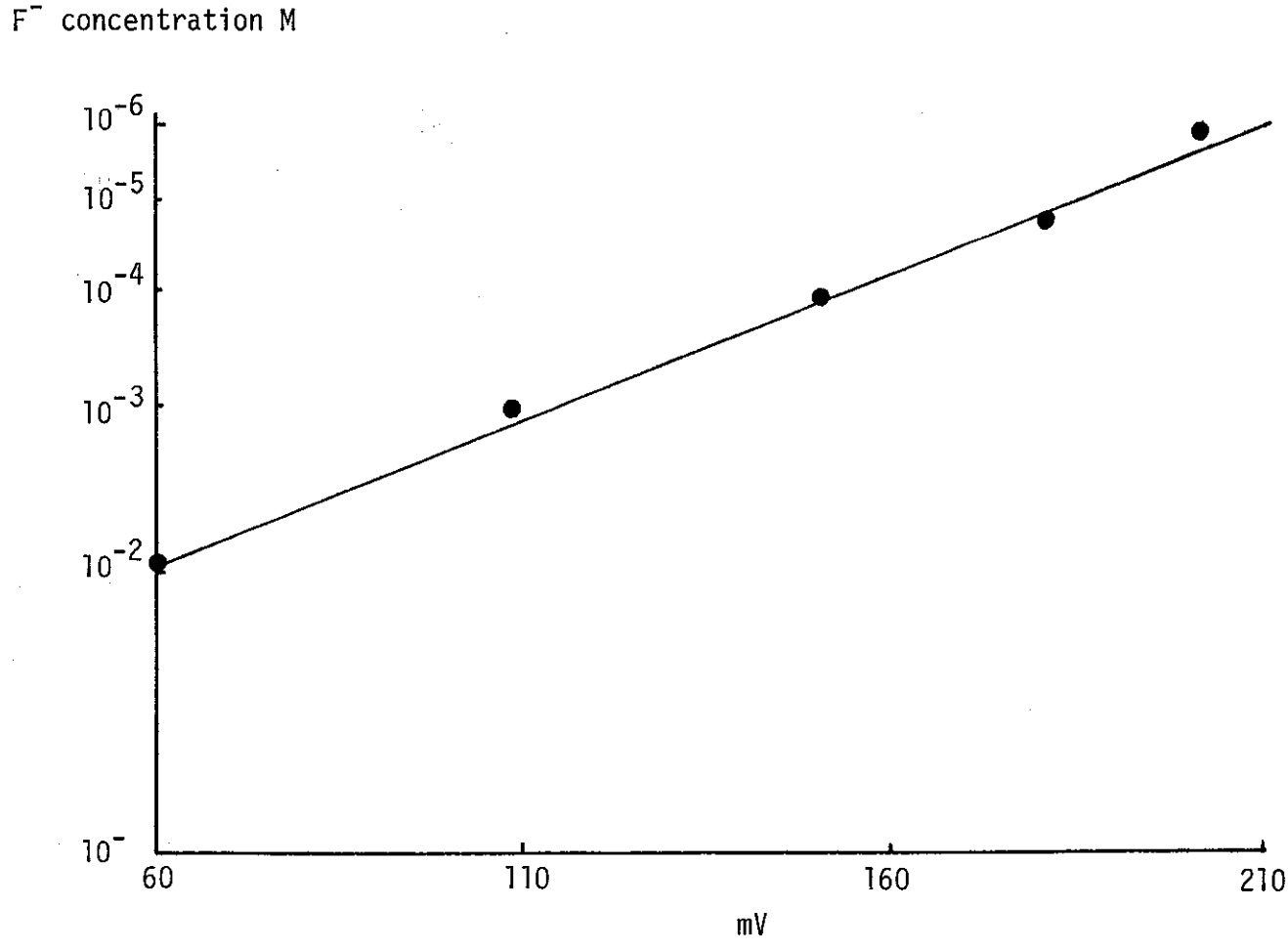


FIGURE 2:1 Electrode response to changes in fluoride concentrations.

The samples, 0.5 m lengths of row, were cut at ground level when the crop was ripe. The ears were removed, counted and oven-dried at 80°C to a constant weight. The leaf laminae were also removed dried and then analysed for total sulphur and fluoride content.

CHAMBER SYSTEMS

This section describes the chamber systems used in this study and how they have been developed to maintain environmental conditions as close as possible to those outside whilst reducing concentrations of pollutants. Various designs of chamber were evaluated in wind tunnel tests and the most successful features incorporated and tested in field chambers. Chambers of two basic designs were used, closed and open-topped types.

Closed Chambers

Closed chambers were used for Experiments 1-3. They were 3 × 5 m and were chosen for their low cost and speed of construction (Plate 1). A framework of metal hoops was covered in 600 gauge polythene sheeting (Clovis Lande Associates). The chambers were operated in pairs; centrifugal fans (London Fan Company) ventilated one with ambient field air and the other with filtered air. Air was cleaned by passing through a filtration unit which had a cross-sectional area of 0.4 m^2. The air first passed through a Vokes VG2 prefilter panel, to remove coarse particulates, followed by a bed of activated charcoal (Norit RBAA) 7.5 cm deep to remove gaseous pollutants. This was followed by a Vokes VG2 prefilter and Vokes B22 absolute filter to remove carbon dust. The air

PLATE 1: Closed chamber with fan and filtration unit.

was then distributed by a plenum and directed into the chambers avoiding turbulent damage to the crops. The rate of air flow in unfiltered chambers was made similar to that of the filtered one by an adjustable damper on the fan inlet.

The chambers were run under a slight positive pressure, air escaping through four 15 cm outlets, at the opposite end of the chambers to the fans. The air flow through the chambers was estimated by recording the flow through the outlets using a vane anenometer (Air Flow Developments UK). Each chamber was ventilated at 2.5 air changes min^{-1}.

Air temperatures and relative humidities were measured at the centre of the chambers 0.5 m above the ground with screened and ventilated dry and wet thermocouples. Photon flux density (PFD) at the same height was measured with quantum light sensors (Lambda).

Open-Topped Chambers

The open-topped chambers used in experiments 2-5 were based on the design of the Boyce Thompson Institute (Mandl et al., 1973). Each chamber was cylindrical 3 m high and 3 m in diameter mode from a framework of galvenized conduit tubing covered in square section Novolux sheeting (I.C.I.). This sheeting was rigid and guaranteed against discolouration for five years. Access to the chambers was by a doorway in the side (Plate 2).

The chambers were operated in pairs, one ventilated with ambient field air, the other with filtered air. Axial flow fans supplied 58 m^3 of

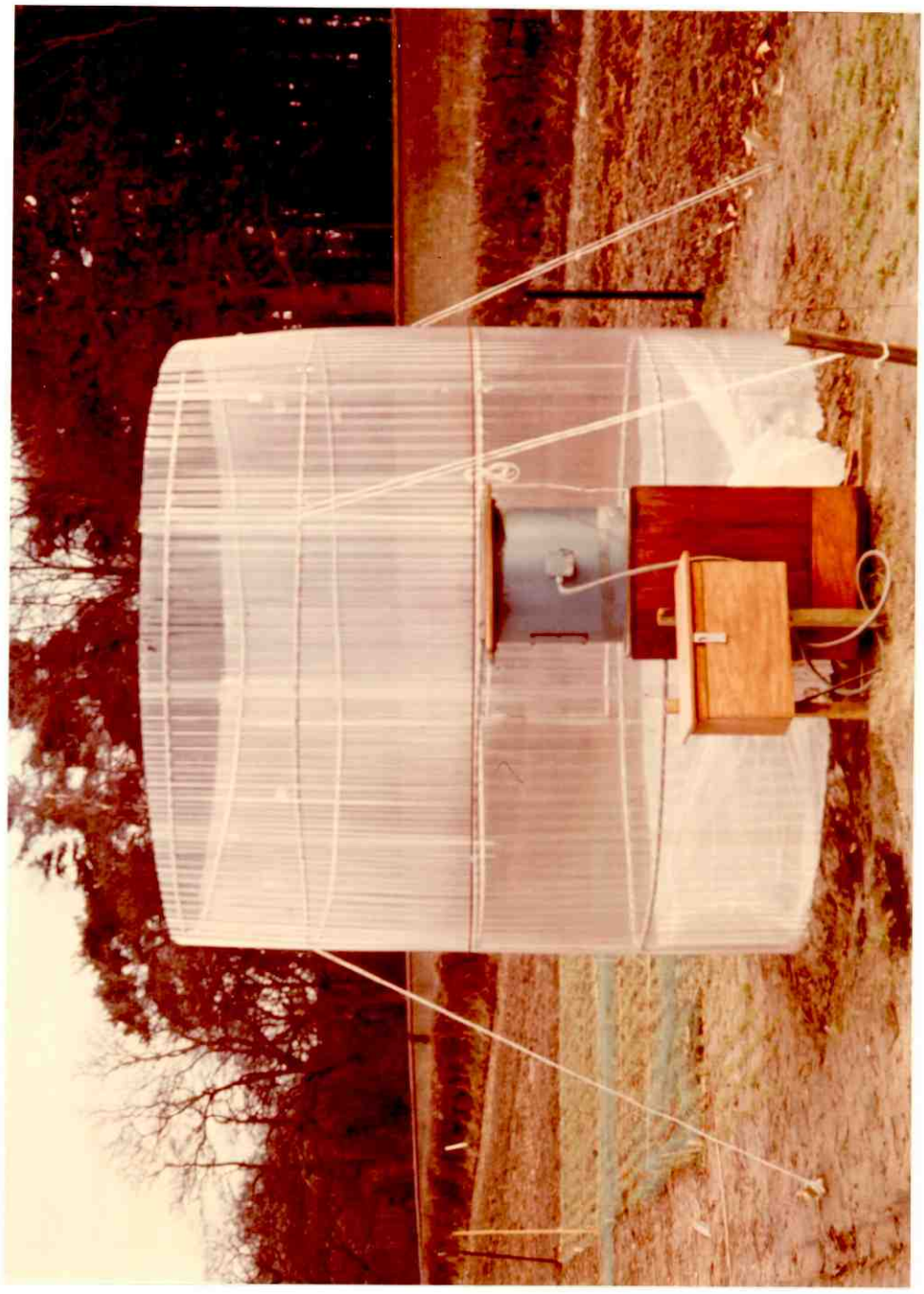


PLATE 2: Unmodified open-topped chamber with fan and filtration unit.

air min^{-1} to each of the chambers to produce 3-4 air changes min^{-1}. The air supply to the filtered chamber passed through a filtration unit of the same design as described for the closed chambers. Air was distributed within each chamber through 1 m wide polythene layflat tubing (Transatlantic Plastics) placed around the inside circumference of the chamber. The air passed out of holes 20-30 cm above ground level, directed upwards and towards the centre of the chamber.

The flow rate was estimated using a vane anenometer in the ductwork. The air flow to the unfiltered chamber was adjusted to equal that of the filtered using an adjustable damper on the fan inlet. The measured flow rates were equivalent to a mean vertical flow of $0.15 m s^{-1}$.

In later experiments this basic open-topped chamber was modified as a result of wind tunnel tests.

Tests of Model Chambers

Models were tested in a small wind tunnel at Rothamsted. It has a cross-section 0.6 m wide and 0.9 m high and wind speed can be varied from $0-5 m s^{-1}$.

A 1:12 scale model of a cylinder 2.3 m high and 2.5 m in diameter was used. This was of a smaller diameter than the field chambers but the size was reduced to increase the vertical flow and air exchange rate. Both the model and floor of the wind tunnel were covered in sandpaper to simulate the turbulence experienced in the field. The model was ventilated by six perforated parallel pipes at the base of the chamber to

give a draught of air moving vertically upwards to give 3.5 air changes min^{-1}. The modifications that were tested in detail are shown in Fig. 2:2.

Tests were conducted in turbulent air flow at five windspeeds between 0 and 5 m s^{-1}. A turbulent air stream was chosen to simulate the conditions likely to be encountered in the field. The flow characteristics of the model were examined by releasing smoke into the air stream upwind of the model and observing the movement of smoke over and into the model. Quantitative measurements of the efficiency of the model in excluding pollutants were made by ventilating it with CO_2-free air and measuring the concentration of CO_2 inside it using an Infra Red Gas Analyser (Analytical Development Corporation). The air in the model was sampled to give a mean concentration through a six port manifold held at 3 cm from the base of the chamber. The efficiency (E) of the chamber is defined as the percentage of CO_2 excluded:

$$E = 100 - \left(\frac{\text{Int. conc.}}{\text{Ext. conc.}} \times 100 \right)$$

The efficiency of the most successful modification was examined in more detail, samples were taken via a T piece independently from seven points around the chamber at a height of 3 cm and at five heights in the centre of the chamber (3, 6, 9, 15 and 18 cm) (Fig. 2:3). All the tests were repeated on three occasions.

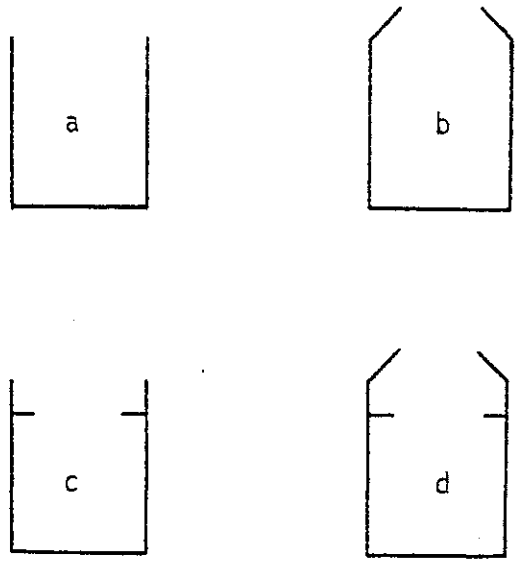


FIGURE 2:2 Vertical sections of chamber designs tested in the wind tunnel:

a unmodified chamber
b chamber + collar
c chamber + inner lip
d chamber + collar + inner lip

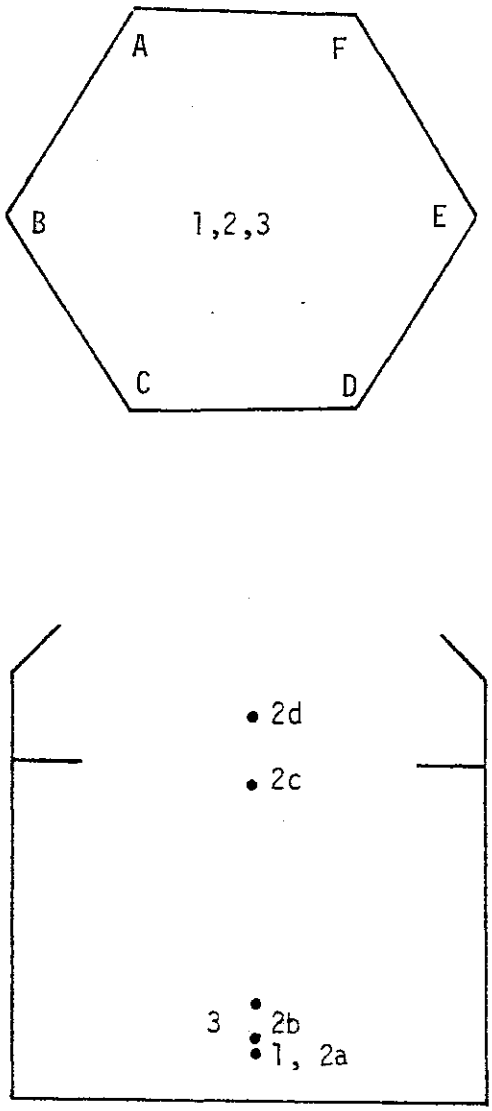


FIGURE 2:3 Sampling points in model and field chambers:

1, air smples via manifold in model chamber

2, air samples via T piece for height vs. efficiency measurements in model chamber.
A-F air samples via T piece for efficiency distribution around the model chamber.

3, air samples, temperature, relative humidity and light intensity measurement in field chambers.

Consultation with the National Physical Laboratory and Warren Spring Laboratory suggested that no formal assessment of Reynolds number (R_e) was required because when smoke was released into the air stream the pattern of flow remained constant over a wide range of wind speeds. Once turbulence had started the flow pattern was not altered by an increase in R_e because the ratio of inertia to mean flow remained constant at increased wind speed.

The efficiency of the chambers in excluding gases was dependent on wind speed, the greatest efficiency being observed in still air (Fig. 2:4). The maximum efficiency of the unmodified model chamber was 40% in still air rapidly decreasing to 4.7% at 5 m s^{-1}. The efficiency of the model was improved by modifying its design. Covering the chamber with a fine mesh net had little effect but the addition of a collar to the top of the chamber (Fig. 2:4, line c) increased efficiency to 81% in still air and 21% at 5 m s^{-1}. Installing a lip on the inside of the chamber had a similar effect. When both the collar and inner lip were used together the modifications increased efficiency to 91% in still air and 46% at 5 m s^{-1}. The wind generated inflows were reduced by the collar because it streamlined the movement of air over the chamber displacing the trailing edge mixing layer and increased the speed of the air leaving the chamber. The inner lip deflected air currents across the mouth of the chamber and prevented them from penetrating as far as the base of the chamber.

In the fully modified model there was little variation in the efficiency with height for samples taken beneath the inner lip (Fig. 2:5) but the efficiency was greatly reduced above the inner lip. Measurements

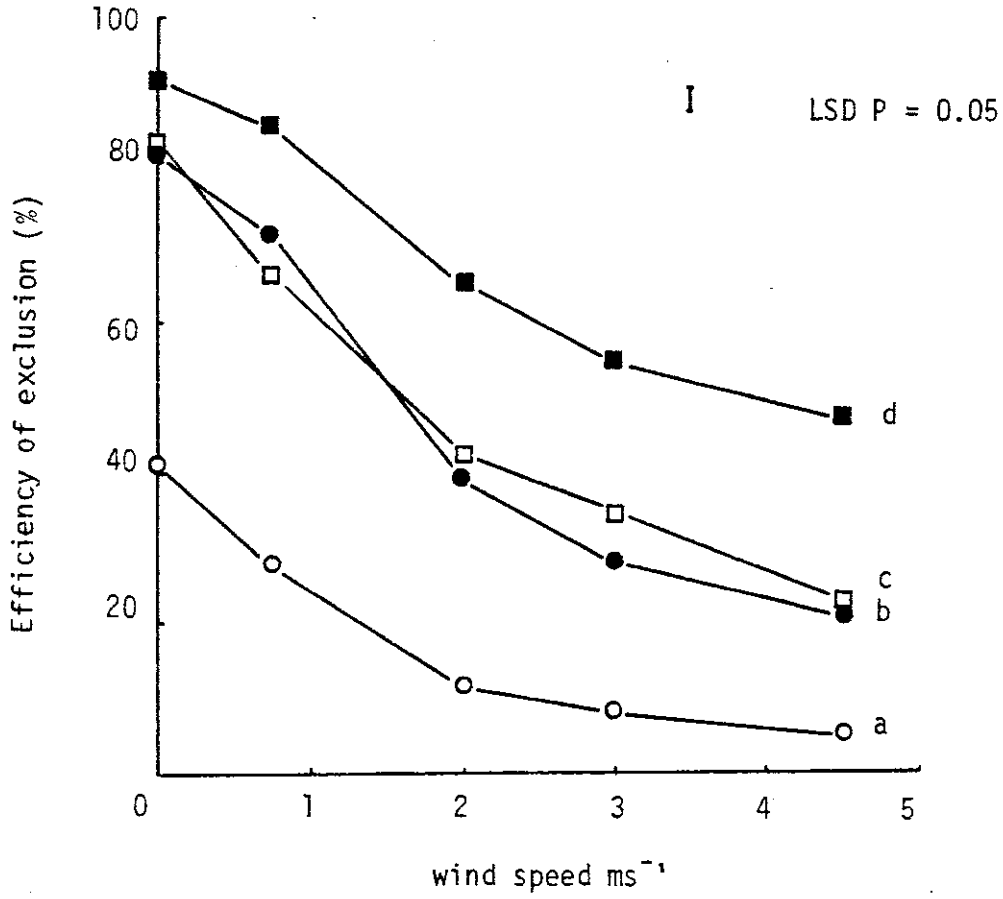


FIGURE 2:4 Efficiency of different modifications to the model chamber:

a no modification

b chamber + collar

c chamber + inner lip

d chamber + collar + inner lip

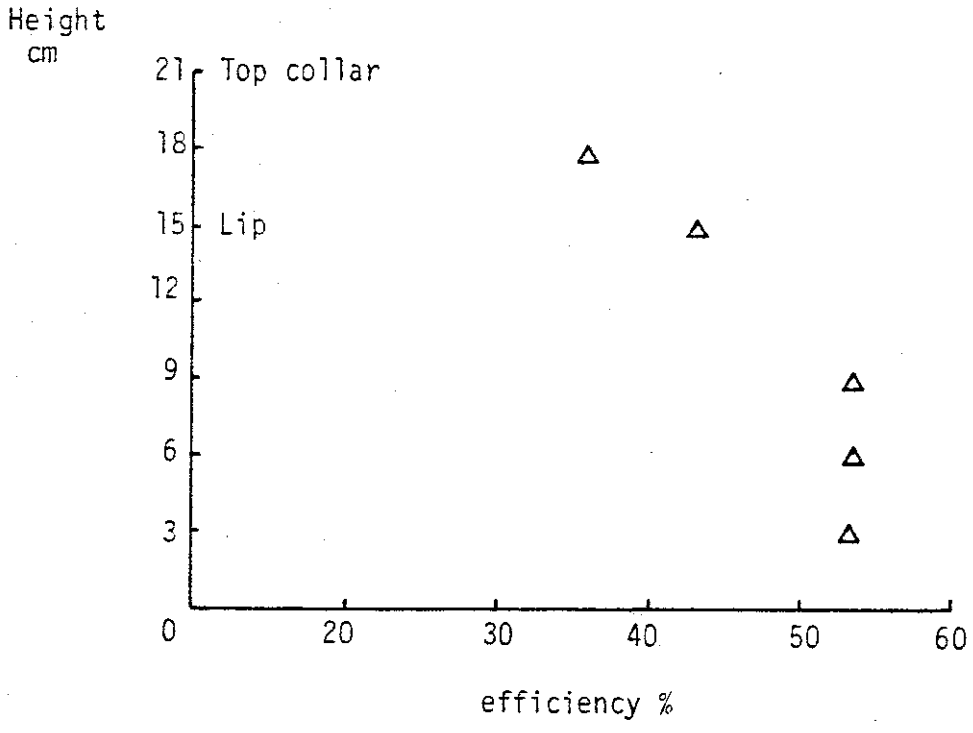


FIGURE 2:5 Efficiency at different heights in the fully modified model chamber.

Fig 2:5

of efficiency taken at points around the chamber were in good agreement and varied within 15% (Fig. 2:6). These results agree with those of other workers who have attempted to streamline the tops of chambers in the field to reduce inflows (Kats et al., 1976; King & Smith, 1975).

Lipped and Collared Open-Topped Chambers

Using the results obtained in the wind tunnel field chambers were designed and constructed (Fig. 2:7, Plate 3). These chambers were hexagonal for ease of construction, 2.3 m in height and 2.4 m in diameter, and incorporated an inner lip and a collar, both of which reduced the opening at the top of the chamber.

A chamber frame was constructed of horizontal bars of 4 cm aluminium right angle and vertical aluminium glazing bars. A cut 0.5 m below the top of the glazing bars allowed them to be bent to support the collar. The lip was made by bolting a piece of aluminium glazing bar 0.3 m long to the horizontal framework. The framework was covered in square-section Novolux sheeting. Access to the chamber was by two sliding doors on opposite sides of the chamber (Baco Ltd.). Large axial flow fans supplied 116 m^3 min^{-1} air to the chambers. The air passed through a Thermiser (Aldridge Air Control) which contained 16 activated carbon filters arranged in parallel. The filters were 1.25 cm thick and had a surface area of 8.9 m^2. The carbon filters were sandwiched between pad filters which removed particles. The fans and filters were installed one metre from the northern corner of the chamber to reduce shading. Two flexible ducts connected the filter unit to the rigid ductwork of the chambers. For row crops each half of the chamber had a 0.25 m diameter pipe along

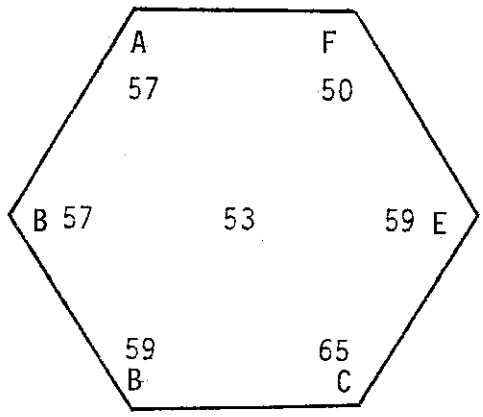


FIGURE 2:6 Efficiency at different points around the fully modified model chamber 3 cm from the base.

Fig 2.6

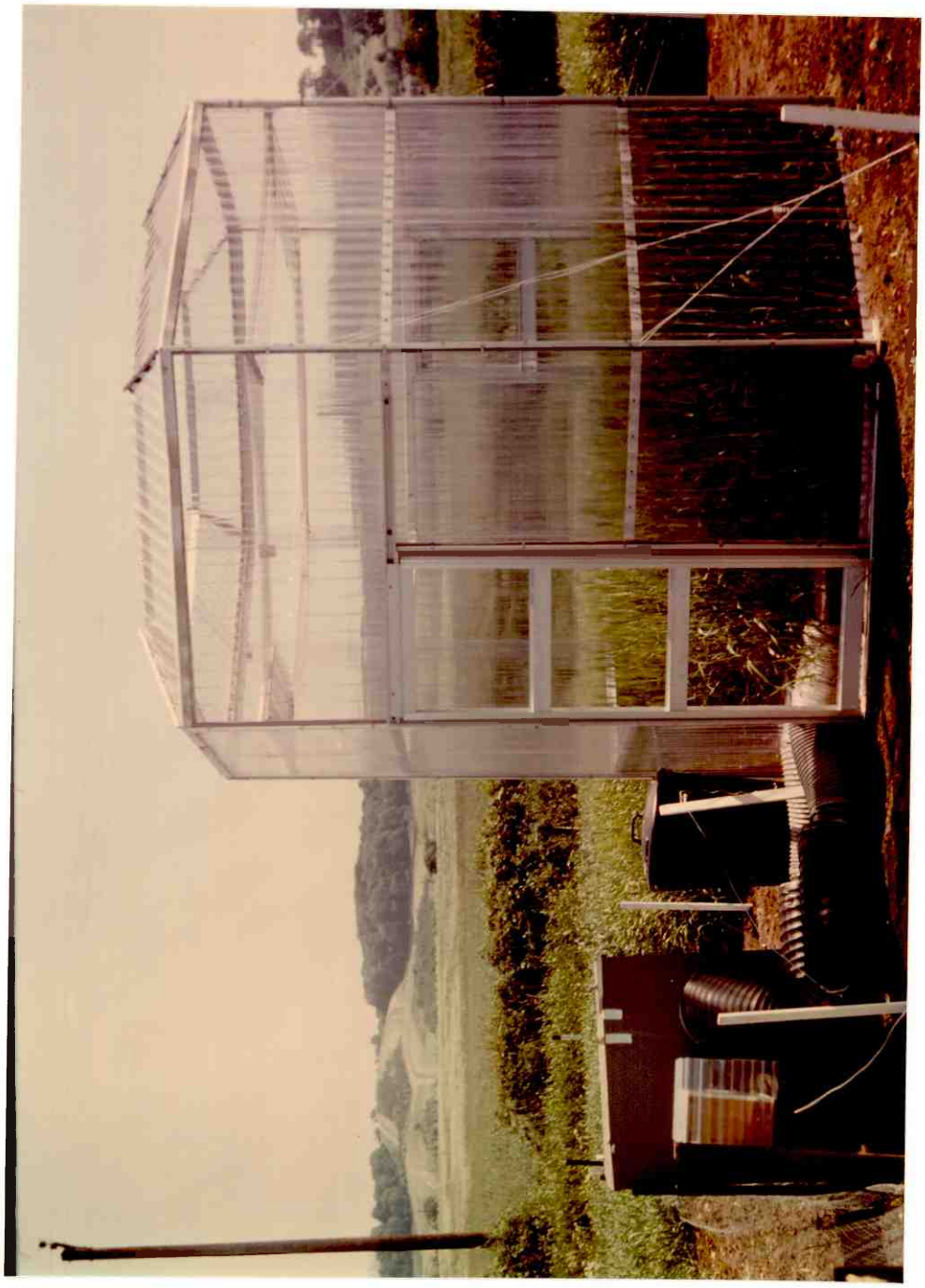


PLATE 3: Collared and lipped open-topped chamber with ventilation and irrigation systems.

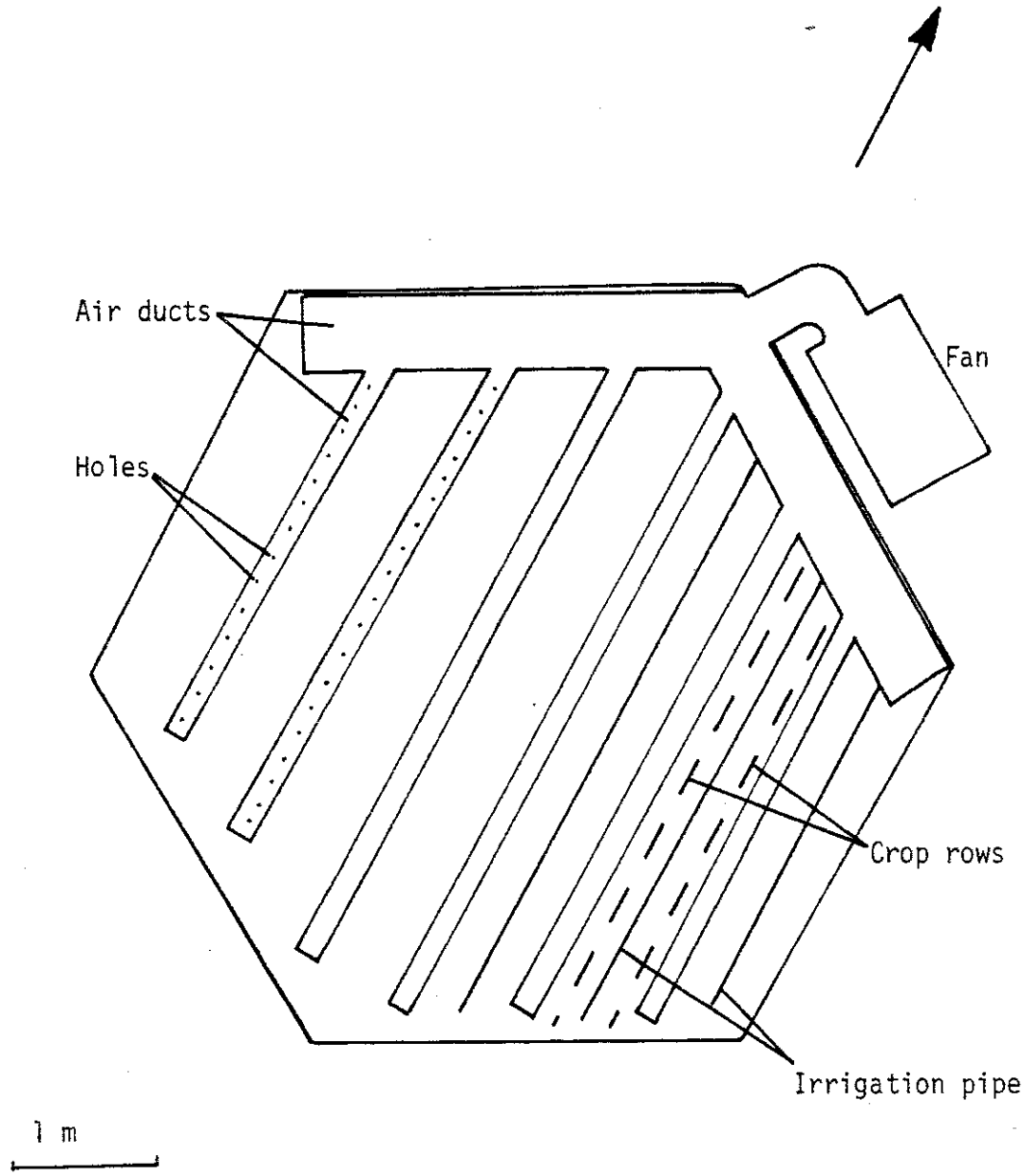


FIGURE 2:7 Field chamber with ventilation and irrigation system for row crops.

one wall of the chamber connected to three parallel pipes 0.1 m in diameter which cross the chamber between the rows of the crop. The small ducts were perforated with holes at 3 cm spacing which directed both up and down.

Air temperatures, relative humidities and PFD were measured as in the closed chambers (p.37).

MEASUREMENT OF POLLUTANTS

Sulphur Dioxide

Volumetric air samples were used to determine mean SO_2 concentrations. An air sample drawn at a known flow rate was bubbled into a neutral solution of H_2O_2 (1 vol). The absorbed SO_2 produced H_2SO_4 which was titrated to pH 4.5 with sodium tetraborate (Anon, 1966). The method is the standard method employed by the National Survey for Air Pollution.

In Experiments 1 and 2 eight port samplers (Glass Developments Ltd.) were used. These operated automatically, samples being changed at 09.00 GMT. Air samples were drawn to samplers along 8 mm internal diameter P.V.C. tubing, less than 12 m long. In experiments (3-5) the eight port samplers were replaced by Dreschel bottles placed at the sampling point. This was done to overcome any possible air line interference (Crittenden, 1976). In this case samples were collected at 10.00 h GMT either every day or at 2-3 day intervals.

Air samples were taken throughout the growing season at crop height both inside the chambers and over the unenclosed plots. There was insufficient equipment to monitor all of the chambers in replicated experiments continuously but each chamber was monitored occasionally at random to check its performance.

In Experiments 3-5 a continuous record showing short-term peak concentrations was obtained using a Meloy SA285 flame photometric analyser (Meloy Lab Inc.) connected to a chart recorder (Rikadenki). Samples were drawn to the Meloy along a less than 3 m length of Teflon tubing.

Sometimes this analyser was connected to a multichannel gas handling unit (Analytical Development Corporation) designed and built to handle pollutants. This unit continually drew air along Teflon tubes from several sampling points. The Meloy sampled each gas stream in turn for 4 min enabling short-term concentrations at different sampling points to be compared.

Fluoride

Fluoride concentration was measured by drawing air through treated filter papers. The prefilter was impregnated with 0.1 M citric acid to retain particulate fluoride and a second paper with 0.1 M Na_2CO_3 to absorb gaseous fluoride (Davison et al., 1973). To prevent acidification and subsequent loss of trapped fluoride two or three papers impregnated with Na_2CO_3 were used for longer sampling periods, e.g. over a weekend. The papers were extracted with acid and then alkali and the fluoride in the extracts measured using a specific ion electrode.

Measurements of fluoride were taken throughout Experiment 5 during the growing season over the unenclosed plots at crop height.

Other Pollutants

Intermittent measurements of NO_x and O_3 were made. Ozone was monitored using an ultra-violet spectrophotometer Dasibi 110 which gave a continuous record of O_3 concentrations. In one experiment NO_x was monitored using diffusion tubes supplied by Harwell Environmental and Medical Science Division (Aitkin, personal communication). Four tubes were mounted vertically in each type of chamber and outside, they were sited to allow free air movement but, as far as possible, out of direct draughts. Sampling periods were of several weeks.

GROWTH EXPERIMENTS

This section describes experiments designed to determine the effect of ambient pollution on crop growth. Unenclosed plots were used to investigate the effects of enclosure on crop growth. Cultivars of Hordeum vulgare L. were selected from the N.I.A.B. list. Experiments were done at two sites providing a range of pollutant concentrations & contrasting soil types.

The site at Woburn Experimental Farm (O.S. sheet 153 G.R. 965358) was 2 km west of Ridgmont brickworks. The soil was sandy pH 7.0. Cereals had been grown for eight years prior to this study. Thrupp End Farm, Lidlington (O.S. sheet 153 G.R. 988397) was 1.8 km NE of the Ridgmont

works and 4.9 km to the SW of Stewartby brickworks. The soil was a clay loam pH 6.8 that had lain fallow during the year previous to each investigation.

Experimental Procedures

The experimental site was rotavated and fertilizer raked in to provide the required amounts of N. P. K. (Table 2:1). Seed was sown by hand at about 1.3 cm intervals in rows 17.8 cm apart to give approximately 430 seeds m^{-2}. The chambers were placed in position immediately after sowing and ventilation started as soon as possible after, usually 2-3 days. The continual development of equipment and experimental techniques resulted in the adoption of several experimental designs which are detailed in the results section.

In Experiments 1-3 water was supplied by a hand-held hose, but in Experiments 4 and 5 irrigation was supplied from pipes laid between the rows of the crop (Fig. 2:7). The crops were watered in amounts approximating to the rainfall on the unenclosed plots.

Mouse traps were used to control the mouse population and the sites were fenced to prevent rabbit damage. The unenclosed plots were covered by a fine net after anthesis to prevent bird damage. In experiment 5, 10 cm mesh netting was used horizontally at 30 cm to prevent lodging.

In Experiments 4 and 5 non-destructive measurements were made at 2-3 day intervals during the development of the crop. Shoots (main stem and tillers) were counted on a 0.5 m length of row on each plot. Six main

shoots from each plot were also tagged and stem height, leaf emergence stage, leaf extension rate and final leaf length measured until anthesis. Leaf extension rates were calculated from changes in the length of the youngest leaf measured from its tip to the ligule of the uppermost fully expanded leaf (Peacock, 1975).

Destructive harvests were taken throughout the growing season, either at developmental stages or harvest dates, see Table 2:1. In all experiments the areas to be sampled were marked out at the start of the experiment. All of the areas sampled were surrounded by single or double guard rows. In Experiments 1-3 the samples were cut at ground level but in the remaining experiments the samples were dug up, washed and remaining roots cut off at the crown node.

For each sample plant and shoot numbers and number of ears were recorded. Green stems and laminae were separated and their areas measured with an electronic planimeter (Paton Industries). Lamina area was taken as the surface area of one side of the leaf and stem area as $\pi/2$ x plan area. The plant parts were oven dried at 80°C to a constant weight.

TABLE 2:1

Experimental Calendar and Details.

Experiment No.	1	2	3	4	5
Year	1976	1977	1978	1979	1980
Experimental Site	Woburn	Woburn	Thrupp End	Thrupp End	Thrupp End
Variety	Abacus	Maris Otter	Porthos	Magnum	Magnum
Seed Rate kg ha^{-1}	157	190	162	150	150
Sowing date	12.3.76	8.10.76	22.3.78	24.4.79	26.3.80
Fertilizer N P K kg ha^{-1}	112/60/60	62/150/150	120/60/60	120/60/60	60/30/30
Final harvest date (unenclosed plots)	5.7.76	10.8.77	23.8.78	21.8.79	26.8.80
No. Chambers: Closed	2	2	2	-	-
Open	4	4	4*	8*	8*

*Collared and lipped chambers - open-topped.

ENZYMOLOGY

Extraction and Purification of RuBP carboxylase

RuBP carboxylase was extracted and purified from wheat (Triticum aestivum var. Maris Dove) and from spinach (Spinacea oleracea). These species were chosen because most published work relates to them. The wheat seeds were sown in seed trays containing a proprietary soilless compost (Eff) enriched with inorganic fertilizer. Seedlings were produced throughout the year and grown for three weeks in a glasshouse. Supplementary lighting (mercury vapour) was used when necessary to give the plants a 16 h day. The laminae of the third and fourth leaves were used. Spinach leaves were obtained from a local greengrocery store and were deribbed and washed with distilled water before use. The leaf material was cut finely and mixed with a 20 mM Tris/Cl buffer pH 8.0 containing 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM EDTA, 10 mM DTT and 0.002% chlorhexidine diacetate (Hibitane ICI) with 1% w/v insoluble polyvinyl polypyrolidone (Sigma). The ratio of buffer to tissue fresh weight was 7:1 v/w.

The leaves were then homogenized for 4 x 15 s periods (with 15 s intervals) at top speed in a Waring blender (MSE Atomix). The homogenate was squeezed through two layers of muslin and the filtrate retained.

For experiments with partially purified extracts the filtrate was centrifuged at 77,000 g av using a 60 Ti rotor in a Beckman L65B centrifuge for 15 or 30 min to remove insoluble debris, and the supernatant used in these experiments.

For complete purification the filtrate containing the carboxylase was 35% saturated with solid $(NH_4)_2SO_4$. After initial stirring it was left for 30 min and the resultant precipitate removed by centrifugation at 20,000 g av for 15 min (MSE HS.21). The supernatant was adjusted to 55% saturation with further additions of $(NH_4)_2SO_4$ to precipitate the RuBP carboxylase protein, then left for 30 min before a further centrifugation at 20,000 g av for 15 min. The precipitate was dissolved in 25 ml of 20 mM Tris/Cl pH 8.0 resuspension buffer containing 1 mM DTT, 1 mM EDTA, 1 mM $MgCl_2$ and 0.002% hibitane and the insoluble material was removed by centrifugation at 77,000 g av for 30 min.

4 ml portions of the supernatant were layered onto eight linear sucrose gradients (8-25% w/v in 32 ml of buffer 2) and centrifuged at 215,000 g av for 2.5 h using a 60 Ti rotor in a Beckman L2 65B centrifuge. The fractions of each gradient containing the RuBP carboxylase were combined and loaded onto a column of DEAE Sephacel Pharmacia (GB) Limited (25 x 360 mm) equilibrated with resuspension buffer. The protein was eluted at 40 ml/h from the column with a linear NaCl gradient (0-0.5 M) and fractions collected. Those fractions exhibiting RuBP carboxylase activity were combined and then desalted in a column of Sephadex G25 (coarse 50 x 310 mm) equilibrated with a 5 mM Hepes buffer pH 8.0. The RuBP carboxylase fractions were collected, combined, shell frozen and then freeze-dried. The freeze-dried enzyme was stored at 5°C over a dessiccant and was found to remain stable for several months.

The protein content was measured by the method of Petersen (1977) and Markwell *et al.* (1978), and bovine serum albumen used as a

standard. From 120 g of leaf material the purification procedure consistently yielded 250-300 mg of purified protein exhibiting a specific activity of 1.0 → 1.6 μmol CO_2 fixed min^{-1} mg $protein^{-1}$ and 0.17-0.27 μmol O_2 fixed min^{-1} mg protein.

Enzyme Activity

To attain maximum catalytic activity the enzyme requires activation involving the binding of CO_2 and Mg^{2+}. The enzyme, in the freeze-dried powder, was dissolved directly into 0.1 M Hepes activation buffer pH 8.2 containing 10 mM $NaHCO_3$ and 20 mM $MgCl_2$. Activation of this form of the enzyme is slow, but can be increased at higher temperatures. Enzyme samples were activated at 40°C for 40 min after which they were found to have reached the maximum activity and could be stored at 25°C without a significant loss of activity over a period of 8 h.

To investigate the time course for activation in the presence and absence of inhibitors different $MgCl_2$ and $NaHCO_3$ concentrations and temperatures were used. Details are given in the Results section.

As CO_2 and O_2 are inhibitors of the oxygenase and carboxylase activities respectively, CO_2 and O_2 free assay solutions were used where appropriate. This was achieved by using boiled-out distilled water, gassing solutions with approximate gas mixtures, and using carbonate-free NaOH to adjust pH of buffers.

The vial assay system used in other studies (Bird et al., 1980) was discarded for several reasons: the preparation of vials was time-

consuming, they had a large gas phase and were cumbersome to use. Rapid sampling of the reaction mixture and simultaneous addition of several chemicals was impossible.

Both carboxylase and oxygenase assays were conducted in a stoppered oxygen electrode (Hansatech U.K. Ltd). This system was simple to use and overcame many of the problems of the vial assay. In addition it allowed the contaminating oxygen to be measured and maintained at a minimum and constant amount ≃ 0.02 mM by bubbling the reaction mixture with O_2-free nitrogen. The elimination of a gas phase removed the need for an equilibration period prior to each assay.

Carboxylase activity was measured in a 0.1 M oxygen-free Bicine buffer pH 8.2 containing 20 mM $MgCl_2$ and various concentrations of $NaH^{14}CO_3$ 1 μCi/μmole and RuBP as stated in the results. A 1.0 ml volume was used and the temperature of the oxygen electrode maintained at 25°C. To ensure the adequate conversion of HCO_3^- to CO_2 during the assays carbonic anhydrase (from Bovine erythrocytes, Sigma) was included (Bird et al., 1980). The course of the reaction was followed in two ways :

(i) by stopping the reaction after 30 s by the addition of 0.1 ml of 2N formic acid and then removing half of the contents, 550 μl, putting it into a scintillation counting vial and drying it in an oven at 60°C.

(ii) by removing 50 µl aliquots from the reaction mixture at different times during the reaction and injecting these into 200 µl of 2N formic acid. Adjustments to the stopper prevented a gas space forming above the reaction mixture. The quenching in formic acid occurred in a counting vial and the contents were oven-dried at 60°C. this technique was used to investigate the time course for the carboxylase reaction in the presence and absence of inhibitors.

The carboxylase activity was calculated from the amount of $^{14}CO_2$ fixed into acid stable products (Bird et al., 1980). Radioactivity was measured by liquid scintillation counting using an Intertechnique 564200 counter. The dried residues in the vials were dissolved in 1 ml of distilled water. 10 ml of scintillator liquid, Tol/triton X-100 (2:1 v/v) were then added (Patterson & Greene, 1965). The scintillator contained 0.4% 2,5-diphenyloxazole and 0.01% (1,4 bis-(2,5 phenyl)oxazolyl benzene) in AR Toluene. The vials were then capped and shaken before being put in the counter. The efficiency of counting was determined using ^{14}C toluene standards, and found to be approximately 85%.

The oxygenase activity was determined polarographically, the initial rate of oxygen consumption and time course followed with an oxygen electrode at 25°C. The Bicine buffer 0.1 M pH 8.2 was equilibrated with CO_2 free air (B.O.C. Special Gases) before use and contained 20 mM $MgCl_2$. Unless otherwise stated the reaction was started by adding the activated enzyme to the assay solution containing 0.06 → 0.6 mM RuBP in a final volume of 1.0 ml. In the presence of high SO_3^{2-} the base line

drifted because of the oxygen consumed in this way did not significantly reduce the SO_3^{2} concentration, and rates were adjusted to allow for the drift in base line.

The kinetic constants K_m and V_{max} were determined using a median method of analysis which has been shown to produce consistently reliable values (Cornish Bowden & Eisenthal, 1978). The FORTAN programs (Cornish Bowden & Eisenthal, 1974) have been adapted for use on a micro computer (Kontron Ltd., St. Albans, U.K.). Other kinetic constants were determined using Dixon and Cornish Bowden plots.

RESULTS

VEGETATION SURVEY

Hawthorn leaves collected in the vicinity of the brickworks at Ridgmont showed an approximately exponential decrease in F concentrations with increasing distance from the brickworks (Fig. 3:1). This fits the expected pattern for the dispersion of gaseous pollutants from a point source (Fig. 3:2). In contrast the sulphur content showed only a small decrease with increasing distance (Fig. 3:3). The different results for sulphur and fluoride may be explained partly by the existence of secondary sources of SO_2 pollution e.g. domestic coal fires but mainly by the sulphur taken up from the soil masking the comparatively small changes in exposure to SO_2 pollution.

Fluoride is taken up from the soil to a very limited extent hence even small differences in the atmospheric fluoride are reflected in the concentrations of fluoride in the leaves. The leaf fluoride concentration at each sampling site were plotted on a map and isopleths drawn (Fig. 3:4). The map indicates the pattern of pollution in the area; the prevailing South Westerly winds increased pollution to the North East. Topographical features show little influence probably because the chimney tops are much higher than the hills in the neighbourhood.

In a transect taken from a field of spring barley, variety Ark Royal, at Stewartby 1 km from the works the fluoride concentrations in the leaves showed a gradual decrease with increasing distance from the works. 39% of the variation in F concentrations was accounted for by a

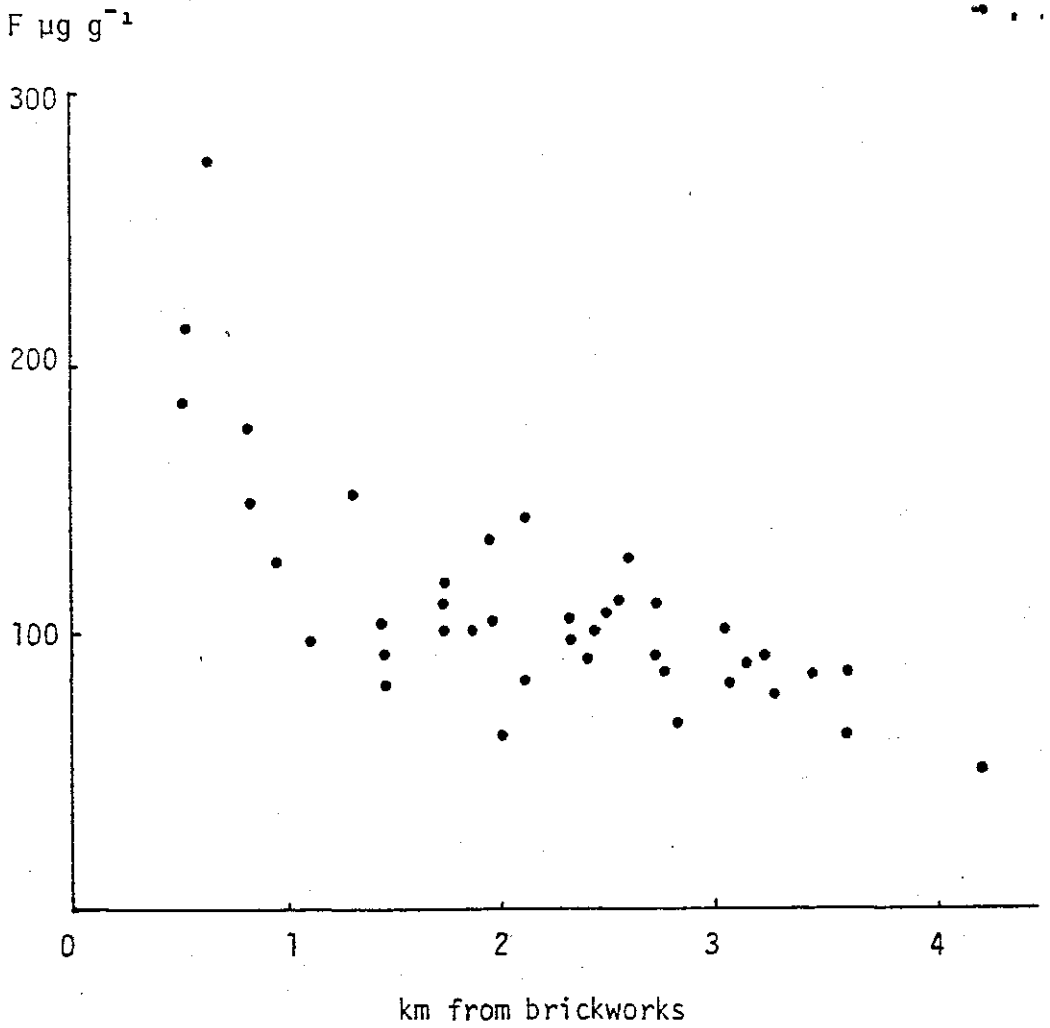


FIGURE 3:1 Fluoride concentrations in hawthorn leaves taken at various distances from the brickworks in 1975.

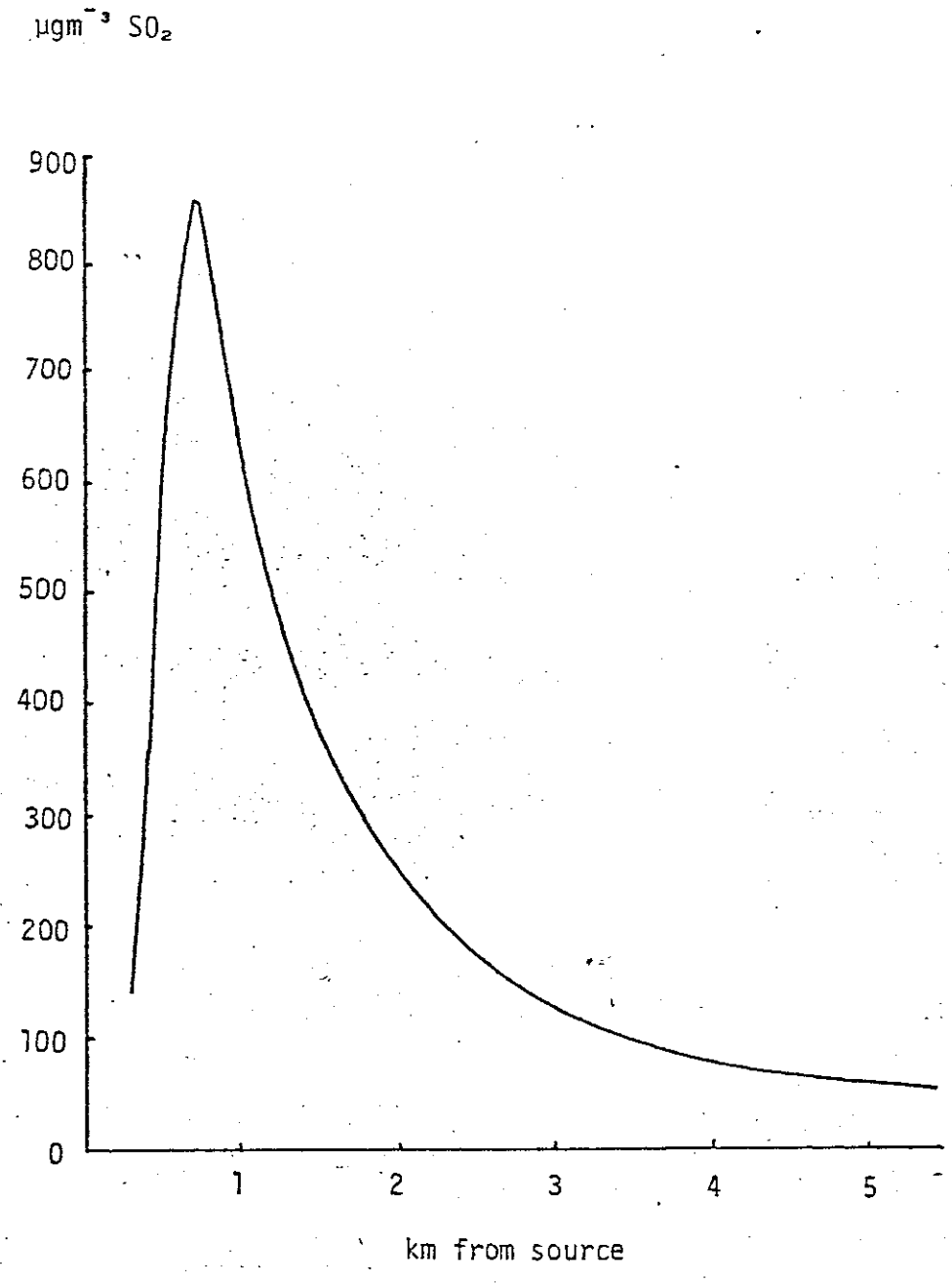


FIGURE 3:2 Profile of ground level SO_2 concentration (daily means) versus distance from the brickworks source. Redrawn from "Air Pollution in the Bedfordshire Brickfields" published by the D.O.E. in 1980.

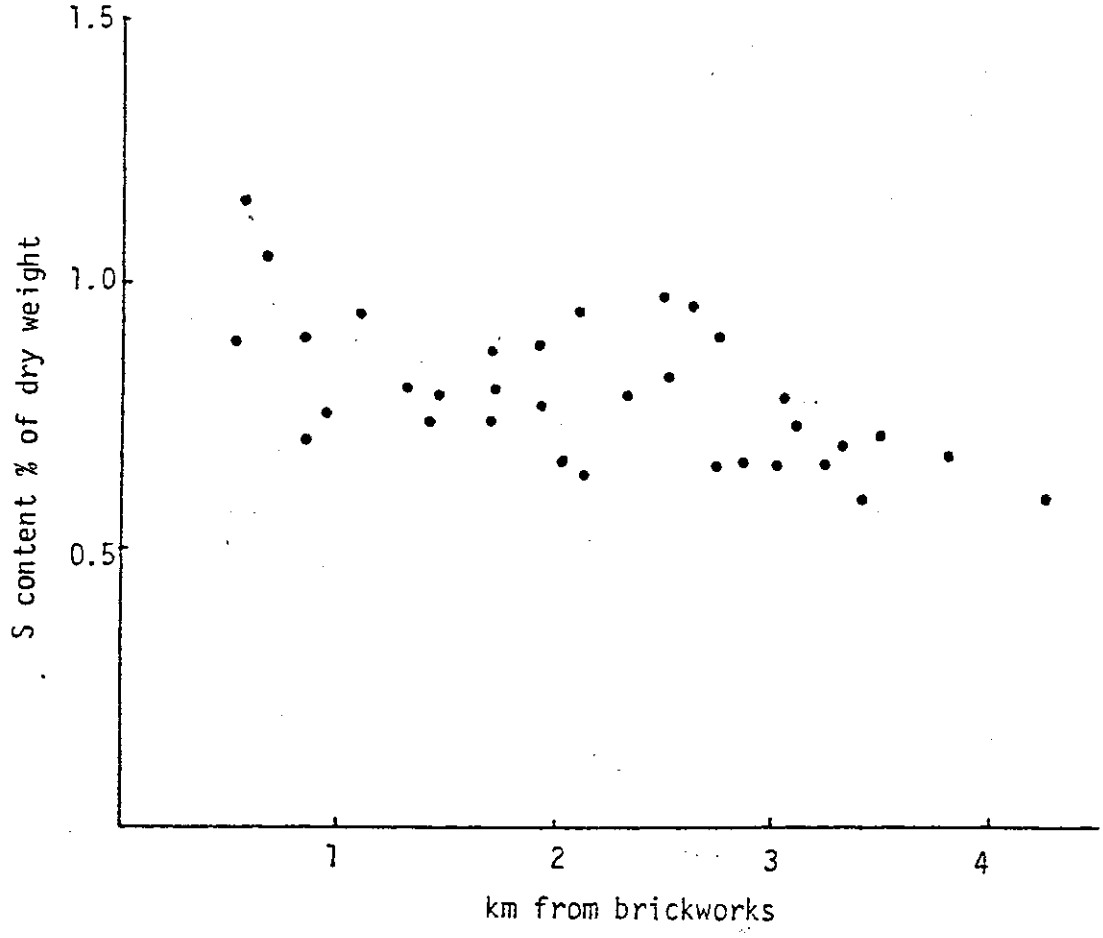


FIGURE 3:3 Sulphur concentrations in hawthorn leaves taken at various distances from the brickworks in 1975.

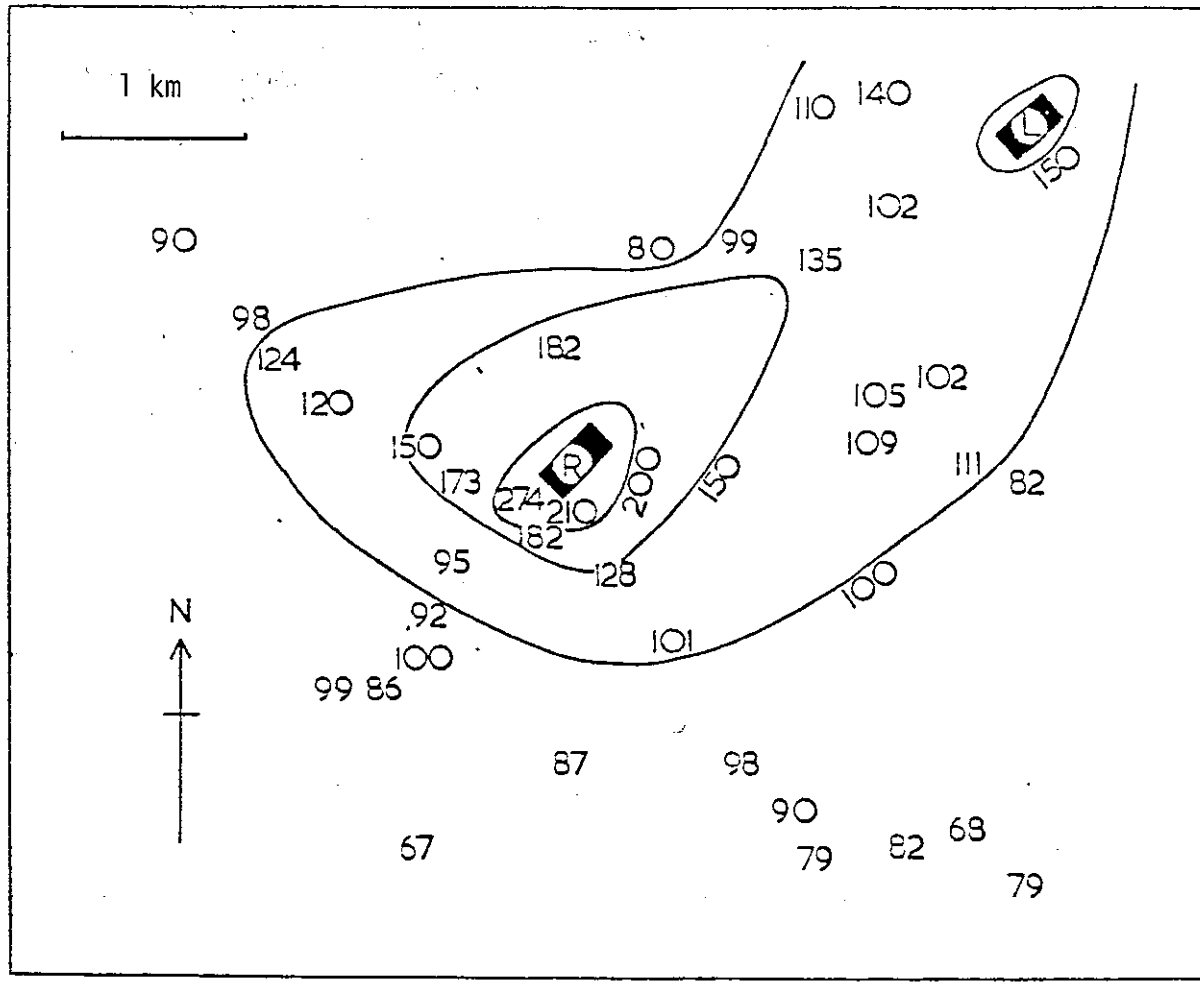


FIGURE 3:4 Fluoride concentrations $\mu g\ g^{-1}$ in hawthorn leaves around Ridgmont and Lidlington brickworks.

linear correlation with distance from the works (Fig. 3:5) ($r = 0.6285$ sig. at $P = 0.005$). The concentrations found were similar to those in hawthorn leaves at a similar distance from the works. The grain yield along the transect was extremely variable and no significant relationship between yield and distance was established (Fig. 3:6). The measurements suggested a small increase in 1,000 grain weight with increasing distance (Fig. 3:7) although this was not significant. Only 12% of the variation in 1,000 grain weight was accounted for by fluoride concentrations in the leaves (Fig. 3:8). The poor correlation suggests that whilst pollutant concentrations may be influencing crop yield, other variables may be more important. Shoot numbers were variable and accounted for 35% of the variation in grain yield (Fig. 3:9). The variation in shoot numbers was probably due to factors other than pollutants. Consequently this method of sampling was too crude to analyse the effects of pollutants on crop yield.

CHAMBER SYSTEMS

Closed Chambers

In initial tests SO_2 concentrations were reduced by 85% in the filtered chambers, but when charcoal thickness was reduced to increase air flow, the reduction in concentration fell to only 60%. This was considered acceptable as it gave a concentration of less than 30 μgm^{-3}, similar to most rural areas. The concentration of SO_2 in the unfiltered chambers was approximately 80% of that outside and fluctuated in a similar way. The reduction in the unfiltered chambers was presumably due to adsorption of SO_2 onto the ventilation system and polythene covers. The concentration in the filtered chambers was approximately 50% of the unfiltered chambers.

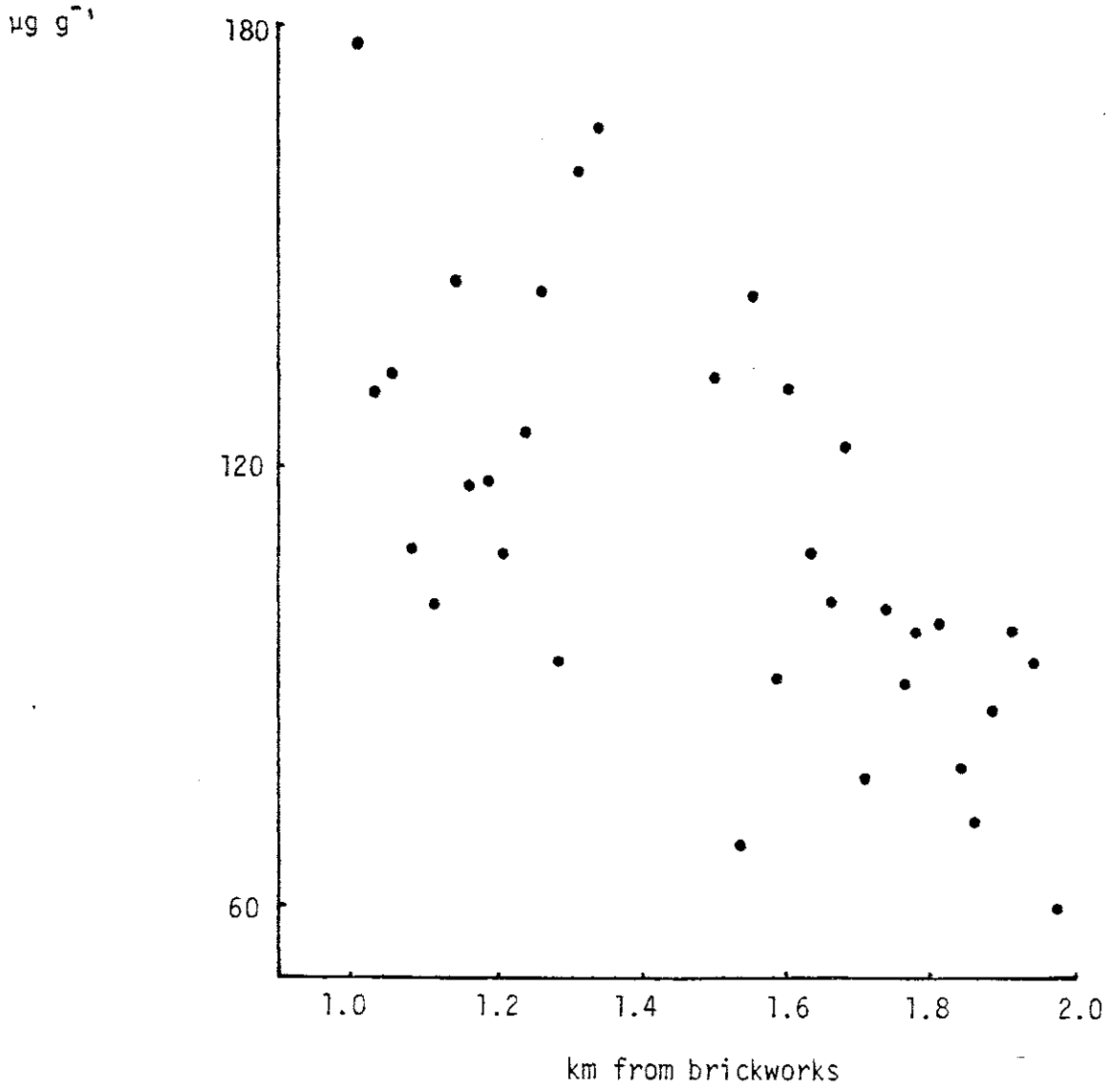


FIGURE 3:5 Fluoride concentrations in spring barley, var. Ark Royal, taken at various distances from the brickworks.

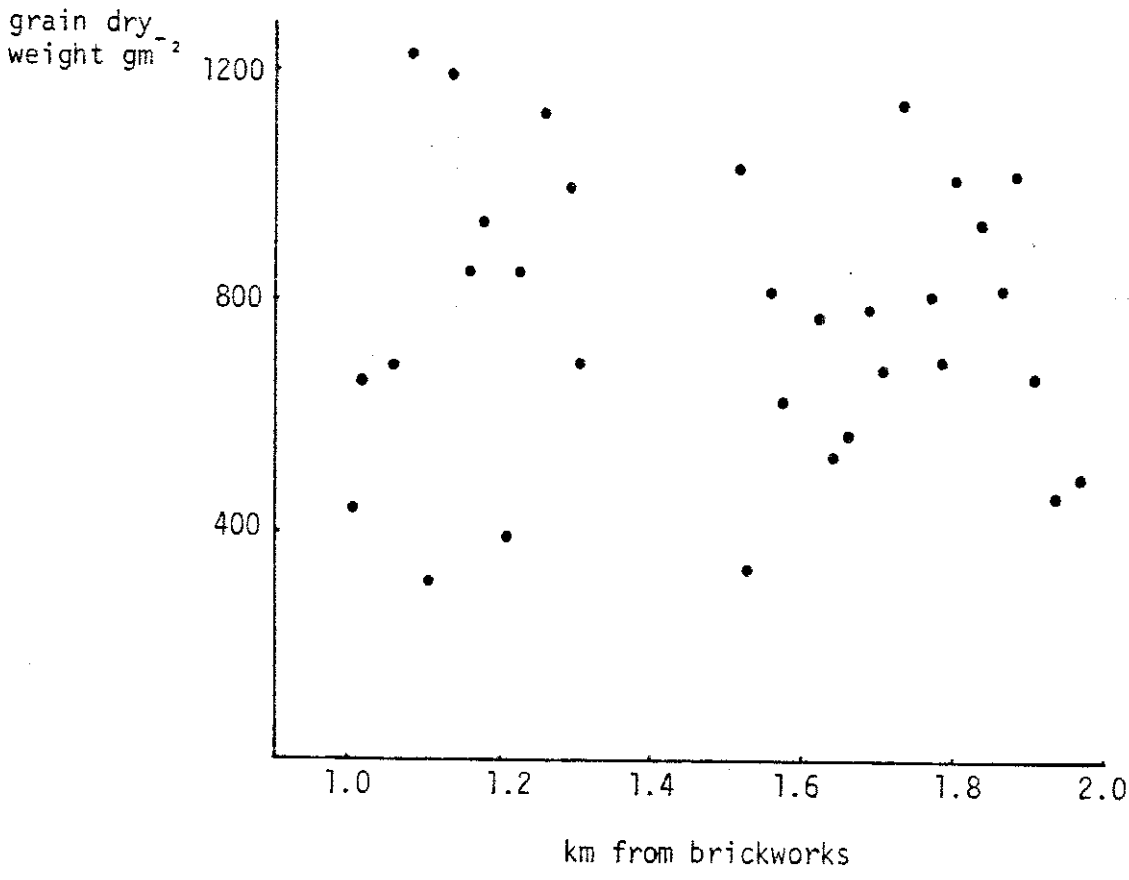


FIGURE 3:6 Grain yield of spring barley, var. Ark Royal, at various distances from the brickworks.

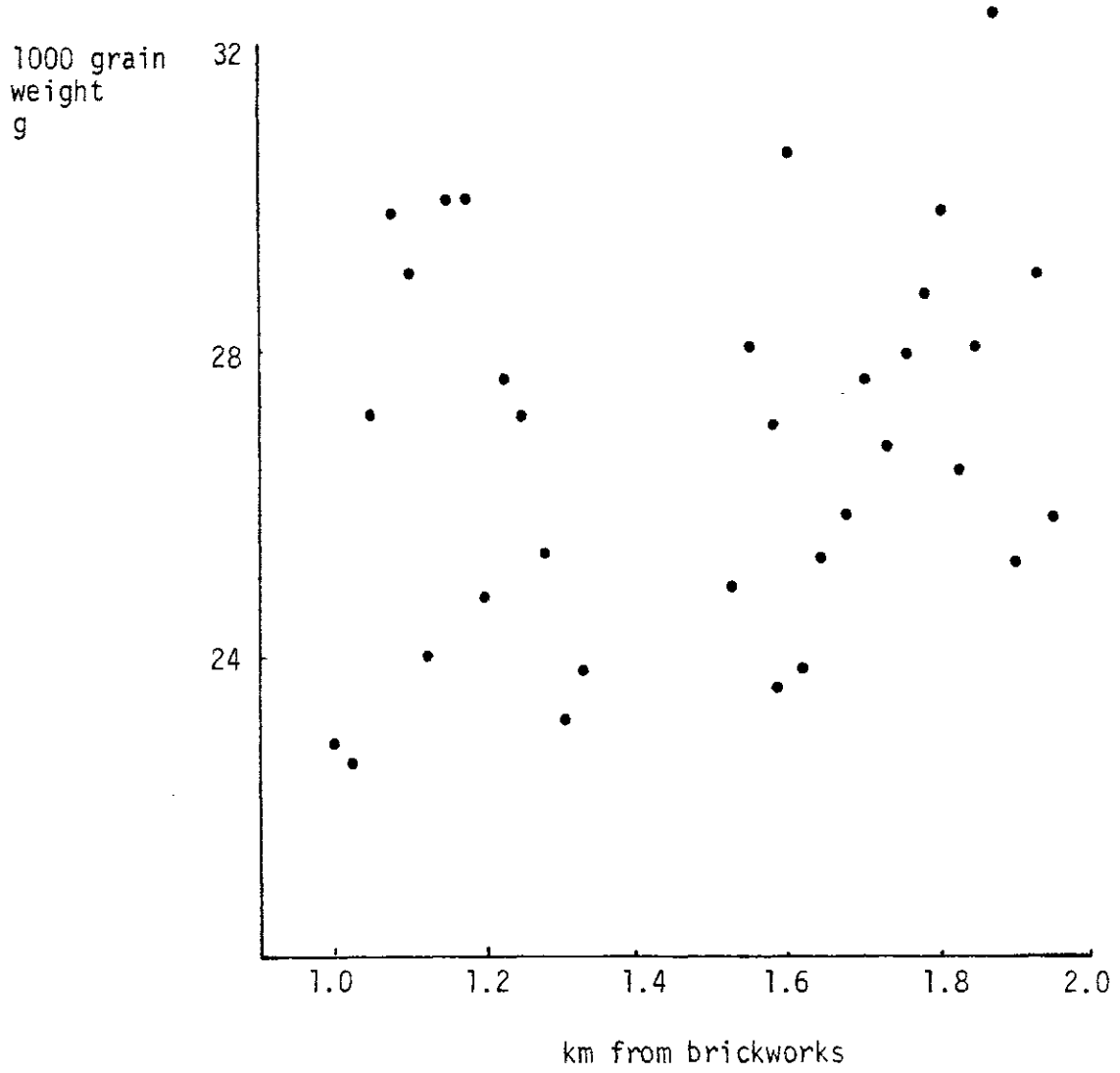


FIGURE 3:7 Relationship between 1000 grain weight of spring barley, var. Ark Royal, and distance from brickworks.

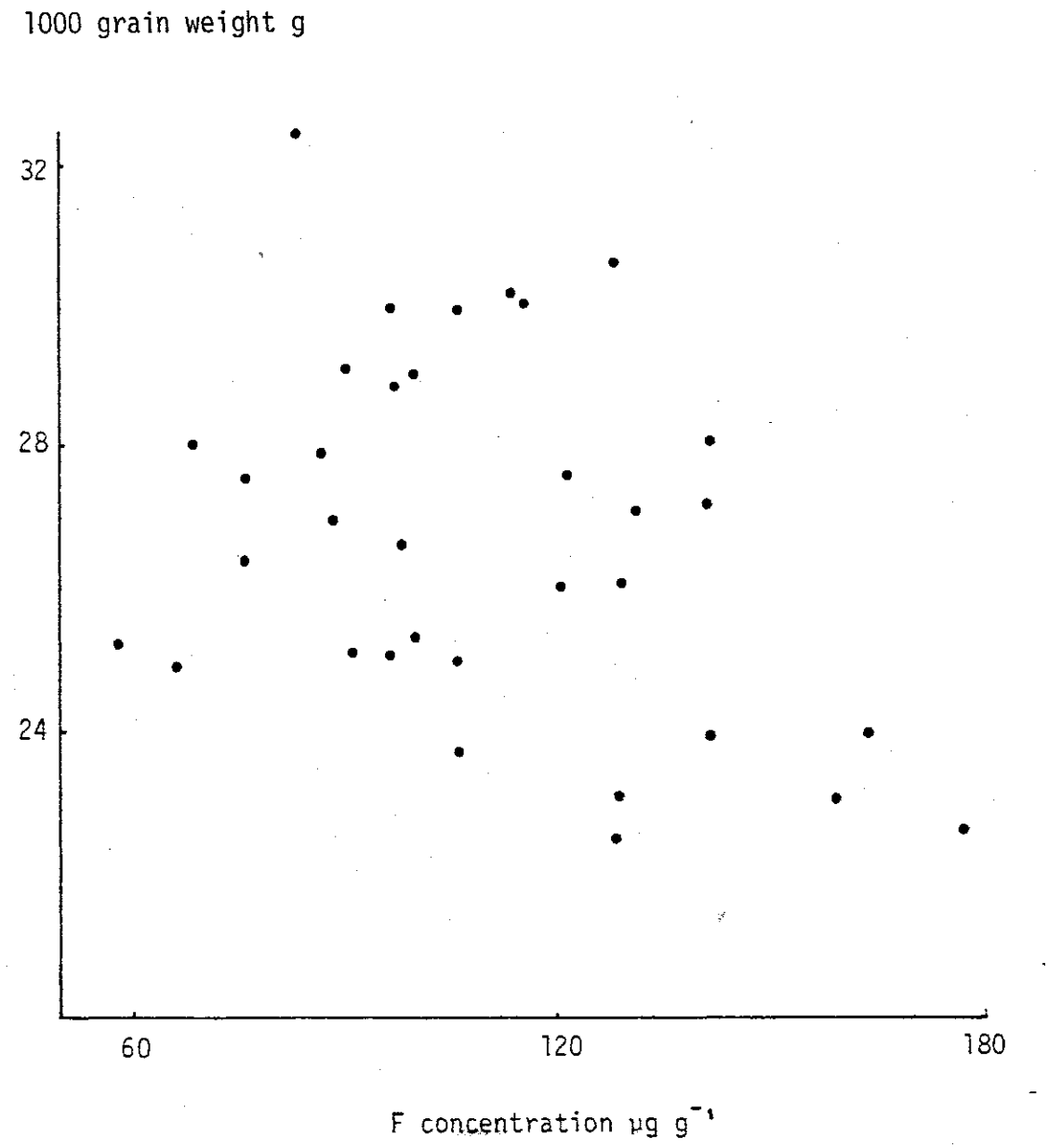


FIGURE 3:8 Relationship between 1000 grain weight of spring barley, var. Ark Royal, and fluoride concentration in the leaves.

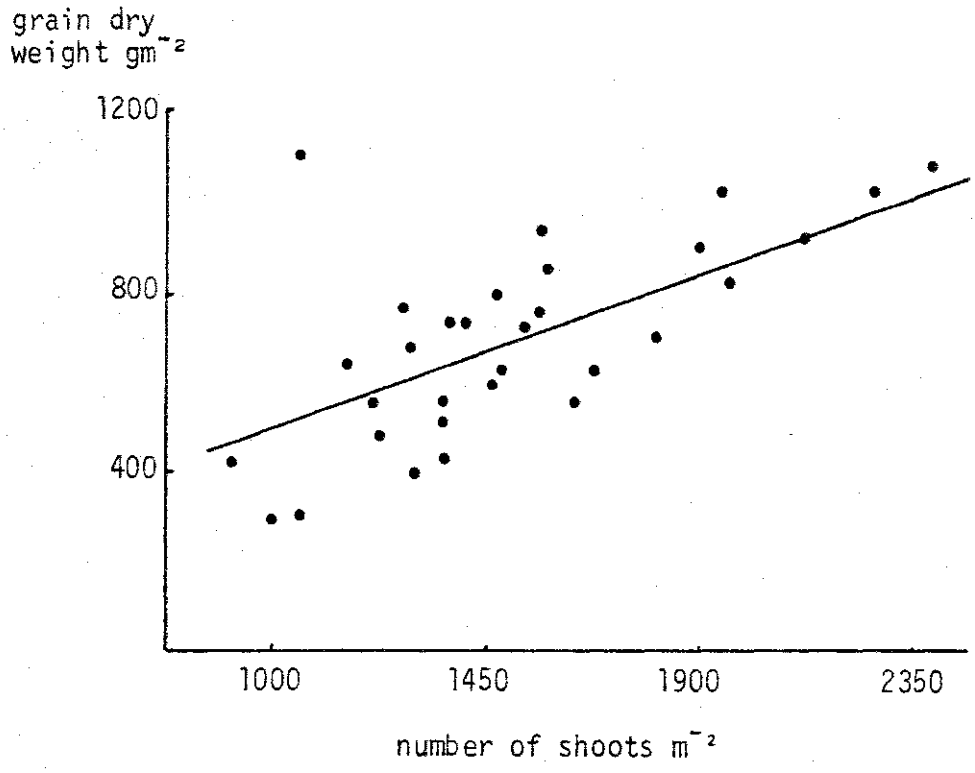


FIGURE 3:9 Relationship between grain yield and shoot number of spring barley, var. Ark Royal.

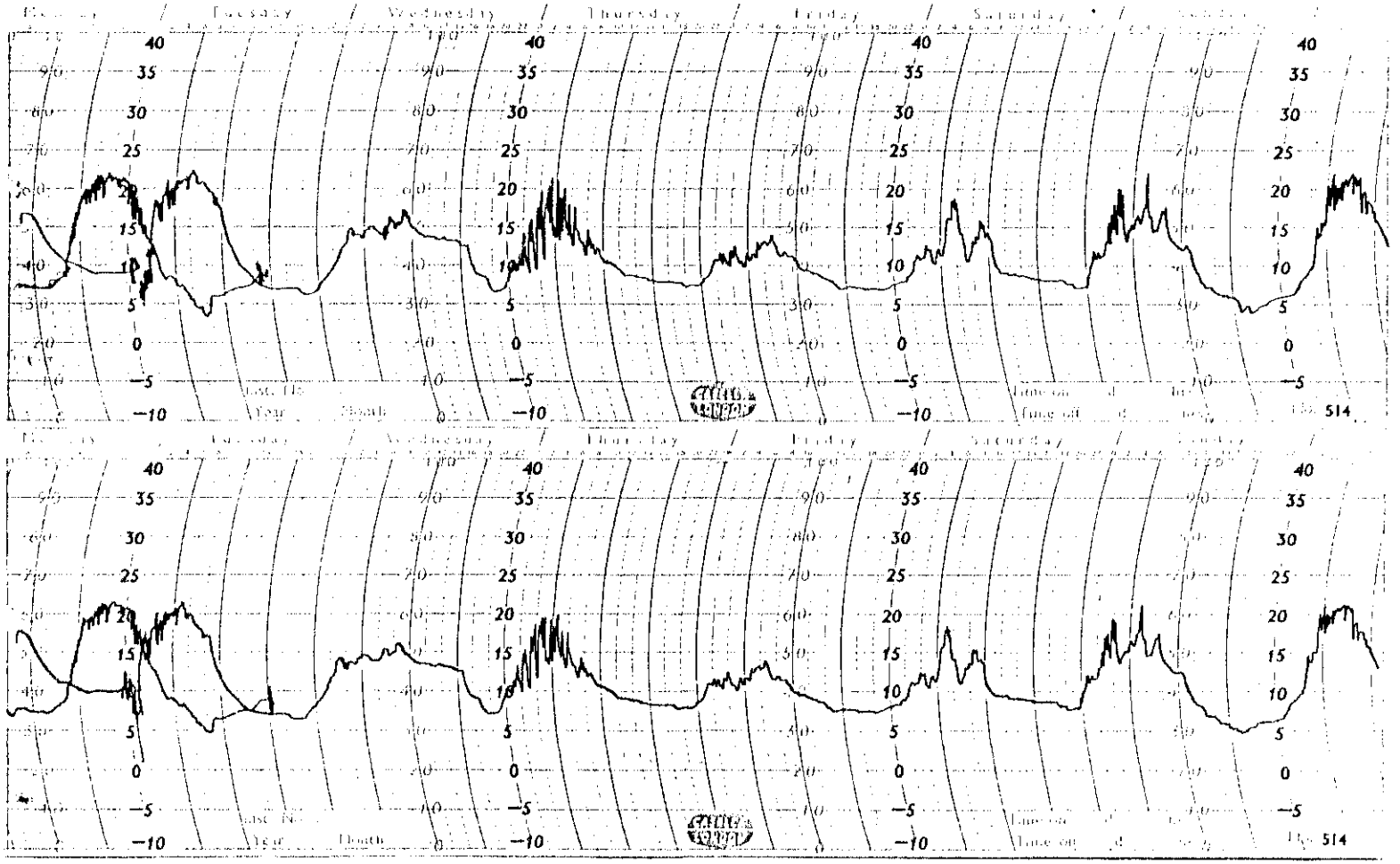
It was not possible to monitor the temperature in the chambers continuously and only occasional measurements were made. Temperatures in the chambers usually average 1-2°C above those outside, but increases of 6-7°C were observed on hot sunny days. No differences were found between the temperatures of filtered and unfiltered chambers (Fig. 3:10).

In diffuse light the PFD through the chambers varied by 10% and was ~ 20% less than outside. In direct sunlight the areas shaded by the framework had a PFD which was 50% less than outside whilst the remainder of the chambers was ≃ 20% less than outside.

Unmodified Open-Topped Chambers

The two main ways in which pollutants may enter through the top of open-topped chambers are by thermal inflows and wind-generated inflows. Chamberlain (personal communication) calculated that a ventilation rate of 0.2 m s^{-1} should be sufficient to prevent thermal inflows caused by convection currents on hot days. The ventilation rate of my chambers was 0.15 m s^{-1}. At low wind speeds the chambers operated satisfactorily but at higher wind speeds wind-generated inflows occurred. Consequently the reduction in SO_2 concentrations in the filtered open-topped chambers was variable but averaged 50% of the outside. Greater efficiencies have been reported for other pollutants, e.g. ozone (Kats et al., 1976) probably because the concentrations of these pollutants is closely correlated with wind speed so that on still days when the concentrations of pollutants is higher wind-generated inflows are reduced. The concentration of SO_2 in the unfiltered chambers closely followed those outside but were on average 12% lower.

FIGURE 3:10 Temperature fluctuations in unfiltered (I) and filtered (II) closed chambers during a typical eight day period during Experiment one.


I
II
514
514

Temperature fluctuations within these chambers are better than in the closed ones and the recorded temperatures never exceeded the outside by more than 2°C. Relative humidity was approximately the same within and without the chamber. The integrated PFD inside the open-topped chambers was about 15% below the outside levels.

Collared and Lipped Open-Topped Chambers

The ventilation rate of these chambers gave a calculated vertical flow of 0.25 m s^{-1} which should prevent thermal inflows caused by convection currents (see p.73). In tests over a 52-day period covering a range of climatic conditions the mean SO_2 concentration was reduced from 39.2 μgm^{-3} outside to 13.1 μgm^{-3} inside. The ambient concentration exceeded 100 μgm^{-3} in 510 out of a total of 7,560 four minute sampling periods, but only did so once in the modified filtered chamber. The efficiency of the chamber in excluding pollutants was maintained on windy days (Fig. 3:11). The concentration of pollutants in the unfiltered chambers closely followed those outside but were on average approximately 10% lower.

Temperatures measured in the centre of the chamber 0.5 m from the ground closely followed those measured at a similar height outside but were on average 1°C warmer (Fig. 3:12). Temperature differences were usually largest in the early morning and smallest in the late afternoon. Measurements of relative humidity made in the chambers closely followed those of the field (± 10%).

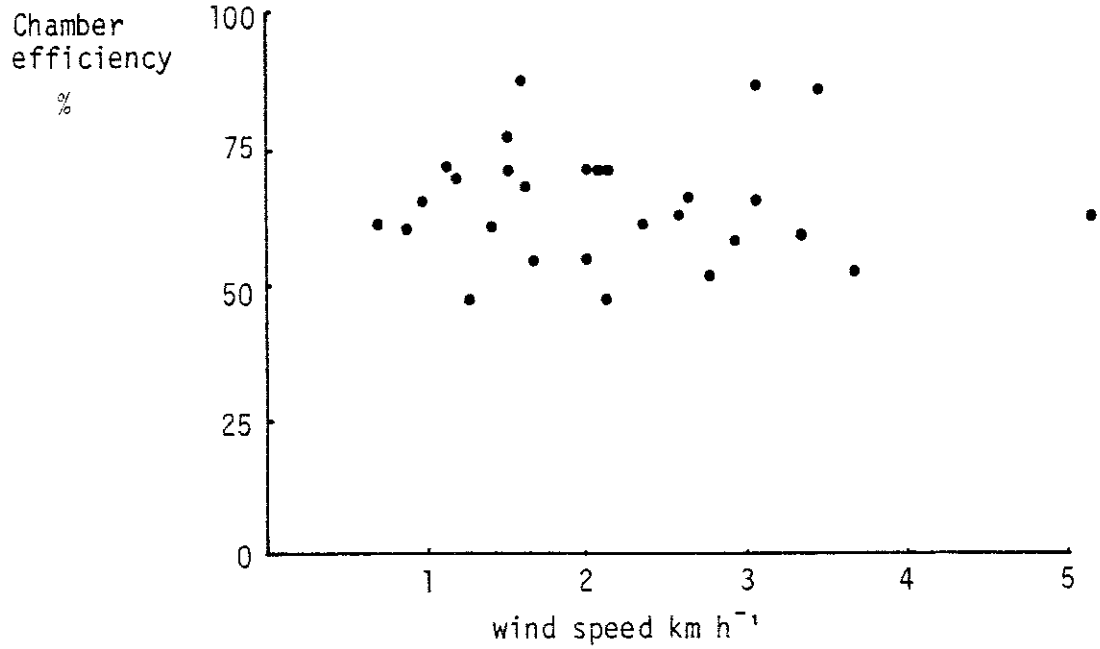


FIGURE 3:11 The efficiency of a collared and lipped open-topped chamber at different wind speeds.

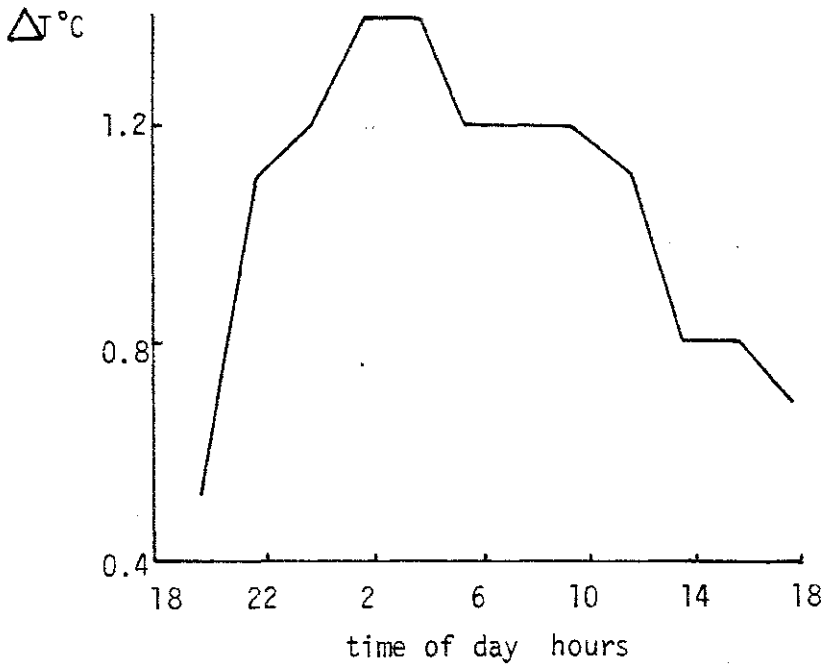


FIGURE 3:12 The average difference in temperature between the inside of a collared and lipped open-topped chamber and outside at different times of day during a 20 day period in April 1978.

Figure 3:13 compares the PFD for the field and in the chamber. Over a wide range the reduction of PFD in the chambers is very small. In direct sunlight the PFD for areas shaded by the framework were 40% less than outside, but the area shaded in this way was small and depended on the time of year and time of day. In diffuse light the PFD throughout the chambers varied by only 6% but was never less than 80% of that outside.

GROWTH EXPERIMENTS

Experiments One and Two

In 1976 and 1977 experiments at Woburn sought to determine the effect of ambient concentrations of pollutants on crop growth and yield. The effects on spring barley, var. Abacus, were examined in 1976 and on a winter barley, var. Maris Otter, in 1977.

Crops were grown in four open-topped chambers, two closed chambers and on two unenclosed plots. Two of the open and one closed chamber were ventilated with charcoal-filtered air the remainder with field air. To facilitate tests of significance the closed chambers were divided into two and each half treated as a separate plot.

Concentrations of pollutants in the air and plants.

Due to practical difficulties pollution monitoring was restricted to measurements of SO_2 for unenclosed plots and filtered chambers in 1977. Filtering the air reduced the mean concentration in both years (Table 3:1) but in 1977 the filtered open-topped chamber only marginally reduced the concentration of SO_2 between anthesis and final harvest.

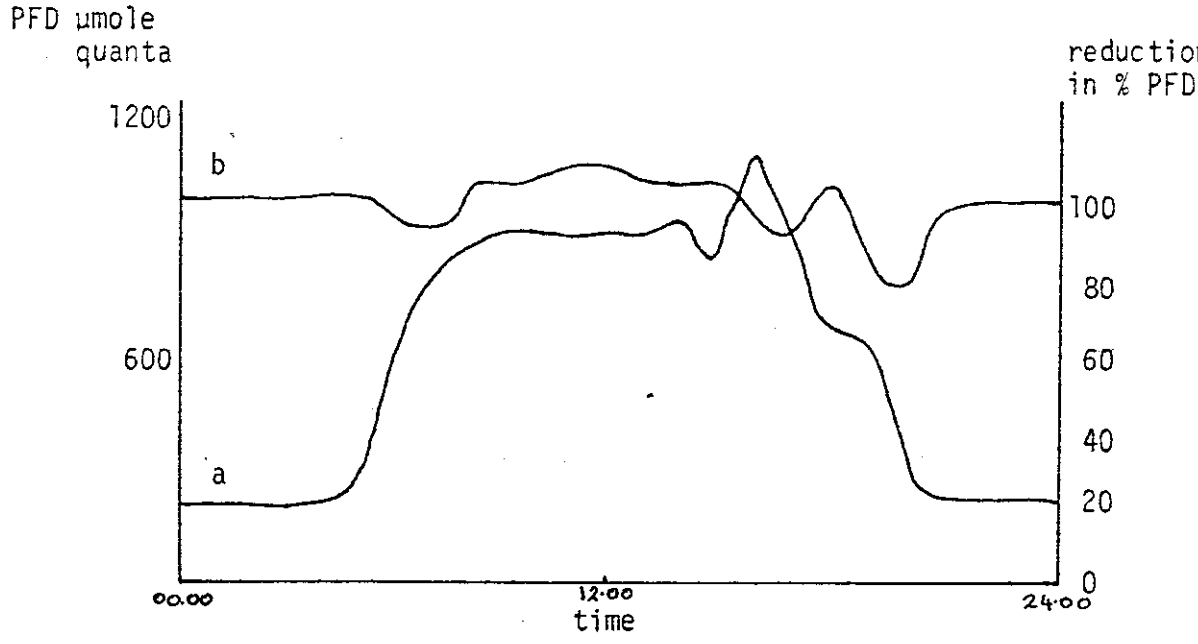


FIGURE 3:13 The PFD outside (a) and the percentage reduction inside (b) a collared and lipped open-topped chamber at different times of day.

TABLE 3:1

Mean SO_2 concentrations (μgm^{-3}) for the unenclosed plots and chambers at Woburn during Experiment 1 (1976) and Experiment 2 (1977).

Year	Unenclosed Plot	Chambers			
		Open-Topped		Closed	
		Filtered	Unfiltered	Filtered	Unfiltered
1976	61	31	54	28	49
1977	54	39	-	22	-

In 1976 the weekly mean concentrations were quite uniform (Table 3:2) although highest during tillering whereas in 1977 there was an episode of pollution at the ear emergence stage (Table 3:3).

Neither enclosure in an open-topped or closed chamber nor cleaning the air consistently altered the sulphur content, as a percentage of dry weight, of the leaves (Fig. 3:14I,II). In both years the fluoride concentrations increased progressively towards maturity. In 1976 the concentration was highest for plants from outside plots and unfiltered open-topped chambers at the final harvest (Fig. 3:15). The fluoride concentrations were lower in the open-topped chambers with filtration than in those without but were least in the closed chambers with filtration. The concentrations in the closed chambers supplied with ambient field air were only a third of those outside.

TABLE 3:2

Weekly mean SO_2 concentrations (μgm^{-3}) for the unenclosed plots and chambers at Woburn during Experiment 1 (1976).

Week Beg.	Unenclosed Plots	Chambers			
		Open-Topped		Closed	
		Filtered	Unfiltered	Filtered	Unfiltered
21/4	65	16	46	13	38
28/4	65	48	54	32	46
5/5	73	33	69	21	65
12/5	55	18	55	23	37
18/5	55	46	58	38	53
25/5	61	38	53	34	49
1/6	55	42	51	34	52
8/6	50	-	-	-	-
15/6	73	25	51	31	54
22/6	60	19	47	28	47

TABLE 3:3

Weekly mean SO_2 concentrations (μgm^{-3}) for the unenclosed plots and filtered chambers at Woburn during Experiment 2 (1977).

Week Beginning	Unenclosed Plots	Filtered Chambers	
		Open-Topped	Closed
10/5	45	39	13
17/5	46	35	20
24/5	62	40	21
31/5	18	17	15
7/6	35	28	23
14/6	128	72	24
21/6	68	18	27
28/6	46	37	16
5/7	54	59	30
12/7	57	55	34
19/7	30	28	17

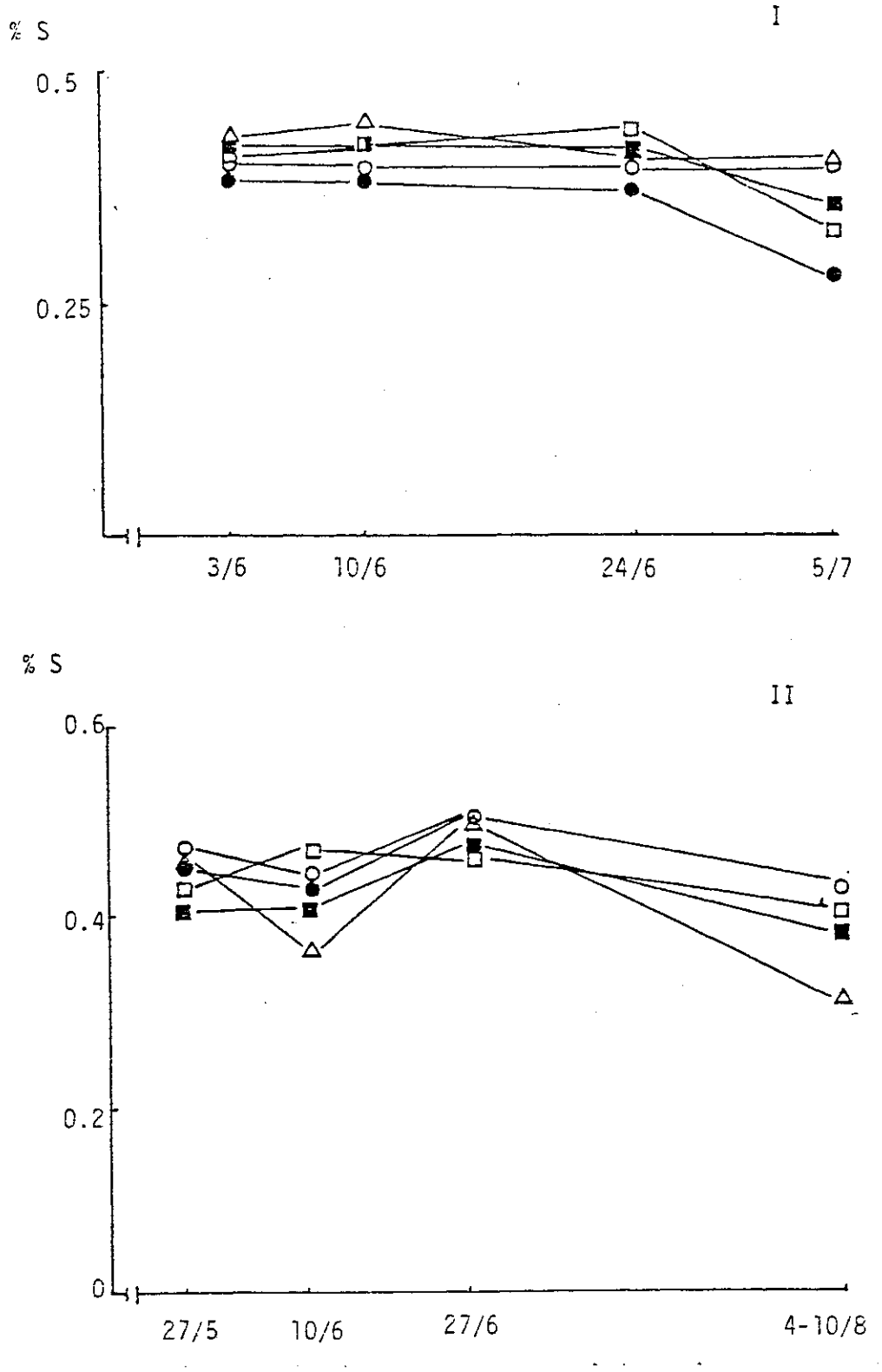


FIGURE 3:14 The sulphur content as a percentage of dry weight of leaves of spring barley at each harvest from unenclosed plots, unfiltered and filtered chambers from Experiment 1 (I) and Experiment 2 (II): △ unenclosed plots

○ unfiltered open-topped chambers
● filtered open-topped chambers
□ unfiltered closed chambers
■ filtered closed chambers

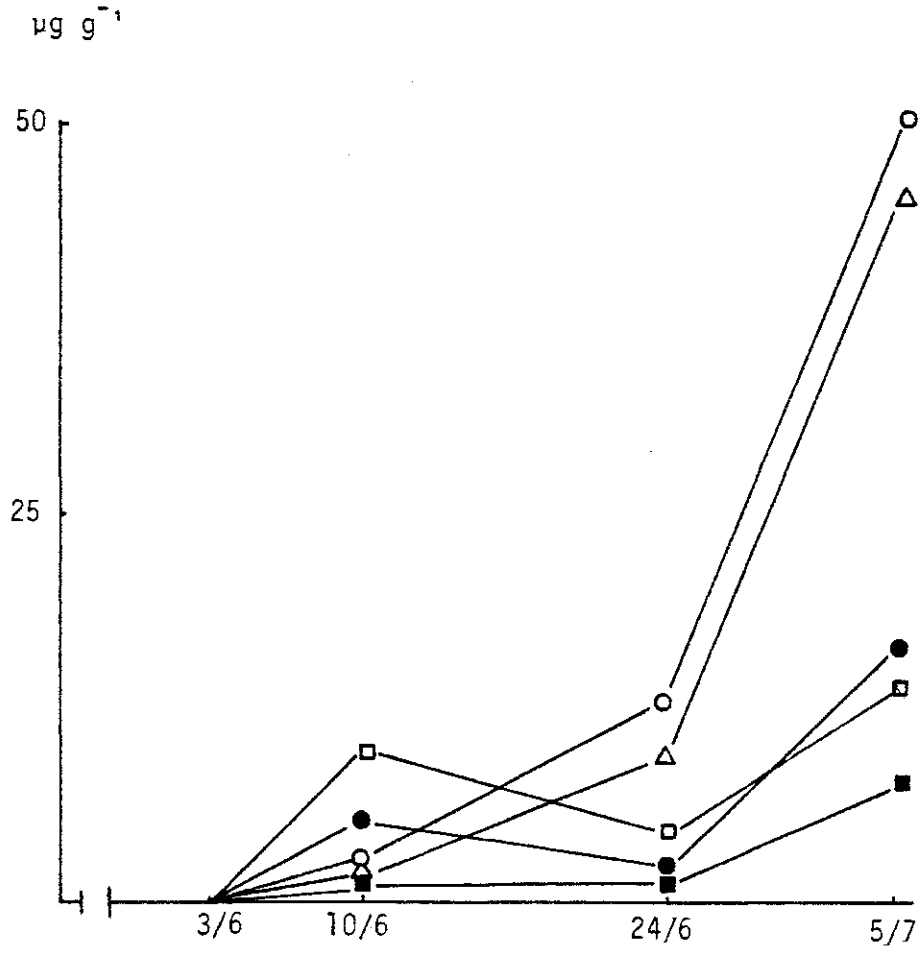


FIGURE 3:15 The fluoride concentration, µg g^{-1}, of leaves of spring barley at each harvest from experiment one:

- △ unenclosed plots
- ○ unfiltered open-topped chambers
- ● filtered open-topped chambers
- □ unfiltered closed chambers
- ■ filtered closed chambers

In 1977 there was no difference between the filtered and unfiltered open-topped chambers at the final harvest (Fig. 3:16) presumably due to the failure to reduce the air concentration during grain filling. Only the concentration in the closed chambers with filtration were lower than the outside.

Growth and Yield.

Grain yields were poor in 1976, 2.9 t ha^{-1} on the unenclosed plots, because the spring and summer were exceptionally dry. In 1977 the grain yields on unenclosed plots were 3.82 t ha^{-1}, appreciably higher than those in 1976.

Effects of Filtration

In 1976 there were no signs of visible injury but in 1977 slight awn scorching occurred on plants in the unfiltered chambers and on unenclosed plots. In both years the numbers of shoots per unit area were greater in the filtered than in the unfiltered chambers of both designs but the increases were only significant in 1976 (Table 3:4). Consequently there were more ears, greater dry weights of straw and ears (Fig. 3:17I) and a larger photosynthetic area in the filtered than in the unfiltered chamber. Grain yields, 1,000 grain weights and grain number per ear were also greater in the filtered than in the unfiltered chambers except in the open-topped chambers in 1977 when filtration did not reduce the concentrations of pollutants in the air (Fig. 3:17II) The increase in 1,000 grain weight etc. in the filtered air is surprising since the unfiltered chambers had fewer shoots m^{-2}, one might have expected an

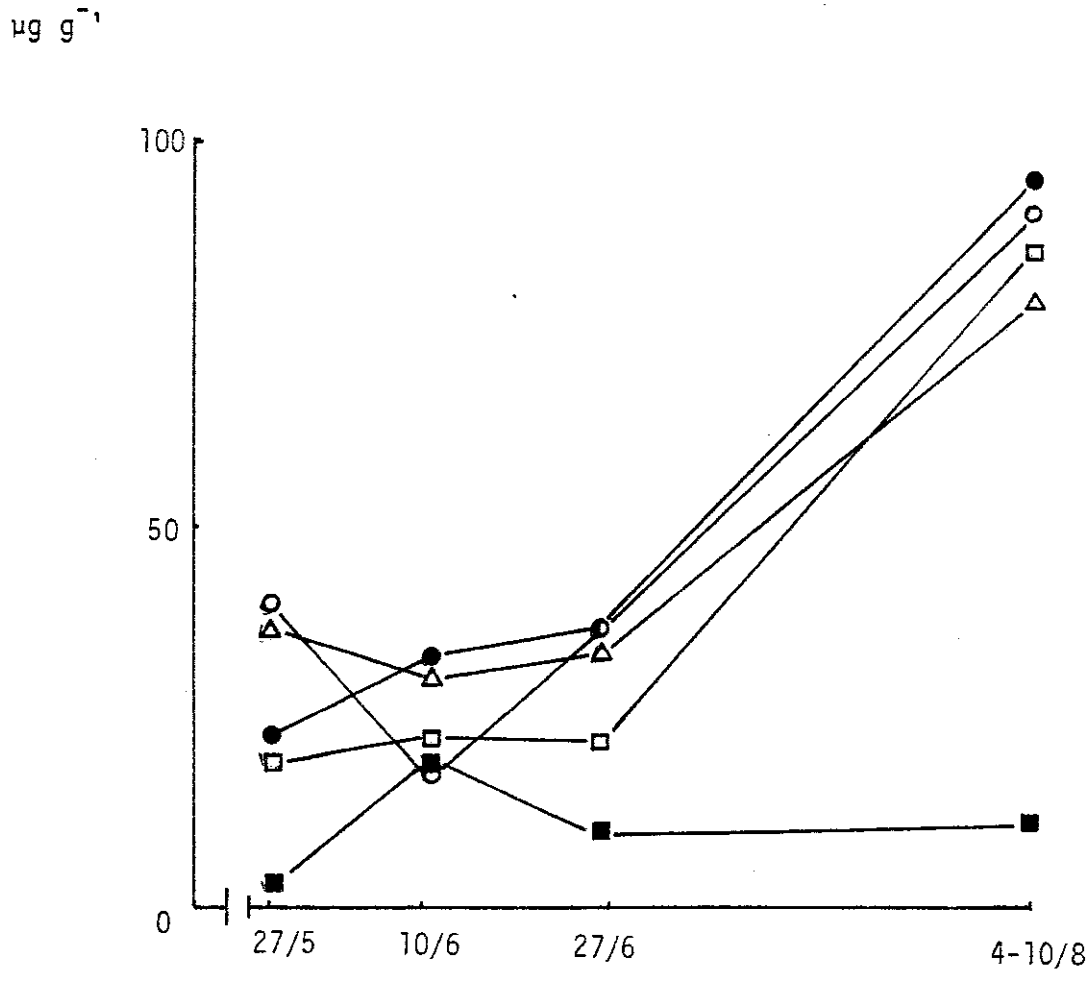


FIGURE 3:16 The fluoride concentration, µg g^{-1}, of leaves of spring barley at each harvest from experiment two:

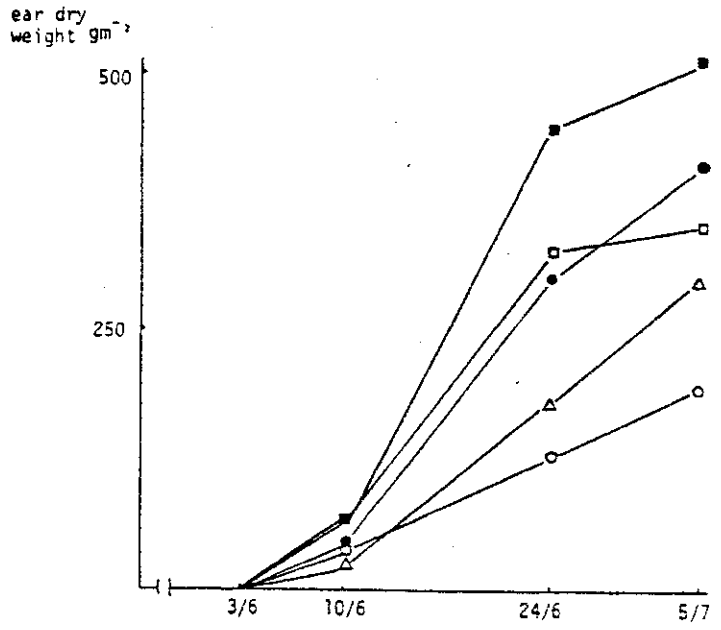
- △ unenclosed plots
- ○ unfiltered open-topped chambers
- ● filtered open-topped chambers
- □ unfiltered closed chambers
- ■ filtered closed chambers

TABLE 3:4 The effect of enclosure and filtration on barley yields and fluoride and sulphur contents in Experiments 1 and 2.

	1976						1977					
	O+C	O-C	UN	CL+C	CL-C	SED n=8	O+C	O-C	UN	CL+C	CL-C	SED n=8
Number of shoots (m^{-2})	715	559	611	988	858	96.4	720	600	933	1107	999	107.7
Straw dry weight (gm^{-2})	382	153	352	425	286	60.9	455	348	515	468	356	53.3
Total dry weight (gm^{-2})	758	349	638	934	644	98.6	777	713	1003	1127	825	115.5
Ear dry weight (gm^{-2})	376	196	286	509	358	54.3	322	364	489	660	471	66.8
Grain dry weight (gm^{-2})	-	-	-	-	-	-	254	300	382	536	386	55.4
1,000 grain dry weight (g)	38.6	33.3	31.3	41.6	29.3	2.7	-	-	-	-	-	-
F concentration ($\mu g\ g^{-1}$)	17	50	46	9	13	6.5	95	91	79	9	85.4	9.6
S content % of leaves	.27	.32	.35	.33	.36	.01	0.5	0.4	0.3	0.3	0.4	0.02

O+C = Open-topped chamber + charcoal filter
O-C = Open-topped chamber - charcoal filter
CL+C = Closed chamber + charcoal filter
CL-C = Closed chamber - charcoal filter
UN = Unenclosed plots

I



II

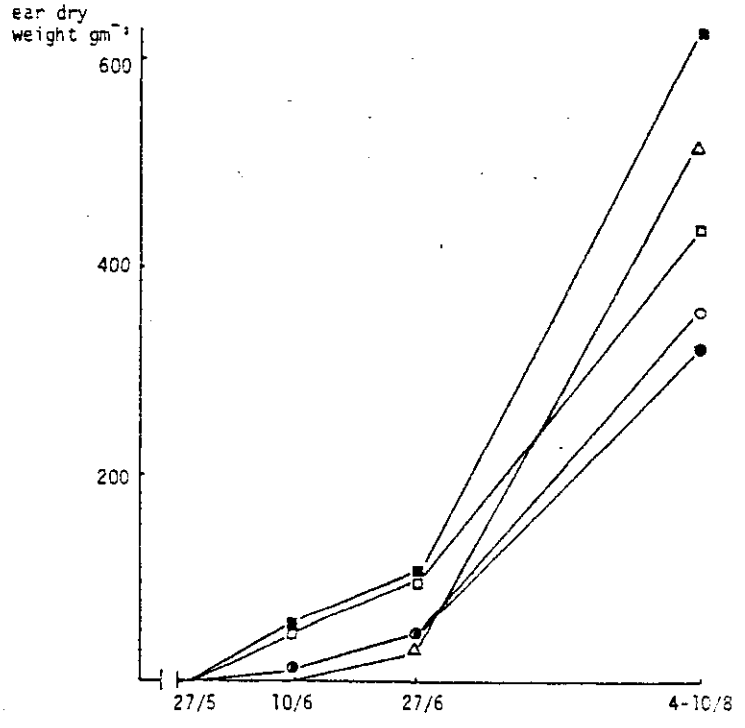


FIGURE 3:17 The ear dry weight of spring barley at each harvest from unenclosed plots, unfiltered and filtered chambers at Woburn in experiment one (I) and experiment two (II):

- △ unenclosed plot
- ○ unfiltered open-topped chambers
- ● filtered open-topped chambers
- □ unfiltered closed chambers
- ■ filtered closed chambers

increase in individual grain size or grains/ear to compensate for a lower shoot number. Cleaning the air significantly increased the total dry matter production by 32% and 28% in the closed chambers in 1976 and 1977 respectively and 54% in the open-topped chambers in 1976.

Effects of Enclosure

The plants grown in the chambers emerged about one day earlier and matured seven to eight days earlier in the open-topped chambers and over 14 days earlier in the closed chambers than outside.

In 1977 the number of shoots m^{-2} on the unenclosed plots was over 50% higher than in 1976 but the values for the chambers did not differ between the two years (Table 3:4).

Straw and ear dry weights were larger on the unenclosed plots than in the open-topped chambers as were shoot numbers. In 1977 although there were more shoots in the closed chambers without filtration than on unenclosed plots, straw and ear dry weights were smaller in the chambers than outside. In both years ear weights increased initially more quickly in the closed chambers (Fig. 3:17I & II) than those outside. This represents a rate of grain growth of 71 and 72 g $week^{-1}$ in the chambers compared to 30 g $week^{-1}$ outside for the period between harvests 2 and 3 in 1976. The increase is short-lived and of no overall benefit being accompanied by premature senescence (see line 7). A similar but less pronounced effect was seen in the open-topped chambers.

Experiment Three

In 1978 the experiment was moved to Thrupp End. The new site had a contrasting soil type to Woburn and historically higher air concentrations of SO_2 and F (information supplied by the London Brick Company). The experimental design was as in previous experiments, but open-topped chambers were of the modified form with collars and lips and all of the chambers had improved fans and filters (Materials and Methods, see p.46).

Spring barley was grown and harvests were taken at specified developmental stages so that effects in yield, independent of changes of rate of development could be determined (Table 3:5).

TABLE 3:5

Harvest dates and developmental stages in Experiment 3

	Closed Chambers	Open-Topped Chambers	Unenclosed Plots
7.6.78	Anthesis	-	-
19.6.78	-	Anthesis	-
26.6.78	Ears emerged	-	Anthesis
10.7.78	-	Grain filling	Ears emerged
2.8.78	Final	-	-
11.8.78	-	Final	-
23.8.78	-	-	Final

Concentrations of pollutants in the air and plants

Shortage of equipment restricted continuous monitoring to the unenclosed plot and chambers supplied with filtered air. Intermittent use of a flame photometric sulphur analyser supplemented these estimations by providing a continuous record of concentrations for periods of up to 48 h The mean ambient SO_2 concentration (49.6 μgm^{-3}) was lower than in previous experiments. The highest concentrations were recorded before tillering when the mean air concentration for one two-day period reached 131 μgm^{-3} (Table 3:6). The flame photometric sulphur analyser showed that peaks of up to 5 x the daily mean values occurred (Fig. 3:18). The improved filters reduced the mean concentration in the closed filtered chamber by over 90% compared to the outside. The filtered open-topped chambers operated most efficiently in lowering the SO_2 concentrations at the start of the experiment; the mean reduction was 66%. The reduced efficiency in the last two weeks of the experiment was not due to failure of the filtration unit, as replacement filters produced no improvement. However this may have been caused by an increasing barrier to air flow offered by the mature crop. In the unfiltered chambers SO_2 concentrations were also reduced probably due to the adsorption of pollutants on to fans and the walls of the chambers.

Intermittent measurements of fluoride were made using impregnated filter papers. Because of the high resistance of the filter papers it was not possible to achieve a high flow rate and initial samples were collected over long periods (Table 3:7) but these accumulated only very

TABLE 3:6

Weekly mean SO_2 concentrations (μgm^{-3}) for the unenclosed plots and chambers at Thrupp End during Experiment 3 (1978).

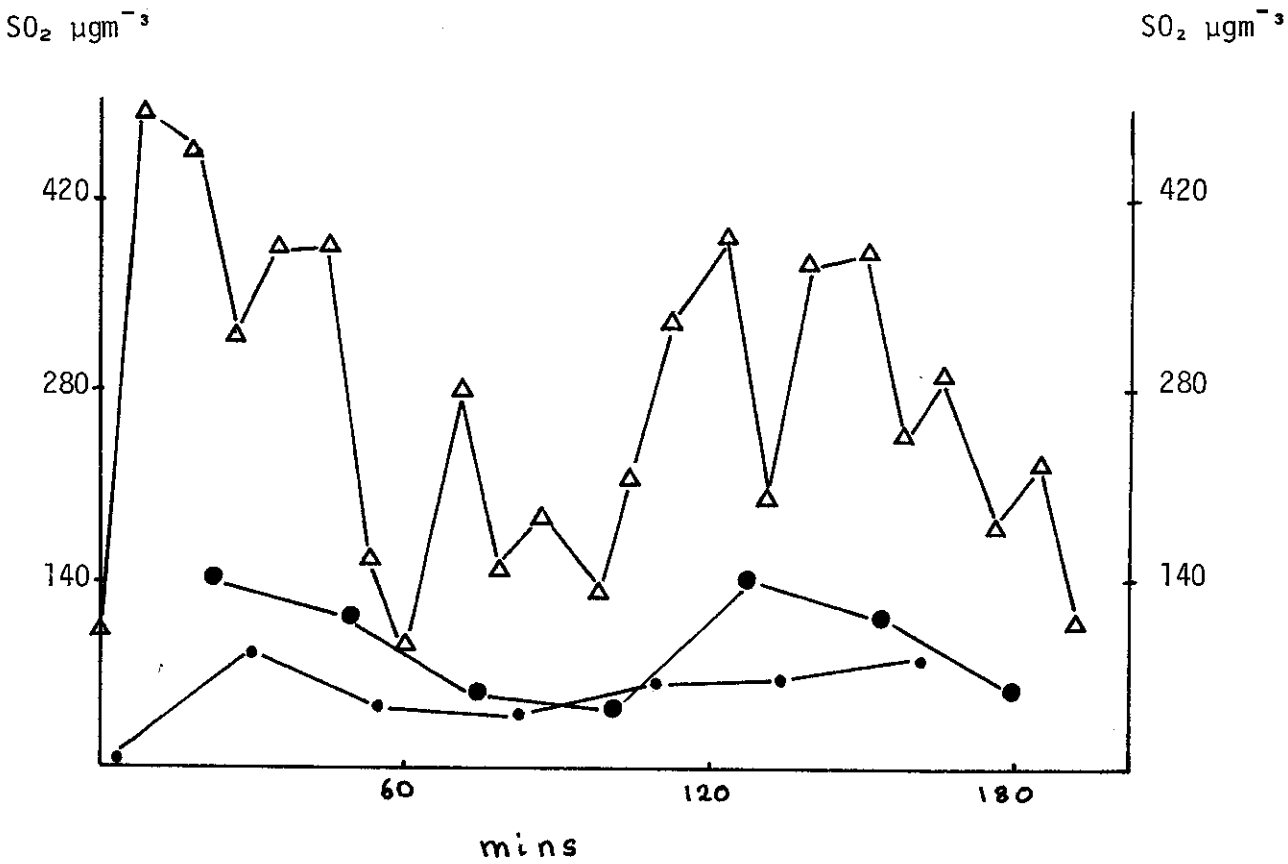
Week Beginning	Unenclosed Plots	Filtered Chambers	
		Open-Topped	Closed
1/5	48	13	10
8/5	50	9	0
15/5	77	9	2
22/5	52	0	0
29/5	48	9	3
5/6	40	4	3
12/6	41	9	2
19/6	42	19	4
26/6	72	37	4
3/7	40	33	3
10/7	55	23	9
17/7	32	16	9
24/7	30	22	10
31/7	52	40	5

FIGURE 3:18 The SO_2 concentration, μgm^{-3}, determined with a flame photometric analyser and sample changer during a pollution episode. Each channel was monitored for four minutes:

△ unenclosed plots

● filtered open-topped chamber 1

• " " " " 2


SO_2 μgm^{-3}
SO_2 μgm^{-3}
420
280
140
420
280
140
60
120
180
mins

low amounts of fluoride. The mean concentrations of F were lower than expected since the ratio for SO_2 to F emitted in the brickworks chimneys was ≈ 100:1.

TABLE 3:7

Concentrations of F (μgm^{-3}) gaseous and particulate for unenclosed plots at Thrupp End in 1978.

Date set up	Period of exposure (Days)	F concentrations (μgm^{-3})	
		Particulate	Gaseous
2.5.78	31	0.012	0.0008
7.6.78	26	0.0096	0.00096
5.7.78	1.5 hrs	1.8	0.45
7.7.78	1	0.054	0.13
12.7.78	2	-	-
7.8.78	9	0.0051	0.019

Nitrogen dioxide was measured for part of the experiment using diffusion tubes (Table 3:8). The results for unenclosed plots must be approximate as the tubes are not suited to use outside as high wind speeds increase the collection rate. The results for open and closed chambers suggest that the reduction of NO_2 in the filtered chambers might be less than for SO_2 and that filters may not be removing NO_2 as efficiently.

TABLE 3:8

Concentrations of NO_2 (μgm^{-3}) for unenclosed plots and chambers at Thrupp End in 1978.

Date set up	Days exposed	Unen-closed plots	Chambers: Open-topped Filtered	Chambers: Open-topped Unfiltered	Chambers: Closed Filtered	Chambers: Closed Unfiltered
28.6.78	16	19	8	10	3	-
14.7.78	14	11	6	-	3	8

Despite the lower SO_2 concentrations in air the percentage sulphur in the leaves was higher than in previous years. This may be caused by differences in soil sulphur at the two sites. Enclosure and filtration had no consistent effect on leaf sulphur content (Fig. 3:19).

Filtration reduced the fluoride concentration of leaves and the reduction of 75 and 40% in the closed and open-topped chambers respectively, at the final harvest, are similar to the reductions in SO_2 concentrations. The concentrations of F in the unenclosed plots are much higher than in the unfiltered chambers probably reflecting a longer period of exposure including an episode of pollution when SO_2 concentrations reached 200 μgm^{-3} after the crop in the chambers has been harvested.

Growth and Yield

Grain yields on unenclosed plots (5.9 t ha^{-1}) were higher than in earlier experiments, but the final harvest was later.

FIGURE 3:19 The sulphur content as a percentage dry weight, (I) and fluoride concentration (II) in leaves of spring barley at each harvest for unenclosed plots, unfiltered and filtered chambers during Experiment 3:

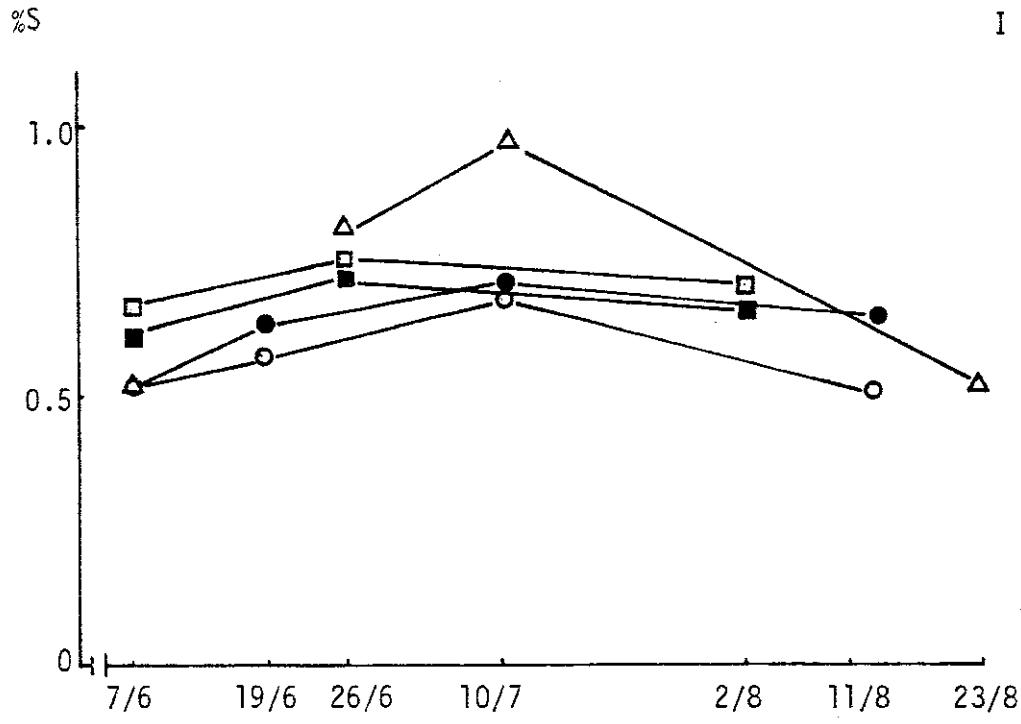
△ unenclosed plots

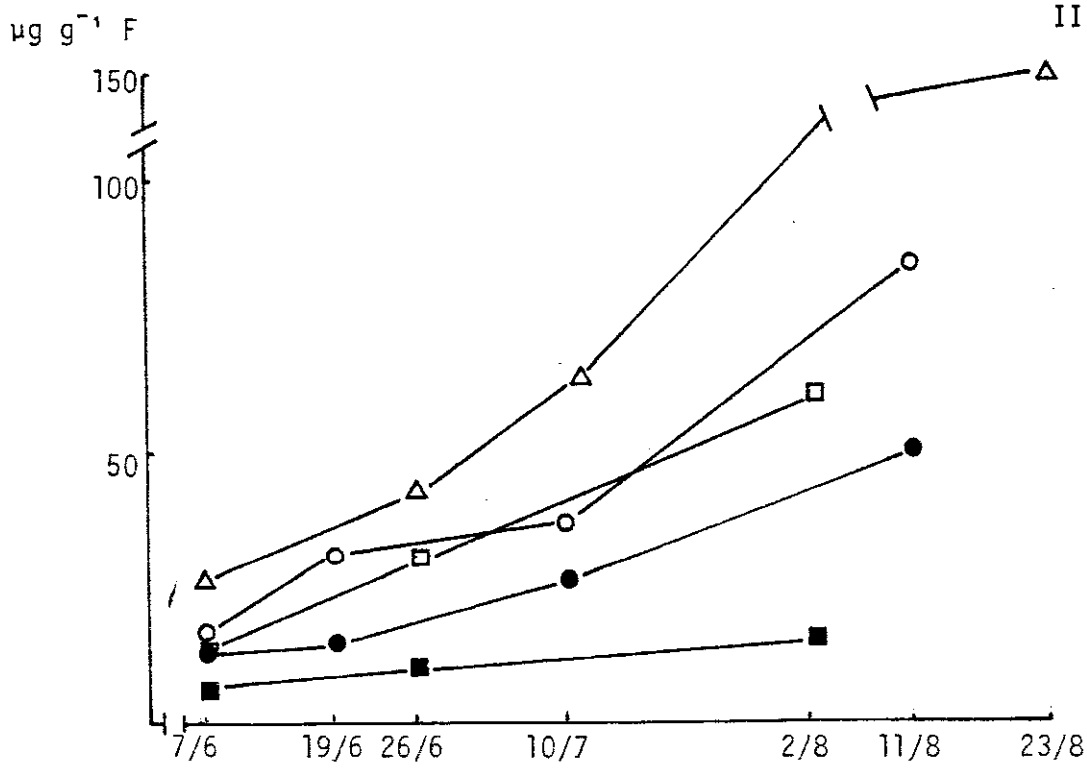
○ unfiltered open-topped chambers

● filtered open-topped chambers

□ unfiltered closed chambers

■ filtered closed chambers


%S
I
1.0
0.5
0
7/6
19/6
26/6
10/7
2/8
11/8
23/8


μg g⁻¹ F
II
150
100
50
7/6
19/6
26/6
10/7
2/8
11/8
23/8

Effects of filtration

Filtration had no significant effect at any harvest on the dry matter production or photosynthetic area per m^{-2} land of plants in the closed chambers. In the open-topped chambers there were no significant differences until anthesis when increased tiller production in the filtered air increased total dry weight and photosynthetic area (Table 3:10). These differences persisted to maturity (Table 3:10) but although the ear dry weight was higher in the filtered air the difference was not significant, however there were no episodes of pollution during grain filling. In the closed chambers filtration significantly increased the weight/ear at maturity.

Effects of enclosure

Plants in the chambers emerged one or two days earlier than those grown outside. Tiller counts show that the accelerated development of plants in the closed chambers increased the number of shoots m^{-2} at the first count but in subsequent counts numbers in the chambers remained much lower than outside (Table 3:9). The maximum tiller number was reduced by 54 and 41% in the open-topped and closed chambers respectively.

Anthesis of the plants in the closed chambers was 12 days ahead of those grown in the open-topped chambers which were again seven days ahead of those grown on unenclosed plots. Development was accelerated more than in previous years possibly because ventilation did not start until ten days after the crop was sown.

TABLE 3:10 The effect of enclosure on spring barley yields 1978 in Experiment 3.

	Anthesis						Maturity					
	O+C	O-C	UN	CL-C	CL+C	SED n=6	O+C	O-C	UN	CL-C	CL+C	SED n=6
Number of shoots (m^{-2})	686	469	716	787	641	82.2 (71.2)	1101	759	937	656	729	163.3 (141.5)
Total dry weight (gm^{-2})	540	345	573	428	311	73.1 (63.3)	1286	915	1080	945	1036	174.4 (151.0)
Straw dry weight (gm^{-2})	374	234	409	265	185	54.0 (46.8)	593	387	492	409	442	78.7 (68.2)
Ear dry weight (gm^{-2})	-	-	-	-	-	-	692	528	588	537	594	(98.8) (85.5)
Photosynthetic area index	9.7	5.8	9.2	10.2	7.6	(1.5) (1.3)	-	-	-	-	-	-

O+C = Open-topped chamber + charcoal filter
O-C = Open-topped chamber - charcoal filter
CL+C = Closed chamber + charcoal filter
CL-C = Closed chamber - charcoal filter
UN = Unenclosed plots
SED's in parentheses are for comparisons between unenclosed plots and chambers

TABLE 3:9

Number of shoots per 0.5 m length of row for the unenclosed plots and chambers at Thrupp End in Experiment 3 (Thrupp End 1978).

Date	Unenclosed Plots	Chambers	
		Open	Closed
17/5	27	28	40
19/5	46	38	47
22/5	75	47	60
24/5	87	50	69
28/5	107	51	69
31/5	109	53	69
2/6	116	54	69

The destructive harvest confirmed the results of the tiller counts but not all of the differences in shoot number were significant. Plants from the unenclosed plots produced 40% more ears per plant than those in the chambers increasing the ear dry weight at anthesis (Table 3:10). However, the difference was small at maturity because the plants in the chambers had produced heavier ears compensating for the smaller number.

Experiments Four and Five

In 1979 and 1980 in experiments at Thrupp End the closed chambers were replaced by four more collared and lipped open-topped chambers. This allowed greater replication each experiment consisting of four blocks each of four plots; one charcoal filtered and one unfiltered chamber and two unenclosed plots. The position of plots within each block was randomized but not allowed to duplicate a position in another block.

PLATE 4: Experimental site during Experiment 4.

Spring barley, var. Magnum, was chosen for its short straw, high yield and resistance to disease. To reduce variability the area sampled was doubled but the number of harvests reduced to two; one at maturity the other at anthesis. To compensate for the loss of harvests at the early growth stages non-destructive measurements were made up to anthesis.

Concentrations of pollutants in the air and plants

The mean ambient SO_2 concentrations were similar for the two growing seasons: 48 μgm^{-3} in 1979 and 52 μgm^{-3} in 1980 (Table 3:11). In both years the highest daily mean concentrations of SO_2 occurred in the period between emergence of the plants and the onset of tillering (Table 3:12 & 3:13). In 1980 the crop was exposed to a further period of high SO_2 concentration just before anthesis. The highest daily mean SO_2 concentration was 224 μgm^{-3} in 1979 and 182 μgm^{-3} in 1980 (Tables 3:12 & 3:13), but short-term peaks (in range of 300-1300 μgm^{-3}), lasting for several minutes, occurred when the experimental site was covered by the plumes of the neighbouring brickworks (e.g. Fig. 3:20).

Filtration satisfactorily reduced the pollutant concentrations in both years by 65-70%. Despite raising the air ducts in the chambers as the crop grew, the efficiency of the chambers decreased between anthesis and maturity in 1979, probably as a result of the crop lodging. In 1979 a shortage of equipment prevented continuous monitoring of the chambers supplied with unfiltered air. In 1980 the concentration in the unfiltered chambers fluctuated the same way as with those of the field and the mean concentration was only 8% less than that outside.

TABLE 3:11

Mean SO_2 concentrations (μgm^{-3}) highest daily mean and number of days above 100 μgm^{-3} for unenclosed plots and chambers at Thrupp end during Experiment 4 (1979) and Experiment 5 (1980).

Year		Unenclosed Plots	Open-Topped Chambers Filtered	Unfiltered
1979	Mean for experiment	48	15	-
	Highest daily mean	224	77	-
	Days above 100 μgm^{-3}	6	0	-
1980	Mean for experiment	52	18	48
	Highest daily mean	182	86	188
	Days above 100 μgm^{-3}	9	0	9

Measurements of fluoride concentrations were not satisfactory in 1979 but were continuous in 1980. The mean F^- concentration in 1980 was 0.15 μgm^{-3} (0.13 μgm^{-3} gaseous and 0.02 μgm^{-3} particulate F^-). The highest daily mean F^- concentration was 1.48 μgm^{-3} (Table 3:12). The pattern of peak concentrations for fluoride closely followed those for SO_2 but the ratio of $F:SO_2$ was ≈ 1:350, considerably less than the reported ratio for stack gases (1:100). This apparent discrepancy may be explained by other sources of SO_2 pollution both locally and imported into the area.

TABLE 3:12

Ambient daily mean SO_2 concentrations μgm^{-3} at crop height during Experiment 4 at Thrupp End in 1979.

Day	Month			
	May	June	July	August
1		86	16	19
2		143	16	34
3		-	10	34
4		-	26	12
5	73	85	31	12
6	72	50	22	12
7	163	31	-	13
8	224	92	-	15
9	71	68	-	7
10	153	68	18	7
11	113	49	26	15
12	42	47	71	19
13	42	50	8	19
14	42	84	22	12
15	41	62	16	78
16	99	29	16	21*
17	93	27	16	18
18	34	25	51	10
19	62	34	14	10
20	62	49	19	19.5
21	66	53	5	
22	13	60	5	
23	31	-	4	
24	40	-	19	
25	85	111	21	
26	34	67	16	
27	34	77	15	
28	34	73	-	
29	34	53	-	
30	39	16	30	
31	86		19	

*To enable comparisons to be made between chambers and unenclosed plots. values after the 15th August were excluded from calculations of mean concentrations.

TABLE 3:13

Ambient daily mean SO_2 and F concentrations, μgm^{-3} at crop height during Experiment 5 at Thrupp End in 1980.

Day	Month									
	April		May		June		July		August	
	SO_2	F	SO_2	F	SO_2	F	SO_2	F	SO_2	F
1			92	0.89	39	0.14	36	0.08	35	0.15
2			172	1.16	39	0.14	36	0.08	46	0.12
3			180	1.13	97	0.58	36	0.15	46	0.12
4			68	0.45	48	0.22	36	0.15	22	0.22
5			68	0.45	65	0.18	27	0.18	70	0.38
6			23	0.16	43	0.09	27	0.18	135	1.02
	17		17	0.15	21	0.09	27	0.18	66	0.31
8	56		52	0.02	31	0.19	30	0.19	20	0.17
9	33		47	0.23	28	0.10	30	0.19	29	0.21
10	47		39	0.11	59	0.58	21	0.10	29	0.21
11	76		51	0.15	40	0.08	30	0.07	29	0.21
12	83		51	0.13	70	0.29	30	0.07	32	0.14
13	-	0	35	0.15	56	0.31	56	0.06	57	0.21
14	79	0.4	18	0.15	66	0.25	56	0.06	28	0.00
15	21	0.22	18	0.13	96	0.86	50	0.21	28	0.00
16	77	0.22	18	0.09	22	0.13	37	0.21	48	0.18
17	83	0.16	27	0.14	121	1.06	53	0.20	48	0.18
18	67	0.34	27	0.14	56	0.45	50	0.43	101	0.24
19	54	0.12	47	0.22	57	0.62	99	0.24		0.12
20	69	0.17	83	0.48	0	0.34	48	0.24		0.76
21	20	0.07	36	0.29	182	1.48	70	0.12		0.76
22	107	0.08	63	0.15	NR	0.47	58	0.17		0.19
23	40	0.04	68	0.36	47	0.47	107	0.11		0.09
24	48	0.17	37	0.12	61	0.39	57	0.52		0.09
25	116	0.88	52	0.18	61	0.39	57	0.20		0.09
26	39	0.21	47	0.10	33	0.25	27	0.09		0.09
27	39	0.21	41	0.10	41	0.19	27	0.09		0.47
28	37	0.12	31	0.13	41	0.20	49	0.06		0.47
29	25	0.05	36	0.07	41	0.20	58	0.33		
30	37	0.26	28	0.07	41	0.20	89	0.66		
31			39	0.14			53	0.09		

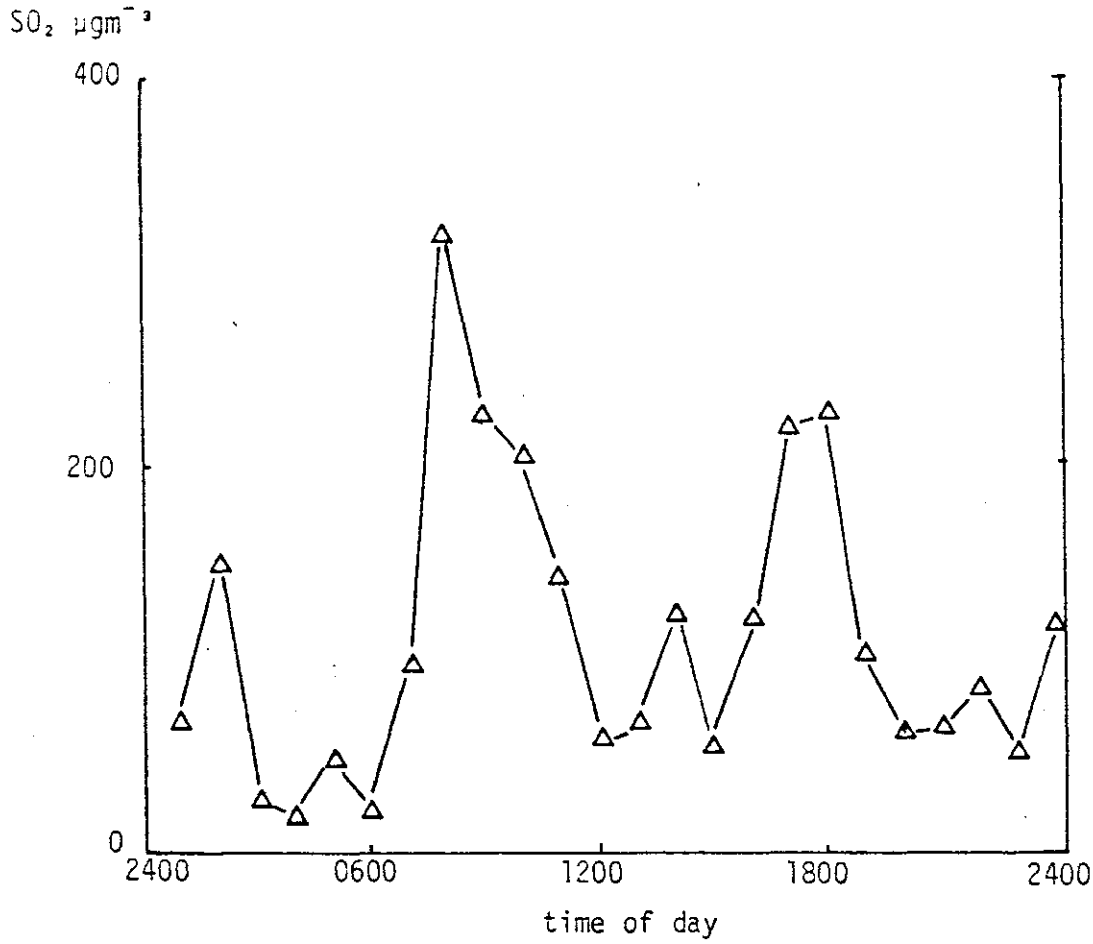


FIGURE 3:20 Hourly mean SO_2 concentrations, μgm^{-3}, for an enclosed plot during a 24 hour period in May 1979 measured using a flame photometric analyser.

In 1980 NO_x concentrations were monitored on occasions using a Meloy Na 530 monitor. It was only possible to operate the monitor for short periods but no increase in concentrations was observed when the experimental site was covered by the plume from the brickworks. This supports the information supplied by the London Brick Company for stack emissions; that the combustion temperature is not high enough for NO_x to be produced (Dr. Bowler, personal communication). Only intermittent measurements of O_3 were made in 1980 as a breakdown in the monitor prevented continuous recording. The highest concentration 150 μgm^{-3} (7 pphm) was recorded in May.

As in previous experiments cleaning the air did not result in a reduction in the sulphur content of the leaves. The fluoride concentration of plants from the filtered chambers was about 50% of those from the unfiltered ones. The concentration of F in the plants in the unfiltered chambers were generally lower than in plants from unenclosed plots, presumably the result of the slightly lower concentrations of pollutants in the unfiltered chambers and the shorter period of exposure compared with plants on unenclosed plots.

Growth and yield

Grain yields were good, 6.4 and 7.2 t ha^{-1} (15% moisture content) in 1979 and 1980 respectively. In 1979 the crop lodged shortly after anthesis.

Effect of filtration

Cleaning the air did not alter the rate of development of the crop.

At anthesis there were no significant differences in tiller numbers but there were more ears m^{-2} in the filtered than in unfiltered chambers. In both years total dry weights and photosynthetic area indices were greater in the filtered chambers but increases were significant only in 1979 (Table 3:14). At maturity straw dry weights were increased in the filtered chambers by 36% in 1979 and 20% in 1980. Grain yields and 1,000 grain weights and grain number per ear were greater in the filtered than in unfiltered chambers in both years but again increases were significant only in 1979. Cleaning the air increased total dry weight by 51% in 1979 and 17% in 1980.

Effects of enclosure

As in previous experiments the crop in the chambers emerged one day earlier than on unenclosed plots. Maximum tiller number was decreased in the chambers by about 50% (Fig. 3:21). Stem elongation began sooner in the chambers than on unenclosed plots. The rates of stem elongation were similar for the chambers and unenclosed plots (Table 3:15) but by virtue of an earlier start the plants in the chambers grew slightly taller than those outside. However, the enclosed plants produced significantly fewer leaves on the main stem (Table 3:15) although the first leaves were significantly larger (Table 3:16).

Anthesis was 7-8 days earlier in the unfiltered chambers than for unenclosed plots. The number of shoots per unit area was less inside the chambers than outside by 36% in 1979 and 54% in 1980 confirming the results on non-destructive harvests. Differences in shoot numbers resulted in few ears, lower straw dry weights and smaller photosynthetic

TABLE 3:14 The effect of enclosure and filtration on spring barley yields, and fluoride and sulphur contents in Experiments 4 and 5 in 1980.

	1979						1980					
	Anthesis			Maturity			Anthesis			Maturity		
Harvest	O+C	O-C	UN	O+C	O-C	UN	O+C	O-C	UN	O+C	O-C	UN
Number of shoots (m^{-2})	859	792 (57.8)	1236 (50.0)	862	668 (92.3)	1089 (79.9)	753	6501 (57.3)	1405 (49.6)	1275	1101 (79.7)	1421 (69.1)
Number of ears (m^{-2})	586	461 (53.2)	1025 (46.1)	541	488 (84.4)	977 (73.1)	536	456 (27.3)	786 (23.6)	715	709 (74.8)	955 (64.7)
Total dry weight ($g\ m^{-2}$)	812	680 (60.5)	1197 (49.0)	1126	745 (82.0)	1278 (71.0)	511	482 (39.0)	804 (33.7)	1357	1159 (59.8)	1529 (51.8)
Grain dry weight ($g\ m^{-2}$)				489	308 (46.4)	559 (40.2)				560	518 (36.1)	631 (31.3)
Straw dry weight ($g\ m^{-2}$)	614	469 (39.9)	898 (31.9)	470	345 (50.9)	597 (44.1)	446	400 (30.0)	692 (26.0)	569	475 (28.5)	675 (24.7)
Photosynthetic area index	8.1	6.6 (0.6)	10.5 (0.4)				7.5	6.4 (0.6)	10.7 (0.5)			
1000 grain dry weight (g)				43.8	37.6 (1.1)	31.7 (1.0)				42.4	42.0 (0.9)	35.0 (0.8)
Number of grains per ear				20.6	16.8	18.1				18.9	17.4	19.1
F concentration ($\mu g\ g^{-1}$)	12.5	20.4 (2.0)	37.7 (1.8)	27.7	53.6 (8.9)	89.4 (7.7)	9.7	19.6 (2.2)	18.9 (1.9)	28.7	42.8 (7.2)	110.6 (6.2)
S concentration % of leaves				0.73	0.70 (0.04)	0.56 (0.07)				0.93	0.69 (0.07)	0.68 (0.07)

Numbers in parentheses are SED's
O+C = Open-topped chamber + charcoal filter
O-C = Open-topped chamber - charcoal filter
UN = Unenclosd plots

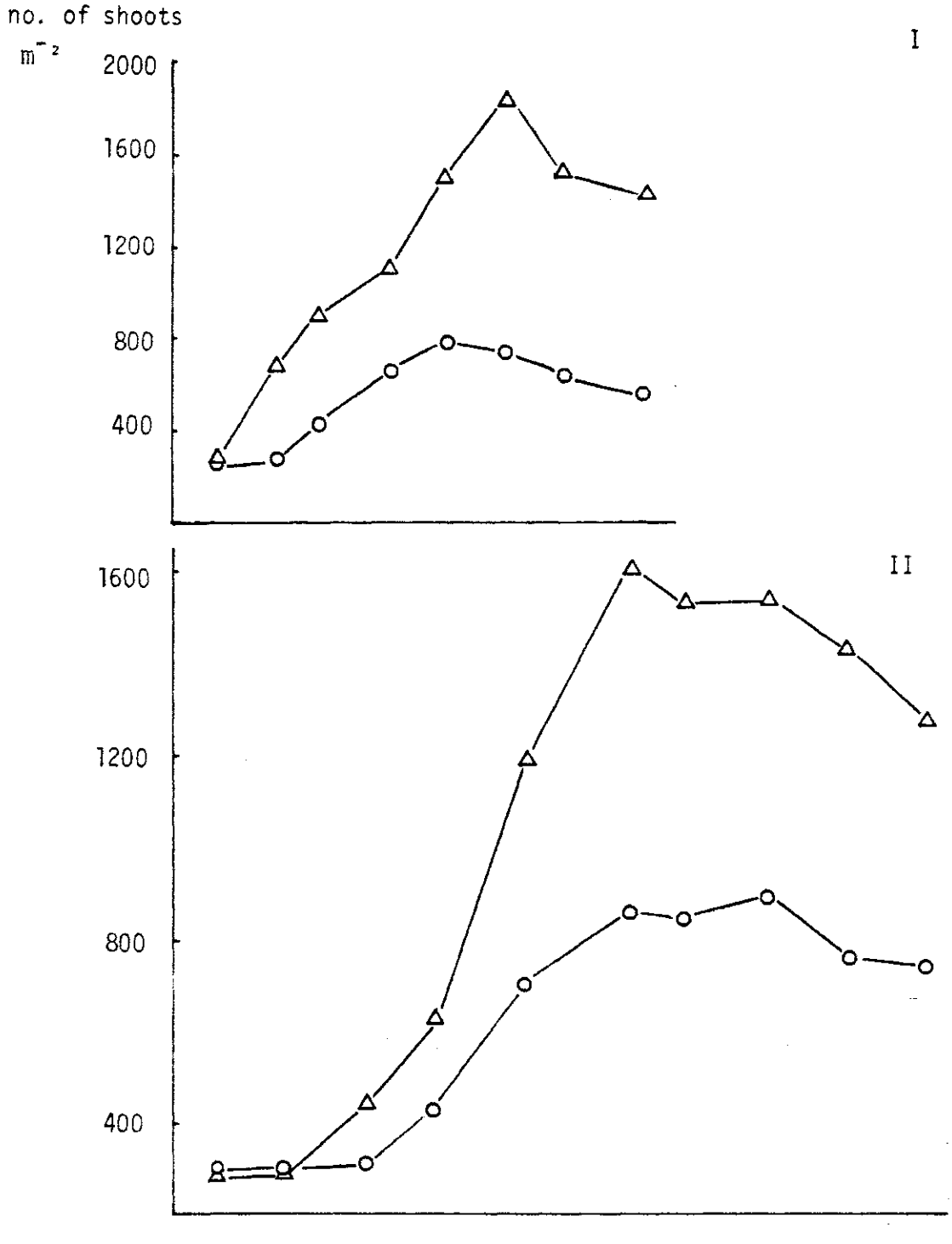


FIGURE 3:21 The number of shoots (m^{-2}) of spring barley grown in unenclosed plots (△—△) and unfiltered (O—O) chambers at Thrupp End during experiment four (I) and experiment five (II).

area inside the chambers at anthesis and the differences present at anthesis persisted to maturity. The crop ripened 7-8 days earlier in the chambers and ear numbers, straw and grain dry weights were less than outside but grain size was larger in the chambers than on unenclosed plots.

TABLE 3.15

Developmental data for plants from unfiltered chambers and unenclosed plots at Thrupp end in Experiment 4 (1979) and Experiment 5 (1980).

		Maximum shoot no. m^{-2}	No. leaves on main stem	Final main stem height mm
1979	O-C	774	8.0	664
	UN	1817	9.0	633
	LSD	306	0.2	22
1980	O-C	889	9.1	745
	UN	1599	10.5	725
	LSD	302	0.2	22

TABLE 3:16

Final leaf lengths for plants from unfiltered chambers and unenclosed plots in Experiment 5 (1980).

Leaf No.	Unenclosed Plots mm	LSD p=0.05	Unfiltered Open-topped chambers mm
1	82	5.5	89
2	105	10.3	132
3	125	12.7	186
4	143	17.5	219
5	150	19.6	234
6	161	23.7	248
7	180	17.0	228
8	203	13.7	213
9	203	19.3	114
10	146	24.3	87
11	96		

ENZYMOLOGY

Research has been demonstrated that pollutants may affect specific processes, by the inhibition of the enzymes catalysing them. Thus the inhibition of RuBP carboxylase has been suggested as a key mechanism by which SO_2 may inhibit photosynthesis. However, research *in vitro* has produced conflicting results and conclusions, probably the result of the conditions used for the assay of the enzyme. Knowing the activation requirements of the enzyme (see Introduction, p.28) this research was conducted to clarify the response of the purified enzyme to SO_2 and F exposures. Fully purified enzyme was used in the study to avoid possible interference from competing processes. Prior to examination of the effects of SO_3^{2-}, SO_4^{2-} and F^- on steady-state catalysis, the effects on the activation and deactivation of the enzyme were studied. The importance of these processes on the *in vivo* response to pollutants is detailed in the discussion.

RuBP carboxylase can exist in several forms. It has been demonstrated that carboxylation and oxidation requires the formation of an active ternary complex involving the carbamylation of an ε amino group of a lysine residue on the enzyme and then the addition of Mg^{2+} to the enzyme CO_2 complex. This process is ordered and reversible. If pollutants interfered with this process variations in carboxylase and oxygenase activities would occur in addition to any inhibition of substrate catalysis. The structure of several oxyanions is such that they resemble CO_2 and could inhibit the carbamylation reaction during enzyme activation. Sulphur dioxide fumigation results in the formation of SO_3^{2-} and SO_4^{2-} in the chloroplast. Therefore their effects and those of other oxyanions have been examined.

Figures 3:22-27 show the activation of a slowly activating form of the enzyme, the freeze-dried powder. All of the experiments were repeated several times and the results were reproducible. The data in the figures and tables are taken from representative experiments. The addition of 10 mM concentration of the anions during activation resulted in a final concentration of 0.4 mM in the assay.

Activation in the absence of added CO_2 but in the presence of saturating Mg^{2+} and several oxyanions was compared with activation in saturating CO_2 and Mg^{2+} (Fig. 3:22). PO_4^{3-}, SO_3^{2-} and SO_4^{2-} produced a slightly enhanced specific activity varying between experiments, whilst NO_3^- and NO_2^- did not affect the amount of activation relative to the control in the absence of added CO_2. The variable increase in activity produced by some oxyanions suggested that CO_2 contamination of the buffers was important. When greater efforts were made to reduce CO_2 contamination the stimulation of activation in response to SO_3^{2-}, SO_4^{2-} and PO_4^{3-}, was reduced suggesting that these oxyanions may be promoting the binding of contamination CO_2.

In low CO_2 (1 mM) concentrations activation was enhanced when incubated with SO_3^{2-} or SO_4^{2-} or PO_4^{3-}, but unchanged in the presence of NO_3^- or NO_2^- (Fig. 3:23). Identical results were found for the carboxylase activity (Table 3:17). The rate and level of activation was similar to that obtained in saturating CO_2 and Mg^{2+}.

In Figure 3:24 data for the activation in 1 mM HCO_3 concentrations and a range of SO_3^{2-} concentrations show that optimum activity was found in response to the highest SO_3^{2-}; however, even the lowest

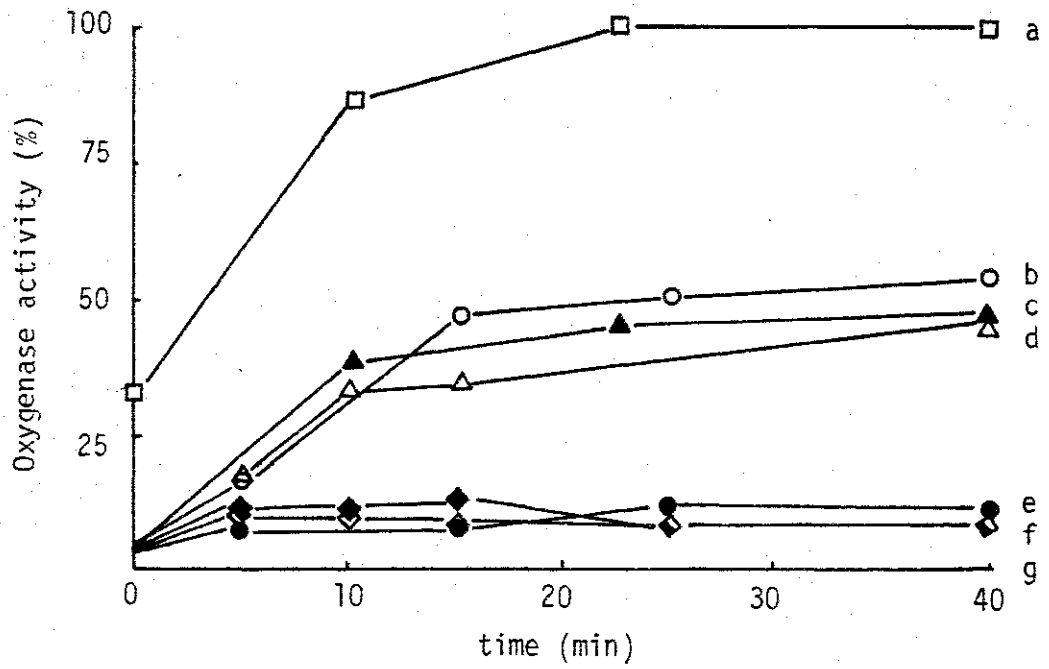


FIGURE 3:22 Activation of the slow activating form of RuBP carboxylase measured by oxygenation at 40°C. The activities are relative to the maximum obtained in (a). The enzyme was activated in 0.1M HEPES buffer pH 8.2 with 20 mM $MgCl_2$ plus these additions:

a 10 mM HCO_3^-
b 10 mM PO_4^{3-}
c 10 mM SO_3^{2-}
d 10 mM SO_4^{2-}
e 10 mM NO_3^-
f 10 mM NO_2^-
g No additions

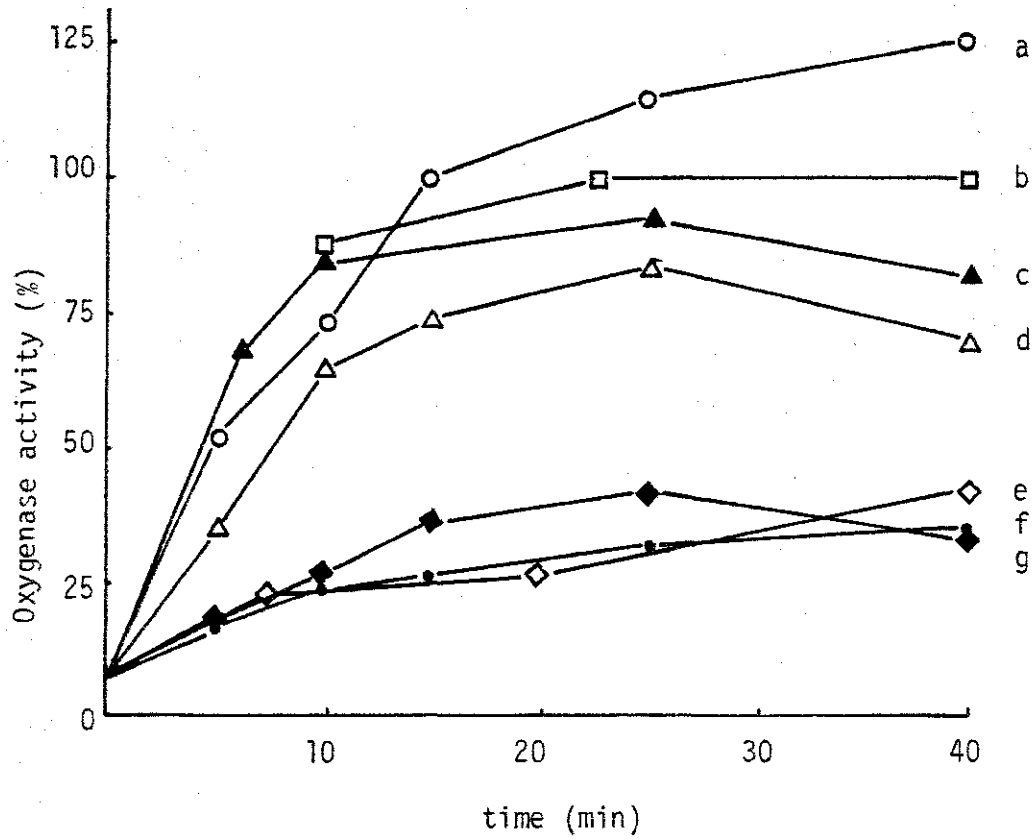


FIGURE 3:23 Activation of the slow activating form of RuBP carboxylase measured by oxygenation at 40°C. The activities are relative to the maximum obtained in (b). The enzyme was activated in 0.1 M HEPES buffer pH 8.2 with 20 mM $MgCl_2$ and 1 mM HCO_3^- plus these additions:

a	10 mM PO_4^{3-}
b	9 mM HCO_3^-
c	10 mM SO_3^{2-}
d	10 mM SO_4^{2-}
e	No additions
f	10 mM NO_3^-
g	10 mM NO_2^-

TABLE 3:17

The effect of anions on the activation of RUBP carboxylase in the presence of different concentrations of activators. Activation for 40' at 40°C in saturating concentrations of HCO_3^- and Mg^{2+} is used as a 100% and is equivalent to 1.3 μmole CO_2 min^{-1} mg^{-1} protein.

		10 mM concentrations						
	No Additions	SO_3^{2-}	SO_4^{2-}	NO_2^-	NO_3^-	PO_4^{3-}	F^-	Cl^-
Activation in:								
10 mM HCO_3^- 20 mM Mg^{2+}	100	130	130	84	81	164	97	101
1 mM HCO_3^- 20 mM Mg^{2+}	45	128	122	45	40	174	43	39
10 mM HCO_3^- 2 mM Mg^{2+}	61	69	62	45	51	82	63	58

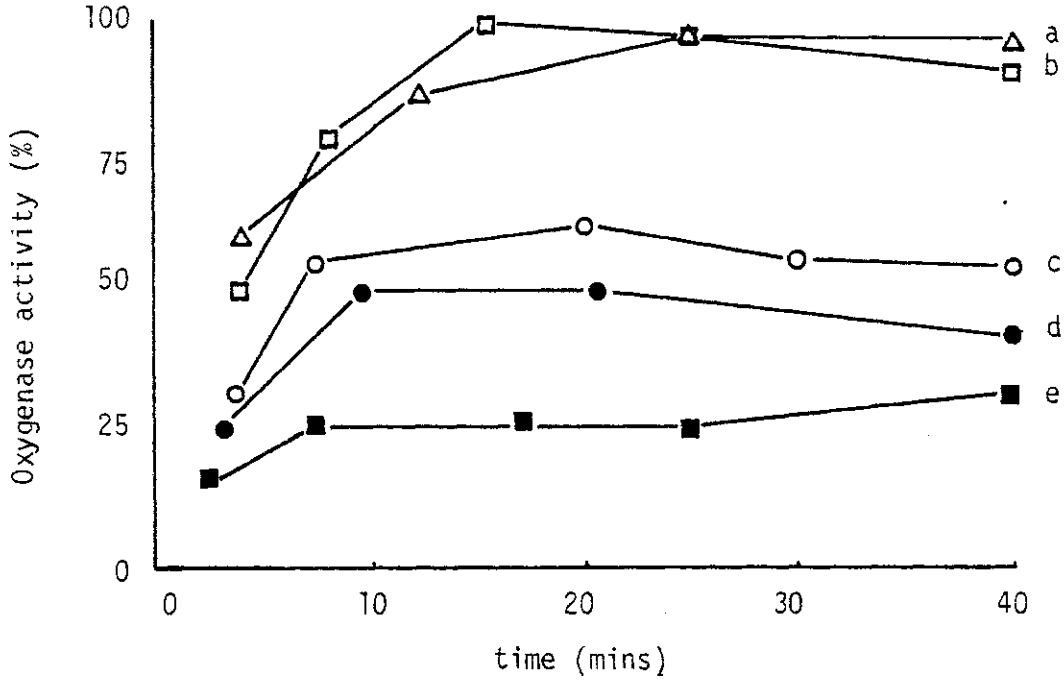


FIGURE 3:24 Activation of the slow activating form of RuBP carboxylase measured by oxygenation at 40°C. The activities are relative to the maximum obtained in (b). The enzyme was activated in 0.1 M HEPES buffer with 1 mM HCO_3^-, 20 mM $MgCl_2$ plus these additions:

a 5 mM SO_3^{2-}
b 9 mM HCO_3^-
c 1 mM SO_3^{2-}
d 0.5 mM SO_3^{2-}
e No additions

concentration (0.5 mM SO_3^{2-}) doubled the activity relative to the control in 1 mM HCO_3. Sulphate was found to be effective over a similar range of concentrations.

The reason for the full activation in low CO_2 concentrations in the presence of such oxyanions is presumably due to these anions binding to and stabilizing the active form of the enzyme trapping the activating cofactors.

In an experiment with low Mg^{2+} concentrations (2 mM) and saturating CO_2 the addition of oxyanions had little effect on the level of activation (Fig. 3:25). This applied to both enzyme reactions (Table 3:17). The main effect of these anions may be in trapping the activating CO_2 rather than Mg^{2+}.

A further experiment was performed to investigate the effect of oxyanions in the presence of saturating CO_2 and Mg^{2+}. Increases in the level of both carboxylase and oxygenase activities occurred in the presence of PO_4^{3-}, SO_3^{2-} and SO_4^{2-} (Fig. 3:26 & Table 3:17) but the rate of activation was similar to that obtained with saturating CO_2 and Mg^{2+}.The magnitude of the increase was found to vary between 20-60% with different enzyme preparations. Such stimulation cannot be explained solely by the trapping of activating CO_2 and Mg^{2+} since a similar stimulation would be seen by increasing the concentration of these co-factors themselves. The addition of oxyanion effectors (SO_3^{2-}, SO_4^{2-} and PO_4^{3-}) to enzyme that had been fully activated in saturating CO_2 and Mg^{2+} for 40 min at 40°C enhanced the enzyme activity (Fig. 3:27). The stimulation occurred when the oxyanions were added to the activated enzyme stored at 25 or 40°C (Fig. 3:27II).

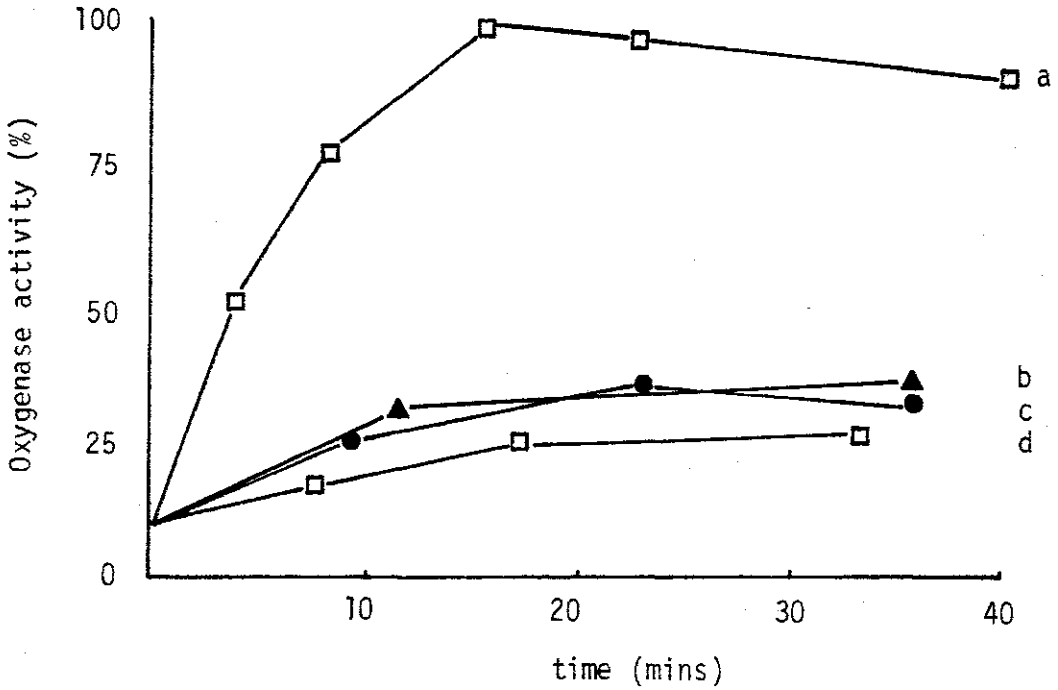


FIGURE 3:25 Activation of the slow activating form of RuBP carboxylase measured by oxygenation at 40°C. The activities are relative to the maximum obtained in (a). The enzyme was activated in 0.1 M HEPES buffer pH 8.2 with 10 mM HCO_3^- and 1 mM $MgCl_2$ plus these additions:

a 19 mM $MgCl_2$
b 10 mM SO_3^{2-}
c 10 mM SO_4^{2-}
d No additions

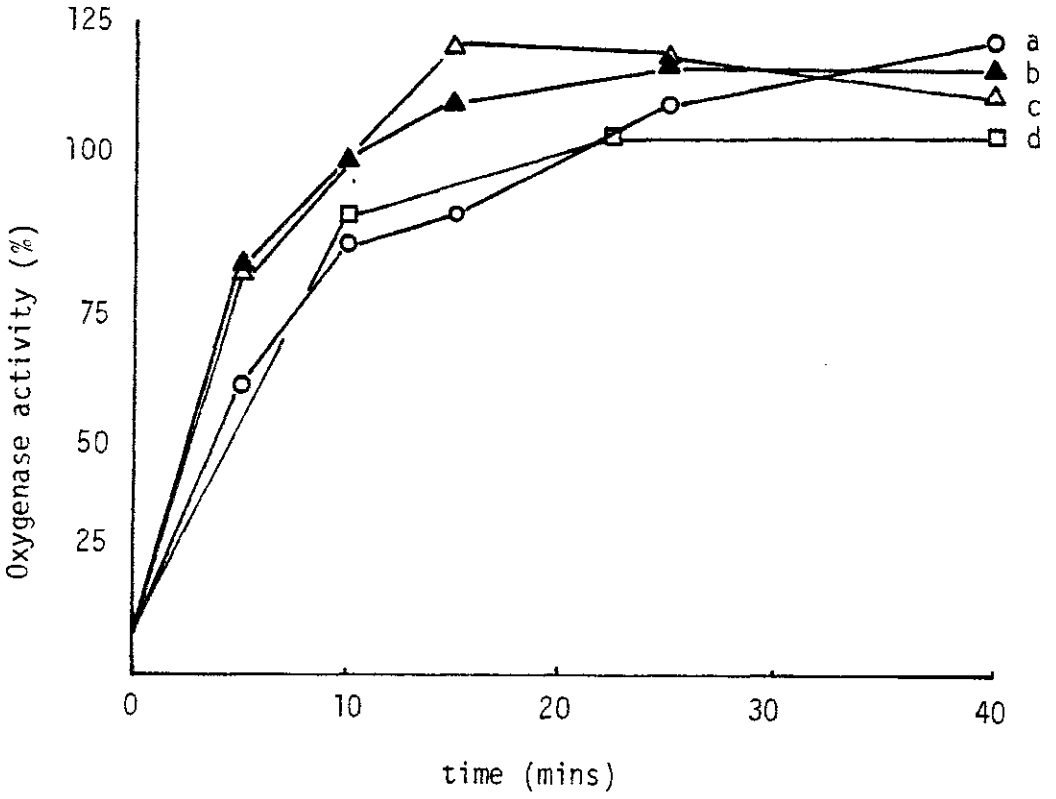


FIGURE 3:26 Activation of the slow activating form of RuBP carboxylase measured by oxygenation at 40°C. The activities are relative to the maximum obtained in standard activation buffer. The enzyme was activated in 0.1 M HEPES buffer pH 8.2 with saturating concentrations of HCO_3^- (10 mM) and $MgCl_2$ (20 mM) plus:

a 10 mM PO_4^{3-}
b 10 mM SO_3^{2-}
c 10 mM SO_4^{2-}
d No additions

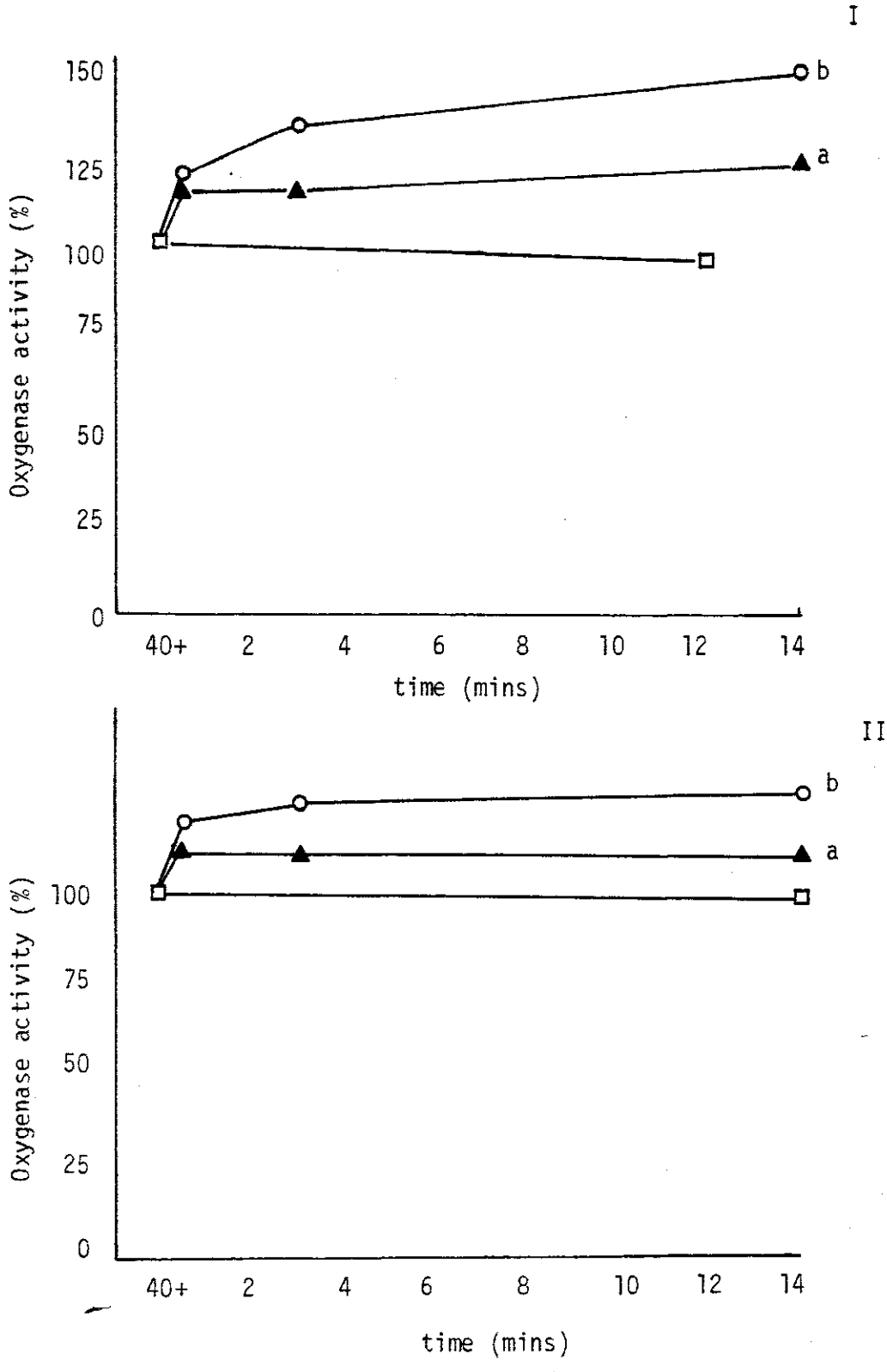


FIGURE 3:27 Stimulation of RuBP carboxylase activity by anions at different temperatures. The activities are relative to the maximum obtained for enzyme activated in standard activation buffer.

1) 40°C a) SO_3^{2-} 10 mM
   b) PO_4^{3-} 10 mM
2) 25°C a) SO_{32-} 10 mM
   b) PO_{43-} 10 mM

The addition of 10 mM HCO_3^- to enzyme that had been pre-incubated in saturating Mg^{2+} for 40 min at 40°C only reached 80% of the activity of normally activated enzyme. However preincubation in the presence of oxyanion effectors resulted in an increased activation on the addition of HCO_3^- similar to that of enzyme activated normally in their presence (Fig. 3:28).

The effect of F^- on the activation was investigated because of its known affinity for Mg^{2+} and was compared with another anion Cl^-. Neither anion affected the rate or level of activation achieved in low or saturating concentrations of CO_2 and Mg^{2+} (Table 3:17) nor on the deactivation of the enzyme.

Deactivation and Reactivation

When fully activated enzyme is transferred to a solution without Mg^{2+} or HCO_3^- there is a rapid loss of the activating cofactors and both carboxylase and oxygenase activities. Deactivation was measured by transferring active enzyme to an oxygen electrode containing CO_2-free buffer and then RuBP substrate added at different times. Even in 20 mM $MgCl_2$ the deactivation is rapid ($t_{0.5}$ = 15 s) and the residual activity afer long periods is that supported by the HCO_3^- and Mg^{2+} carried over from activation (Fig. 3:29). Deactivation of enzyme activated in PO_4^{3-}, SO_3^{2-} or SO_4^{2-} had the same residual activity as normal enzyme, but because of the higher initial specific activity of enzyme incubated with PO_4^{3-}, SO_3^{2-} or SO_4^{2-} the percentage loss in activity is therefore greater. Deactivation in the presence of 10 mM PO_4^{3-}

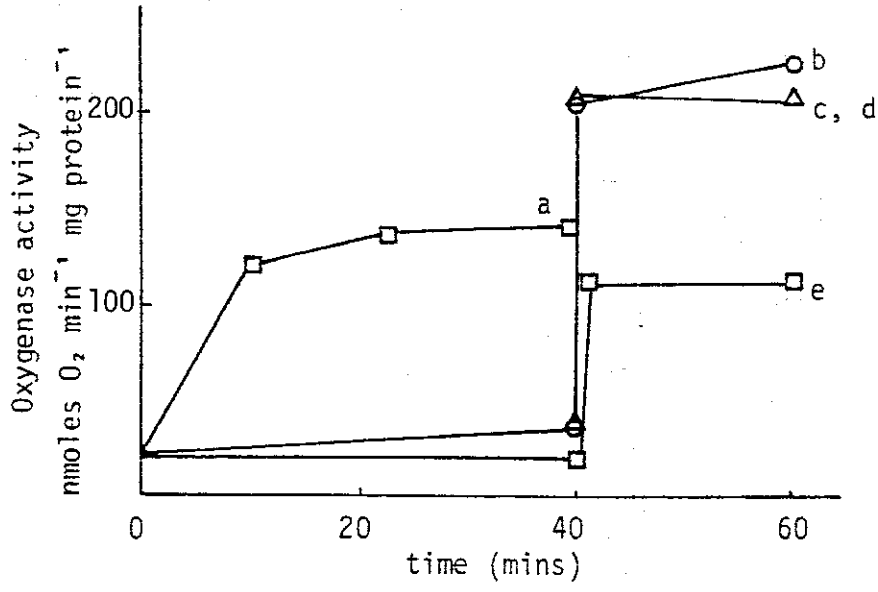


FIGURE 3:28 Activation of RuBP carboxylase by 10 mM HCO_3^- following pretreatment in 0.1 M HEPES pH 8.2 with 20 mM $MgCl_2$ and oxyanions for 40' at 40°C:

a standard activation
b 10 mM PO_4^{3-}
c 10 mM SO_3^{2-}
d 10 mM SO_4^{2-}
e no additions

SO_3^{2-} or SO_4^{2-} is inhibited relative to the control in the absence of these effectors. However, none of the oxyanions were very effective in maintaining activity and high concentrations were needed to reduce deactivation significantly (Fig. 3:29). The percentage deactivation ws unaltered in the presence of NO_3^- or NO_2^-.

Since deactivation is the result of the loss of the activating co-factors oxygenase and carboxylase activities can be restored by the addition of HCO_3^- and Mg^{2+}, even at 25°C. Reactivation is extremely rapid, e.g. $t_{0.5}$ in 5 mM HCO_3^- and 20 mM Mg^{2+} is ≃ 12 s. Reactivation was followed after heat activation by the dilution of the activating cofactors in an oxygen electrode containing CO_2-free buffer and subsequent addition of HCO_3^- for different times.

The reactivation of enzyme activated in saturating cofactors (20 mM Mg^{2+}, 10 mM HCO_3^- and PO_4^{3-} or SO_3^{2-} or SO_4^{2-} regained a higher specific activity than the control reactivated only in saturating cofactors (Fig. 3:30). However, the percentage recovery of the samples activated in oxyanions was slightly reduced, the greatest reduction occurring for the phosphate-activated enzyme which had the highest specific activity (Fig. 3:31). This supports the idea of anions binding at the active site and their dissociation during deactivation, i.e. $E_A \rightarrow E$.

When reactivation was conducted in the presence of SO_3^{2-} and SO_4^{2-} and in low (1 mM) HCO_3^- the activity was only slightly enhanced and closely followed that of the control reactivated in 1 mM HCO_3^- only (Fig. 3:32). In contrast the presence of PO_4^{3-}

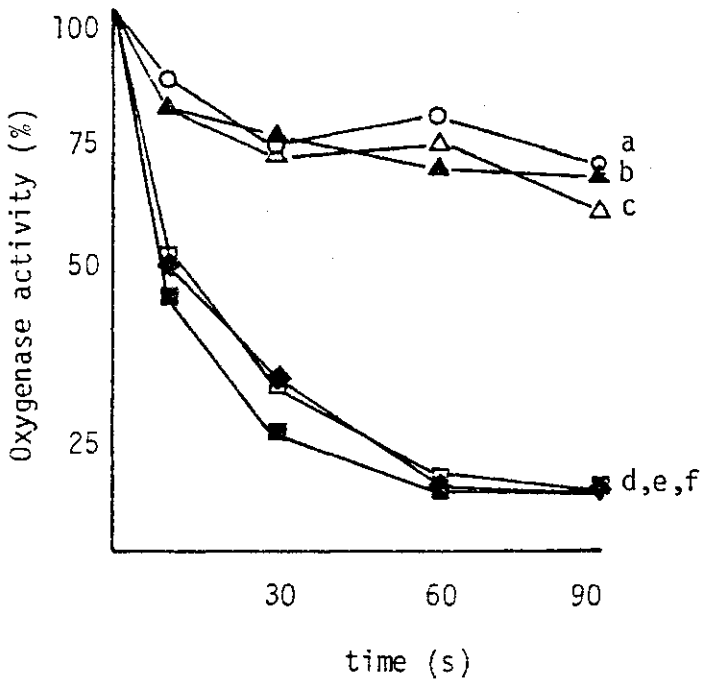


FIGURE 3:29 Deactivation of RuBP carboxylase measured by oxygenation. Remaining activity was determined by introducing RuBP at the indicated times. The 100% value was the maximum activity of the enzyme without oxyanions (d) or the inhibited activity. Deactivation was done in:

a 10 mM PO_4^{3-}
b 10 mM SO_3^{2-}
c 10 mM SO_4^{2-}
d no additions
e 10 mM NO_2^-
f 10 mM NO_3^-

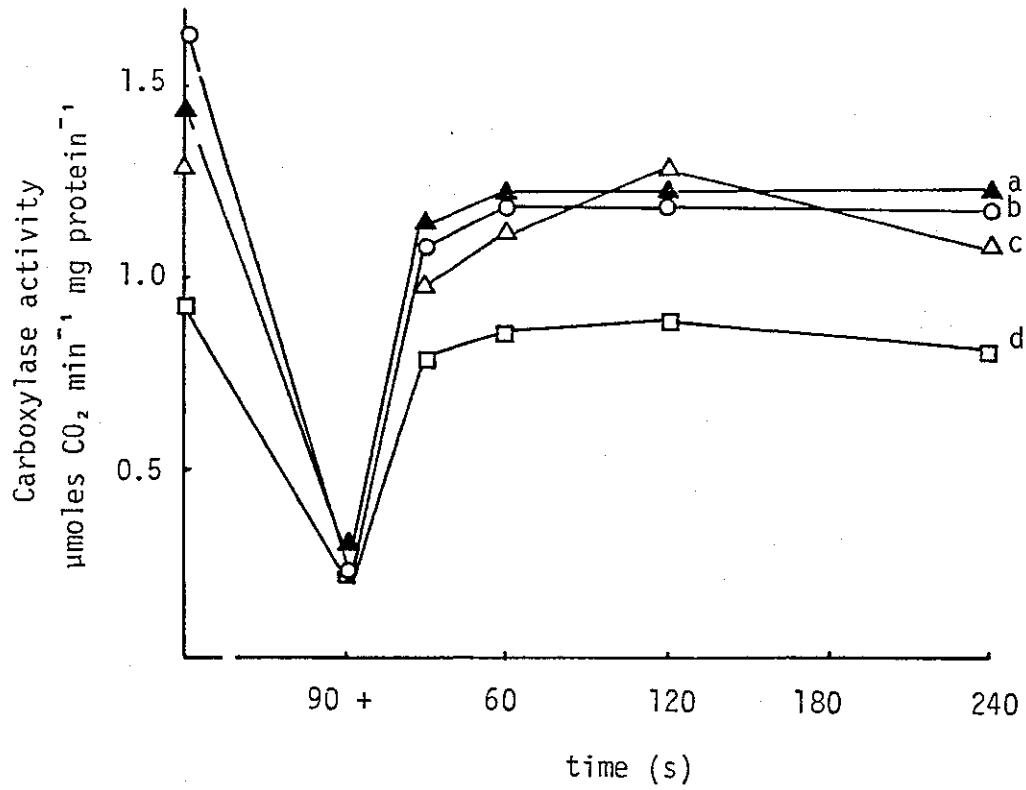


FIGURE 3:30 The reactivation of RuBP carboxylase originally activated in the presence of oxyanions. The rapid reactivation of the carboxylase at 25°C was obtained by deactivating the enzyme over 90 s in CO_2 free buffer. 5 mM HCO_3^- was then added for reactivation. All assays were conducted in 5 mM HCO_3^-. The enzymes were originally activated in 10 mM HCO_3^-, 20 mM $MgCl_2$ plus these additions:

a 10 mM SO_3^{2-}
b 10 mM PO_4^{3-}
c 10 mM SO_4^{2-}
d no additions

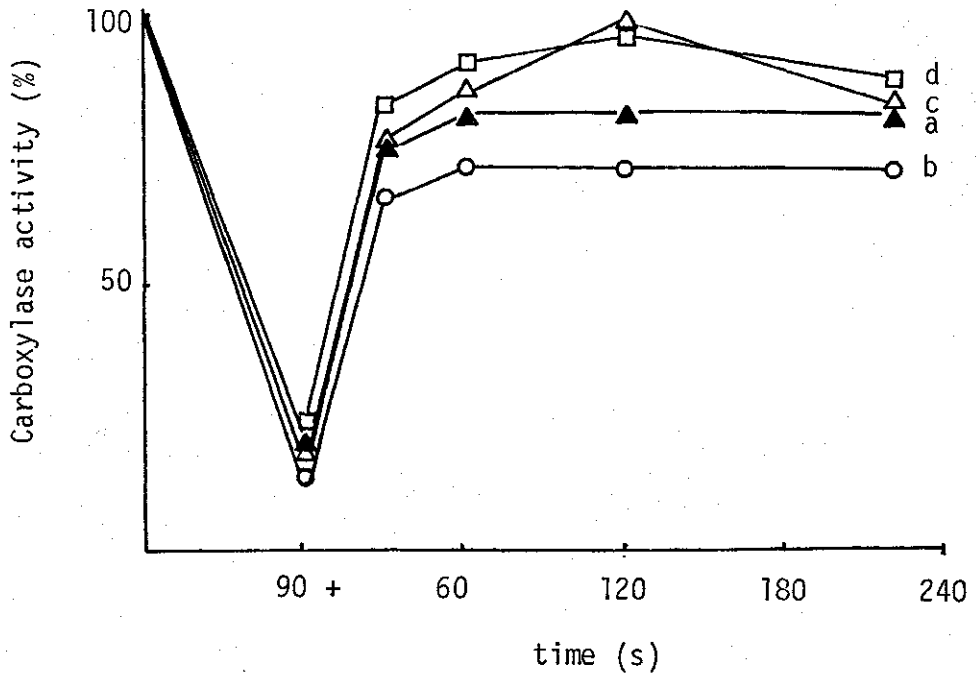


FIGURE 3:31 The % reactivation of RuBP carboxylase originally activated in the presence of oxyanions. 100% for each line is the enzyme activity after 40' at 40°C:

a 10 mM SO_3^{2-}

b 10 mM PO_4^{3-}

c 10 mM SO_4^{2-}

d normal activation

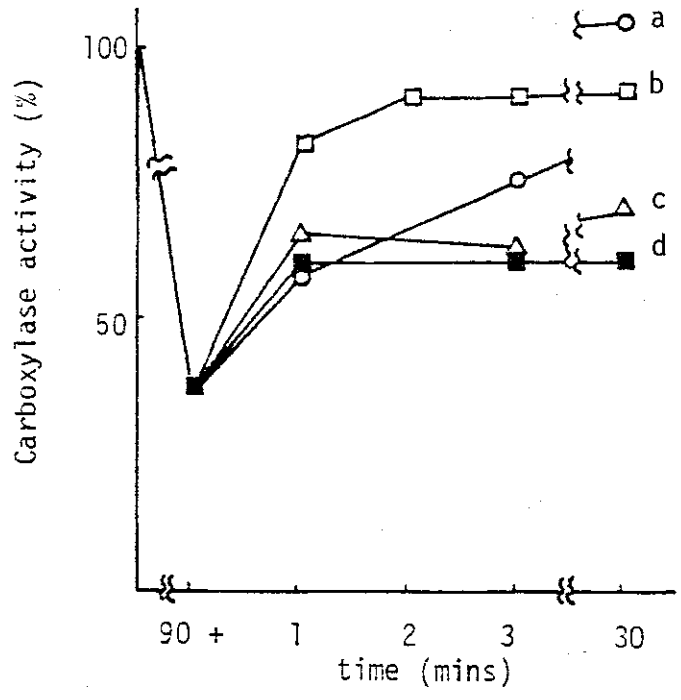


FIGURE 3:32 The effect of anions on the rapid reactivation of carboxylase. The rapid reactivation of the carboxylase at 25°C was obtained by first deactivating the enzyme over 90 s in CO_2 free buffer. HCO_3^- was then added for reactivation. All assays were conducted in 10 mM HCO_3^- . In a) 2 mM HCO_3^- and 10 mM PO_4^{3-} were used for reactivation and compared with normally activated enzyme assayed in the presence of 10 mM PO_4^{3-} as 100%, b) 10 mM HCO_3^- was used for reactivation, c) 2 mM HCO_3^- and 10 mM SO_3^{2-} or SO_4^{2-} were used for reactivation and compared with activated enzyme assayed in the presence of 10 mM concentration of oxyanion as 100% d) 2 mM HCO_3^- was used for reactivation and compared to activated enzyme as 100%.

reactivation was apparently slower but finally reached a much enhanced rate, similar to that found with the slow activating form of the enzyme. The small stimulation observed in response to SO_3^{2-} and SO_4^{2-} contrasts with the results for the slow activating form.

The differences in response to SO_3^{2-} and SO_4^{2-} of the slowly and rapidly activating forms were investigated further. Both anions enhanced the activity of the slow activating form in low HCO_3^- even at 25°C (Fig. 3:33).

The results demonstrate that the differences between the slow and rapid activating forms response to SO_3^{2-} and SO_4^{2-} which are not able to stabilize the active site of the enzyme form produced on deactivation. These oxyanions are able to stabilize the active site of the slow activating form.

Inhibition of carboxylase and oxygenase activities

SO_4^{2-} inhibition

2mM SO_4^{2-} and higher concentrations caused significant inhibition of RuBP carboxylase and oxygenase activity. The inhibition of carboxylase activity in 30 s assays was measured at various RuBP and SO_4^{2-} concentrations but at a constant concentration of 41 μM CO_2 (5 mM HCO_3^-). This CO_2 concentration was chosen because although slightly less than saturating, higher concentrations were found to produce substrate inhibition. Double reciprocal plots (Fig. 3.34I) show that the pattern of inhibition was close to competitive with respect to RuBP. Dixon and Cornish Bowden plots (Fig. 3:34II & III) confirmed the

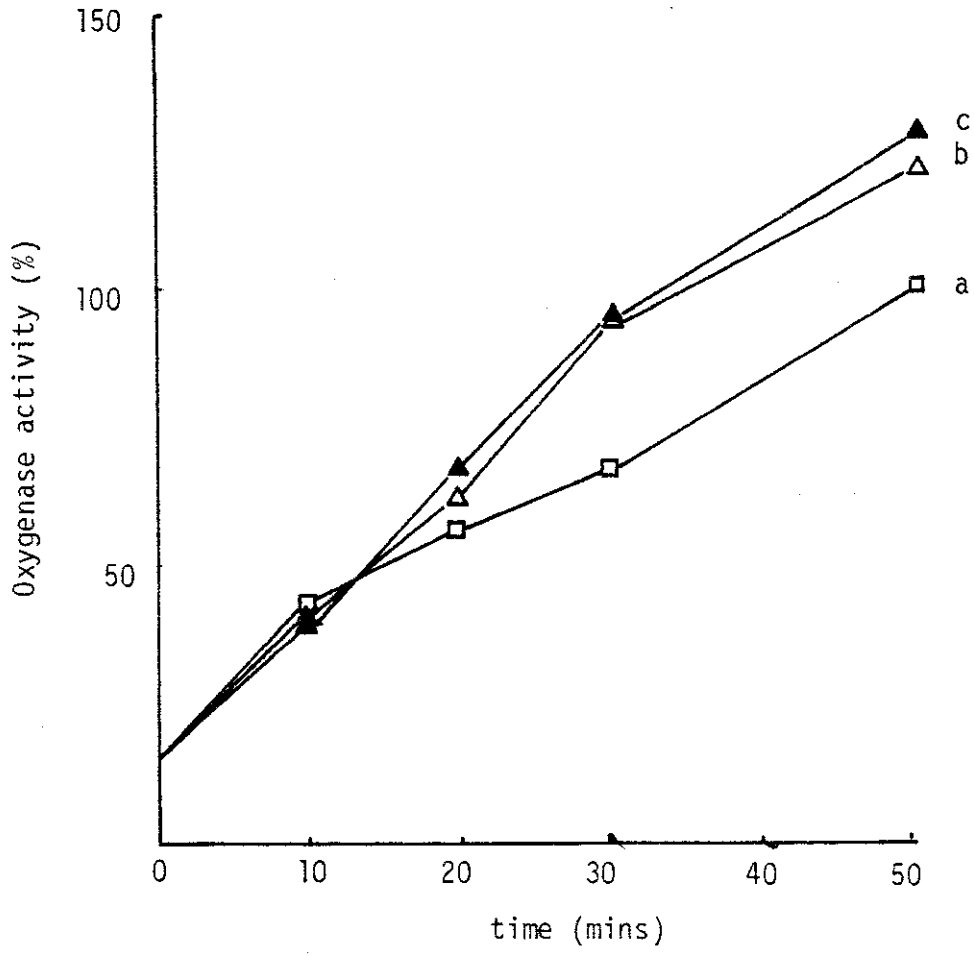


FIGURE 3:33 Activation of the slow activating form of RuBP carboxylase at 25°C. The activities are relative to the maximum obtained in (a) the enzyme was activated in 0.1 M HEPES buffer pH 8.2 with 20 mM $MgCl_2$ plus these additions

a 10 mM HCO_3^-
b 1 mM HCO_3^- + SO_3^{2-}
c 1 mM HCO_3^- + SO_4^{2-}

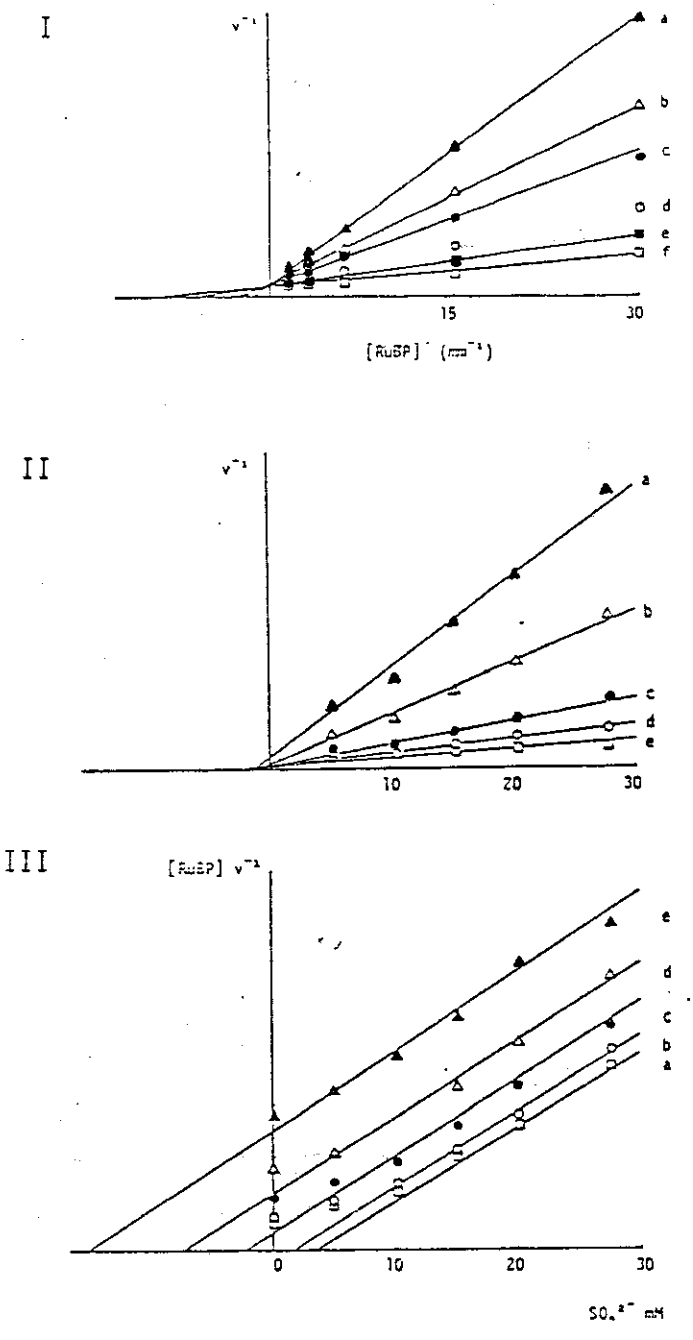


FIGURE 3:34 SO_4^{2-} inhibition of RuBP carboxylase with respect to RuBP illustrated in three different ways:
(I) Double reciprocal plots where SO_4^{2-} concentrations are a) 27.7 b) 20.3 c) 15 d) 9.2 e) 5.5 and f) 0 mM
(II) Dixon plots, and (III) Cornish Bowden plots, where RuBP concentrations are a) 0.033 b) 0.066 c) 0.165 d) 0.33 and e) 0.66 mM

inhibition pattern and were used to obtain inhibition constants (Table 4:3). When CO_2 and SO_4^{2-} concentrations were varied and RuBP maintained at maintained at 0.6mM double reciprocal plots (Fig. 3:35I) show the pattern of inhibition of the carboxylase activity, measured over 30 s produced a non-competitive pattern of inhibition by SO_4^{2-} with respect to CO_2. Dixon and Cornish Bowden plots were used to confirm the inhibition pattern and obtain kinetic constants (Fig. 3:35II & III, Table 4:3).

The inhibition of the oxygenase activity was measured at various RuBP and SO_4^{2-} concentrations whilst oxygen was maintained at air concentration. Double reciprocal plots showed that the oxygenase activity was inhibited almost competitively with respect to RuBP. The kinetic constants were obtained by secondary plots (Table 4:3).

In the Dixon plots the zero inhibitor rate for all substrate concentrations was lower than lines fitted through the inhibited rates. This suggested that the inhibited rates may also be stimulated by the SO_4^{2-}; when SO_4^{2-} activated enzyme was examined, this proved to be the case with zero inhibitor values on the Dixon plots falling close to lines fitted through the inhibited value.

SO_3^{2-} inhibition

Sulphur dioxide largely enters the chloroplast as an uncharged molecule and then dissociates to form HSO_3^- and SO_3^{2-} ions. Since the PKa for the equilibrium is 7.2 at a pH of 8.2, the chloroplast pH in the light, the ratio of SO_3^{2-} to HSO_3 is 9:1. The effects of SO_3^{2-} have therefore been extensively examined. Published work

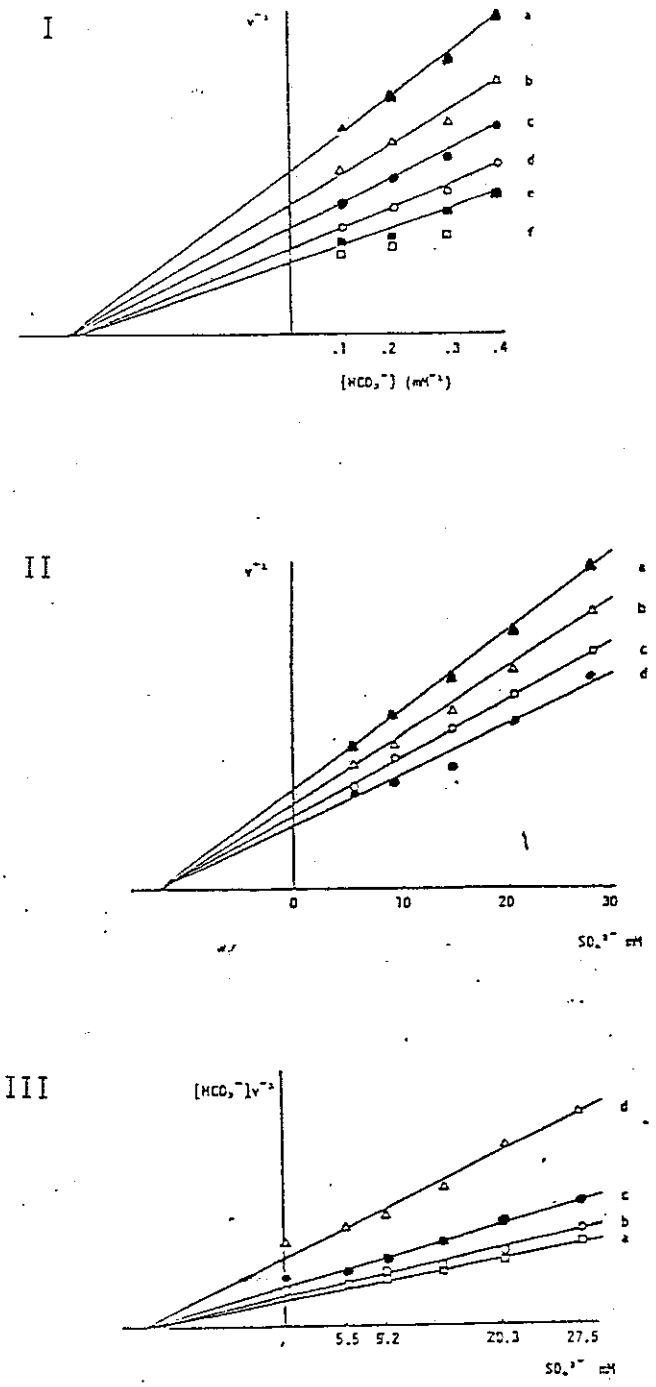


FIGURE 3:35 SO_4^{2-} inhibition of RuBP cabroxylase with respect to HCO_3^- illustrated in three different ways:
(I) Double reciprocal plots where SO_4^{2-} concentrations are a) 27.7 b) 20.3 c) 15 d) 9.2 e) 5.5 and f) 0 mM
(II) Dixon plots, and (III) Cornish Bowden plots where HCO_3^- concentrations are a) 2.6 b) 3.4 c) 5.2 and d) 10.2 mM HCO_3^-.

relating to the spinach and pine enzyme has produced conflicting results.

The inhibition of the carboxylase activity in 30 s assays was measured at various RuBP and SO_3^{2-} concentrations, but at a constant 41 μM CO_2 (5 mM HCO_3^-). A double reciprocal plot shows that the pattern of inhibition was mixed with respect to RuBP (Fig. 3:37). Kinetic constants were determined from secondary plots.

When CO_2 and SO_3^{2-} concentrations were varied and RuBP maintained at 0.6 mM a double reciprocal plot (Fig. 3:36) showed a mixed pattern of inhibition of SO_3^{2-} with respect to RuBP. These inhibition patterns conflict with those reported in earlier publications. The oxygenase reaction leads to the loss of the activating CO_2 and reduced enzyme activity which can be seen as a loss in linearity in enzyme turnover as the assay proceeds. In experiments examining the effect of SO_3^{2-} on the oxygenase activity, a progressive inactivation of the enzyme becomes apparent (Fig. 3:38). The time course for the inactivation was investigated for the carboxylase reaction by removing aliquots from the reaction mixtures at different times (see Method, p.60). The carboxylase reaction was followed to void the deactivation of the enzyme which occurs in the oxygenase activity. Figure 3:39 shows that SO_3^{2-}, even at 5 mM concentrations has little effect on the initial rate and may stimulate it, but this is replaced by a progressive inactivation of the enzyme to a much reduced rate. The time course for both carboxylase and oxygenase activities was biphasic, the initial stages were not linear and made the estimation of initial rates for the reaction difficult. The second phase, which developed from the first, was linear when the reaction proceeded over a period of several minutes but the rate was

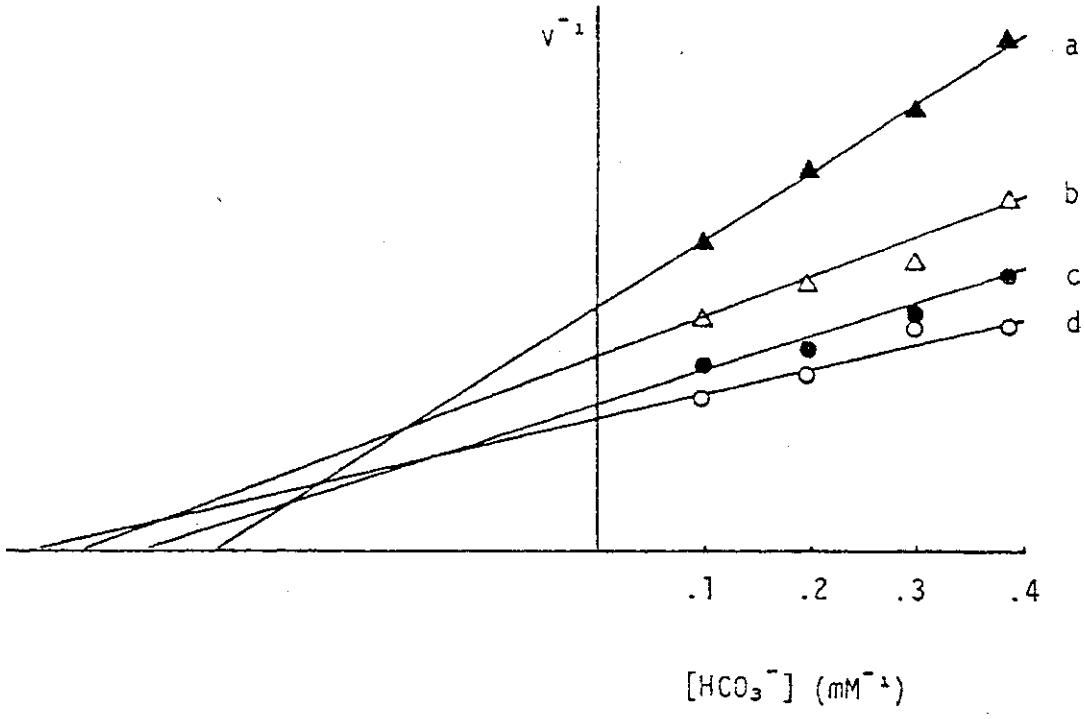


FIGURE 3:36 SO_3^{2-} inhibition of RuBP carboxylase with respect to HCO_3^-. A double reciprocal plot where SO_3^{2-} concentrations are a) 20 b) 10 c) 5 and d) 0 mM.

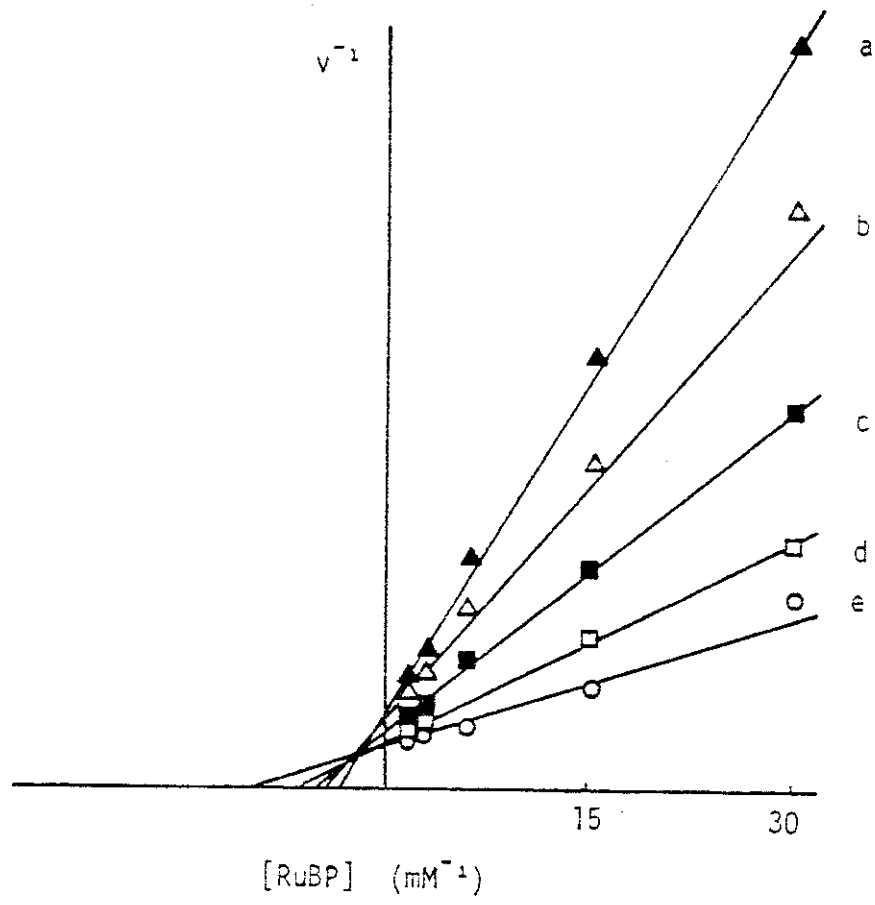


FIGURE 3:37 SO_3^{2-} inhibition of RuBP carboxylase with respect to RuBP. A double reciprocal plot where SO_3^{2-} concentrations are a) 20 b) 10 c) 5 d) 2.5 and e) 0 mM.

FIGURE 3:38 The effect of SO_3^{2-} on the oxygenase activity.
a) 0 and b) 10 mM SO_3^{2-}.

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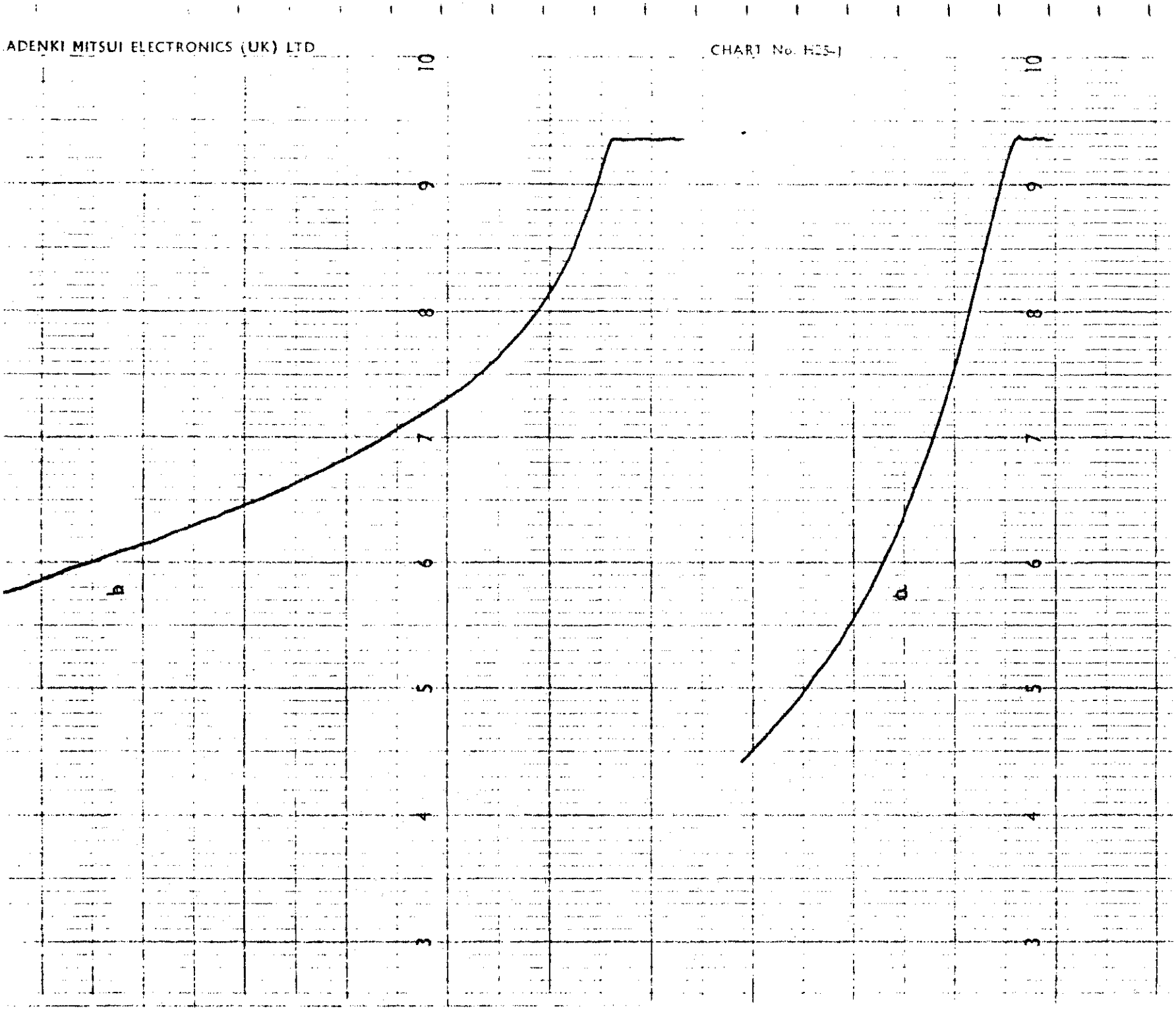
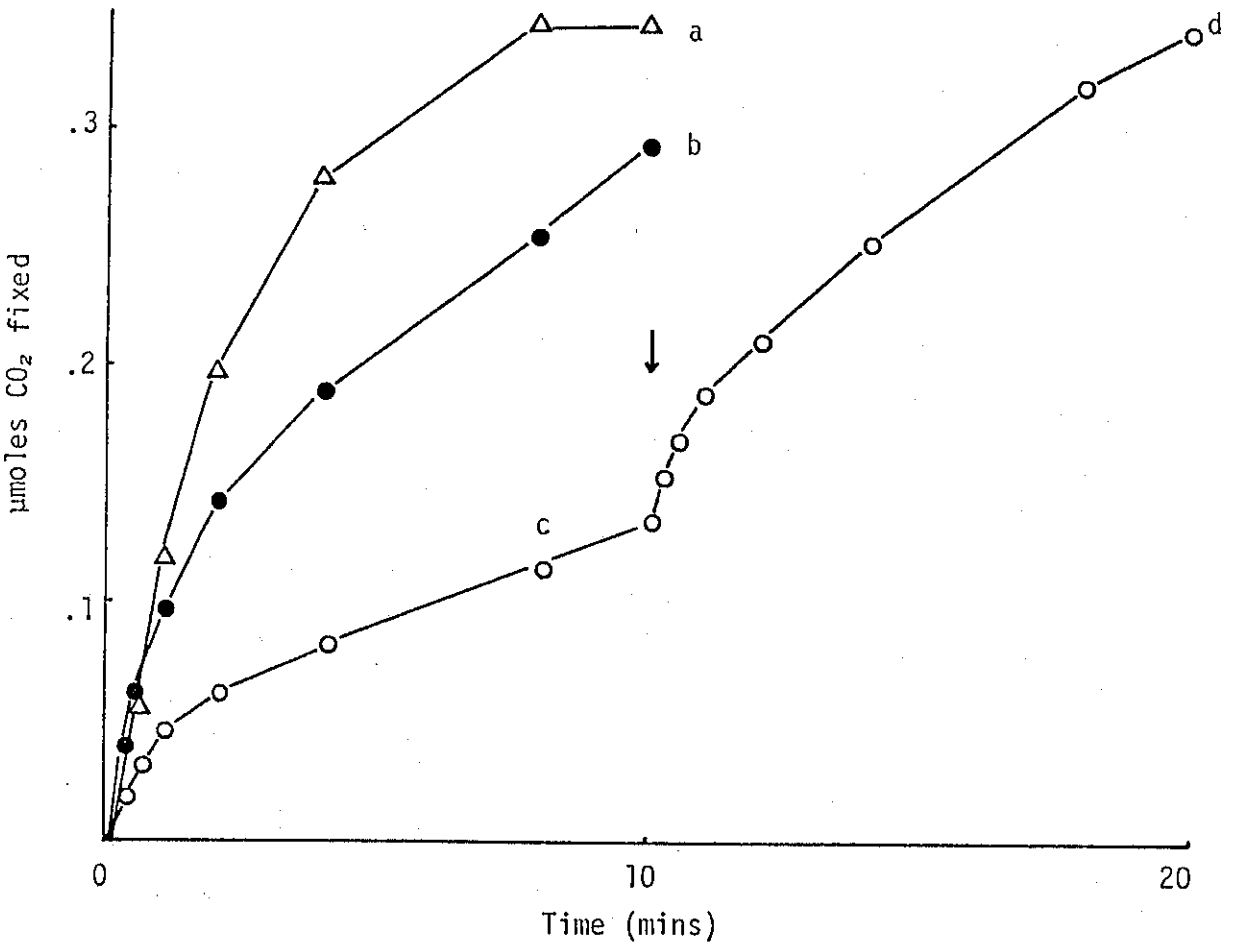


FIGURE 3:39 The biphasic inhibition of carboxylase activity by SO_3^{2-} is shown using 5 mM HCO_3 and 0.6 mM RuBP. The concentrations of SO_3^{2-} used were a) 0 b) 5 and c) 20 mM. The enzyme concentration was 0.1 mg/ml and in d) a second addition of the enzyme has been made (arrowed).


a
b
c
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.3
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0
10
20
Time (mins)

significantly lower than in the absence of SO_3^{2-}. Neither preincubation of the enzyme with SO_3^{2-} or RuBP with SO_3^{2-} reduced enzyme activity demonstrating that enzymes SO_3^{2-} and RuBP are all required for the inactivation to occur. The addition of RuBP to the assay during the second phase did not increase the enzyme activity. But the addition of more enzyme resulted in an identical two phase curve superimposed on the first (Fig. 3:39, line c), suggesting that no potent inhibitor is released during enzyme turnover. Moreover if the enzyme was allowed to consume all of the RuBP substrate a further addition of substrate resulted in a further two phase curve similar to the first which shows that the inhibition is reversible.

Research has demonstrated that RuBP carboxylase can become irreversibly inactivated during enzyme turnover (Paech et al., 1978) such that the addition of RuBP does not restore activity. In these studies enzyme and substrate concentrations were adjusted so that the addition of RuBP to the control at the end of the assay did significantly increase its activity.

The effect of inactivation on inhibition patterns and kinetic constants was examined by taking samples at intervals during an eight minute assay. The oxidation of SO_3^{2-} to SO_4^{2-} was followed in air saturated buffer in the oxygen electrode and found to be negligible during this period.

The inhibition patterns with respect to RuBP changed over the duration of the assay. The inhibition pattern was mixed at 15 s and non competitive after 4 min whilst the K_i increased from 2.5 mM SO_3^{2-}

at 15 s to 9 mm SO_3^{2-} at 4 min. The inhibition pattern versus HCO_3^- was mixed throughout the assay period but the K_i decreased from 8 mm at 15 s to 1.25 mM at 2 min. The progressive inactivation of the enzyme means that Michaelis Menton kinetics are not applicable to initial rates and may explain the discrepancy in published results. Since the second phases are linear these were used to identify inhibition patterns. The inhibition versus HCO_3^- was mixed (Fig. 3:40) and had a K_i of 1.5 mM SO_3^{2-}. Although the data points for inhibition versus RuBP were not linear they could best be described as uncompetitive (Fig. 3:41) with a K_i' of 17.5 mM SO_3^{2-}.

Since exposure to pollutants can result in a decrease in the pH of the stroma the effects of SO_3^{2-} were examined at pH 7.6 when the concentration of HSO_3^- was increased four fold. At this pH SO_3^{2-} was a more potent inhibitor of carboxylase activity (Fig. 3:42) and even after the first 15 s of the assay significant inhibition occurred rather than the stimulation in activity at pH 8.2. It was not possible to determine whether this was due to the distribution of SO_3^{2-} or HSO_3^- species or some change in the enzyme.

F^- inhibition

When F^- inhibition was examined, 0.5 mM and higher concentrations caused significant inhibition of both carboxylase and oxygenase reactions. The inhibition of carboxylase activity in 30 s assays was measured at various RuBP and F^- concentrations but at a constant 41 μM CO_2 (5 mM HCO_3^-) concentration. Double reciprocal plots (Fig. 3:43) show that the pattern of inhibition was atypical and the transformed data

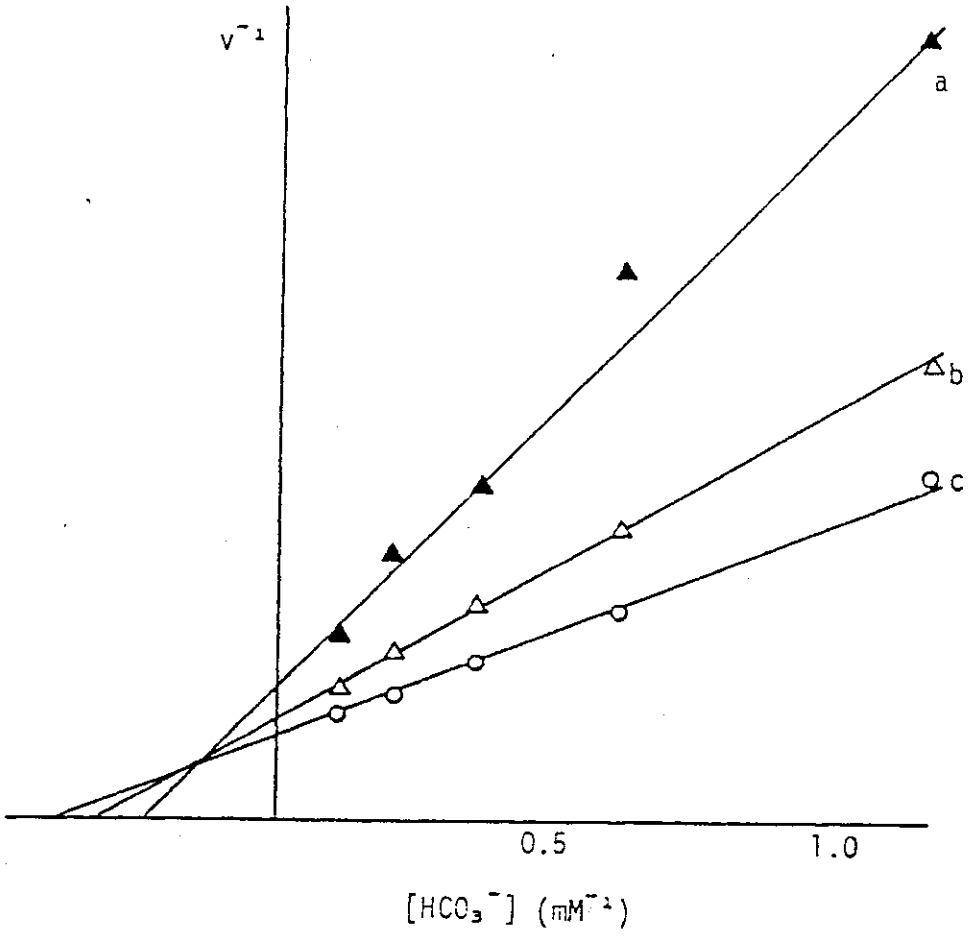

FIGURE 3:40 SO_3^{2-} inhibition of RuBP carboxylase second phase rates with respect to HCO_3^-. A double reciprocal plot where SO_3^{2-} concentrations are a) 10.0 b) 5.0 and c) 2.5 mM.

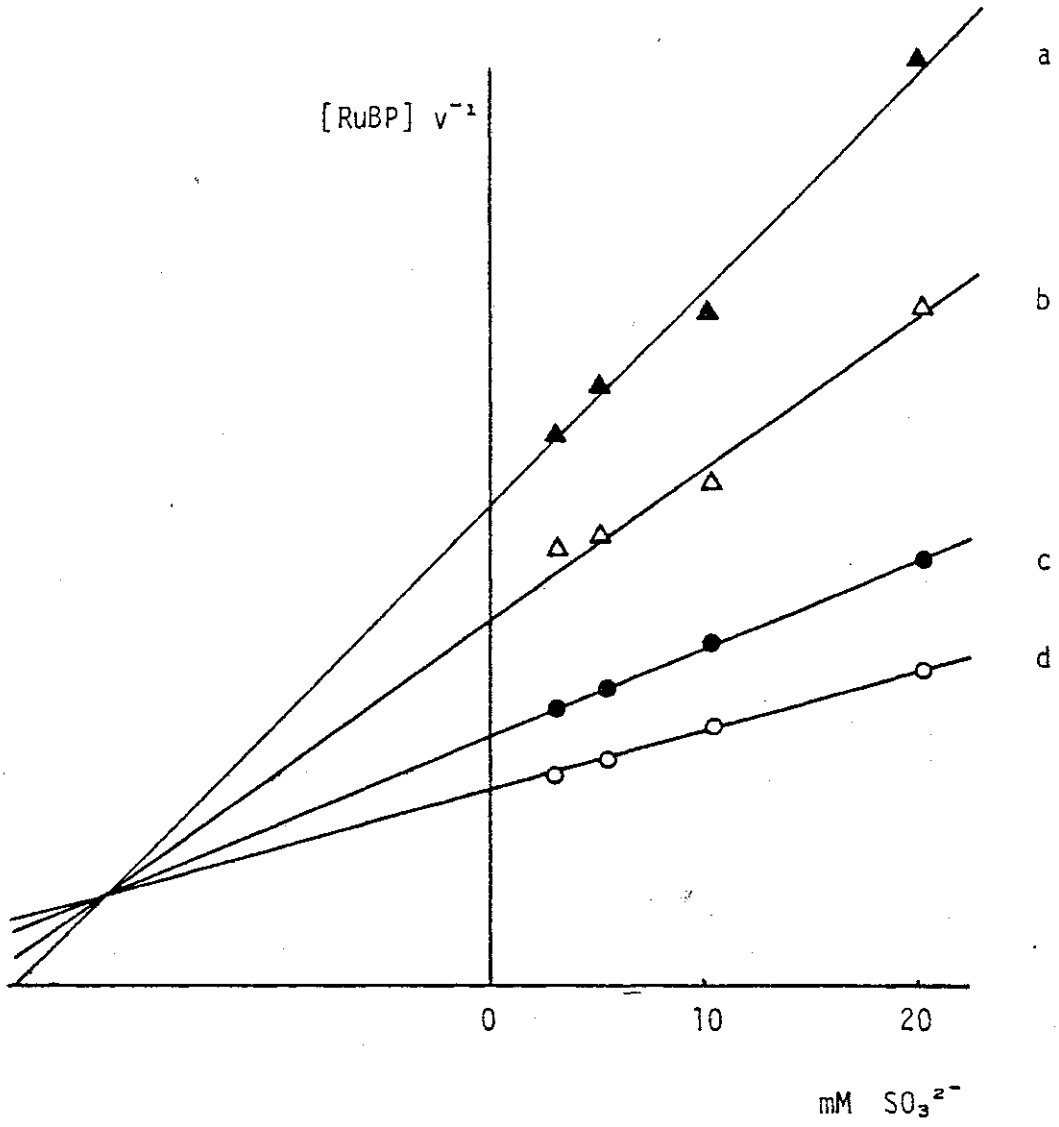

FIGURE 3:41 SO_3^{2-} inhibition of RuBP carboxylase second phase rates with respect to RuBP. A Cornish Bowden plot where RuBP concentrations are a) 0.33 b) 0.165 c) 0.066 and d) 0.033 mM.

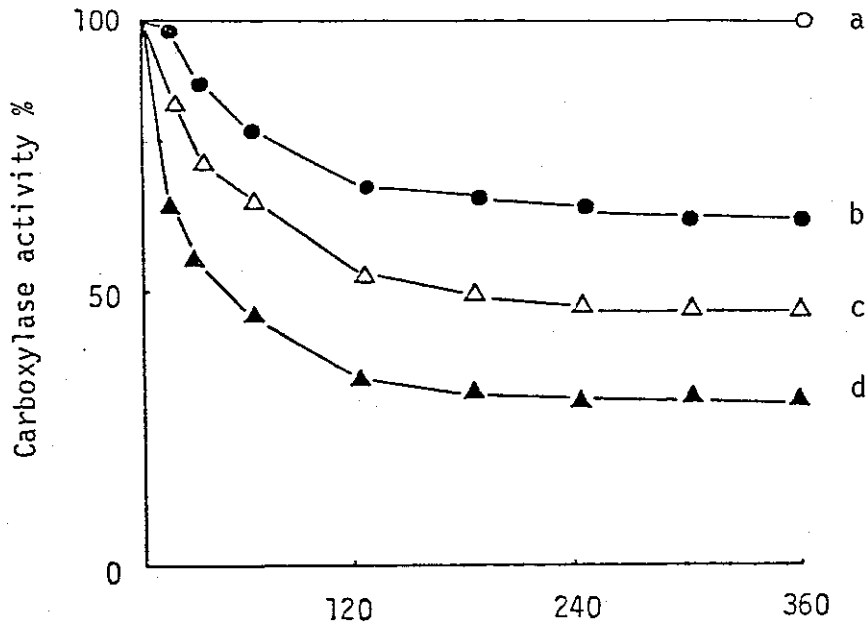

FIGURE 3:42 The inhibited rate relative to the uninhibited rate of the enzyme is plotted to show the degree of inhibition in initial rates. The SO_3^{2-} concentrations used were: a) 0, b)2.5, c) 5.0 and d) 10.0 mM.

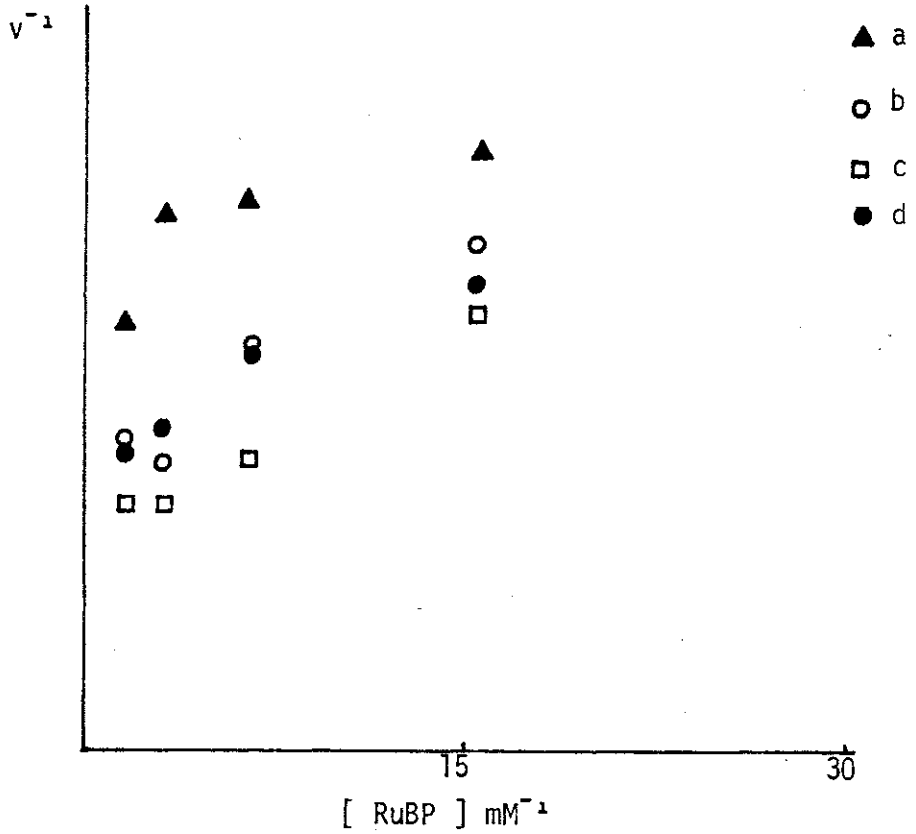

Fig. 3.43I: F^- inhibition of RuBP carboxylase with respect to RuBP. A double reciprocal plot where a) 2.5 b) 1.0 c) 0.5 and d) 0.0 mM F^-.

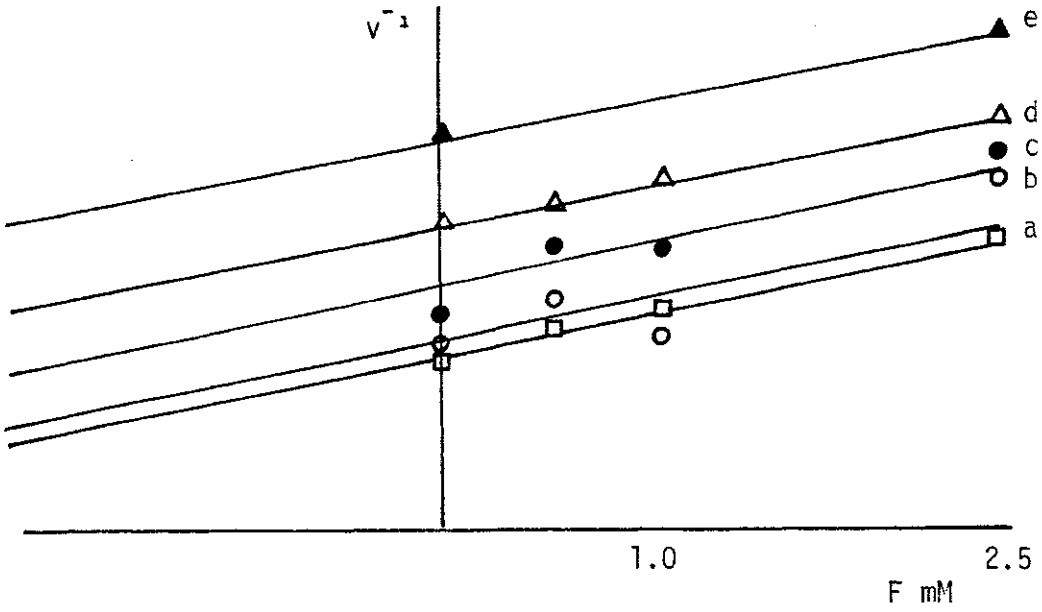

Fig. 3.43II: F^- inhibition of RuBP carboxylase with respect to RuBP. A Dixon plot where a) 0.66 b) 0.33 c) 0.165 d) 0.066 and e) 0.033 mM RuBP.

was not linear and suggests that the degree of inhibition was dependent on both RuBP and F^- concentrations. However, both this plot, and the Dixon plot suggest that the inhibition pattern may be uncompetitive. When CO_2 and F^- concentrations were varied and RuBP maintained at 0.6mM double reciprocal plots show the pattern of inhibition by F^- with respect to CO_2, with a K_i of 0.75 mM F^- (Fig. 3:44).

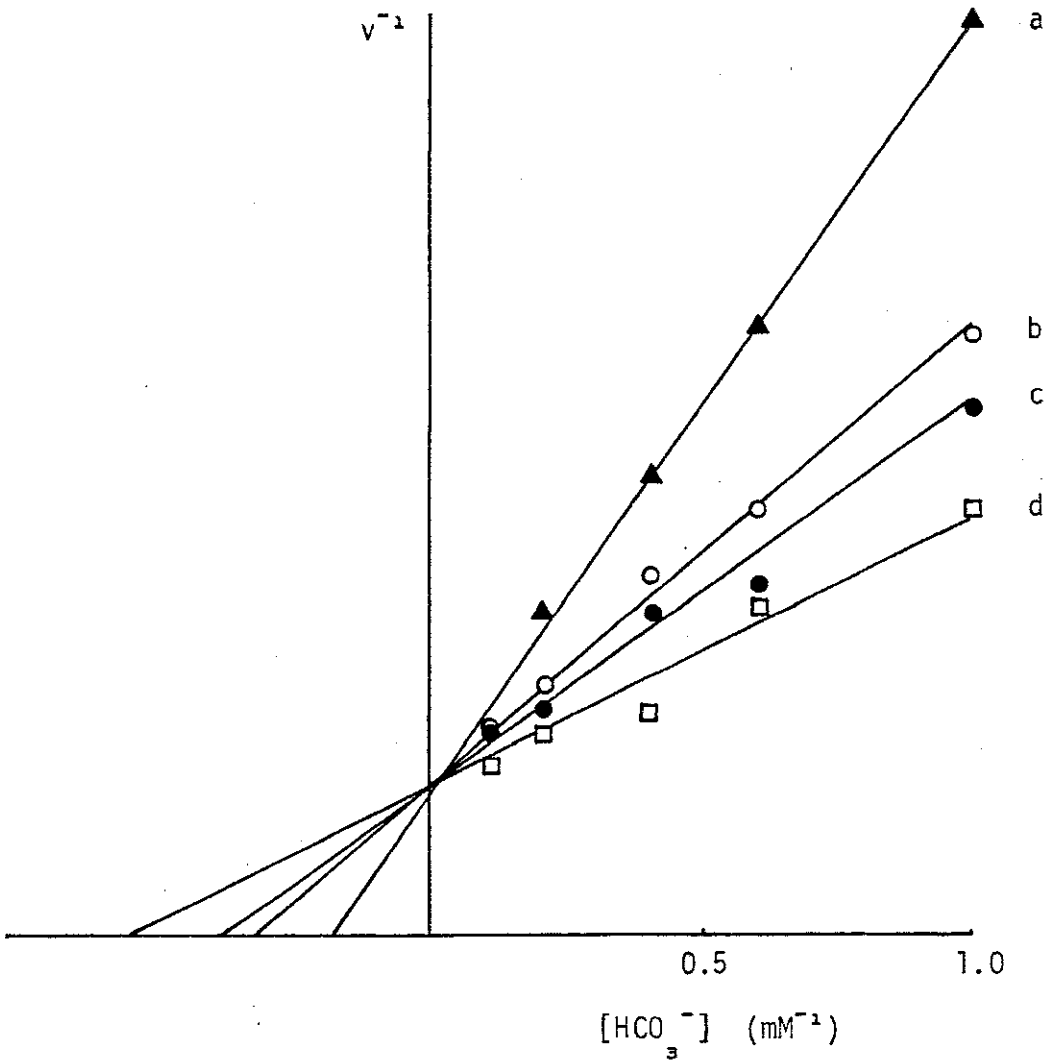

FIGURE 3:44 F^- inhibition of RuBP carboxylase with respect to HCO_3^-. A double reciprocal plot where a) 2.5 b) 1.0 c) 0.5 and d) 0.0 mM F.

DISCUSSION

A major object of this study was to determine the effect of aerial pollution, in the vicinity of the brickworks, on the development and yield of barley. The study raised two main questions:

(1) The nature and degree of pollution, and
(2) How the effects on crop growth were established and how large were they.

I shall deal with the second question first.

The effects of pollutants on crop growth were determined in a series of experiments in which the growth of crops in charcoal-filtered air and unfiltered air were compared. These were the first experiments of this type to be conducted in the UK on a cereal or using chambers whose microclimate was close to that of the field. In chambers both straw and grain yields in barley were consistently higher when the air supply was filtered than when it was not. However, enclosure was also shown to affect growth and yield. If the results of ambient versus filtered air experiments are to be used to assess the effects of pollutants in the field then ideally the plants in the unfiltered enclosures and unenclosed plots should have identical rates of development and yield. This did not happen even in the open-topped chamber despite the similarity between the major environmental variables inside these chambers and in the field. This highlights a problem inherent in the use of fumigation chambers, namely how representative of unsheltered field conditions are the effects observed in them. It is therefore important to determine the effects of

enclosure and separate them from the response of a crop to air filtration. Before discussing the effects of cleaning the air in more detail the effects of enclosure within a chamber will be considered.

Enclosure

The data describing the microclimate of the closed chambers, used in Experiments 1-3, is limited because of the lack of appropriate equipment for continual monitoring. The results reported are similar to those reported by other workers for closed chambers, who for example found temperature increases of 2-3°C at night and up to 10°C on sunny days (Farrar et al., 1977; Crittenden & Read, 1978). In addition these chambers share the problems common to other closed chambers including reduced photon flux density (PFD), exclusion of rainfall and pests.

Despite the partial closure of the collared and lipped open-topped chambers the microclimates of both types of open-topped chambers were similar. Only minor and inconsistent differences in temperature were ever recorded between the chambers. However, the temperature in these chambers was consistently hotter than those of ambient plots ($\simeq$ 1°C). Temperature differences were usually largest in the early morning but smallest in the late afternoon. Similar increases in temperature have been reported for other open-topped chambers (Heagle et al., 1973; Mandl et al., 1973; King & Smith, 1975; Olszyk et al., 1980 and Roberts et al., 1983).

The PFD in the open-topped chambers average 10-20% less than outside which is better than reports for some other designs of open-topped

chambers. Both the amount of framework and characteristics of the covering are critical: King and Smith (1975) reported that chambers made from glass fibre panels reduced PFD by 30% whilst Roberts et al. (1983) and Olszyk et al. (1980) found PFD reduced by up to 20% compared with ambient plots.

Enclosing plants within a chamber resulted in a faster development than on unenclosed plots. The effect was most pronounced in closed chambers in which maturity was up to three weeks earlier than outside, but even in open-topped chambers maturity was a week earlier than outside. This may be due to the increased temperature inside the chambers. Hutcheson and Quantz (1917) found that a 2°C 'increase' in temperature caused anthesis to occur 12 days earlier in barley. The warmer temperatures may also hasten ear initiation thus accounting for the production of one less leaf on the main stem in the chambers, as was found in Experiments 4 and 5 when developmental measurements were made. When leaf emergence stage was calculated on the basis of thermal time, °Cd, (Gallagher, 1979) effects of enclosure were still apparent (Fig. 4:1). This might reflect an inappropriate basis for thermal time or an effect other than that due to temperature.

Plants inside the chambers generally produced fewer tillers per plant. Although nutrient supply is known to influence tiller number in barley (Aspinall, 1961) this is unlikely to have varied in the experiments. However, Cannell (1969a & b) reported that both warmer temperatures (5-10°C) and reduced light intensity (≃ 10%) reduced tillering in barley by up to 60% in growth room experiments. Friend, et al. (1962) have shown that warmer temperatures increase assimilate

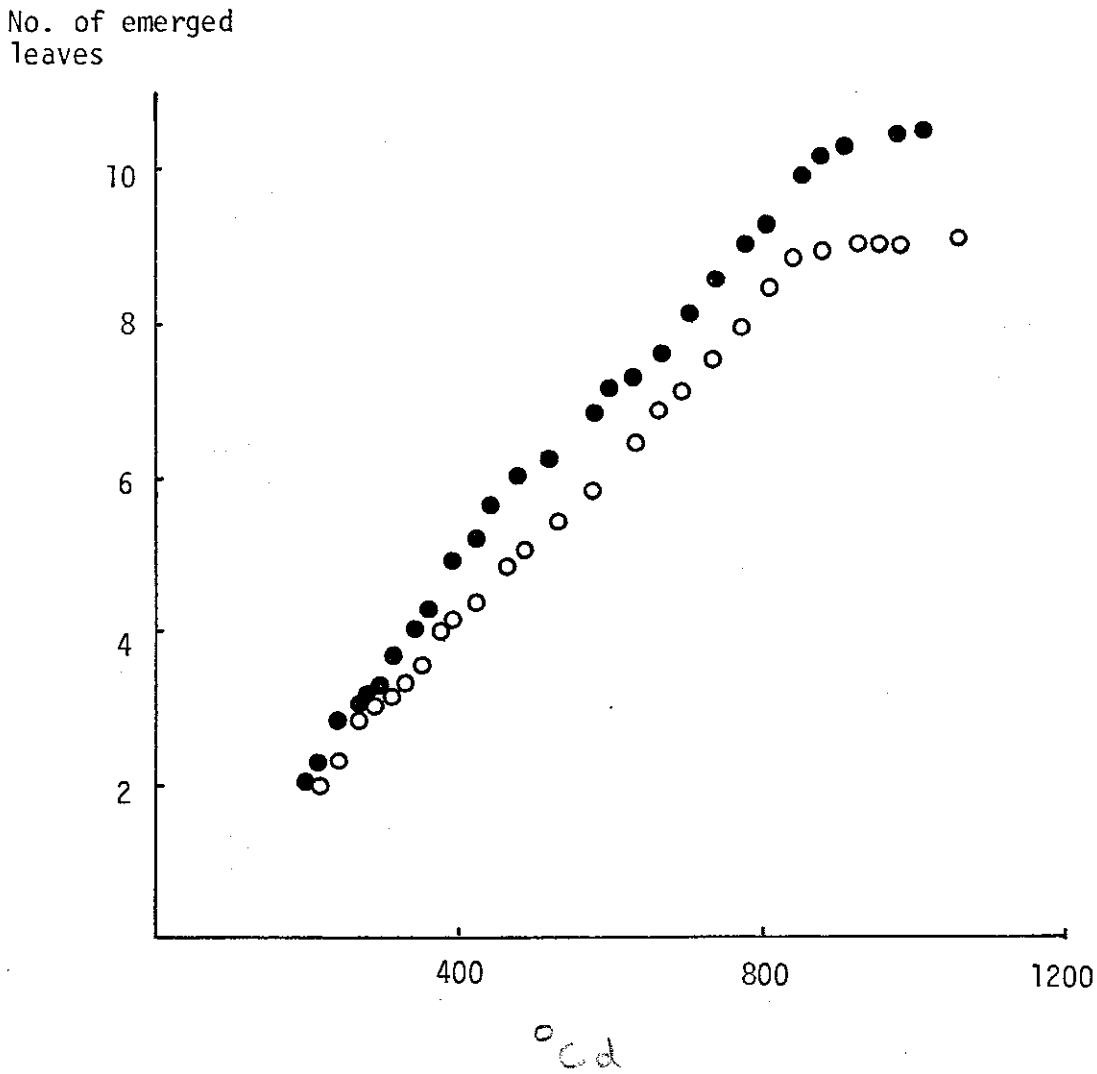

FIGURE 4:1 The number of leaves emerged, with thermal time, for barley main stems grown in chambers (O) and on unenclosed plots (●).

demand on the main axis by accelerating cell division and the formation of leaf primordia, thus reducing tillering growth.

Faster development and less dry matter growth in the chambers resulted in fewer ears and fewer grains per ear. However, dry weight per grain was generally larger in the chambers than outside. This could be the consequence of the smaller number of ears and grains per ear or because of a greater proportion of ears on main stems. Cannell (1969b) found that the main shoot ears of barley had the heaviest grains.

Even in the open-topped chambers where the environment was least altered, the changed environment did, over a growing season, significantly affect the growth and development of crops. Despite the likely magnitude of the effect of enclosure, often much greater than the effect of filtration, previous experimenters only rarely included comparisons with plants grown outside and even they have produced conflicting results. Heggestad et al (1980) found plants of several species grew about 10% taller and had more foliage in open-topped chambers than in field plots. Olszyk et al. (1980) found the height of alfalfa plants increased in open-topped chambers when compared to plants in the field, but dry weight was unaffected by enclosure. In contrast Howell et al. (1979) found plants enclosed in open-topped chambers had yields reduced by up to 30% when compared to plants grown outside. Roberts et al. (1983) found the yield of ryegrass grown in mini open-topped chambers was not reduced in the winter but was reduced during the summer when compared with unenclosed plots.

It has been suggested that enclosure in chambers may predispose plants to pollutants. Davies (1980) reported that in a growth tunnel experiment reducing PFD from 480 to 120 μmole quanta increased plant sensitivity to SO_2. She found that fumigation with 343 μgm^{-3} SO_2 decreased yield by 50% at the low PFD but had no effect at the high PFD. This work was confirmed by Jones and Mansfield (1982) who also found that sensitivity to SO_2 was increased as temperatures were reduced. They postulated that plants were more sensitive to SO_2 under conditions that are unfavourable to growth. In the experiments the PFD in the chambers was 10-20% lower than outside, which may have increased sensitivity to pollutants, but this was accompanied by a small increase in temperature which, according to Jones and Mansfield's results, should decrease sensitivity. Moreover, the differences in PFD between the chambers and unenclosed plots are much smaller than those used in the growth tunnel experiments (Davies, 1980; Jones & Mansfield, 1982) and are therefore unlikely to substantially increase sensitivity to pollutants, whereas the accelerated growth and development of plants in the chambers may indicate decreased sensitivity.

Olszyk et al. (1980) found the evaporative water loss reduced in open-topped chambers and suggested that associated changes in stomatal movement may alter pollutant uptake. The changes in water loss that they observed may reflect the low air flow rate in their chambers ~ 0.1 m s^{-1} and accumulative effect of changes in soil moisture as none of their plots were irrigated. In contrast Roberts et al (1983) found some indication of a greater uptake of SO_2 in unfiltered chambers than on unenclosed plots. Although sulphur content was increased by 10-20% in their experiments the differences were not significant. They suggested an

increased uptake of pollutants may result from the high air flow over the crop, 0.5 → 0.8 m s^{-1}, but this is unlikely as horizontal wind speeds of 5 m s^{-1} are common in the field (Lawlor, personal communication). In the experiments the plots were irrigated and the chambers had an air flow of 0.25 m s^{-1}. There was no evidence of increased uptake of pollutants in the chambers. Fluoride concentrations in the leaves of plants from both closed and open-topped chambers were not increased by enclosure. Whilst sulphur concentrations in plants were sometimes higher in the unfiltered chambers than outside this is unlikely to be due to differences in pollutant flux because of the greater proportion of sulphur taken up from the soil than from the air.

The effects of enclosure may limit the conclusions that can be drawn from chamber experiments with respect to the effects of pollutants. The retention of ambient field conditions and the reduction of pollutant concentrations are incompatible, but the conditions in the collared and lipped open-topped chambers offer a compromise between the two objectives. By including the comparison with unenclosed plots the effects of enclosure can be quantified and separated from the response of the crop to filtration.

Effects of filtration

Filtration increased grain yield in four out of five experiments but the increases were variable and not all were statistically significant. The increases occurred without visible damage or chlorosis of leaf laminae in any experiment. This is contrary to the observations of Katz (1949) and Thomas and Hill (1937) who concluded that yield reductions in

barley, wheat and alfalfa occurred only when SO_2 exposure produced more than 5% necrosis. Similarly Benedict et al (1964) found no significant effects of fluoride on the growth of several species in the absence of visible injury. These conclusions were based on short-term experiments with high concentrations of pollutants in chambers with low flow rates, and high temperatures and humidities. In contrast Stoklosa (1923) suggested that plant yields may be depressed by low SO_2 concentrations in the absence of visible injury (Bell & Clough, 1973; Lockyer et al., 1976; Ashenden, 1979; Bell et al., 1979; Ayazloo et al., 1980; Davies, 1980; Jones & Mansfield, 1982; Roberts et al., 1983). MacLean et al. (1977) demonstrated that long-term exposure to HF at concentrations below those causing visible injury reduced yields.

In my experiments the effects of filtration were greatest in 1976 and 1979 (Table 4:1) the years with the highest and lowest SO_2 concentrations respectively. In both years the highest episodes of pollution occurred before tillering whereas in other experiments, e.g. Experiment 5, in 1980, there were episodes of pollution close to anthesis but small effects on yield. This contrasts with the results of van Haut (1961) who found the greatest yield losses when plants were exposed to SO_2 during flowering. Similarly Pack (1971), Pack and Sulzbach (1976) demonstrated that HF fumigation inhibited fertilization. The varied response to filtration in seasons with similar pollutant concentrations but different timing in relation to crop development indicates the probable interaction of pollutants with environmental and developmental conditions (Davies, 1980; Jones & Mansfield, 1982). The relationship between the yield of the unenclosed plots and the magnitude of the

TABLE 4:1

Summary of Results from Field Experiments 1-5

Year			Open-topped Chambers + Charcoal	Open-topped Chambers - Charcoal	Unenclosed Plots
1976	Grain dry wt.*	gm^{-2}	376	196	286
	Straw dry wt.	gm^{-2}	382	153	352
	Fluoride	$\mu g\ g^{-1}$	17	50	46
	Sulphur	%	.27	.32	.35
1977	Grain dry wt.	gm^{-2}	254	300	382
	Straw dry wt.	gm^{-2}	455	348	515
	Fluoride	$\mu g\ g^{-1}$	95	91	79
	Sulphur	%	.50	.40	.30
1978	Grain dry wt.*	gm^{-2}	692	528	588
	Straw dry wt.	gm^{-2}	593	387	492
	Fluoride	$\mu g\ g^{-1}$	52	84	150
	Sulphur	%	.63	.53	.49
1979	Grain dry wt.	gm^{-2}	489	308	559
	Straw dry wt.	gm^{-2}	470	345	597
	Fluoride	$\mu g\ g^{-1}$	28	54	89
	Sulphur	%	.73	.70	.56
1980	Grain dry wt.	gm^{-2}	560	518	631
	Straw dry wt.	gm^{-2}	569	475	675
	Fluoride	$\mu g\ g^{-1}$	29	43	111
	Sulphur	%	.93	.69	.68

*Ripe ear dry weight

filtration effect (Fig. 4:2) suggests that the effects of filtration are largest in years when yields are already low. Moreover when yields on the unenclosed plot fall below ~ 600 gm^{-2} there is a sharp increase in the yield reductions due to pollutants. Above 600 gm^{-2} there appears to be sufficient photosynthate produced to mask the effects of pollution. These results suggest that the effects of pollutants will be largest in years when yields are already poor.

In 1976 and 1979 filtration increased the number of shoots and also increased both the weight of individual shoots and the 1,000 grain weights. Moreover in 1979 filtration increased the number of grains per ear but it is not known whether this was due to more grains being initiated or fewer grains aborting. It appears that if vegetative growth is adversely affected prior to anthesis leading to a reduced photosynthetic area and decreased straw dry weight then this may reduce grain yield at maturity. There will be less photosynthate produced for grain filling and less assimilate reserves for translocation to the grain.

Since filtration also increased yield by increasing ear number, but enclosure decreased tiller production this effect of filtration may only be relevant in a situation where tiller number is already small. This may be the case for example with crops sown late or at low density when tillering might be terminated before it could compensate for the low plant density.

Whilst the effects of enclosure may limit the assessment of effects of filtration on tiller or ear number filtration also increased the

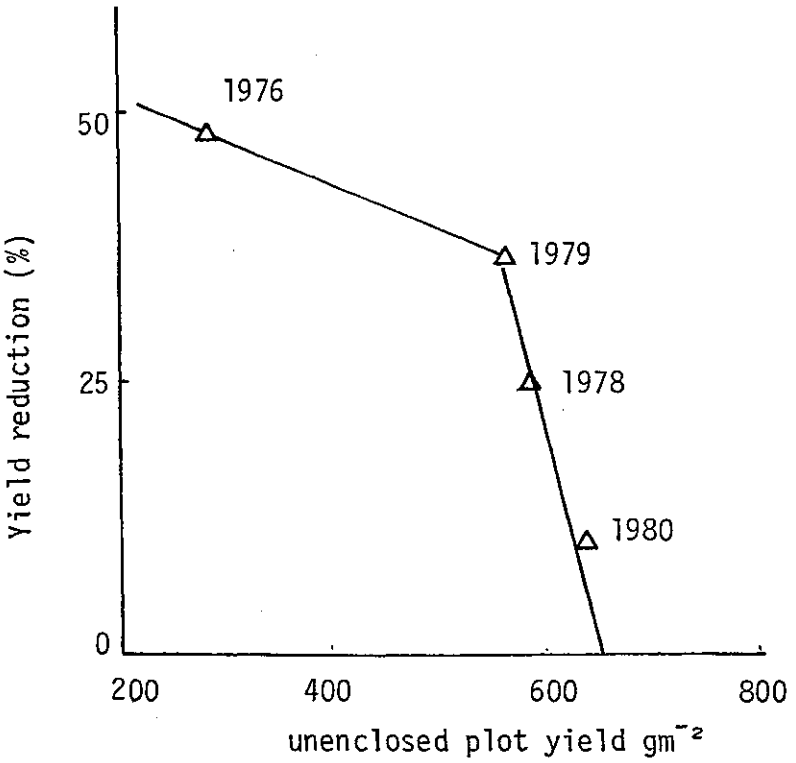

FIGURE 4:2 The percentage yield reductions in filtered compared to unfiltered open-topped chambers versus the yield of unenclosed plots in Experiments 1, 3, 4 and 5. The data for Experiment 2 is excluded because of the failure to reduce pollutant concentrations in the filtered air.

weight per shoot, number of grains per ear and 1,000 grain weights. These parameters can clearly be separated from those related to enclosure and be used to measure pollutant effects.

Pollutants

Having established that filtration increased yields it is necessary to determine the nature and degree of pollution producing such effects. The mean ambient SO_2 concentrations were between 48 and 61 μgm^{-3} and were consistent with mean SO_2 concentrations for Warren Spring's national survey sites in the area (Table 4:2).

TABLE 4:2

Mean SO_2 concentrations (μgm^{-3}) between April and August at monitoring sites in the Marston Valley.

	Woburn	Thrupp End	Husbourne Crawley[a]	Lidlington[a]	Aspley Heath[a]
1976	61	-	44	66	61
1977	54	-	55	66	49
1978	-	50	72	57	65
1979	-	48	74	71	56
1980	-	52	54	77	n

a) figures from WS
-) no monitoring at site
n) not enough data points

Although in the UK only 10% of the land area is exposed to a mean SO_2 concentration exceeding 50 μgm^{-3} over 30% of the UK is exposed to

between 30-50 μgm^{-3} (Fowler & Cape, 1982). The more polluted areas, > 30 μgm^{-3}, includes most arable farm land, hence concentrations of SO_2 similar to those recorded in my experiment may occur over ≃ 23% of arable land.

Unfortunately fluoride concentrations in the air were not monitored continuously except in the final experiment, at Thrupp End, when the mean concentration of F was 0.15 μgm^{-3}. However since the pattern of peak fluoride concentrations closely followed those of SO_2 in 1980 it is reasonable to assume that this was also the case in earlier experiments and mean F concentrations between 0.14 and 0.17 μgm^{-3} can be calculated for earlier experiments, although the ratio of SO_2:F may be different at the two sites. These concentrations are only 14% of those reported for an LBC monitoring site close to the brickworks, 1·05 μgm^{-3} (D.O.E., 1980) but are consistent with the concentrations predicted by computer models for this site (Cremer & Warner, 1979; D.O.E., 1980). Davies (1982) reported an ambient fluoride concentration in excess of 97 μgm^{-3} for a site within 0.5 km of Thrupp End Farm. Unfortunately no details of sampling period are given. She reports similar concentrations at two other sites in the area but her results are inconsistent with previous published data for ambient fluoride concentrations, the ratio of SO_2:F in stack gases and the accumulated fluoride concentrations in lichens from the same site and are therefore unlikely to be correct.

The fluoride concentrations that I measured at Thrupp End are only three times those reported in rural areas (Thompson et al., 1971; Davison, et al., 1973).

The dispersion of fluoride in the area of Ridgmont brickworks can be mapped by the fluoride concentrations in hawthorn leaves. The dispersal is wind-dependent and largely unaffected by local topography. Similar patterns for fluoride dispersion from Ridgmont and other brickworks have recently been published for both air concentrations (D.O.E., 1980; Cremer & Warner, 1979) and the concentrations in lichen thalli (Davies, 1982). In contrast sulphur concentrations in hawthorn leaves were not simply related to the dispersal of SO_2. Some researchers have found a relationship between SO_2 exposure and sulphur content but this has been when the sulphur supply is deficient (Faller et al., 1970; Cowling & Lockyer, 1976) or after exposure to high SO_2 concentrations (Khan & Malhotra, 1982). While F is only taken up in very small amounts from the soil except at extreme pH's, (Hansen et al., 1958) sulphur is an essential nutrient and is normally taken up from the soil (Loughman, 1964). Sulphur uptake is regulated in such a way that even in a 3000-fold range of external sulphate concentrations only small changes occurred in the internal concentration of sulphur (Datko et al., 1978). Whilst little is known about the regulation of sulphur assimilation in plants this may occur via some effect of cysteine on APS sulfatransferase or emission of H_2S from the leaves (Anderson, 1980). In my study it is probable that any differences that may be attributed to SO_2 exposure are masked by the system of sulphur regulation.

As expected gaseous SO_2 concentrations were higher in the unfiltered chambers than in the filtered but were slightly lower than those outside. The SO_2 concentrations in the filtered chambers were similar to those in remote parts of the UK (Fowler & Cape, 1982), i.e.

< 30 μgm^{-3}. Since charcoal filters reduce the concentrations of O_3 and HF more effectively than SO_2 (M. Roberts personal communication) greater reductions in the concentrations of these pollutants are expected; fluoride should be reduced to approximately 0.05 μgm^{-3} F. However, the limited measurements of NO_x concentrations made in the chambers suggest that reductions of these may be smaller than were found for SO_2. This is consistent with the removal of only 20% of the NO and 50% of the NO_2 by the charcoal filters reported by Roberts et al (1983).

In relation to other research

I am not aware of any other air filtration experiments conducted in areas with a similar combination of low concentrations of both SO_2 and F. Most ambient versus filtered air experiments have been used to determine the effects of photochemical oxidants (Heggestad, 1980) and relatively few in which SO_2 or F were believed to be the main pollutants. In Germany, Guderian and Schoenbuch (1970) used ambient versus filtered air experiments to demonstrate that fluorides are an important source of injury near to a chemical factory. Other experiments (Bleasdale, 1973; Crittenden & Read, 1978; Navara, 1978 and Roberts et al., 1983) have been conducted in urban areas where SO_2 is a major pollutant, but studies have been concerned exclusively with ryegrass where vegetative growth is important not grain production. Crittenden and Read (1978) found the yields of ryegrass cv. S23 were reduced by 30 and 40% when it was grown in chambers ventilated with air containing mean concentrations of SO_2 of 50 to 90 μgm^{-3} respectively, compared with a clean air control. Roberts et al. (1983) reported a 20% increase in

the yield of ryegrass when it was grown in chambers ventilated with filtered air even though the SO_2 concentration was only reduced from 92-61 μgm^{-3}. But in later experiments (Colvill et al., 1983) there was no significant difference between the yield of ryegrass grown in unfiltered or filtered air despite the greater reduction in SO_2 concentrations in these experiments, i.e. from 73 to 21 μgm^{-3} SO_2 and from 104 to 47 μgm^{-3} in the two experiments. Similar variable results have been found for fumigation experiments with low SO_2 concentrations, some showing decreases in yield with SO_2 other increases. Bell et al. (1979) reported a yield depression of 68% in ryegrass exposed to a mean concentration of 43 μgm^{-3} SO_2 for 173 days in winter. Setterstrom et al. (1938) and Cowling and Lockyer (1976 & 1978) have reported no adverse effects and also sometimes increased yield with SO_2 concentrations of less than 100 μgm^{-3}.

In contrast to the detailed information concerning SO_2 fumigation little is known about the effects of long-term exposure to low, < 1.0 μgm^{-3}, F concentrations. Most reports concern short-term exposures to high concentrations of HF (Davison, 1982). Whilst Thompson and Taylor (1969) found no effect of 0-1.2 μgm^{-3} F on the growth of citrus, MacLean et al. (1977) found a reduction of almost 25% in the yield of pods in bean Phaseolus vulgaris L. cv. Tendergreen, exposed to 0.6 μgm^{-3} F for 43 days. But in another experiment the same authors found no effect of 0.6 μgm^{-3} F on the growth or fruiting of tomato Lycopersicon esculetum Mill cv. Fireball.* I know of no experiments in which the effects on barley of prolonged exposure to HF have been investigated.

* see errata 1

Fumigation experiments tend to use constant concentrations of pollutants whereas in the field the average concentrations conceal fluctuations with some large peaks. In my experiments peaks of over 300 μgm^{-3} SO_2 were frequently recorded (> 1 μgm^{-3} F implied) for periods of several hours. Moreover in the field it is rare for plants to be exposed to a single pollutant, and mixtures of pollutants may produce a more than additive effect on plant growth. Field observations made in areas where SO_2 and F concentrations were sufficiently high to injure plants indicated neither antagonistic nor synergistic effects (Weinstein, 1977). However, fumigation of barley and corn with the two pollutants together reduced F uptake compared with uptake from F fumigation alone (Mandl et al., 1975; McCune, 1980). In my experiments it was not possible to identify which pollutant or combination of pollutants was causing the yield reduction. But my experiments do suggest that the brickworks emissions, as the primary source of F, are involved as indicated by the negative correlation between the F concentration in the leaf and the straw dry weights of plants grown in the chambers (Fig. 4:3). However this correlation only accounts for about 25% of the variation.

These experiments were the first air filtration experiments to be conducted in the UK with field-grown crops. If the results are typical for areas exposed to over 50 μgm^{-3} SO_2 the pollution may be reducing barley yields by up to 48% for over 23% of the arable land in the UK, equivalent to an annual loss of £127M.* Moreover the effects appear greatest when yields are already low. It is clearly important to continue

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*Calculation using production figures for 1980 in FAO yearbook assuming a barley price of £117/tonne. 23% of barley crop produced in areas with over 50 μgm^{-3}, maximum yield reduction 48%.

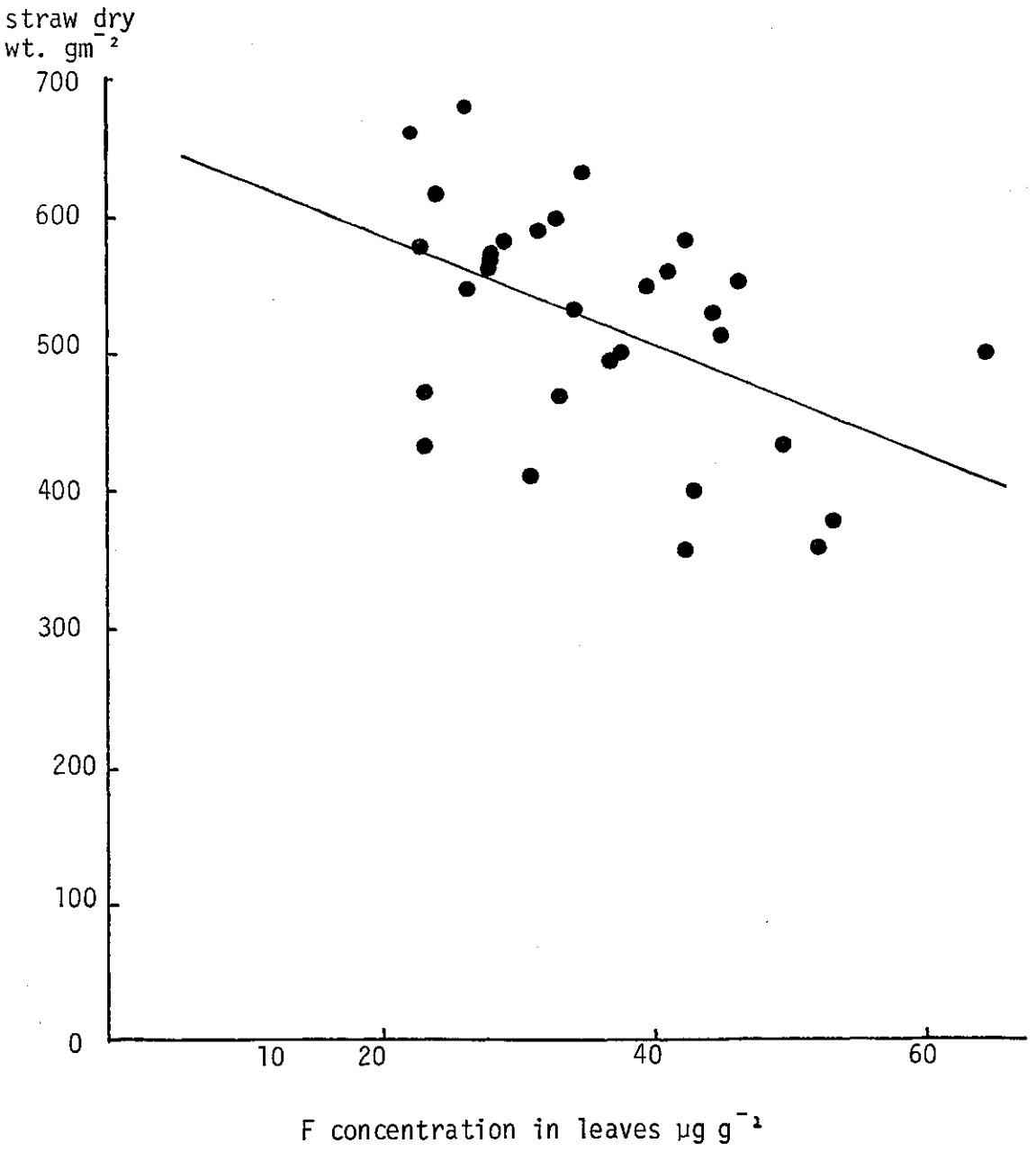

FIGURE 4:3 Correlation between straw dry weight and fluoride concentration in the leaves at maturity:
r = -0.486 p = 0.005

such research in other areas and on other crops, the true cost of pollution damage can then be assessed in relation to pollution control measures.

Enzymology

Despite detailed research no single mechanism of action can explain the effects of both SO_2 or F on plant growth. Exposure to low concentrations of SO_2 results in the reversible inhibition of photosynthesis possibly because of an effect on the enzymes involved in CO_2 fixation. There have been only a few studies of the effects of pollutants on these enzymes *in vitro*. Moreover most studies have concentrated on the important enzyme concerned with primary reaction of CO_2 fixation, RuBP carboxylase and the competitive inhibition of this enzyme by SO_3^{2-} with respect to HCO_3^- has been proposed as a key mechanism by which SO_2 interferes with photosynthesis. I know of no research examining the effect of F on this enzyme. In my research the effect of SO_2 and F was examined, on the activation and both catalytic activities of RuBP carboxylase.

Whilst F^- had no effect on the activation of the enzyme the response to SO_3^{2-} and SO_4^{2-} is complex. With the slow activating form of the enzyme (E_S) both anions enhance activation, particularly at low (1 mM) HCO_3^- concentrations such that the enzyme attains activity similar to that attained with saturating concentrations of HCO_3^- alone. Incubation of the enzyme with NADPH and 6-phosphogluconate has been shown to produce similar effects (Gutteridge *et al.*, 1982; Badger & Lorimer, 1981; McCurry *et al.*, 1981) as a result of binding

to and stabilizing the activated ternary complex E_S - CO_2 - Mg. The stabilization of this complex by SO_3^{2-} and SO_4^{2-} was confirmed by the ability of these oxyanions to reduce the rate of deactivation, presumably by inhibiting the dissociation of the activating CO_2 and Mg^{2+}.

However, when the activation process was in the presence of saturating CO_2 concentration but low concentrations of Mg^{2+} (1 or 2 mM) there was only a small enhancement of activity. This suggests that either the carboxylation cannot proceed at low Mg^{2+} concentrations or that the anions can only stabilize the enzyme-CO_2 complex.

In contrast to the results with the slow activation species the rapidly activating species of the enzyme (E_R) produced only a small increase in activity after incubation with SO_3^{2-} and SO_4^{2-} irrespective HCO_3^- concentration used for activation. SO_3^{2-} and SO_4^{2-} appear to be unable to effect the equilibrium between the rapidly activating form of the enzyme, E_R - CO_2 - Mg. In contrast the mode of action of PO_4^{-3} another oxyanion is more straightforward. With both the slow and rapidly activating species the anion produced a marked enhancement of the activity of the enzyme when present during activation both in sub-optimal and saturating concentrations of activating CO_2. The reason for the different response of the two forms of the enzyme to SO_3^{2-} and SO_4^{2-} compared with PO_4^{3-} is unclear. Oxyanions may operate in two ways by stabilizing the activated ternary complex and by an enhancement effect on catalytic activity. The stimulation would explain the increase in specific activity of the enzyme above that obtained in saturating concentrations of HCO_3^- and Mg^{2+}. Whether this stimulation,

presumably due to the binding of the oxyanion to the enzyme, results from the formation of new active sites in normally inactive enzyme or an increase in the reaction rate of existing sites has yet to be established. But the effect is relatively specific since other anions, NO_3^-, NO_2^-, F^- and Cl^- did not produce the response.

Research with mutants has demonstrated the importance of inhibitors of the activation of this enzyme but the activation is not thought to be involved in photosynthetic induction (Leegood & Walker, 1981; Robinson & Walker, 1980; Sicher, 1982). Thus the effects of SO_3^{2-} and SO_4^{2-} on activation *in vivo* are unlikely to be significant particularly because of the presence of other potent effectors such as NADPH, PO_4^{2-} fructose bisphosphate and 6-phosphogluconate. For example PO_4^{3-} can be present at up to 6 mM concentration in the stroma (Portis *et al.*, 1977; Heldt *et al.*, 1978). This may explain why Gezelius and Hallgren (1980) were unable to detect any effect of SO_3^{2-} on the activation of partially purified plant extracts. However, my research emphasizes the importance of examining the effects of pollutants on activation prior to inhibition studies.

Inhibition by SO_4^{2-} was non competitive versus HCO_3^- and competitive versus RuBP. Although Trown (1965) reported non competitive inhibition versus RuBP he used very high (100 mM) concentrations of ammonium sulphate and his results may have been influenced by the high concntration of ammonium ions. My results are consistent with more recent research (Table 3). Paulsen and Lane (1966) found competitive inhibition with respect to RuBP using up to 20 mM ammonium sulphate and Khan and

Malhotra (1982) reported non competitive inhibition by SO_4^{2-} versus HCO_3^-. Similar results have been reported for PO_4^{3-} inhibition (Laing & Christeller, 1980; Bhagwat, 1981).

TABLE 4:3

Nature of inhibition due to SO_4^{2-} reported by different authors.

Author	Inhibitor	Type of Inhibition	HCO_3^- varied K_i (mM)	K_i'	RuBP varied K_i (mM)	K_i'
Parry	$NaSO_4$	Non competitive	13	13	———	
		Competitive	———		1.5	
		Competitive/mixed*	———		1.5	
Trown	$(NH_4)_2SO_4$	Non competitive	———		n	
Paulsen & Lane	$(NH_4)_2SO_4$	Competitive	———		8.1	
Khan & Malhotra	n	Non competitive	17.5	17.5	———	

*Oxygenase activity n Information not given

The inhibition of catalytic activity by SO_3^{2-} is complex. Previous reports of the effect of SO_3^{2-} on carboxylase activity have produced conflicting conclusions concerning both the potency and patterns of inhibition (Table 4:4). A number of factors may have contributed to this. The results of Gezelius and Hällgren (1980) may have been influenced by the oxidation of SO_3^{2-} to SO_4^{2-} by the partly purified extracts during activation and assay since plant extracts may contain very active SO_3^{2-} oxidizing systems. This would explain the non competitive pattern of inhibition and the high K_i that they reported.

The results of Ziegler (1972) and Khan and Malhotra (1982) are in better agreement, but could take no account of the biphasic curves found in my experiments. My results demonstrate the importance of following the progress of the reaction in the presence of SO_3^{2-} rather than attempting to deduce initial rates after a set reaction time. SO_3^{2-} does interfere with substrate binding to the enzyme but over and above this effect there appears to be an effect on the chemistry of the catalytic reactions that results in the biphasic progress curves.

TABLE 4:4

Nature of inhibition due to SO_3^{2-} reported by different authors

Author	Plant Species	Inhibitor	Type of Inhibition	HCO_3^- varied K_i (mM)	K_i'	RuBP varied K_i (mM)	K_i'
Parry	Wheat	Na SO_3	Uncompetitive			-	17.5
			Mixed	1.5	12.5		
Ziegler	Spinach						
Gezelius & Hällgren	Spinach	n	Non competitive	9	9	———	
	Pine			11	11	———	
	Pine	n	Non competitive	———			
	Pine		Non competitive/ mixed	13	13	———	
	Spinach			7	7	———	
Khan & Malhotra	Pine	n	Competitive	2.2	2.2	———	

n = information not given

The SO_3^{2-} may react with an enzyme generated intermediate of the RuBP substrate e.g. an enedial or 3 keto derivative (Siegel & Lane, 1973; Schloss & Lorimer, 1982; Saver & Knowles, 1982) or with a group at the active site which is essential for catalysis. Control experiments demonstrated that neither enzyme nor substrate react with SO_3^{2-} prior to substrate turnover and that no potent inhibitor is released during enzyme turnover.

SO_3^{2-} ions are known to react with prosthetic groups and amino acid residues in proteins, e.g. flavin groups, disulphides or cysteine residues (Massey et al., 1969). RuBP carboxylase does not contain flavin groups and cysteine residues usually require oxidizing conditions to react (Cecil, 1963) and are therefore unlikely explanations for the biphasic reactions. In contrast disulphide groups are very susceptible reacting readily and reversibly to give a S-sulphonate derivative of one of the cysteines (Cecil, 1963). If SO_3^{2-} was reacting with an amino acid essential for enzyme activity cyanide ions may be expected to produce similar biphasic reaction curves. Although such biphasic curves have been reported (Lorimer et al., 1973) cyanide, unlike SO_3^{2-}, also reacts with RuBP. Consequently any effect of cyanide on essential enzyme groups will be masked by the reaction with substrate.

From my results it is not possible to conclude whether SO_3^{2-} is reacting with a substrate intermediate or an essential enzyme group. But the biphasic reaction curves during both carboxylase and oxygenase reactions provide strong evidence that the two reactions do share a common site or substrate intermediate.

The expression of the inhibition of RuBP carboxylase by low concentrations of SO_3^{2-} and SO_4^{2-} will depend on many factors. Of great importance is the concentration that these anions attain in the chloroplast. This is dependent on both the rate and amount of SO_2 taken up and also the relative rates of detoxification in the chloroplast. Clearly the concentrations of these pollutants in the cell and cellular compartments must be determined if their effects on plant metabolism are to be interpreted. Saunders (1966) reported a relationship of ~ 1000:1 for atmospheric to aqueous concentrations but Nieboer et al. (1976) demonstrate that, at least at high SO_2 concentrations, the relationship was not linear. However, Khan and Malhotra (1982) demonstrated that the total S content in a leaf can double as a result of exposure for 48 h to 0.34 ppm. From the figures they reported, assuming the increase in S was only in the form of SO_3^{2-} and SO_4^{2-} and a 50% water content, an upper limit of ~ 15 mM can be estimated. Thus the concentrations of anions used in this study, 0-20 mM, should approximate to those in plants in the field. The inhibitor constants, K_i = 1.5 for inhibition by SO_4^{2-} and SO_3^{2-} are lower than the previously reported values which suggests that the potential effects of SO_2 on this enzyme may have been underestimated. The mixed inhibition versus SO_3^{2-} suggests that with an increased concentration of CO_2 SO_2 inhibition may be overcome. Black (1982) and Carlson (1983) found that increased CO_2 concentrations did protect plants from SO_2 and that the protection was independent of stomatal movement . If this is the case, it follows that plants with the C_4 type of fixation and an increased CO_2 concentration in the bundle sheath cells should be less affected by SO_2 (see also Introduction p.28). It is therefore important to isolate enzymes from such species.

Recent research (Cerovic, Kalezic-Plesincor, 1982) has suggested that the inhibition of non-cyclic photophosphorylation by SO_3^{2-} may be responsible for the inhibition of photosynthesis. The K_i for SO_3^{2-} inhibition of this process (0.8 mM) was similar to that for carboxylase. The regulation of the pentose phosphate reaction sequence is complex and under certain conditions, low illumination, the generation of phosphorylative capacity may be important. However under optimal illumination and with high PO_4^{3-} concentrations (Portis et al., 1977; Heldt et al., 1978) the reactions catalysed by RuBP carboxylase will regulate the cycle. Under such conditions the inhibition of RuBP carboxylase and oxygenase activities by SO_3^{2-} and SO_4^{2-} will be of greater importance.

I know of no other study in which the effects of fluoride on RuBP carboxylase have been investigated. Inhibition of carboxylase was competitive with respect to HCO_3^- but both carboxylase and oxygenase reactions were inhibited in an uncompetitive way with respect to RuBP. Fluoride inhibition often is the result of the formation of F- PO_4^{3-} - Mg complexes and in this form has been shown to inhibit phosphoglucomu-mutase (Najjar, 1948) 5'-nucleotidases (Heppel & Hilmore, 1951) pyrophosphatases (Noganna et al., 1955) and enolase (Chang. 1975). Whilst the two PO_3^{2-} groups of RuBP could be incorporated into such complexes in control experiments incubation of RuBP with Mg^{2+} and F^- for different periods did not increase the degree of inhibition.

There is little information concerning the concentrations of fluoride in the chloroplasts. However, concentrations of 200 ppm in dry material can be calculated to produce up to 5 mM concentrations in the

leaves. Research has demonstrated that fluoride accumulates at the tips and margins of leaves (Weinstein, 1977) thus only small areas of leaf may be exposed to very high concentrations of fluoride. The remainder to background concentrations. The inhibition of RuBP carboxylase may therefore play an important role *in vivo* in fluoride polluted atmospheres, but *in vivo* investigations are needed to confirm this.

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ERRATA

See p 107

1. Mandl et al. (1975) found that short-term exposure to 0.5—0.7 μg m^{-3} had no effect on the dry weight of barley. MacLean and Scheider (1981) reported that a four day exposure to 0.9 μg m^{-3}F reduced grain size and yield.

MacLEAN, D.C. & SCHNEIDER, R.E. (1981). The effects of gaseous H F on the yield of field-grown wheat. *Environ. Pollut. Series A*, 24, 39-44.

AERIAL POLLUTANT EFFECTS ON THE GROWTH OF CEREALS AND ON RIBULOSE BISPHOSPHATE CARBOXYLASE IN VITRO

A thesis submitted for the degree of
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in the University of London

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AN IMPROVED OPEN-TOPPED CHAMBER FOR POLLUTION STUDIES ON CROP GROWTH

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ABSTRACT

This paper describes an improved design of open-topped chamber, based on wind tunnel tests, which reduces wind-generated inflows. The design incorporates a collar on top of the chamber and an inner lip. The conditions within chambers under field conditions approximate to the ambient but still show a detrimental effect on plant growth.

INTRODUCTION

Methods used to determine yield losses due to atmospheric pollutants include fumigation in controlled environment cabinets, field surveys and field chamber experiments in polluted, ambient and clean air. It is well recognised that it is difficult to relate the results of fumigation experiments to observations in the field (Mukammal, 1976).

Both the response of plants and the uptake of pollutants are dependent on a number of environmental variables (e.g. light, temperature, humidity etc.). Moreover, most fumigations arrange for a continuous exposure at a constant concentration and do not simulate the pattern of peaks observed naturally. Also, mixtures of pollutants, often varying independently, are found in the field—a situation difficult to simulate in fumigation chambers.

The most direct approach to studies in the field is to compare plants grown in the field with ambient air or in air that has been cleaned. Some attempts to do this without using chambers are now being developed (Jones *et al.*, 1977). If chambers are used it is essential that the conditions within them are as close as possible to those in the field. Much effort has been put into the design of chambers that satisfy this requirement (Miller & Yoshiyama, 1973; Thompson & Taylor, 1966).

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Open-topped chambers were developed by Mandl *et al.* (1973) and Heagle *et al.* (1973) in which temperature, relative humidity and light intensities were close to, and fluctuated in the same way as, values in the surrounding field. The new chambers were considered to be a great improvement over closed chambers. The open-topped chambers were designed, and have been extensively used for, fumigation work but were less successful as exclusion chambers. The levels of pollution can only be satisfactorily reduced in calm weather because of wind-generated inflows (King & Smith, 1975; Kats *et al.*, 1976). Although preferable to closed chambers, open-topped chambers can affect the growth of some crops (Mukammal, 1976; Lewis & Brennan, 1977; Brough *et al.*, 1978).

This paper describes our most recent chamber design which overcomes some of the deficiencies of the original chambers. The main objectives were to improve the design for exclusion studies and, in particular, to overcome the problem of incursions of field air in windy weather whilst maintaining environmental conditions as close as possible to those outside. Various modifications were evaluated in wind tunnel tests and the most successful features incorporated and tested in field chambers.

MATERIALS AND METHODS

Tests of model chambers

Models were tested in the small wind tunnel at Rothamsted Experimental Station, which has a cross section of 0·6 m wide and 0·9 m high and allows wind speed to be varied from 0–5 m s^{-1}.

The basic design of an open-topped chamber was taken as a cylinder, 2·3 m high and with a 2·5 m diameter. A model chamber, 1:12 scale, was constructed of cardboard and covered in sand-paper to simulate the turbulence experienced in the field. The model was ventilated by six perforated parallel pipes to produce a draught of air moving vertically upwards to give 3·5 air changes a minute. The chamber modifications that were tested in detail are shown in Fig. 1.

Tests were conducted in turbulent air flow at five wind speeds between 0 and 5 m s^{-1}. The flow characteristics of the model were examined by releasing smoke into the air-stream upwind and observing the movement of smoke over and into the model. Quantitative measurements of the efficiency of exclusion of pollutants were made by ventilating the model with CO_2-free air and measuring the concentration of CO_2 inside the model using an infra-red gas analyser (Analytical Development Corporation). The mean concentration for the chamber was obtained by drawing air through a six-port manifold held at 3 cm from the base. The efficiency (E) of the chamber is defined as a percentage of CO_2 excluded:

$$E = 100 - \frac{\text{Internal concentration}}{\text{External concentration}} \times 100$$

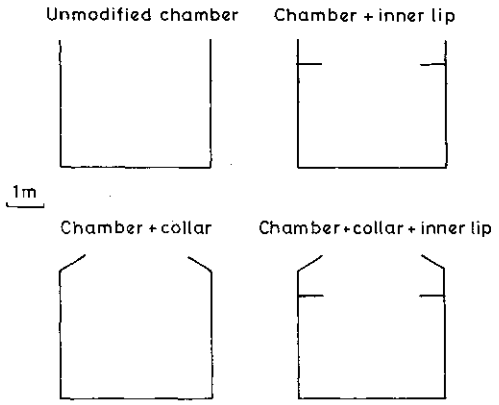

Fig. 1. Vertical sections of chamber designs tested in the wind tunnel.

The efficiency of the most successful modification was examined in more detail; samples were taken via a T-piece independently from seven points—the six corners and the centre of the chamber at a height of 3 cm—and at five heights within the centre of the chamber (3, 6, 9, 15 and 18 cm) (Fig. 2). All of the tests were repeated on three occasions.

Construction and testing of field chambers

The field chambers were designed and constructed in the light of the results obtained in the wind tunnel (Fig. 2). The chambers were hexagonal in shape, 2·3 m in height and 2·4 m in diameter. A collar at 30° partly closed the top, as did a lip which projected into the chamber 0·5 m below the collar.

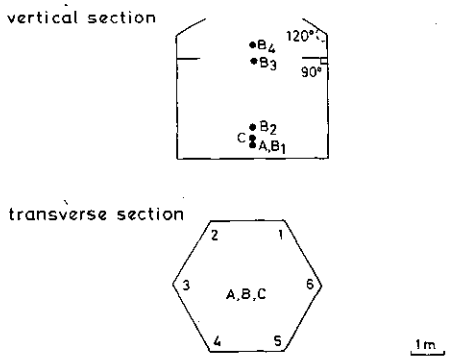

Fig. 2. Sampling points in model and field chambers. A, air samples via manifold. In model chamber: B, air samples via T-piece for height vs. efficiency measurements; 1–6, air samples via T-piece for efficiency distribution around the chamber. In field chamber: C, air samples, temperature, relative humidity and light intensity measurements.

A chamber frame was constructed of horizontal bars of 4 cm aluminium right angle and vertical aluminium glazing bars. A cut 0·5 below the top of the glazing bars allowed them to be bent to support the collar. The lip was made by bolting a piece of aluminium glazing bar, 0·3 m long, to the horizontal framework. The framework was covered in square section Novolux (ICI) sheeting. This sheeting was rigid and guaranteed against discoloration for five years. Access to the chambers was by two sliding doors on opposite sides of the chamber (BACO Ltd).

Large axial flow fans supplied 116 m^3 of air a minute to the chambers. The air passed through a Thermister filter unit (Aldridge Air Control) which contained sixteen activated carbon filters arranged in parallel. The filters were 1·25 cm thick, with a surface area of 8·9 m^2, and were sandwiched between pad filters which removed particulates. The fans and filters were mounted 1 m from the northern corner of the chamber to reduce shading. Two flexible ducts connected the filter units to the rigid ductwork of the chambers. For row crops each half of the chamber had a 0·25 m diameter pipe along one wall of the chamber connected to three parallel pipes, 0·1 m in diameter, which ran across the chamber between the rows of the crop. The small ducts were perforated by 1 cm holes at 3 cm spacing which directed air both up and down (Fig. 3).

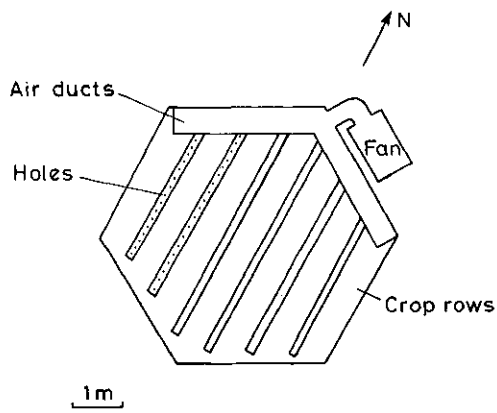

Fig. 3. Field chamber with ventilation system for row crops.

The internal area was 5·5 m^2 but plants were sampled from only 2·5 m^2 to reduce possible edge effects.

Air samples were obtained by drawing air from the centre of the chamber at a height of 0·5 m. The samples were then bubbled through one volume of peroxide which was titrated with sodium tetraborate to give daily mean SO_2 concentrations. Instantaneous SO_2 concentrations were determined using a Meloy SA285 flame photometric sulphur analyser. Air temperatures and relative humidities were measured at the centre of the chamber at a height of 0·5 m using screened and

ventilated dry and wet thermocouples. Light at a height of 0·5 m was measured with quantum light sensors (Lambda).

RESULTS AND DISCUSSION

Wind tunnel tests

Smoke distribution showed that the pattern of flow remained constant over a wide range of wind speeds. No formal assessment of Reynolds number (Re) was required. Once turbulence had started the flow pattern was not altered by an increase in Re because the ratio of inertia to mean flow remained constant at increased wind speeds.

The efficiency of the chambers in excluding gases was dependent on wind speed, the greatest efficiencies being observed in still air (Fig. 4). The maximum efficiency of the unmodified chamber was 40 % in still air, decreasing rapidly to 4·7 % at 5 m s^{-1}.

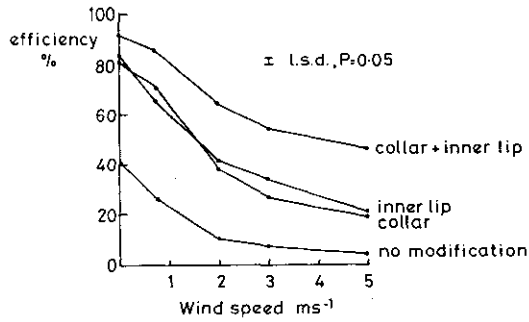

Fig. 4. Efficiency of different modifications to the model chamber.

The efficiency of the model was improved by modifying its design. The addition of a collar to the top of the chamber increased efficiency to 81 % in still air and 21 % at 5 m s^{-1}. Installing a lip on the inside of the chamber had a similar effect. The best results were obtained by using both the collar and the inner lip. Used together the modification increased efficiency to 91 % in still air and 46 % at 5 m s^{-1}. The wind generated inflows were reduced by the collar which streamlined the movement of air over the chamber, displaced the trailing edge mixing layer and increased the speed of the air leaving the chamber. The inner lip deflected currents of air across the mouth of the chamber and prevented them from penetrating as far as the base of the chamber.

In the fully modified model there was little variation in the efficiency with height of samples taken beneath the inner lip (Fig. 5), but the efficiency was greatly reduced above the lip. Measurements of efficiency based on samples taken at points around the chamber were in good agreement and varied within 15 % (Fig. 6).

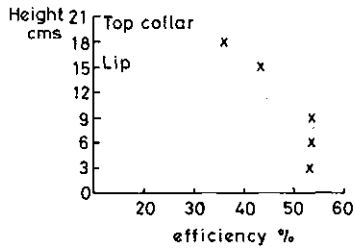

Fig. 5. Efficiency at different heights in the fully modified model.

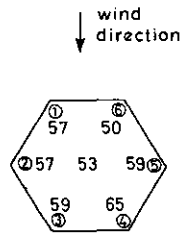

Fig. 6. Efficiency at different points around the fully modified model at 3 cm from the base.

These two modifications produced a substantial improvement in efficiency. If the modified chambers were used for fumigation studies the reduced wind operated inflows would facilitate the control of pollutant concentrations.

The results from the wind tunnel tests agree with those of other workers who have attempted to streamline the tops of chambers in the field to reduce wind generated inflows (King & Smith, 1975; Kats *et al.*, 1976).

Field tests on modified chambers

There are two main ways in which pollutants can contaminate the air in open-topped chambers—by thermal inflows and by wind-generated inflows. Unless heating due to radiation is very strong, a ventilation rate of $0{\cdot}2\,m\,s^{-1}$ should be sufficient to prevent thermal inflows (Chamberlain, 1976). The ventilation rate of the modified chambers gave a calculated vertical flow of $0{\cdot}25\,m\,s^{-1}$.

At low wind speeds the unmodified chambers operated satisfactorily but at higher wind speeds wind generated inflows occurred (Mandl *et al.*, 1973; Kats *et al.*, 1976). Under field conditions the occurrence of moderate winds meant that it was often only possible to reduce SO_2 pollution by about 50% (Brough *et al.*, 1978).

Over a 52-day period covering a range of climatic conditions, the mean SO_2 concentration was reduced from $39{\cdot}2\,\mu g\,m^{-3}$ outside to $13{\cdot}1\,\mu g\,m^{-3}$ inside the filtered modified chamber. The concentration exceeded $100\,\mu g\,m^{-3}$ for 510 out of a total of 7,560 4-min sampling periods outside but it only did so once in the modified filtered chamber. The efficiency of the chamber in excluding pollutants was not reduced on windy days (Fig. 7).

Temperatures measured in the centre of the chamber at 0·5 m from the ground closely followed those measured at a similar height outside but were, on average, 1 °C warmer. Temperature differences were usually largest in the early morning and smallest in the late afternoon (Fig. 8). Similar increases in temperature have been reported for the unmodified design (Heagle *et al.*, 1973; Mandl *et al.*, 1973; King & Smith, 1975).

Measurements of relative humidity made in the chambers closely followed those of the field ($\pm 10\%$) as in unmodified chambers (King & Smith, 1975).

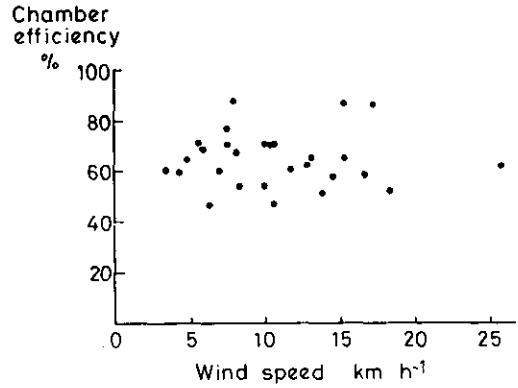

Fig. 7. The efficiency of the field chamber at different wind speeds.

In diffuse light the intensity throughout the chambers varied by only 6 % but was 10–20 % less than outside. In direct sunlight the light intensities in areas shaded by the framework were 40 % less than outside. The intensity in the remainder of the chamber was uniform and 10–20 % less than outside. The area shaded by the framework was small and depended on the time of year and time of day. The unmodified chambers made from glass-fibre panels reduced light levels by 30 % (King & Smith, 1975) whilst the reductions reported for open-topped chambers glazed in Novolux (Brough *et al.*, 1978) were similar to those reported above.

Although the major environmental variables inside open-topped chambers are close to those of the field, these chambers adversely affect the growth of some crops. There are few reports in the literature where the growth of plants in unfiltered open-topped chambers and on outside plots are compared (Howell *et al.*, 1979). In the case of spring barley growing in the UK the development of the crop was accelerated by 7–8 days and the yield was reduced in both unmodified and in modified open-topped

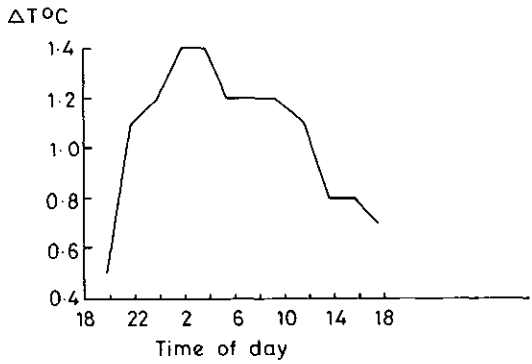

Fig. 8. The average difference in temperature between the inside of a chamber and outside at different times of day during a 20-day period in April.

chambers. The magnitude of the effect varied from year to year and may be related to water stress, although the chambers were watered to approximate rainfall. In 1976 and 1977 yields were reduced by 21 % and 29 % in the unmodified design whilst in 1978 and 1979 yields were reduced by 10 % and 45 % in the modified chambers. The large seasonal variations make it impossible to compare modified and unmodified chambers in this respect. The reasons for the yield reduction of some crops require further investigation (details to be published later).

CONCLUSIONS

The chamber described is a significant improvement over the unmodified design. It allows the chambers to be used as exclusion chambers with plants in the filtered chambers being exposed to only about 30 % of the ambient concentrations of pollutants. The modifications produced a more stable air flow which would make the control of pollutant concentrations in fumigation studies easier. These improvements were achieved without further adversely affecting the way in which the chamber environment differed from ambient.

The retention of ambient field conditions and the reduction of pollution levels are incompatible. The modified chambers offer a compromise between the two objectives and are a valuable tool in determining yield loss due to pollution.

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Effects of aerial pollutants on the growth and yield of spring barley

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(*Accepted 10 August 1981*)

SUMMARY

Open-topped chambers were used to determine the effect of field concentrations of aerial pollutants on the growth and yield of spring barley. Experiments were conducted in the Marston Valley, Bedfordshire, where sulphur dioxide and fluoride are the major pollutants. The charcoal filtered chambers enabled pollutant concentrations to be reduced by 60–70%. Cleaning the air increased straw and grain yields. The filtration was non-selective and did not identify the injurious agent. The chambers were found to accelerate the crop's development by 7–8 days and reduce yield by suppressing tillering.

INTRODUCTION

High concentrations of aerial pollutants can visibly damage plants resulting in a quantifiable loss of photosynthetic area (Webster, 1967; Jacobson & Hill, 1970). The effects of lower concentrations are more difficult to establish. Some investigators have stated that in the absence of visible damage aerial pollutants do not decrease growth or yield (Thomas & Hill, 1937; Katz, 1949; Zahn, 1961). Indeed, under certain conditions, low concentrations of some pollutants, e.g. sulphur dioxide have been shown to increase the growth of crops (Faller, Herwig & Kuhn, 1970). However, stomatal movement and photosynthesis have been shown to be affected even by low concentrations which do not produce visible damage (Biscoe, Unsworth & Pinckney, 1973; Black & Unsworth, 1979). There are also reports from some workers that, even in the absence of visible injury, growth and yield have been reduced as a result of long-term exposure to low concentrations of pollutants (Bell, Rutter & Relton, 1979; Bleasdale, 1973; Davies, 1980).

In 1979 we began a study in the primarily agricultural area of the Bedfordshire brickfields of the effect of existing levels of pollutants (mainly S and F compounds) on the growth and yield of spring barley. There are some historical reports of air pollutant damage to plants and animals in the area (Thorold, 1968; Blakemore, Bosworth & Green, 1948; Burns & Allcroft, 1964) but no incidence of visible damage to agricultural crops has occurred in recent years (Brough, Parry & Whittingham, 1978). This paper describes experiments in which open-topped chambers (Buckenham, Parry, Whittingham & Young, 1981) were used in 1979 and 1980 to compare the growth of crops in charcoal-filtered 'clean' air and unfiltered field air. Unenclosed plots were used to estimate the effect of the chambers on crop growth. Experiments using open-topped chambers have only rarely included comparisons with plants grown outside (Leone & Green, 1974; Thompson, Kats & Cameron, 1976). However, any enclosure may affect the growth and development of plants (Buckenham & Parry, 1979; Howell, Koch & Rose, 1979) and it is important to determine the magnitude of this effect and separate it from the response of a crop to air filtration.

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AERIAL POLLUTANT EFFECTS ON THE GROWTH OF CEREALS AND ON RIBULOSE BISPHOSPHATE CARBOXYLASE IN VITRO

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by

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SEPTEMBER 1983

MATERIALS AND METHODS

Spring barley, *Hordeum vulgare* L. cv. Magnum was grown inside open-topped chambers of a design described by Buckenham *et al.* (1981) and on unenclosed adjacent plots. The cultivar was selected for its short-straw, high yield and resistance to fungal diseases. The experiment was sited at Thrupp End Farm, Lidlington (O.S. Sheet 153, GR. 988397) with Stewartby brickworks 4·9 km to the N.E. and Ridgmont brickworks 1·8 km to the S.W. The prevailing winds were from the S. and S.W. The soil was a clay loam pH 6·8 that had lain fallow during the year previous to each investigation.

The chambers used were hexagonal, 2·4 m in diameter and 2·3 m in height. A collar inclined 30° above the horizontal partly closed the top of the chamber as did a lip projecting into the chamber 0·5 m below the collar. Each chamber was ventilated at 3·5 air changes min^{-1} to produce an air stream moving vertically upwards. This largely excluded ingress of air from the top.

In each year the experiment consisted of four blocks each of four plots: one charcoal filtered and one unfiltered chamber and two unenclosed plots. The area within the chambers was 5·5 m^2 and the unenclosed plots squares of 7·6 m^2.

The experimental site was rotavated and fertiliser raked in to provide 120/60/60 kg ha^{-1} in 1979 and 60/30/30 kg ha^{-1} in 1980 of NPK respectively. Seed was sown by hand at about 1·3 cm intervals in rows 17·8 cm apart to give approximately 430 plants m^{-2}. Chambers were placed over the plots immediately after sowing and ventilation started 2–3 days later. Trickle irrigation pipes were laid between the rows of the crop so that they could be watered to approximate the rainfall to the unenclosed plots. The site was fenced to prevent rabbit damage and the plants covered with a fine mesh net after anthesis to prevent bird damage. In 1980, 10 cm mesh netting was used horizontally at about 30 cm to stop lodging.

The concentration of SO_2 in the air was measured using a flame photometric sulphur analyser (Meloy SA285) and the titrimetric method of the National Survey (Anon., 1966). Measurements were taken throughout the growing season at crop height both inside the chambers and over the unenclosed plots. The titrimetric method gave daily mean concentrations whilst the photometric sulphur analyser gave a continuous record showing short term peak concentrations. The daily mean ambient fluoride concentration was measured by drawing air through a prefilter consisting of a filter paper impregnated with 0·1 M citric acid to retain particulate fluoride, and through two papers impregnated with 0·1 M Na_2CO_3 to absorb gaseous fluoride (Davison, Rand & Betts, 1973). The papers were then extracted with acid and then alkali and extracts measured using a specific ion electrode (Jacobson & Heller, 1970). In 1980 ambient levels of ozone were also monitored using an ultra-violet spectrophotometer (Dasibi 110).

Plant samples were taken both when most of the main stem ears had reached anthesis and the tiller ears were emerging (1980) or 1 wk after this stage (1979) and also at maturity (Table 1). For sampling each plot was divided into four sub-plots and two half-metre lengths of two adjacent rows removed from the four quadrants of each plots. All of the sample areas were surrounded by single guard rows. Plant and shoot numbers, and number of ears/sample were recorded. Stem and green laminae were separated and their area measured with a planimeter

Table 1. *Calendar*

		1979	1980
Sowing date		24 April	26 March
Anthesis harvest	Chambers	10 July	23 June
	Unenclosed plots	18 July	2 July
Maturity harvest	Chambers	15 August	19 August
	Unenclosed plots	21 August	26 August

(Paton Industries). Leaf area was taken as the surface area of one side of the leaf and the stem area as $\pi_2 \times$ plan area. The dry weights of the plant parts were then measured. Analyses were made for both fluoride extractable in acid and alkali (Jacobson & Hill, 1970) and total sulphur in dried samples of green and dead leaf laminae at anthesis and at final harvest. Sulphur was analysed by X-ray fluorescence spectrometry (Brown & Kanaris-Satiriou, 1969) and fluoride determined with a specific ion electrode.

Non-destructive growth analyses were carried out at intervals of 2–3 days during the development of the crop. Shoots (main stem and tillers) were counted on a 0·5 m length of row on each plot. Six main shoots from each plot were tagged and stem height, leaf emergence stage, leaf extension rate and final leaf length measured until anthesis. Height was measured from the ground to the ligule of the uppermost fully expanded leaf. Leaf extension rates were calculated from changes in the length of the youngest leaf measured from its tip to the ligule of the uppermost fully expanded leaf (Peacock, 1975).

RESULTS

Concentrations of pollutants in the air and plants

The mean ambient SO_2 concentrations were similar for the two growing seasons: 48 $\mu g\ m^{-3}$ in 1979 and 52 $\mu g\ m^{-3}$ in 1980. Only in 1980 was fluoride monitored continuously when a mean concentration of 0·15 $\mu g\ m^{-3}$ F^- was recorded (0·13 $\mu g\ m^{-3}$ gaseous and 0·02 $\mu g\ m^{-3}$ particulate F^-).

In both years the highest daily mean concentrations of SO_2 occurred in the period between the emergence of the plants and the onset of tillering. In 1980 the crop was exposed to a further period of high SO_2 concentrations just before anthesis. The pattern of peak fluoride concentrations closely followed that of the SO_2. The highest daily mean SO_2 concentration was 224 $\mu g\ m^{-3}$ in 1979 and 182 $\mu g\ m^{-3}$ in 1980 (Table 2). The highest daily mean fluoride concentration in 1980 was 1·48 $\mu g\ m^{-3}$. Short term peaks (10 min) in the range of 300–1300 $\mu g\ m^{-3}$ SO_2 occurred when the experimental site was covered by the plumes of neighbouring point sources.

Intermittent measurements of ozone were made in 1980 and the highest concentration 150 $\mu g\ m^{-3}$ (7 pphm) recorded in May. In the filtered chambers the SO_2 concentration was reduced to 37% of that in the unfiltered chamber which was 92% of the outside concentration.

Cleaning the air did not result in a reduction in the sulphur content of the leaves. The fluoride concentration in plants from the filtered chambers averaged 57% of those from the unfiltered ones. The concentration in the plants in the unfiltered chambers was always less than for outside plants except at anthesis in 1980 (Table 3).

Growth and yield

Grain yields were good, 6·4 and 7·2 t ha^{-1} (15% moisture content) in 1979 and in 1980 respectively on the unenclosed plots. In 1979 the crop lodged after anthesis.

Table 2. *Ambient SO_2 concentrations $\mu g m^{-3}$ at crop height*

	Chambers								
	+ filter			− filter			Outside		
Year	Mean for experimental duration	Highest daily mean	Days with mean above 100 $\mu g m^{-3}$	Mean for experimental duration	Highest daily mean	Days with mean above 100 $\mu g m^{-3}$	Mean for experimental duration	Highest daily mean	Days with mean above 100 $\mu g m^{-3}$
1979	15	77	0	—	—	—	48	224	6
1980	18	86	0	48	188	9	52	182	9

Table 3. *The effect of enclosure and filtration on spring barley yields, and fluoride and sulphur contents*

	1979						1980					
	Anthesis			Maturity			Anthesis			Maturity		
Harvest	O + C	O − C	UN	O + C	O − C	UN	O + C	O − C	UN	O + C	O − C	UN
Number of shoots (m^{-2})	859 (57·8)	792	1236 (50·0)	862 (92·3)	668	1089 (79·9)	753 (57·3)	650	1405 (49·6)	1275 (79·7)	1101	1421 (69·1)
Number of ears (m^{-2})	586 (53·2)	461	1025 (46·1)	541 (84·4)	488	977 (73·1)	536 (27·3)	456	786 (23·6)	715 (74·8)	709	955 (64·7)
Total dry wt ($g\ m^{-2}$)	812 (60·5)	680	1197 (49·0)	1126 (82·0)	745	1278 (71·0)	511 (39·0)	482	804 (33·7)	1357 (59·8)	1159	1529 (51·8)
Grain dry wt ($g\ m^{-2}$)				489 (46·4)	308	559 (40·2)				560 (36·1)	518	631 (31·3)
Straw dry wt ($g\ m^{-2}$)	614 (39·9)	469	898 (31·9)	470 (50·9)	345	597 (44·1)	446 (30·0)	400	692 (26·0)	569 (28·5)	475	675 (24·7)
Photosynthetic area index	8·1 (0·6)	6·6	10·5 (0·4)				7·5 (0·6)	6·4	10·7 (0·5)			
1000-grain dry wt (g)				43·8 (1·1)	37·6	31·7 (1·0)				42·4 (0·9)	42·0	35·0 (0·8)
Number of grains per ear				20·6	16·8	18·1				18·9	17·4	19·1
F conc. $\mu g\ g^{-1}$	12·5 (2·0)	20·4	37·7 (1·8)	27·7 (8·9)	53·6	89·4 (7·7)	9·7 (2·2)	19·6	18·9 (1·9)	28·7 (7·2)	42·8	110·6 (6·2)
S content % of leaves				0·73 (0·04)	0·70	0·56 (0·07)				0·93 (0·07)	0·69	0·68 (0·07)

Numbers in parentheses are S.E.D.'s.
O + C = open-topped chamber + charcoal filter.
O − C = open-topped chamber no filter.
UN = unenclosed plots.

Effect of filtration. At anthesis there were no significant differences in tiller numbers, but there were significantly more ears per unit area in the filtered than in the unfiltered chambers. In both years total dry weights and the photosynthetic area index were greater in the filtered chambers but the increases were only significant in 1979 (Table 3). At maturity, straw dry weights were increased in the filtered chambers by 36% in 1979 and 20% in 1980. Grain yields, 1000 grain weights and grain number per ear were greater in the filtered than in the unfiltered chambers in both years but the increase was only significant in 1979. Cleaning the air increased total dry matter production by 51% and 17% in 1979 and 1980 respectively; it did not alter the rate of development of the crop.

Effect of enclosure. Anthesis was 7–8 days earlier in the unfiltered chambers than for the unenclosed plots. The number of shoots per unit area was less inside the chambers than outside by 36% in 1979 and 54% in 1980. This resulted in fewer ears, lower straw dry weights and a smaller photosynthetic area inside the chambers at anthesis. The differences present at anthesis

Table 4. *Developmental data for plants from unfiltered chambers and unenclosed plots*

	1979			1980		
	O − C	UN	L.S.D.	O − C	UN	L.S.D.
Maximum shoot number (m^{-1})	774	1817	306	889	1599	302
Number of leaves on main stem	8·0	9·0	0·2	9·1	10·5	0·2
Final main stem height (mm)	664	633	22	745	725	22

O − C = open-topped chamber, no filter.
UN = unenclosed plot.

persisted to maturity. The crop ripened 7–8 days earlier in the chambers and ear numbers, straw and grain dry weights were less than outside. But the grain size was larger in the chambers than on the unenclosed plots.

Measurements made before anthesis showed that the crop emerged one day earlier in the chambers than on the unenclosed plots and tiller production was decreased in the chambers. The enclosed plants produced significantly fewer leaves on the main stem (Table 4).

DISCUSSION

In chambers barley produced higher straw and grain yields if the air supply was filtered than when it was not. The grain yield of plants grown outside was always significantly greater than plants grown in chambers with unfiltered air.

Before discussing the effect of cleaning the air in more detail the effect of enclosure within a chamber will be considered. The reduced yield in the chambers compared to outside plots was caused by reduced tillering resulting in fewer ears which was only partly compensated for by larger grains.

The effect of enclosing plants within a chamber resulted in faster development and anthesis occurring 7–8 days earlier than outside. This may be due to the increased temperature inside the chambers. Buckenham *et al.* (1981) reported an average increase in temperature of between 0·5 and 1·4 °C at different times of day. Hutcheson & Quantz (1917) found that a 2 °C increase in temperature caused anthesis to occur 12 days earlier in barley. The warmer temperature may also hasten ear initiation and so account for the production of one less leaf on the main stem in the chambers. However, if leaf emergence stage is calculated on the basis of thermal time (Gallagher, 1979), there still remains a difference between the chambers and unenclosed plots (Fig. 1).

Plants inside the chambers produced fewer tillers per plant which may be due to reduced irradiance (10–20%) as well as the increase in temperature. Cannell (1969*a*) reported reduced tillering in warmer temperatures and reduced light intensity in barley. The warmer temperatures increase the assimilate demand of the main axis due to faster leaf primordia formation and cell division, so reducing tiller growth (Friend, Helson & Fisher, 1962).

Faster development and less dry matter growth in the chambers resulted in fewer ears and gain

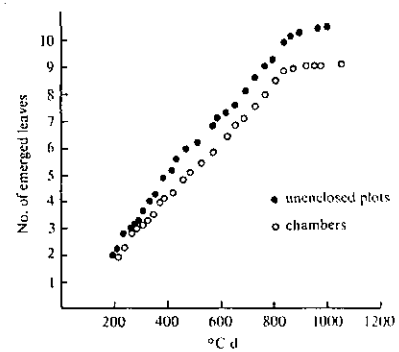

Fig. 1. The number of leaves emerged, with thermal time for barley main stems grown in chambers (○) and on unenclosed plots (●).

per m^2, and fewer grains per ear. However, mass per grain was about 16% heavier in the chambers. This could be a compensatory effect, or it may have been due to the greater proportion of ears on main stems. Cannell (1969*b*) found that main shoot ears in barley had the heaviest grain.

It is apparent from the results that although the differences in temperature and light intensity between the chambers and outside are small; they are able, over the growing season, to affect significantly the growth of the crop.

The effect of filtration on the plants was much greater in 1979 than in 1980, which indicates the probable interaction of pollutants with varying environmental conditions (Davies, 1980). However, the results do confirm those of an earlier study in the same area in which the yield of barley was decreased by up to 40% in unfiltered chambers (Brough *et al.*, 1978). Experiments with barley in Bedfordshire have indicated grain yield losses in 4 out of 5 years, but not all were statistically significant.

In 1980 there was only a small decrease in unfiltered chambers in straw dry weight per m^2 at anthesis, and final grain dry weight per m^2 was not significantly decreased despite fewer grains per ear. However, in 1979 there was a large depression in straw dry weight per m^2 at anthesis and also decreased grain dry weight per m^2 at maturity due to smaller mass per grain and fewer grains per ear. It is uncertain whether the decreased number of grains per ear in the unfiltered chamber was due to fewer grains being initiated, or more grains aborting. It appears that if vegetative growth is adversely affected before anthesis, leading to a smaller photosynthetic area and decreased straw dry weight, then this may cause reductions in grain yield at maturity. There will be less photosynthate produced for grain filling, and less assimilate reserves for translocation to the grain. The rate of photosynthesis may also be reduced by the presence of pollutants. SO_2 and F^-, given singly have both been shown to affect carbon metabolism in the absence of visible injury (Black & Unsworth, 1979; Bennet & Hill, 1973) and also to alter chloroplast structure (Malhotra, 1976; Wallis, Miller, Psenak & Shieh, 1974) and change other physiological processes (Biscoe, Unsworth & Pinckney, 1973; Weinstein, 1977).

Filtration did not reduce the sulphur content of the leaves. It is believed that any differences that may be attributed to the comparatively small changes in exposure to aerial SO_2 are masked by the much greater proportion of sulphur taken up from the soil. Fluoride is taken up from the soil to a very limited extent; hence even small differences in atmospheric fluoride are reflected in the concentration of fluoride in the leaves.

We know of no other field experiment with a combination of low concentrations of both SO_2 and F^-. Ambient versus filtered air experiments have been conducted in areas where SO_2 is the major pollutant, but most have been confined to studies on rye grass where vegetative growth is important, not grain production. Crittenden & Read (1978) found that yields of *Lolium perenne* L. cv. S23 were reduced by 30–40% when it was grown in chambers ventilated with air containing mean SO_2 concentrations of 50–90 μ gm^{-3} respectively, compared with a clean air control. There have been many fumigation experiments with SO_2 some showing decreases in yield with SO_2, and other increases. Bell *et al.* (1979) reported a yield depression of 68% in *L. perenne* exposed to a mean concentration of SO_2 of 43 μgm^{-3} for 173 days in winter. Setterstrom, Zimmerman & Crocker (1938) and Cowling & Lockyer (1976 & 1978) have reported no adverse effects, and also sometimes increases in yield, with SO_2 concentrations of less than 100 μgm^{-3}. However, fumigation experiments tend to use constant levels of pollutant, whereas in the field the average concentration can conceal fluctuations with some relatively large peaks. In our study, short-term peaks of over 500 μgm^{-3} were frequently recorded for periods of several hours. In the field it is rare for plants to be exposed to only one pollutant, and it is possible that pollutants may produce a more than additive effect on plant growth. Field observations made in areas where SO_2 and F^- concentrations were sufficiently high to physiologically injure plants indicated no synergistic or antagonistic effects (Weinstein, 1977).

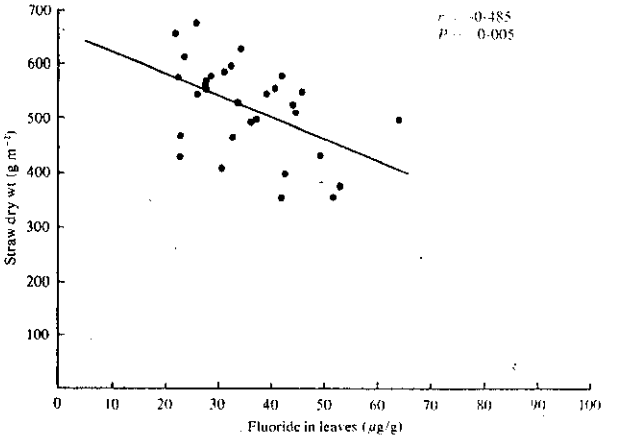

Fig. 2. Correlation between straw dry weight and fluoride concentration in the leaves at maturity. $r = -0{\cdot}485$ $P = 0{\cdot}005$.

However, fumigations of barley and corn with the two pollutants demonstrated that F^- uptake is reduced in the presence of SO_2, but that the two have a synergistic effect with respect to visible injury (Mandl, Weinstein & Keveny, 1975).

In the present experiment it is not possible to identify which pollutant or combination of pollutants is causing the yield reduction as the filtration was unselective. However, F^- does seem to be involved as indicated by the negative correlation between F^- concentration in the leaf and straw dry weights of plants grown in chambers (Fig. 2), but this correlation only accounts for 25% of the variation.

CONCLUSIONS

Although open-topped chambers can provide an environment closely resembling outside field conditions, the crops grown in them show increasingly different characters from those grown outside. The main effect of enclosing barley was to reduce yield by suppressing tiller production and ear number. The effect of filtration, although much smaller in 1980 than in 1979, was to increase final grain yield per m^2 by increasing the number of grains per ear. In 1979 filtration also decreased the dry weight of the shoots and the weight per grain.

In both years the conditions for grain growth were favourable (i.e. a long cool grain filling period), but in 1979 the plants in the unfiltered chambers were unable to compensate fully for the large reductions in straw dry weight and photosynthetic area apparent at anthesis. In 1980, effects at anthesis were smaller and did not greatly alter final grain yield.

Despite the difference in magnitude of the effect of filtration in the 2 years, results suggest that a reduction in the levels of pollution in the Bedfordshire brickfields would increase straw and grain yield of barley.

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