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ENVIRONMENTAL MODIFICATION OF THE RESPONSES OF <u>Vicia</u> <u>faba</u> TO SULPHUR DIOXIDE

by

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-i-

ABSTRACT

The responses of gas exchange processes in two varieties of the field bean <u>Vicia faba</u> (CV. Aquadulce Claudia and Dylan) to a range of sulphur dioxide concentrations (0 - 600 ppb) were measured under optimum conditions of light and temperature (500 μ E m⁻² s⁻¹, 20°C). This was carried out in an open gas exchange system, built to monitor both SO₂ treated and control plants simultaneously, and permit measurements of stomatal resistance, transpiration rates, dark respiration rates, net photosynthetic rates and sulphur dioxide fluxes to the plant to be made.

Varietal differences in the sensitivity of <u>Vicia</u> <u>faba</u> to SO_2 were observed which were related, in part, to differences in pollutant uptake. However, when SO_2 fluxes to each variety were equal, net photosynthetic inhibition was significantly greater in Aquadulce plants indicating their photosynthetic mechanism to be more sensitive to SO_2 .

The responses of both varieties of <u>Vicia faba</u> to SO_2 were shown to be modified by added environmental stress such as low light and cold temperature. The duration of the cold (10°C) treatment prior to fumigation with SO_2 was found to be an important factor governing the extent of the change in pollutant sensitivity of the plant.

Respiratory rates, SO_2 fluxes, stomatal responses and resistances to gaseous exchange were also altered in responses to added environmental stress which could explain some of the observed differences in photosynthetic inhibition: However, the environmentally stressed plants were less responsive to the actual amount of SO_2 entering the plant, indicating a fundamental change in the operation of some internal mechanism. Examination of the components of the resistance pathway discerned an added internal resistance to SO_2 uptake which was largely negative under optimum environmental conditions, thus facilitating SO_2 uptake, but which was altered significantly following periods of added environmental stress.

Significant varietal differences in total chlorophyll, protein and carbohydrate contents and Hill reaction activity of isolated chloroplast suspensions were observed; such varietal differences persisted following exposure to sulphur dioxide and/or environmental stress.

In this study, differential sensitivity to SO_2 between plants of the varieties Aquadulce and Dylan appeared to result from a combination of avoidance and tolerance mechanisms depending on SO_2 concentrations and prevailing environmental conditions.

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LIST OF SYMBOLS

C - gas concentration at leaf surface.
C _o - gas concentration inside leaf.
$C_{g}H_{2}O$ - water content of the air entering the chamber system
recorded as dew point temperature.
C_0H_2O - water vapour content of air leaving the chamber
recorded as dew point temperature.
ea – ambient water vapour pressure in millibars calculated from
$[\mathbf{e}_{\mathbf{o}} - \mathbf{e}_{\mathbf{g}}] \div 2.$
ee - water vapour pressure in millibars of air entering
chamber.
e water vapour pressure in millibars of air leaving chamber.
es - water vapour pressure in millibars inside the leaf
assuming saturation at leaf temperature, TL.
E - evaporation rate of water vapour through the stomata.
FR - the air flow rate through the chamber.
g_s - stomatal conductance, the reciprocal of stomatal
resistance.
LA - leaf area.
P_{FCRLC} - calculated SO ₂ flux from analogy to water vapour
diffusion, the ratio of atmospheric SO_2 concentration to
gas phase resistance to SO_2 .
PFmeas - measured flux determined from mass balance calculations,
the rate of uptake of SO_2 per unit leaf area.
Pmax - gross photosynthetic rate.
Pnet - net photosynthetic rate.
Rd - dark respiration rate.
r _a - aerodynamic resistance.
rc - cuticular resistance.
rint - internal resistance.
r _m - mesophyll resistance
r_r - residual resistance (or internal or mesophyll resistance).
r _s - stomatal resistance.

- rt the total resistance to gas exchange which is composed of the sum of the aerodynamic, stomatal and residual (mesophyll) resistances.
- S_i the SO₂ concentration of the air entering the exposure chamber, ppb.

 S_0 - the concentration of SO_2 in the air leaving the chamber.

- S_{sor} the amount of SO₂ adsorbed onto the walls of the chamber.
- T transpiration rate (equal to evaporation rate, E).

T_a - temperature of ambient air ie. chamber temperature.

 T_{L} - leaf temperature.

- χ_{0} water vapour concentration outside leaf (ie. in chamber) in g cm⁻², calculated from $e_{a}(T_{a}) \div T_{a}$.
- χ_i water vapour concentration inside leaf in g cm⁻², assuming saturation at leaf temperature, T_L, calculated from $e_s(T_L) \div T_L$.

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CHAPTER ONE

GENERAL INTRODUCTION

Plant pollution research over the past two decades has been directed towards determining pollutant induced effects on agricultural crops and forests. As well as defining effects on growth and yield, it has been recognised that a study of biochemical and physiological responses to pollutants, is vital in order to understand the mechanisms of pollutant action. Many of these studies have been laboratory based and have of necessity, concentrated on pollutant effects in plants exposed under nonstressful environmental conditions to identify mechanisms of pollutant action and have used short-term exposure to pollutants. However, there are inherent difficulties in extrapolating such data to determine plant responses in the field where environmental parameters are constantly fluctuating. Field exposures and the use of open top chambers have enabled plant responses to long-term pollutant exposures to be studied under natural environmental conditions. Such studies, particularly in the UK, have examined perennial crops or annuals that are grown during the autumn, winter and early spring when environmental conditions are suboptimal. This has led to the growing recognition that, in the field, the effects of air pollutants are modified by suboptimal environmental conditions. The converse has also been seen to occur, in that exposure to pollutants may predispose plants to injury from environmental stresses such as winter injury and drought. Because, the significance of such interactions have only recently been highlighted, very little is known at present regarding the nature of environmental/pollutant interaction on economically important species and the mechanisms contributing to such interaction.

This study was undertaken in the laboratory to determine the influence of low light and low temperature stresses (typical winter conditions) on plant responses to sulphur dioxide and to identify possible mechanisms of action and interaction. Sulphur dioxide was chosen because it is a ubiquitous atmospheric pollutant which is produced during the combustion of sulphur-containing fossil fuels and the smelting of sulphur-containing ores. Natural SO₂ emission can result from the oxidation of hydrogen sulphide (H₂S), carbon disulphide (CS₂) and carbonyl sulphide (COS) emitted from soils, plants and the oceans although the relative

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contribution of natural emissions are small in comparison to anthropogenic emissions which are the dominant SO_2 source in the atmosphere of Europe and the industrialised areas of North America (Unsworth, Crawford, Gregson & Rowlatt, 1985).

SO₂ may be deposited directly onto surfaces in its gaseous form ie. dry deposition, the rates of which depend on concentration, atmospheric mixing and surface affinity for SO₂ (Unsworth et al., 1985). Apart from the effects of its dry deposition, SO₂ is also a primary constituent of acid rain since atmospheric SO₂ can be incorporated into rain drops or snow flakes. Alternatively, wind blown cloud or fog droplets can be captured by vegetation via 'occult deposition' whereby the drops are too small to be adequately captured by conventional rain gauges (TERG, 1988). Rates of transfer and transformation of sulphur from gas-phase SO_2 to liquid drops and particles are relatively slow and, generally, the longer the gaseous pollutant is retained in the atmosphere the greater the chance of chemical reaction and its subsequent wet deposition. Thus close to SO_2 emission sources, dry deposition is greatest whilst wet deposition is of greater significance in areas remote from sources. Dry deposition is thought to remove over 50% of the SO_2 emitted annually over the UK, the remainder is oxidised to sulphate and removed in precipitation; the atmospheric residence time for sulphur is thought to be about five days (Garland, 1978).

Sulphur dioxide pollution has a long history in the UK, particularly in London where levels were seen to rise progressively during the 1700s with the increased burning of coal (Laxen & Thompson, 1987). Several thousand premature deaths were attributable to the infamous London smog of 1952 and as a result of public outcry, the Clean Air Act was introduced in 1956. Prior to 1956, concentrations of SO₂ in the UK were high enough to cause visible injury to plants. Since this time there has been a marked decrease in smoke emissions and a consequent decline in SO₂ concentrations, although this is partly due to improved dispersal with the building of 'tall stacks' (Roberts, Bell, Horseman & Colvill, 1983). The Clean Air Act had little immediate impact on SO₂ levels which increased steadily until 1964/65. At this time, annual mean sulphur dioxide levels generally lay between 300 and 400 μ g m⁻³ (15 ppb) even in highly industrialised regions (Laxen & Thompson, 1987).

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However, the general tendency to express SO₂ concentrations in terms of annual means does not reflect the marked seasonal, diurnal and even hourly differences in concentration which may occur. Typically SO₂ concentrations are greater in winter due to greater consumption of fossil fuels predominantly for heating. Consideration of Laxen & Thompson's data for the highest daily SO_2 concentrations in the London area show records of daily winter peaks of around 1500-2000 µg m⁻³ (560-750 ppb) up to 1964/65. These levels have declined steadily in the last few years and the highest daily winter peaks during 1984/85 were around 200-250 µg m⁻³ (75-94 ppb) (Laxen & Thompson, 1987). However, SO₂ continues to be a problem and there were numerous reports in the National Press during the winter of 1988 of 'killer smogs' in London. SO₂ levels of 670 μ g m⁻³ (250 ppb) were recorded in London during November 1988 concomitant with particularly thick fog. Such short periods of large concentrations of gaseous pollutants, 'episodes', are known to occur if there are nearby sources of primary pollutants and the weather favours the buildup of secondary pollutants (TERG, 1988).

Researchers at University of Nottingham, School of Agriculture at Sutton Bonyington have monitored ambient sulphur dioxide concentrations at this rural site over the last few years and determined current ambient annual means to be around 40 μ g m⁻³ (15 ppb) (Baker, Fullwood & Colls, 1987). However, daily means of up to 80 μ g m⁻³ (30 ppb) have been recorded and hourly means in excess of 240 μ g m⁻³ (90 ppb) are not infrequent (Geissler *pers. comm.*). Such episodic increases in SO₂ concentration at Sutton Bonyington may be attributed to the presence of three coal-powered electricity generating plants in the immediate vicinity.

The concentration of sulphur dioxide above which it is injurious to plants has been the subject of much debate. As is outlined in chapter 3, until the 1970's it was widely considered that SO_2 did not damage plants at concentrations below that which induced visible injury symptoms such as necrotic lesions and chlorosis. A threshold concentration of 300 ppb (800 µg m⁻³) was not considered to be detrimental to plant life (Katz, 1949). However, in recent years there has been irrefutable evidence that low ambient levels of SO_2 may affect physiological processes without visible injury being seen to occur. Short-term exposures to concentrations as low as 35 ppb (93 µg m⁻³) have been shown to inhibit net photosynthetic rates (Black & Unsworth, 1979b). A review of reported SO_2 effects in the absence of visible injury is given in \$3.1.

As described above, episodic increases in SO_2 to levels far and above normal ambient concentrations are not uncommon. Such episodes may have very important effects on vegetation but it is difficult to predict the effects of such episodes on the growth and yield of plants overall. The experimental design used in this study was intended to simulate such short episodes in that SO_2 exposure periods were for only 4 h. It was important to determine if the observed SO_2 effects were readily reversible or were permanent thus having potentially important consequences on plant yield.

The field bean, <u>Vicia faba</u> was chosen for this study because it is a commonly grown agricultural crop. It is also easily grown in artificial conditions and its rapid growth ensured a readily available supply of material for experimentation. Because there is a wealth of information in current literature to suggest that intra- and inter-specific differences exist in pollutant responses, two cultivars of <u>Vicia faba</u>, Dylan and Aquadulce Claudia were chosen for study. By using these two cultivars, it was hoped to assess whether the existence of intra-specific differential sensitivity to pollutants was maintained under conditions of environmental stress.

In order to identify the modifying influence of environmental stresses on plant pollutant responses it was first necessary to determine the responses of both varieties of <u>Vicia faba</u> to sulphur dioxide under optimum environmental conditions to provide a base of data for comparison purposes. Similarly if possible mechanisms of environmental/pollutant stress interaction were to be elucidated it was necessary to determine plant responses to both low temperature and low light stress alone. Thus the effects of a range of sulphur dioxide concentrations on the gaseous exchange mechanisms of both Dylan and Aquadulce plants under optimum environmental conditions are detailed in chapter 3. Chapter 4 details the effects of low light and low temperature on gas exchange mechanisms and the resulting responses to SO₂. Chapter 5 details analyses of plant pigments and metabolites which were undertaken to identify factors contributing to environmental/pollutant induced changes in photosynthetic rates in both varieties.

Plant resistance to pollutant stress may be the result of stress avoidance, stress tolerance or a combination of both factors. Similarly, the imposition of environmental stress may modify the absorption of pollutants into the leaf or, alternatively, modify the response of the plant to absorbed pollutant. Thus particular attention was paid to actual pollutant fluxes to the plant and the relationship between actual flux and the degree of plant response under optimum environmental conditions and following the imposition of environmental stress.

Exposure to pollution is usually defined in terms of ambient concentrations or 'ambient dose', the product of concentration and time (Unsworth, 1982). Such studies assume that the pollutant dose to which the plant is exposed (ie. ambient dose) is a direct quantitative measure of the dose that causes a physiological response (Taylor, McLaughlin & Shriner, 1982) and that actual pollutant flux (uptake) is proportional to ambient concentration. However, it is now widely recognized that a proportional relationship between ambient and effective pollutant dose does not always exist (Taylor, McLaughlin & Shriner, 1982; Unsworth, 1982). The effective pollutant dose is a function of the rate at which pollutant/derivative molecules arrive at perturbation sites within the leaf interior. This rate is controlled primarily by the conductivity of the gas-to-liquid pathway which is known to be vary with prevailing environmental conditions and plant genotype (Taylor et al., 1982). Gas-phase resistance, principally at the stomata is thought to be the predominant factor limiting the diffusion of most pollutant gases, including SO₂ (eg. Mansfield & Majernik, 1970; Winner & Mooney, 1980b; Black, 1985). The importance of leaf resistances in controlling pollutant flux are discussed in greater detail in chapter 3. The importance of the effects of environmental stress factors on gas-phase resistances are considered in detail in chapter 4.

Flux has also been shown to be related to the solubility of the gaseous pollutant in water thus reflecting the importance of pollutant movement into and through a water-dominated cell environment. This is particularly true of sulphur dioxide which is a highly soluble and reactive gas and fluxes have been shown to be underestimated when estimates are based on the product of leaf conductance and ambient concentration (Taylor, McLaughlin, Shriner & Selvidge, 1983). Thus the importance of relating plant responses to actual uptake has become very clear in recent years (Runeckles, 1974; Cowling, Lockyer, Chapman & Koziol, 1981; Roberts, 1984). The relative sensitivities of plant species can be more accurately determined in terms of threshold concentrations and dose-response relationships. However, such thresholds can not be absolute values since they will change dramatically according to the prevailing environmental conditions (Bell, 1985).

When SO_2 enters the leaf, predominantly via the stomatal pores, it dissolves in the water surrounding the cells of the substomatal cavity. In solution, SO_2 establishes the following equilibria:

> $SO_2 + H_2O \Leftrightarrow H_2SO_3$ $H_2SO_3 \Leftrightarrow H^+ + HSO_3^ HSO_3^- \Leftrightarrow H^+ + SO_3^{2-}$

(Malhotra & Hocking, 1976).

The sulphite $(SO_3^{2^-})$ and bisulphite (HSO_3^-) ions and any dissolved SO_2 which has not reacted with water are then available for transport and/or diffusion across the membranes of the surrounding epidermal and mesophyll cells into the cytoplasm where their accumulation can interfere with normal plant metabolic processes. The relative quantities of sulphite and bisulphite ions are dependent on pH so that although it is impossible to measure the concentration of these ions within the cell, a knowledge of cell pH allows some sensible deductions to be made. Since the pH of the plasma is around $6\cdot 8$, approximately 70% of dissolved SO_2 occurs as bisulphite (Ziegler,1975). Similarly, the pH of the aqueous stromal phase of chloroplasts is pH 8 -9 and sulphite ions are the major product of SO_2 dissolution, thus many <u>in</u> <u>vitro</u> studies of SO_2 at likely sites of action have been carried out using sulphite (Wellburn, 1987). A review of some of the observed subcellular effects of dissolved SO_2 is given in chapter 5.

Sulphite and bisulphite ions are both much more toxic than sulphate ions and their detoxification can involve oxidation to sulphate which is then incorporated into normal plant metabolism (TERG, 1988). Oxidation of sulphite to sulphate in plant cells is thought to occur by both enzymic and non-enzymic mechanisms. The enzyme sulphite oxidase is located in mitochondria and is thought to play an important part in detoxification mechanisms. Sulphite has also been shown to be oxidised in the light in a reaction induced by the electron transport system (Malhotra & Khan, 1984). An alternative detoxification mechanism is the reduction of sulphite to sulphide and the emission of H_2S is thought to be a mechanism that confers resistance to SO_2 pollution (Tingey & Olszyk, 1985).

Experiments using ³⁵SO₂ have shown that SO₂ adsorbed by plant

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Chapter 1

leaves does not remain fixed at the site of absorption but has a substantial degree of mobility (Ziegler, 1975; Garsed & Read, 1977) and is translocated from leaves to roots, from old leaves to young leaves and from roots to the surrounding medium (Malhotra & Khan, 1984). SO₂ fumigations result in an accumulation of sulphur containing products such as SHcontaining amino acids, in the plant which suggests that SO₂ can be utilised by plants in the reductive sulphur cycle (Ziegler, 1975). Such activity may be of benefit to plants in conditions where soil sulphur supplies are limited.

The high degree of mobility of SO_2 products within the leaf results in their interaction with several biochemical processes. SO₂ may react with any number of metabolites along its course of migration through the cell and as a consequence numerous reaction sites may be involved. Thus elucidation of the exact mechanism of SO_2 action in inhibiting net photosynthetic rates and reducing growth and yield is very difficult. Further complications arise from the action of environmental stresses such as low light and low temperature on plants since environmental stress factors can influence numerous biochemical processes and different reaction sites may be preferentially affected. Since both pollutant and environmental stresses have such wide ranging effects individually, elucidation of mechanisms of their interaction is likely to be highly problematic and be further complicated by intra- and inter-specific variability in response and the fact that gaseous pollutants seldom occur alone but rather in mixtures. However, such studies must be undertaken if an understanding of plant responses to ambient pollutants in the field is to be gained.

CHAPTER TWO

EXPERIMENTAL PROCEDURE

2.1 EXPOSURE SYSTEM DESIGN

2.1.1 Chamber Design

Initial work involved the building of two exposure chambers, one for use as a control for the treatment of plants with 'clean' air and one for use as a pollutant chamber in which plants were treated with air containing sulphur dioxide. The chambers had to be reasonably airtight and allow light of sufficient quality and quantity to pass through for maximum plant photosynthetic activity to occur. The chambers were constructed from perspex (polymethyl methacrylate) cylinders 32 cm in height and a radius of 13 cm. Two perspex sheets were glued to the ends of each cylinder using 'Tensol' cement No.12 (dichloromethane mixture) rendering the seals air tight. Perspex was used as it does not significantly alter the quality of light passing through it. The cylindrical shape reduced the 'dead space' volume characteristic of the more conventional cuboid chamber design and thus ensured more efficient mixing of the air flowing through the chambers (Fig. 2.1 & Plate 2.1). The chamber size enabled a maximum of two air changes per minute to be achieved and was sufficient to accommodate one shoot of Vicia faba at the four pairs of leaves stage. These characteristics permitted immediate measurement of plant responses via measurement of the inlet and outlet air composition.

A hole was cut into the bottom of each chamber 8 cm in diameter and sealed with a rubber bung. A small hole was drilled in the centre of each bung and the bungs cut into half, the holes were lined with closed cell sponge allowing the two halves to be fitted around the base of the stem of the plant thus sealing the shoot in the chamber. This technique differed from that of Black & Unsworth (1979a) who placed the whole plant in the chambers with the pots and roots contained in polythene bags to prevent water loss and gas exchange between the soil and the plant roots and the chamber air. However, this was found to result in plant stress, possibly resulting from root over-heating if plants were kept in the



Figure 2.1. Schematic of Plant Exposure Chamber.

<u>KEY</u>

A =	Air inlets	E = Air outlets
B =	Fan	F = Perspex chamber
C =	Rubber bung	G = Support block
D =	Shielded thermocouple	H = Leaf thermocouple



Plate 2.1. Plant Exposure Chamber chambers for long periods. Stressed plants have been shown to produce ethylene (Abeles & Rubinstein, 1964) which may have a significant effect on the nature of plant responses to sulphur dioxide. These problems of root over-heating were overcome in this study since only the plant shoot was placed in the chamber allowing the plant to be watered normally and to remain unstressed for periods longer than four days.

2.1.2 Air Supply

2.1.2.1 Chamber

Five perspex tubes 3 cm in length and 0.4 cm in diameter were fitted into the sides of each chamber, three at the top and two near the bottom. The top three tubes were the air inlet pipes, the split flow encouraged good circulation and mixing of the air within the chamber, the lower two tubes were the air outlets. Having three air inlets and only two air outlets induced a slight positive pressure within the chamber thus minimising the possibility of air leakage into the chamber. The air inlet and outlets were positioned on opposite sides of the chamber. Sulphur dioxide is heavier than air and so the positioning of the air inlets and outlets in this manner, in conjunction with the internal fans, aided a uniform concentration of SO_2 within the chamber (§2.1.2.4).

2.1.2.2 Gas Exchange System

An open system of ventilation was used, which, unlike a closed system, involved no recycling of the air passing through the chambers. In an open system an air stream of known composition is passed through the chamber at a measured constant flow rate. As there is no recycling, the system does not have to be completely leak proof or require a leak free chamber. In addition, since the air flow circuit is arranged so that air is pushed rather than drawn through the chamber, slight leakage is unimportant. The open system allowed continuous recording of small rapid fluctuations and changes in plant photosynthetic rate with considerable accuracy (Sestak, Catsky & Jarvis, 1971).

This has a number of advantages over a closed system. Firstly, in a closed system the chamber, tubing, pump and gas analyser must be completely air tight and this is not always easy to achieve. Secondly, in a closed system one gas analyser is required for each chamber which was

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financially impracticable in this case. Finally, in a closed system, the concentration of gases such as H_2O and CO_2 change, either depleting or rising to concentrations that have significant physiological effects on the plant independent of the pollutant treatment. Thus closed systems may only be used accurately over small and very slow changes in carbon dioxide concentration (Sestak, Catsky & Jarvis, 1971).

Figure 2.2 and Plate 2.2 show the layout of experimental system used in this study. Air was drawn in by an air pump through a polythene tube fixed above the roof outside the laboratory and passed through a sealed 230 litre (fifty gallon) mixing tank to dampen fluctuations in gas concentration in the ambient air. An activated charcoal filter was incorporated into the air inlet line to remove the majority of sulphur dioxide from the air entering the system. After the air had passed through the pump the lines were split into two to supply both chambers. The air supply line to the control chamber (no SO₂) was connected to a second activated charcoal filter to ensure that SO₂ supplied to the pollutant chamber was not drawn back through the sample lines and into the control chamber. The supply line to the pollutant chamber was connected to a glass mixing vessel to allow SO₂ to be introduced in to the system. The air was then pushed through two flow meters in parallel for each chamber to achieve flow rates of up to 8 j min⁻¹ through the chambers.

Samples of the air supplied to both chambers were drawn off before the flow meters and passed through a dew point hygrometer and a carbon dioxide analyser for analysis of H_2O and CO_2 concentrations. All joints were rendered air tight by the use of silicon rubber resin (Silicoset, Fisons U.K.). Samples of air from the outlet lines from the chambers were drawn off and passed to the carbon dioxide analyser and the dew point hygrometer and excess air was vented outside by passing the outlet lines through the laboratory window.

2.1.2.3 Humidity Control

The humidity of the incoming air varied from day to day depending on the weather (Relative Humidity, RH, ranged from 10 to 80%) but it was possible to adjust the humidity within limits. The humidity could be increased by means of connecting the air inlet line to a water bottle placed in a water bath set at 20°C, the air being drawn over the water in the bottle and thereby increasing the water vapour concentration.



KEY

M	=	230 litre mixing tank	W = water bath	
H	=	water bottle or water bath	S = gas tight switch	
P	=	electric pump	M1 = glass mixing vessel	1
CF	=	charcoal filter	C1 = control chamber	
F	=	flow meter	C2 = pollutant chamber	
т	=	thermocouple		

Inlet and Outlet samples: to dew point hygrometer and carbon dioxide analyser. Arrows indicate direction of gas and air flow.

Figure 2.2

Schematic of the Open Gas Analysis System Used in This Study.



Plate 2.2 Experimental System. The humidity of the incoming air was decreased by passing the air inlet line through a cold water bath (4°C) and incorporating a collecting bottle to trap the condensation so formed. Using this system the relative humidity could be maintained between 50-70%.

2.1.2.4 Fans

In order to ensure adequate mixing of the gases within each chamber a small electric fan was fitted into the sides of both chambers directly below the air inlet tubes. The fans were powered by a small motor fixed to the outside chamber wall connected to a power pack. Fan speed could be altered by reducing or increasing the voltage from the power packs. The fans were also necessary to lower leaf boundary layer resistance of the plants within the chamber. A model of copper wire and green blotting paper leaves was used to measure aerodynamic resistances with varying fan speeds and air flow rates. It was necessary for the boundary layer resistances (r_a) to be small in comparison to resistances within the leaf as high r_a values have been shown to reduce rates of net photosynthesis (Pnet) and transpiration (E) and thus affect the rate of pollutant uptake by the plant (Black & Unsworth, 1979a).

The aerodynamic resistance in the control chamber was 0.32 s cm⁻¹ whilst that of the pollutant chamber was 0.6 s cm⁻¹. This discrepancy in values was a function of the voltage available from both power packs set at minimum levels and both values were considered to be low enough not to influence gas exchange between the plants and the atmosphere.

2.1.2.5 Sulphur Dioxide Supply

Sulphur dioxide was supplied to the pollutant chamber from a cylinder (100 vpm in nitrogen) through teflon tubing to a flow meter and entered the air inlet supply to the chamber via a small glass mixing bottle. Teflon was used as it has been shown to have a negligible uptake of SO_2 (Garland, from Black & Unsworth, 1979a). Concentrations of SO_2 from 0 to 1000 ppb could be achieved in the chamber. The concentration of SO_2 in the air entering and leaving the chamber was monitored by drawing off samples of the air directly before entering and immediately after leaving the chamber through teflon tubing connected to the SO_2 analyser, which was in turn connected to the SO_2 analyser via an air tight valve (Whitey,

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SS 41XS 2 with Swagelock fittings) facilitating the switching of the air supply to the SO₂ analyser to monitor either SO₂ entering or leaving the chamber. The concentration of SO₂ entering the chamber was controlled by two needle values at the top and bottom of the flow meter.

2.1.3 Lights

The light source was a 400 W metal halide lamp suspended from a framework above the chambers, the light quality from the lamp closely resembling that of natural sunlight (Gaastra, 1959). Maximum irradiance within the chambers at plant height was 500 μ E m⁻² s⁻¹ and was sufficient for light saturation of photosynthesis in the species used. The framework supporting the light was covered with sheets of aluminium foil to minimise light scattering and to concentrate the light on the chambers. The light intensity could be reduced by the use of neutral filters, such as muslin, placed over the top of the chambers.

2.1.4 Temperature

2.1.4.1 Measurement

The temperature within both chambers was measured by means of copper/constantan thermocouples (AWG .35). The thermocouples were placed inside each chamber through a small hole drilled in the chamber tops and were shielded by aluminium foil to prevent the heating effect of direct radiation. Leaf temperature was monitored using copper/constantan thermocouples (AWG .30) which were inserted under the lower epidermis of one of the leaves on each plant.

The four thermocouples were connected to adjacent inputs in the back of an electronic thermometer (Comark, Type 1625) which allowed the temperature in degrees celsius to be read directly for each thermocouple by means of an in-built selector unit.

2.1.4.2 Temperature Regulation

The metal halide lamp used as a light source was found to result in a rise in chamber temperature. In order to prevent the plants becoming heat stressed during the exposure period, a perspex water bath was built into the framework directly under the light source. The water acted as an infra-red filter and reduced the amount of radiant heat from the metal halide lamp reaching the chambers. The water bath was connected to the mains water supply to give a continuous flow of water through the bath during the experimental period. An electric fan was fitted to the framework between the water bath and the tops of the exposure chambers to further reduce the amount of heat reaching the chambers.

Chamber temperature was 23 ± 3 °C throughout the year; leaf temperature was never more than 1 °C above that of the chamber thus indicating that the plants were not heat stressed.

2.2 GAS ANALYSIS

2.2.1 Water Vapour Content (Humidity)

Water loss from plant leaves within the exposure chamber alters the water vapour content of the air as it passes through the chamber. This change in water vapour content can be used to calculate the rate of transpiration and stomatal resistance is. the degree to which the stomata are open. The equations used in this calculation are presented in §2.3. The humidity of the air entering and leaving both the control and the pollutant chamber was monitored using a dew point hygrometer (General Eastern System 1100DP). The readings from the dew point hygrometer were converted to water vapour pressure in millibars with the use of tables.

Polythene tubes leading from the air inlets and outlets of both chambers were connected to the dew point sensor and the air flow through the sensor was switched from one sample to another by means of clamps. The clamps enabled an optimum flow rate of 0.5 - 1 f min⁻¹ to be directed over the dew point sensor.

It was necessary to sheath the polythene tubes leading to the sensor with insulating foam to prevent condensation forming in the pipes, leading to spurious readings.

The transpiration rate and stomatal resistance were calculated using the difference in water vapour pressure of the air entering and leaving the chambers with knowledge of the aerodynamic resistance, leaf & chamber temperature, leaf area and the rate of flow of air through the chamber (\$2.3).

2.2.2 Carbon Dioxide

The difference in carbon dioxide concentration of the air entering and leaving both chambers was needed for calculation of net photosynthetic and dark respiration rates. This was measured using an infra-red gas analyser (ADC 225 Mk3) set in the differential mode. The air sample lines were connected to the analyser via a gas sampler unit (ADC WA161). The CO_2 analyser was in turn connected to a chart recorder which had been calibrated previously.

The chart recorder was calibrated by taking repeated readings from the analyser scale and marking the distance moved by the pen on the chart recorder. A regression equation relating changes in carbon dioxide concentration (ppm) to distance moved by the pen from a central zero was calculated (r = 0.997, p < 0.001). Thus changes in carbon dioxide concentration could be calculated via measurements from the chart recorder trace. The system could be left in operation overnight allowing measurements of dark respiration rates to be made.

The analyser was calibrated every week using span gas from a cylinder (300 ppm CO_2 in air). When set in the absolute mode, the analyser gave the absolute concentration of CO_2 in the sample air from 0 to 500 ppm. However, for the experiments in this study, the analyser was used in the differential mode giving the difference in ppm CO_2 between the reference air and the samples supplied over a range of +25 to -25 ppm.

The CO₂ concentration of the ambient air entering the control chamber was used as the reference supply to set the analyser to zero when in the differential mode. The CO₂ concentration of the ambient air varied throughout the day and also from day to day about a mean value of 330 ppm (\pm 20 ppm). These fluctuations were unimportant when the analyser was operating in the differential mode as only the difference in concentration of CO₂ between the incoming and outgoing air flow was being measured, enabling the calculation of net photosynthetic and respiratory rates.

When SO_2 is added to the air line to the pollutant chamber the CO_2 concentration of the air is reduced relative to that of the control. It was therefore necessary to monitor the difference in CO_2 concentration of the two air inlet supplies to the chambers when SO_2 was in use.

The gas sampler unit had the capacity to compare six samples to a reference supply, switching from one to another every five minutes. It was Experimental

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only necessary to use three sample inlets in this study. The air inlet to the control chamber was used as the reference supply and was compared in turn with the air inlet to the pollutant chamber, the air outlet from the control chamber and the air outlet from the pollutant chamber.

In five minute cycles, the sampler switched the air supply to the CO_2 analyser allowing the comparison of each of the three samples, in turn, to the reference supply and gave the CO_2 depletion in ppm. The analyser was connected to a chart recorder and left running for the duration of the plant exposure period (up to three days).

Net photosynthetic (Pnet) and dark respiration rates (*Rd*) were calculated with knowledge of change in CO_2 concentration, leaf and chamber temperature, leaf area and the rate of flow of air through the chambers. The equations used in the calculation of Pnet and *Rd* are presented in §2.3.

The flow rate of air through the chambers was regulated to ensure that depletion of CO_2 from the air passing through the chamber was never more than 25 ppm as this was beyond the range of the analyser and, more importantly, a greater depletion of CO_2 could lead to alteration in plant responses to SO_2 . As significant changes in CO_2 concentration have been shown to alter photosynthetic rate and leaf resistances to gas exchange, the responses of the plants to low CO_2 concentrations rather than SO_2 would be observed. The importance of CO_2 concentration and leaf resistances to gas exchange when considering plant pollutant responses are discussed in §3.7.

2.2.3 Sulphur Dioxide

Sulphur dioxide concentrations were measured using a Meloy labs. SA 285E sulphur dioxide analyser. To measure sulphur dioxide fluxes to the plant it was necessary to monitor the concentration of SO_2 in both the air entering and leaving the pollutant chamber. This was done initially without the plant in the chamber to calculate the amount of SO_2 adsorbed on to the walls of the perspex chamber at varying incoming SO_2 concentrations. The data obtained from this experiment are shown in Figure 2.3. Regression analysis of the data shows a quadratic relationship between ambient SO_2 concentrations and chamber adsorption, r = 0.942 (p < 0.001). This plot was used to determine the degree of adsorption of SO_2 onto the chamber walls for each experiment performed.



Figure 2.3. The Relationship Between Sulphur Dioxide Concentration in the Exposure Chamber and SO₂ Adsorption onto Chamber Walls (r = 0.942, p < 0.001).

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The flux of SO_2 to the plant could then be calculated with knowledge of the SO_2 content of the air entering and leaving the chamber, the amount of SO_2 adsorbed onto the chamber walls, leaf area and flow rate through the chamber. The equations used in the calculation of SO_2 fluxes to the plant are presented in §2.3.

The sulphur dioxide concentrations of the air entering and leaving the pollutant chamber were measured as described in 92.1.2.5. The SO₂ analyser was connected to a chart recorder in order to record SO₂ concentrations throughout the duration of the exposure period. The system was run predominantly with the analyser measuring the SO₂ concentration of the air leaving the chamber and was switched manually every thirty minutes to monitor the sulphur dioxide concentration in the incoming air for five minute periods. It may be assumed that, in a well mixed chamber, the SO₂ concentration of the air surrounding the plant is approximately that of the air leaving the chamber. This concentration was maintained close to the predetermined level throughout the duration of each exposure period.

The SO₂ analyser was calibrated regularly using sulphur hexafluoride which is not readily absorbed onto the surface of the chamber or the teflon tubing. The fine flow meter controlling the pollutant entry into the air supply to chamber 2 was first calibrated using a burette and a soap bubble to give accurate measurement of flow rate in cm³ min⁻¹. The concentration of sulphur hexafluoride in the span cylinder was known (100 vpm in nitrogen) and with knowledge of the air flow rate into into the chamber and the flow rate of sulphur hexafluoride into the system, the actual concentration in the chamber was calculated. This value was then compared with the reading on the analyser and the meter adjusted where necessary. The analyser was first set to zero using air passed through a fresh charcoal filter then the concentration of sulphur hexafluoride was varied from 10 to 700 ppb and the meter reading on the analyser checked for each gas concentration.

The experimental protocols for plant material and growth conditions for each environmental regime are presented in \$3.3 and \$4.3.

2.3 CALCULATION OF GAS EXCHANGE PARAMETERS

2.3.1 Evaporation Rate (Transpiration Rate)

The evaporation rate, E, of water vapour through the stomata in the leaf was calculated from the following equations:

$$E = \frac{FR \times \Delta C}{LA}$$
(2.1)

where, FR is the flow rate through the chamber, LA is leaf area and ΔC is the change in the water vapour content of the air entering and leaving the chamber.

To calculate E in the appropriate units of g H₂O m⁻² h⁻¹, the following measurements are required:

- (i) $C_e H_2 O$, water content of the air entering the chamber system. This is recorded as dew point temperature and converted to millibars (mb) by referring to saturation vapour pressure over water tables, (e_e)
- (ii) C_0H_2O , water vapour content of air leaving the chamber, also converted to millibars, (e₀)
- (iii) T_a, temperature of ambient air ie. chamber temperature measured in °C and converted to Kelvin by adding 273.3
 (iv) T_L, leaf temperature, converted to Kelvin
- (v) FR, Flow rate of air through system, measured in 1 min⁻¹
- (vi) LA, leaf area measured in cm²

Evaporation rate (g H₂O m⁻² h⁻¹) is then expressed as:

$$E = \frac{e_{s} - e_{e}}{T_{a}} \times 217 \times 10^{-4} \times FR \times 10000 \times 3600 \times 1000}{60 \times LA}$$
(2.2)

The conversion factors involved are:

- (i) multiplication by 217×10^{-6} converts millibars to g cm⁻².
- (ii) multiplication by 1000 converts litres to cm³.

(iii) × 10,000 converts from cm^{-2} to m^{-2} .

(iv) × 3600 converts from seconds to hours.

A shorter version of equation 2.2 can be achieved as follows:

Since $217 \times 10^{-4} \times 10,000 \times 3600 = 7812$ therefore,

 $= \frac{e_{\circ} - e_{\circ}}{T_{a}} \times \frac{FR \times 1000}{60 \times LA} \times$

Evaporation rate, E = transpiration rate, T.

Е

2.3.2 Calculation of Stomatal Resistance, rs

The passage of water vapour transfer from the leaf is proportional to the resistances encountered such that:

$$E = -\frac{\Delta C}{\Gamma_1}$$

where r_t is the total resistance to water vapour transfer and r_t is composed of the sum of the aerodynamic and stomatal resistances to water vapour transport ($r_a + r_s$). ΔC is the difference between the water vapour concentration (wvc) inside the leaf, assuming saturation at leaf temperature (χ_i), and the water vapour concentration outside the leaf (χ_e).

$$\chi_{0} = \frac{e_{a} (T_{a}) \times 217 \times 10^{-6}}{T_{a}}$$
(2.5)

where e_{a} is the ambient water vapour content in the chamber air in millibars calculated from

and T_A is the ambient temperature in Kelvin.

(2.3)

(2.4)

7812
The water vapour concentration inside the leaf, χ_i , is calculated from:

$$\chi_{i} = \frac{e_{s} (T_{L})}{T_{i}} \times 217 \times 10^{-4}$$
(2.6)

where e_s is the water vapour content inside the leaf assuming saturation at leaf temperature, T_L .

 $\chi_i - \chi_o$ is then the concentration gradient of water vapour in g cm⁻² between the saturated leaf interior and the chamber air. If,

$$E = \frac{\chi_i - \chi_o}{r_i}$$
(2.7)

then,

$$\mathbf{r_t} = \frac{\chi_i - \chi_o}{E} \tag{2.8}$$

Both E and $\chi_i - \chi_0$ are known and if E is expressed in g cm⁻² s⁻¹ and both χ_i and χ_0 are expressed in g cm⁻², then the total resistance to gas transfer, r_t, (equation 2.8) is expressed in s cm⁻¹.

For water vapour, total leaf resistance to transfer through the stomata is equal to the sum of the aerodynamic resistance, r_a , and the stomatal resistance, r_s .

The aerodynamic resistance, r_a , is calculated using model leaves of green blotting paper which have no internal resistance to water vapour transfer. Hence, r_s can be calculated from:

$$\mathbf{r}_{s} = \mathbf{r}_{t} - \mathbf{r}_{a} \tag{2.9}$$

The stomatal conductance is the reciprocal of resistance such that,

$$g_s = 1 / r_s$$
 (2.10)

the units for stomatal conductance, g_s , being cm s⁻¹.

2.3.3 Calculation of Net Photosynthetic Rate, Pnet

Net Photosynthetic rate, Pnet, is calculated as follows:

$$Pnet = \frac{FR \times \Delta CO_2}{LA}$$
(2.11)

where, FR is the flow rate of air through the chamber (1 min⁻¹), LA is the leaf area (cm²) and ΔCO_2 is the difference in the carbon dioxide concentration of the air entering and leaving the chamber (ppm).

In order to express Pnet in the appropriate units of g CO_2 m⁻² h⁻¹, equation 2.11 becomes:

Pnet =
$$\frac{\Delta CO_2 \times 0.00183 \times 60 \times 1/1000 \times FR}{LA \times 1/10000}$$

The conversion factors employed are:

(i) ppm CO₂ × 0.00183 gives g m⁻³.
(ii) minutes to hours, × 60.
(iii) cm⁻² to m⁻², × 1/10000.
(iv) litres to m⁻³, × 1/1000.

Equation 2.12 may be shortened to:

Pnet = $\frac{\Delta CO_2 \text{ (ppm)} \times 1.83 \times 60 \times FR \text{ (} I \text{ min}^{-1}\text{)}}{LA \text{ (cm}^2) \times 100}$ (2.13)

 $= g CO_2 m^{-2} h^{-1}$.

2.3.4 Calculation of Sulphur Dioxide Fluxes to the Plant

Sulphur dioxide fluxes to the plant were assessed in two ways. Firstly, flux was determined from mass balance calculations giving the rate of uptake of sulphur dioxide per unit leaf area, this value for flux being termed, measured flux, P_{fmeas}.

(2.12)

(2.14)

Thus,

$$= \frac{\Delta SO_2 \times FR}{LA}$$

PFREAS

where, FR is the flow rate of air through the chamber (1 min^{-;}), LA is leaf area (cm²) and ΔSO_2 is the change in gas concentration of the air entering and leaving the chamber, corrected for adsorption onto the chamber surface (ppb).

 $\Delta SO_2 = S_i - S_o - S_{sor}$

where, S_i is the SO_2 concentration of the air entering the chamber measured in ppb, S_0 is the concentration of SO_2 in the air leaving the chamber and S_{sor} is the amount of SO_2 adsorbed onto the walls of the chamber, this latter value being pre-determined.

To convert from ppb to μg m⁻³, SO₂ concentration is multiplied by 2.86. Thus,

 $P_{fmeas} = \frac{\Delta SO_2 \ (\mu g \ m^{-3}) \times FR \ (1 \ min^{-1}) \ \times \ 1/60 \ \times \ 1/1000}{LA \ (cm^2) \ \times \ 10^{-4}}$ (2.15)

= flux in $\mu g m^{-2} s^{-1}$.

Conversion factors employed convert from minutes to seconds, litres to cubic metres and cm^2 to m^2 .

The flux measured this way comprises both deposition of SO_2 onto the leaf surface and into the leaf. If the former deposition is large this method will significantly over estimate the amount of pollutant entering the plant.

Secondly, sulphur dioxide flux may be calculated by analogy to water vapour diffusion and is defined as the ratio of the atmospheric sulphur dioxide concentration to gas phase resistance to SO_2 . This value for flux is termed, calculated flux, P_{fcalc} Flux is given as:

$$P_{FCALC} = \frac{C - C_0}{r_a + r_s + r_i}$$

(2.16)

where, C is the sulphur dioxide concentration at the leaf surface ($\mu g m^{-3}$), C₀ is the sulphur dioxide concentration inside the leaf ($\mu g m^{-3}$), r_a aerodynamic resistance to SO₂ transfer (s m⁻¹), r_s is the stomatal resistance to SO₂ transfer and r_i is the internal leaf resistance to SO₂ uptake.

It is assumed in equation 2.16 that sulphur dioxide is rapidly oxidised to sulphite on the walls of the substomatal cavity by dissolving in the surface water present on the cell walls. This sulphite is thought to be converted to sulphate at the same rate at which it was formed. Therefore C_0 and r_i are assumed to be negligible (Unsworth, Biscoe & Black, 1976).

Both r_a and r_s have previously been determined for water vapour transfer and corrections for differing molecular diffusivities of the gases through the stomatal pore must be made to determine r_aSO_2 and r_sSO_2 . Resistances are inversely proportional to molecular diffusion coefficients such that:

 $r_{a}SO_{2} = 1.57 r_{a}H_{2}O$

$$r_{4}SO_{2} = 1.98 r_{4}H_{2}O_{2}$$

and

(Unsworth <u>et al.</u>, 1976).

Therefore equation 2.16 becomes:

$$F_{fcalc} = \frac{C}{(r_a H_2 O \times 157) + (r_s H_2 O \times 198)}$$

where r_a and r_s are measured in s cm⁻¹ and C is the concentration of SO₂ perceived by the plant ($\mu g m^{-3}$).

Surface deposition of SO_2 is not a factor considered in the estimation of P_{FCALC} , only flux into the stomatal cavity is measured and flux is regarded as being proportional to water loss through the same aperture. This measure of flux, ideally, requires the substomatal cavity to be a perfect sink for SO_2 and the assumption that the presence of SO_2 does not influence the stomatal mechanism (Fowler, 1985). It also assumes insignificant SO_2 transport via cuticular pathways.

(2.17)

2.3.5 Computer Calculation of Gas Exchange Parameters

Calculation of all the parameters outlined in this section was simplified by the use of a specially adapted computer programme which enabled these calculations to be performed directly and speedily. The programme is presented in the Appendix.

2.4 STATISTICAL ANALYSES OF DATA

All linear regressions and correlations were performed on the BBC microcomputer using 'MICROTAB' statistics package. All polynomial regressions were performed using 'AMSTAT3' on the Amstrad 8256 PCW. Multivariate Analysis of Variance was performed on the Honeywell Multics System using 'GENSTAT'. Analysis of covariance tests were performed using the data obtained from 'MICROTAB' and 'AMSTAT3' and all significance levels were obtained from statistical tables (Murdoch & Barnes, 1974).

3.1 INTRODUCTION

The effects of sulphur dioxide on plants were first reported in the late nineteenth century and there is, therefore, a great deal of existing literature which has often been reviewed. Many researchers have concentrated on visible injury symptoms such as leaf necrosis and agree that these occur at high pollution concentrations (Ting & Dugger, 1968; Bell & Clough, 1973; Tingey, Fites & Wickliff, 1973; O'Connor, Parbery & Strauss, 1974; Murray, Howell & Wilton, 1975; Crittenden & Read, 1978; Sanders & Reinert, 1982a,b). However, there have been many conflicting reports over the years with regard to the validity of the theory of hidden or non-visible injury to plants from sulphur dioxide which was proposed initially by Sorauer & Ramann in 1899 and Wislicenus in 1901. Wislicenus held the opinion that SO_2 in the absence of visible symptoms caused an inhibition of the assimilatory processes of plants and poisoned other cellular functions, these physiological effects forming the basis of hidden injury. He further suggested that "... if one accepts that hidden injury does occur then its presence must be acknowledged wherever atmospheric pollutants can be detected ... ".

However, Haslehoff & Lindau (1903) concluded that there was no need for the term "invisible injury" as even anatomical changes not visible to the naked eye could be detected under the microscope. These workers suggested that it was not possible to extrapolate from cellular effects to growth reduction as growth reduction must be determined by actual measurements ie. depression in net photosynthetic rate due to pollutants can not be taken as an indication that yield reductions will result.

In later years the strongest opponents of the theory of hidden injury were Katz and Thomas. Thomas (1943, 1951 & 1956) extended the study of hidden injury, from data on growth and yield, to cover the effects on photosynthesis under low SO_2 fumigations and concluded that where there was no visible damage, there was no hidden injury. This result was verified by Katz (1949) and his co-workers (Katz <u>et al.</u>, 1939 a,b) in studies that included measurements of carbohydrate and protein levels. When no invisible injury was detected Katz stated "...it is hoped that the 'invisible injury' theory has now been disposed of once and for all time and will not be resurrected again in problems involving sulphur dioxide damage to plant life". This statement has proved to be somewhat premature in the light of current research studies. Katz suggested a threshold concentration of less than 300 ppb SO_2 with continuous exposure as not being detrimental to plant life, whereas Thomas suggested a threshold of less than 450 ppb for 4 h daily fumigation.

McCune, Weinstein, Maclean & Jacobsen in 1967 reviewed the current literature concerning plant injury in response to gaseous pollution and concluded also that the term 'hidden injury' should be abandoned.

Therefore, from the early 1950's to the 1970's very little research was carried out on sulphur dioxide effects on crops and forest species. This was due largely to the work of Thomas and of Katz and the generally accepted perceptions that the effects of SO_2 were understood (Heck, Heagle & Shriner, 1986). However, Bleasdale published work in 1952 (a,b) showing that low concentrations of SO_2 (100 ppb for 192 days) caused a reduction in the dry matter production of <u>Lolium perenne</u> CV. Aberystwyth S23 in relation to unpolluted plants. No visible signs of damage were detected but this work received little attention until the early 1970's when the data were republished (Bleasdale, 1973).

Since then more work has been done to examine the effects of low concentrations of air pollutants on plants in the absence of visible injury, on growth and yield effects and more specifically, the effects on gas exchange mechanisms (eg. Ziegler, 1973, 1975; Ashenden & Mansfield, 1978; Bell, 1980; Tingey & Reinert, 1975; Suwannapinunt & Kozlowski, 1980; Buckenham, Parry & Whittingham, 1982) and there is now deemed to be irrefutable evidence for the existence of 'invisible injury' in plants.

The definition of direct effects of SO_2 on gaseous exchange mechanisms was made possible with the development in 1933 of new infra-red gas analysers (IRGA's) which could continuously, automatically and directly measure CO_2 concentrations. IRGA's were used initially to measure the respiratory and photosynthetic rates of unpolluted plants grown under field conditions (Thomas & Hill, 1937; Egle & Ernst, 1949; Thomas, 1951 & 1956; Sestak, Catsky & Jarvis, 1971) but in the 1970's their use was extended to monitor the direct action of air pollutants on CO_2 exchange on plants. This was an important advance as carbon dioxide fixation proved to be a process of primary importance in pollution studies. This process, which is known to provide 90 - 95% of plant dry weight (Zelitch, 1975), was regarded as one of the initial sites of action of gaseous pollutants on plant metabolism, and inhibition of photosynthetic rate was thought to be one of the prime explanations of SO_2 -induced reductions in plant growth (Ziegler, 1973). However, more recent studies have recognised that pollutant interference with assimilate partitioning and transport may be of equal importance in determining pollutant induced reductions in plant growth (eg. Noyes, 1980; Freer-Smith, 1985; Pell, Pearson & Vinten-Johansen, 1988; Marie & Ormrod, 1988). Interference with assimilate distribution can result in reduced export to plant roots, leading to a decrease in root:shoot ratio thus having severe effects where water supplies are limited. Similarly, reduced export of assimilates to developing fruits would contribute to decreased fruit and seed yield (TERG, 1988).

Numerous reports have been made as to the effects of sulphur dioxide on net photosynthesis, respiration, transpiration and growth and yield in plants. There have been several comprehensive reviews published (Mudd, 1975; Hällgren, 1978; Heath, 1980; Black, 1982; Heck <u>et al.</u>, 1986).

However, despite these advances, it is still difficult to define the exact effect of sulphur dioxide on photosynthetic activity in plants as there is a wide range of conflicting data in the literature. It has been shown several factors, including environmental parameters are important in governing photosynthetic response to SO_2 (Ashenden & Mansfield, 1977; Matsuoka, 1978; Davies, 1980; Jones & Mansfield, 1982). More especially, research over the last four years has highlighted the significance of the interaction between environmental parameters and plant pollutant responses (CEC/COST, 1986; TERG, 1988) and this topic will be dealt with more fully in the following Chapter (S4.1).

In addition, different experimental protocols have been used to study the effects of SO_2 on photosynthesis on a wide range of plant species and the results obtained are very variable and may arise from the range of sulphur dioxide concentrations used, the differing lengths of the fumigation periods and the different plant species. Table 3.1 gives a brief summary of selected studies on photosynthetic responses to sulphur dioxide. Generally, photosynthesis was inhibited in all plants at the SO_2 TABLE 3.1

The Effects of Sulphur Dioxide on Net Photosynthetic Rates

Test Plant	Exposure Characteristics	Degree of Inhibition	Reference
<u>Vicia faba</u> L. CV. Dylan	0,35,87.5,175, 320,500 & 700 ppb upto 3 days.	upto 40% (dark resp. enhanced).	Black & Unsworth 1979b
<u>Glycine</u> max.	117,300,786 ppb 24 × 4.5 h	high, 53% medium, 17% low, -18%	Muller Miller & Sprugel 1979
<u>Diplacus</u> <u>aurantiacus</u> <u>Heteromeles</u> <u>arbutifolia</u>	96 ppb 8 h 1710 ppb 8 h	73% 40%	Winner Mooney 1980a,b
<u>Phaseolus</u> <u>vulgaris</u>	0.1 ppm 1.0 ppm 3.0 ppm	0% 13% 73%	Noyes 1980
<u>Glycine max.</u>	0.25 ppm 0.50 ppm 0.75 ppm	-18 to -28% 17-23% 17-23%	Takemoto & Noble 1982
<u>Glycine max</u> .	0.4 ppm 2 h	70%	Carlson 1983b
<u>Phaseolus</u> <u>vulgaris</u>	2.9 ppm 2 h	75%	Tch & Swanson 1982
<u>Phaseolus</u> <u>vulgaris</u>	15-1170 μg m ^{-s} 3 × 4 h 5 d a week for 4-5 weeks	25-70% when SO ₂ above 250 μg m ⁻³	Saxe 1983a
<u>Pinus</u> <u>sylvestris</u>	2-0 ppm 18 h	Inhibition of Pnet & photo- respiration (dark resp. enhanced).	Lorenc- plucinska 1983a,b
<u>Pinus</u> sylvestris	0.7 ppm, 5 h 1.33 ppm, 3 h 1.64 ppm, 3 h	25% 30% 35%	Hällgren & Gezelius 1982

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Test Plant	Exposure characteristics	Degree of Inhibition	Reference
<u>Oryza</u> <u>sativa</u>	0.5 ppm 5 h per day 21 days	None. But yield was reduced.	Matsuoka 1978
<u>Glycine max</u> .	0·2 - 1·0 ppm	10 - 80%	Carlson 1983a
<u>Vicia faba</u>	Flux: 0·1 - 2·0 µg m ⁻² s ⁻¹	up to 40%	Black 1982
<u>Pinus</u> <u>sylvestris</u>	1.0 ppm 6 h per day 3 days 2 dajent	20-124	Lorenc- plucińska 1982
(6 clones)	3 tolerant 3 susceptible	57-70% (dark resp. enhanced : sensitive clones only).	in
<u>Zea mays</u> <u>Sorghum</u> <u>yulgare</u> <u>Amaranthus</u> <u>tricolor</u>	0·1 - 1·0 ppm 5 - 8 h	20-30% at 1.0 ppm only "	Katase, Ushijima & Tazaki 1983
<u>Oryza sativa</u> <u>Avena sativa</u> <u>Helianthus</u> <u>annus</u>		60% at 0·2 ppm 90% at 1·0 ppm 100% at 0·4 ppm	
<u>Zea mays</u>	10-20 ppm 30 mins (successively)	20% only at 20 ppm!	Yamauchi Choi & Yamada
<u>Lycopersicon</u> <u>esculentum</u>	0-10 ppm 15 mins (successively)	60% only at 10 ppm	1983
<u>Pleurozium</u> <u>scheberi</u> <u>Hylocomium</u> <u>splendens</u> <u>Ptilium</u> <u>crista-</u> <u>castrensis</u> (3 feather mos	0.7, 2.7, 7 & 9.2 ppm 18 mins sses)	Inhibition. (no effect on dark resp.)	Winner & Bewley 1983
<u>Sphagnum</u> spp.	0.05 - 0.1 mM bisulphite sol.	50% in Pnet but enhancement in 14C fixation.	Fergusen & Lee 1979
<u>Vicia faba</u>	O ₃ and SO ₂ mixt. 50 - 300 ppb O ₃ ± 40 ppb SO ₂	8 - 82% dependent on 0_3 conc.	Black, Ormrod & Unsworth 1982

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concentrations used. However, several workers have reported a temporary enhancement in net photosynthetic rate (Pnet) at low SO₂ concentrations (Black & Unsworth, 1979b; Winner & Mooney, 1980c). From the table, it can be seen that the sulphur dioxide concentrations used ranged from 0.035 ppm to 20 ppm and fumigation periods varied from 18 minutes to 3 weeks. It can be seen that species differ in their sensitivity to SO₂, the magnitude and threshold of photosynthetic responses to SO₂ being extremely variable (Black, 1982), ranging from 100% inhibition in <u>Helianthus annus</u> at 0.4 ppm within 3 h (Katase <u>et al.</u>, 1983) to no observed response in <u>Oryza sativa</u> after 21 days at 0.5 ppm (Matsuoka, 1978).

One of the reasons for variability in reported response to SO₂ is that most of the published dose/response studies have been performed in specially designed plant growth chambers equipped for controlling and monitoring SO₂. The design of these chambers has been very variable, some having poor ventilation rates and velocity of air movement across the leaf surface (Mansfield, 1983). The importance of air movement across the leaf surface in relation to pollutant uptake cannot be over-stated. The boundary layer resistance ra must be overcome before a pollutant gas can enter the leaf. In the field, this resistance is usually very low in comparison with other leaf resistances and does not play a large part in governing entry of the pollutant into the plant. However, many fumigation chambers have air flow systems that do not provide the necessary wind speed over the leaf surface and as a result have high boundary layer resistances. Dose/response relationships determined under these conditions show threshold tolerances of SO₂ to be much higher than may be expected (Mansfield, 1983). The influence of boundary layer resistance on the entry of SO₂ into the plant is discussed in more detail later in this chapter (\$3.7.1). This may be one of the reasons that Katz (1949) was unable to detect any invisible injury in either barley or alfalfa in response to SO₂. Katz selected field plots and covered the field plots with a cabinet for SO_2 fumigations. As no aid to air circulation is mentioned, it is doubtful that the boundary layer resistance was low enough to facilitate any SO₂ uptake by the plants, the cabinets being an effective wind break, stopping the flow of air across leaf surfaces.

Another factor relating to variability in published dose/response data is the manner in which pollutant exposure concentration is defined (Koziol, 1980). The concentration of SO_2 in the air entering a fumigation

chamber may not be assumed to be that of the air surrounding the leaf surface. Allowances must be made for gas deposition onto the chamber and leaf surfaces and for the entry of gas into the plant. It is now more usual to assume that, in chambers with efficient air flow rates and mixing, the SO_2 concentration of the gas leaving the chamber is that at the leaf surface ie. the actual concentration perceived by the plant. Much of the information in the literature does not make clear how the pollution concentrations referred to are defined.

It is well documented that environmental factors such as light. humidity and temperature influence plant metabolism in the absence of air pollution (Gaastra, 1959; Brun & Cooper, 1967; Hofstra & Hesketh, 1969a,b & 1975; Hofstra, 1972; Downton & Hawker, 1975; Zelitch, 1975; Sharkey & Raschke, 1981; Graham & Patterson, 1982; Oquist, 1983). It may be assumed that plant responses to sulphur dioxide will also be affected by these environmental parameters as components of the resistance pathway to gas exchange will be altered. The influence of changes in the resistance pathway components on gas exchange are discussed in §3.7. Much of the published data does not give the required information detailing the conditions under which the pollution fumigations were performed. The interactions of these environmental factors on pollutant response are discussed fully in Chapter 4. It is apparent that any data relating plant responses to gas pollutant concentration must be analysed very carefully with the growing knowledge that a wide variety of factors can modify plant responses to pollutants.

The mechanisms of SO_2 action on plant metabolic processes have been investigated by many workers but there is still conflicting information as to how essential processes are affected. However, there has been general agreement that the effects of SO_2 are more damaging when the stomata are open (Juhren, Noble & Went, 1957; Majernik & Mansfield, 1970, 1971), suggesting that the stomata are the chief means of entry of SO_2 to the interior of the leaf. The behaviour of the stomata, is then, of great importance in determining the sensitivity of plants to sulphur dioxide as the diffusive resistance of the stomata is one of the major factors controlling gas exchange between the leaves and the atmosphere. Any changes in stomatal resistance as a result of SO_2 exposure may indirectly affect plant growth by changing fluxes of carbon dioxide and water vapour ie. photosynthesis and transpiration (Biscoe, Unsworth & Pinckney, 1973).

Since the early 1970's there have been many studies of the effect of sulphur dioxide on stomatal aperture, resistance (or conductance) and transpiration rates. Table 3.2 gives a brief summary of selected studies on stomatal response to SO₂. It can be seen that stomatal effects induced by SO₂ vary in both magnitude and direction. Enhanced stomatal opening or closing may occur depending on the species examined, the SO₂ concentration, the length of the exposure period and the prevailing environmental conditions (Black, 1982). The majority of workers find enhanced stomatal closure or increases in stomatal resistance and transpiration rates at high SO₂ concentrations but stomatal responses at lower gas concentrations are not usually examined (eg. Omasa et al., 1985). Those workers who examined stomatal responses at low SO_2 concentrations found either no response (Temple et al., 1985) or enhancement of stomatal opening (Majernik & Mansfield, 1970, 1971; Biscoe et al., 1973; Black & Black, 1979a,b). Furakawa et al. (1980a) studied the effects of short SO₂ funigations on 29 plant species including 25 herbaceous and 4 woody species and found significant inter-specific differences in changes in transpiration rates due to SO₂.

There is still a considerable degree of controversy about whether the effects of SO_2 on stomatal action are temporary or permanent (Black, 1982). There are many conflicting reports in the literature, some workers have found the observed stomatal responses to be reversible immediately following the removal of the pollutant (Unsworth, Biscoe & Pinckney, 1972) or a return to pre-fumigation levels after several days or hours (Majernik & Mansfield, 1970 and 1971). During long term fumigations at low SO_2 concentrations, recovery to pre-fumigation levels has been observed by some authors, during the exposure period (Bell, Rutter & Relton, 1979; Winner & Mooney, 1980c).

The effects of sulphur dioxide on transpiration and stomatal conductance have been reviewed several times, Ziegler (1975), Hällgren (1978), Heath (1980) and Black (1982) give comprehensive reviews on the then current literature. All researchers agree that environmental factors such as humidity (Black & Unsworth, 1980) or carbon dioxide concentration (Carlson, 1983a) have a profound effect on stomatal responses to pollutants, and the prevalent environmental conditions must be considered when comparing responses to SO₂ observed by different workers.

While it is now recognized that variation in plant response may

TABLE 3.2

The Effects of Sulphur Dioxide on Stomatal Resistance

Test Plant	Exposure Characteristics	Response	Reference
<u>Vicia</u> <u>faba</u>	0.25 - 1.0 ppm continuous	Stomatal opening, Recovery after 6 h but not 3 d.	Majernik & Mansfield 1970
<u>Vicia faba</u> CV. Windsor Harlington	0.25 - 1.0 ppm continuous	Stomatal opening early in photoperiod at 0.25. At 1.0ppm, stimulation of opening continues into dark period.	Majernik & Mansfield 1971
<u>Vicia faba</u> CV. Great Green Longpod	25 - 500 ppb	Stomatal opening, r _s down by 20%. Minimum response at 25 ppb. E up by 23%	Biscoe, Unsworth & Pinckney 1973
<u>Vicia faba</u> CV.Dylan	17·5 - 175 ppb 2 h	Stomatal opening, r _s down 20-25%. (Destruction of epidermal cells.)	Black & Black 1979a
<u>Vicia faba</u> CV. Dylan	175 ррb 2 h	rs down 20%	Black & Black 1979b
<u>Vitis</u> labrusca	0.5 ppm SO₂ 0.5 ppm O₃ 0.5 ppm SO₂ + 0.5 ppm O₃	rs up 30% rs up 190% rs up.	Rosen, Musselman & Kender 1978
<u>Arachis</u> <u>hypogea</u> <u>Lycopersicon</u>	2.0 ppm 2 h "	10 - 50% reduction in E within 20 mins. """	Kondo & Sugahara 1978
<u>Raphanus</u> <u>sativus</u> <u>Spinacia</u>	41 10	gradual decline in E.	·
<u>oleracea</u> <u>Perilla</u> <u>frutescens</u>	10	E declined only after 70 mins.	
<u>Acer</u> <u>saccharinum</u> <u>Robinia</u> <u>pseudoacacia</u>	0.75 ppm 2 - 16 h "	Increase in E, the extent of increase lessening with length of exposure period.	Suwanna- pinunt & Kozlowski 1980

Test Plant	Exposure Characteristics	Response	Reference
<u>Vicia faba</u> <u>Raphanus</u> <u>sativus</u> <u>Phaseolus</u> vulgaris	17 – 350 ppb 35 ppb "	gs down 20 - 30% gs increased, regardless of vpd.	Black & Unsworth 1980
<u>Nicotinia</u> tabacum	15 10	g_s increased at low vpd but g_s decreased at high vpd.	
<u>Pisum</u> <u>sativum</u>	0 - 1.8 ppm 2 h or 8 h	Stomatal closure. Degree of closure same up to 1.4 ppm. Above this, 8h more closure than for 2h.	Olszyk & Tibbitts 1981a,b
<u>Populus</u> <u>tremuloides</u> (5 clones 2 sensitive 3 tolerant)	0·2 / 0·5 ppm 8 h	0.2 ppm decreased g_s in 2 sensitive clones only. 0.5 ppm g_s down, all 5 clones.	Kimmerer å Kozlowski 1981
<u>Pisum</u> <u>sativum</u>	0·2 / 0.5 ppm 3 h 0·2 / 0.5 ppm 2 d	no effect. decrease in g _s	Rao et al. 1983
<u>Phaseolus</u> <u>vulgaris</u>	0.5 ml i^{-1} , lh 0.25 2 h 0.15 3.34 h 3 times per week for 2 weeks then 5 times a week for 2 weeks.	g: down 39% g: down slightly no effect	Temple, Fa & Taylor 1985
<u>Helianthus</u> <u>annus</u>	1.5 ppm 1 h	decrease in r _s within 10 min. Stomata closed completely after 45min Re-open after 1h.	Omasa et al. 1985
<u>Vicia faba</u> CV. Exhibition Long Pod	SO ₂ solutions in citrate buffer. 10 ⁻¹⁰ - 10 ⁻⁷ M >10 ⁻⁵ M	rs decrease rs increase	Taylor, Reid & Pharis 1981
<u>Phaseolus</u> <u>vulgaris</u> 4 cultivars (2 sensitive 2 resistant)	134 ± 10 pphm Ozone 1 h	stomatal opening stomatal closure	Butler & Tibbitts 1979b,c

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arise from environmental parameters, differing SO₂ concentrations or lengths of exposure period, it is becoming increasingly obvious, from recent literature, that differences in response to SO₂ also occur within species, in different clones or varieties of certain plants. This intra-specific variation in response to SO₂ has not been extensively studied by many researchers, although, Reinert, Heggestad & Heck (1982) compiled a comprehensive summary of cultivar screening results up to 1980. Much more work seems to have been done on the relative susceptibility of plant cultivars to ozone rather than SO₂ but, if such variation occurs in response to O₃, it is reasonable to assume that cultivars will vary in their response to SO₂.

O'Connor, Parberry & Strauss (1974) examined the relative susceptibility to acute SO_2 injury of seedlings of 131 Australian tree and shrub species widely used in urban plantings. a range of SO_2 concentrations from 0.3 to 3.0 ppm were used for exposure periods of 0.5 to 6 h. These workers developed a sensitivity scale from 0 (unaffected by SO_2) to 6 (sensitive to SO_2) based on fumigation times, SO_2 concentration and % leaf tissue destroyed by SO_2 . A wide range of sensitivities to SO_2 was found within the species examined and differences in sensitivity between seeds of the same variety, from different districts, were found. Murray, Howell and Wilton (1975) examined seventeen cultivars of <u>Poa pratensis</u> for sensitivity to ozone and sulphur dioxide and found that cultivars differed significantly in sensitivity to both phytotoxicants. However, the range of sensitivity to SO_2 was not as great as for O_3 .

The evolution of pollutant tolerance has been shown to result in differential sensitivity to pollutants (Wilson & Bell, 1985). In a recent study Gould & Mansfield (1989) examined the relative sensitivities of three cultivars of winter wheat (<u>Triticum aestivum L.</u>) to SO_2 and NO_2 mixtures; results showed the modern cultivar, Avalon to be significantly more tolerant to pollutants than two old cultivars, Little Joss and Holdfast which were introduced early in this century. These authors postulated that the mechanisms behind this differential sensitivity may be attributed to differing physical characteristics but are now exploring possible physiological mechanisms.

There have been a wide range of plant species studied for differential sensitivity to ozone and sulphur dioxide mixtures (eg. Furakawa <u>et al.</u>, 1980a). Both Beckerson, Hofstra & Wukash (1979) and Butler

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& Tibbitts (1979a,b,c) screened <u>Phaseolus vulgaris</u> using up to 33 cultivars. Sanders & Reinert screened & cultivars of <u>Azalea</u> (1982a) for sensitivity to NO₂, SO₂ and O₃, in combination or singly. A number of tree species have been studied, <u>Populus</u> spp. (Kimmerer & Kozlowski, 1981; Furakawa <u>et al.</u>, 1983), <u>Pinus</u> spp. (Lorenc-plucinska, 1982 & 1983a,b; Kress, Skelly & Hinckleman, 1982a; Kress, & Skelly, 1982). Differential sensitivity in populations of <u>Lolium perenne</u> L. have been widely investigated by a number of workers (Crittenden & Read, 1978 & 1979; Ayazloo & Bell, 1981; Ayazloo, Garsed & Bell, 1982; Ashmore, Bell & Godzik, 1984; Koziol, Shelvey, Lockyer & Whatley, 1986).

All workers have demonstrated intra-specific variability in pollutant response, although, the proposed mechanisms of this variability are unclear. Klein et al. (1978) determined that differential SO₂ uptake was partly responsible for variation in pollutant response, but biochemical mechanisms must be involved. Kondo & Sugahara (1978) also determined that less SO₂ was absorbed by resistant plants than sensitive plants and correlated low pollutant uptake with highest abscisic acid (ABA) content. Ecological responses of plant types to SO_2 have been addressed in several studies. Winner & Mooney (1980c) studied two ecologically matched Atriplex species and related greater sensitivity in the C₃ species to higher stomatal conductance (in part due to SO_2 stimulation) and to a more sensitive photosynthetic mechanism as compared to that of the C_4 species. Levitt (1972) and Taylor (1978) suggested that resistance to pollutant stress is a consequence of two general mechanisms (i) pollution stress avoidance and (ii) pollutant stress tolerance. Plant species are known to adapt to SO₂ stress (Horseman & Wellburn, 1977; Horseman, Roberts & Bradshaw, 1978, 1979; Ayazloo & Bell, 1981). From morphological and physiological studies Ayazloo et al. (1982) suggested that acute differences in injury may be primarily due to to avoidance (stomatal control), whereas differences in response to chronic injury may have a biochemical mechanism. Heck, Heagle & Shriner (1986) give a comprehensive update of the genetic factors affecting plant responses to air pollutants. Furakawa et al. (1980a) also attributed the degree of foliar injury in 29 plant species to the amount of SO_2 taken up by the plant, sensitive plants having greater SO_2 flux.

More recently, Alscher, Bower & Zipfel (1987) examined the basis for different sensitivities of two cultivars of pea to SO₂. These authors found more sulphite to accumulate in the leaves of the sensitive than in those of the insensitive cultivar and identified the relative abilities to detoxify exogenous sulphite as one of the causes of differential sensitivity; however, differential responses of photosynthetic enzymes to SO_2 were also observed. More importantly, these authors concluded that the ability to detoxify SO_2 -induced increases in hydrogen peroxide was a contributor to differential sensitivity to metabolic stresses such as SO_2 .

To understand the responses of plants to air pollution, and to compare results from different experimental systems, it is necessary to define the actual pollutant exposure that the plants receive. Much of the work carried out in observing plant responses to pollution have been related to ambient gas concentration and time (Unsworth, 1982). Recently many more researchers have concentrated on the actual amount of pollutant taken up by the plant and related this to observed injury. Unsworth (1982) reviewed the various designs of exposure systems for air pollution research showing how systems may be analysed to predict pollutant uptake and carbon dioxide exchange in plants. Taylor, McLaughlin & Shriner (1982) described the relationships between ambient sulphur dioxide concentrations and actual flux to the leaf and found that a proportional relationship did not exist between ambient SO₂ and effective pollutant dose. When SO₂ effects on net photosynthesis, dark respiration and other biochemical mechanisms are observed, it is as a direct result of sulphur dioxide entering the leaf, ie. the actual flux. Obviously, the amount of pollutant entering the plant is of prime importance because if flux is not proportional to ambient SO₂ then observed plant responses can not readily be expressed as a function of pollutant concentration when the true relationship will not be seen.

Gas phase resistance, principally at the stomata is thought to be the predominant factor limiting the diffusion of most pollutant gases, including SO₂ (Mansfield & Majernik, 1970; Winner & Mooney, 1980b). The importance of diffusive resistances in gas exchange between the leaf and the surrounding air have been determined by many workers, Gaastra (1959) and Sestak <u>et al.</u> (1971) give detailed accounts of such leaf resistances. The flux of SO₂ into the leaf can be measured from resistance analogues using Ohm's Law, relating SO₂ transfer to that for CO₂ and H₂O (Unsworth, Biscoe & Black, 1976; Black & Unsworth, 1979a,c; Taylor & Tingey, 1983; Schut, 1985). Sulphur dioxide flux to the plant may also be determined from mass balance equations where SO₂ flux is measured per unit leaf area of

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plant tissue (Unsworth, 1982). The importance of leaf resistances and pollutant flux in relation to ambient SO_2 concentration are discussed in \$3.7 and \$3.9.

It can be seen that the nature of plant responses to SO_2 are very complex. Several factors must be considered when interpreting results of plant responses as a function of ambient sulphur dioxide concentration including the prevailing environmental conditions and air flow across the leaf surface. Most importantly, actual pollutant flux into the leaves and the relationship between flux and ambient SO_2 must be considered if comparisons are to be made within or between species.

3.2 AIMS

The aim of this section of the experimental work was to establish the effects of sulphur dioxide on the gaseous metabolism of two varieties of <u>Vicia faba</u>.

The responses under optimum environmental conditions were required as a basis for comparison with pollutant responses of plants subject to additional environmental stress.

The influence of a range of sulphur dioxide concentrations on net photosynthesis, transpiration, stomatal resistance, dark respiration and pollutant fluxes to the plant for both varieties was determined.

Evidence for varietal differences in pollutant response was also investigated.

3.3 EXPERIMENTAL PROTOCOL

3.3.1 Plant Material

Seeds of <u>Vicia faba</u> L. CV. Dylan and Aquadulce Claudia were grown in plastic growth bags (10 cm diameter and 10 cm high) containing potting compost (John Innes No.2). Seeds of the variety Dylan were planted three to a pot whilst the much larger seeds of the variety Aquadulce were planted two to a pot.

The plants were placed in a Fisons environmental growth cabinet set at a constant temperature of 22°C. The photoperiod was 16 h light and 8 h dark and the photon flux density was 210 μ E m⁻² s⁻¹. The relative humidity was 70 ± 5%.

When the plants had developed three fully expanded leaf pairs, usually 10-17 days after germination, two pots of the same variety were removed from the growth cabinet. The plants in each pot were compared and two plants of similar size and development were selected for fumigation experiments, the other plants in the pot being discarded.

Leaf area was measured and the plants placed in the experimental chambers described in chapter 2, one being the control plant subject to charcoal filtered air and the other being the treated plant.

3.3.2 Exposure Chamber Parameters

The photoperiod for the experimental chambers was 14 h light and 10 h dark. Chamber temperature was 23 \pm 3°C. Leaf temperature was never more than 1°C above chamber temperature indicating the plants were not heat stressed. Photon flux density in both chambers during the light period was 500 μ E m⁻² s⁻¹ and was sufficient for light saturation of photosynthesis in the species used. Relative humidity was measured each time the chambers were in use and was controlled to maintain RH between 50 and 75%. It was necessary to control relative humidity as it is well documented that stomata respond to changes in humidity (eg. Meidner & Mansfield, 1968; Mansfield & Majernik, 1970; Black & Unsworth, 1980) and as a consequence, alter rates of carbon dioxide exchange. It was necessary to minimise the effects on the plants due to changes in humidity so that these effects would not be confused with the action of sulphur dioxide on gas exchange mechanisms.

The air flow through the chambers was closely monitored and gave between 60 and 100 air changes per hour in both chambers. Small electric fans, fitted inside the chambers, aided efficient mixing and air circulation and ensured boundary layer resistances were maintained below 0.65 s cm^{-1} for both chambers.

The plants were left in the chambers for 24 h to acclimatise. During this time the difference in carbon dioxide content of the air entering and leaving both chambers was monitored regularly (for a five minute period every fifteen minutes) in order to enable rates of net photosynthesis and dark respiration to be calculated. Air flow rates, leaf temperature, chamber temperature and water vapour concentrations of the air entering and leaving both chambers were measured every thirty minutes. These data were used to calculate relative humidity, transpiration rate and stomatal resistance.

3.3.3 Pollutant Application

After the 24 h acclimatisation period sulphur dioxide was introduced into the funigation chamber for four hours from a span gas cylinder of 100 vpm SO₂ in nitrogen. The gas was controlled by means of two needle flow valves connected to a flow meter and was added directly in to the air supply for chamber 2 via a small mixing bottle. Chamber 1 was the unpolluted chamber and the air inlet supply was passed through two activated charcoal filters before reaching the plant. The sulphur dioxide concentrations used ranged from 90 - 620 ppb and the exposure period in all cases was 4 hours.

The sulphur dioxide concentration of the air entering and leaving the treatment chamber was monitored continuously over the four hour exposure period using a Meloy SA 285E SO₂ analyser connected to a chart recorder. The flux of SO₂ to the plant was calculated using a previously established correction factor allowing for adsorption of SO₂ onto the chamber walls during the exposure period (§2.2.3).

At the end of four hours SO_2 exposure the gas was switched off and the plants left for a further 24 h to permit recovery to be monitored. After the recovery period the plants were removed from the chambers and the leaf area again measured.

3.3.4 Data Collation

The data gathered over the three day experimental period were analysed and used to calculate rates of net photosynthesis, dark respiration and transpiration. Stomatal resistances and pollutant flux into the plant were also calculated. The percent inhibition of net photosynthesis and changes in stomatal resistance in the treated plant could then be calculated in relation to the control plant.

Plants of both Dylan and Aquadulce Claudia were exposed to a range of sulphur dioxide concentrations from 0 - 620 ppb and dose/response relationships for both varieties were established.

3.4 NET PHOTOSYNTHESIS

3.4.1 Time-Response Data

Two examples of the time-response data for net photosynthesis in two varieties of <u>Vicia faba</u> exposed to either 'clean' air or air containing sulphur dioxide are shown in Figures 3.1 & 3.2. Experiments were performed, on plants of both varieties, over a range of sulphur dioxide concentrations from 92 to 620 ppb, each experiment being run with a concomitant control supplied with charcoal filtered air. It was necessary to run control plants parallel to those treated with SO_2 due to natural and diurnal variation in photosynthetic and dark respiration rates throughout the experimental period.

Figure 3.1 shows the typical data for plants of the variety Dylan exposed to 500 ppb (1430 μ g m⁻³) for four hours. At the onset of fumigation it can be seen that the rate of net photosynthesis (Pnet) decreased immediately but this was followed by an increase in Pnet 15 minutes later. This brief increase was followed by a rapid decline in Pnet which continued for the duration of the SO₂ exposure and resulted in an inhibition in net photosynthesis of 44% in relation to the control plant.

However, when the SO_2 treatment ceased, recovery of the treated plant was rapid, this beginning within 30 minutes and continuing until the end of the light period. Full recovery had occurred by the start of the next photoperiod when Pnet was not significantly different from that of the control plant.

It can be seen that during the dark period following SO_2 exposure, the respiratory rate of the treated plant was more than double that of the control plant. This increase in dark respiration reduced steadily in the early hours of the morning and by 0600 h the dark respiration rate of the treated plant had returned to pre-fumigation rates.

This response at 500 ppb for Dylan plants was typical of the responses observed at all SO_2 concentrations above 450 ppb. Below 450 ppb the responses to SO_2 followed similar trends to those already described but the magnitude of response was lessened. These data are presented in §3.4.2.





Typical time-response data for <u>Vicia</u> <u>faba</u> CV. Dylan showing rates of net photosynthesis and dark respiration (g $CO_2 m^{-2} h^{-1}$) in two plants, one control (O) and one exposed to 500 ppb SO_2 for 4 h (\bullet).





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Typical time-response data for <u>Vicia faba</u> CV. Aquadulce Claudia showing rates of net photosynthesis and dark respiration ($g \text{ CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) in two plants, one control (O) and one exposed to 400 ppb SO₂ for 4 h (\bullet).

The responses of dark respiration to SO_2 , in Dylan plants, were similar irrespective of the sulphur dioxide concentration supplied. Dark respiration responses of both varieties are discussed in \$3.5.

Figure 3.2 shows the time-responses of Aquadulce Claudia plants to 400 ppb (1144 μ g m⁻³) SO₂ for four hours. It can be seen that the rate of net photosynthesis (Pnet) declined sharply following the onset of fumigation. One hour after the start of the fumigation period the rate of decline in Pnet slowed and at the end of the four hour treatment, net photosynthetic rate in the plant had been reduced by 51% in relation to the control plant.

Recovery began soon after the end of the treatment but the plant had not reached pre-fumigation rates 24 h after the SO_2 exposure ceased.

The response described here was typical for all plants exposed to SO₂ concentrations above 400 ppb, the extent of the photosynthetic inhibition showing a positive linear correlation with SO₂ concentration. At 550 ppb, the inhibition of Pnet was 77% in relation to the control plant. At all treatments above 400 ppb, the fumigated plants had not reached pre-fumigation levels for Pnet up to 24 h after treatment. The data for photosynthetic responses to a range of sulphur dioxide concentrations are presented in §3.4.2.

In all cases, exposure to sulphur dioxide, had no observable effect on the rates of dark respiration in Aquadulce Claudia plants. These difference in dark respiration responses between the varieties are discussed more fully in \$3.5.

3.4.2 Response of Net Photosynthesis to a Range of Sulphur Dioxide Concentrations.

Both varieties of <u>Vicia faba</u> were subjected to a range of sulphur dioxide concentrations, from 0 to 620 ppb, in the manner previously described. Figures 3.1 & 3.2 showed examples of plant responses to SO_2 with time. The data from these experiments were collated enabling concentration/response relationships to be plotted for % change in net photosynthetic rate of the polluted plants in relation to control plants monitored simultaneously. It was necessary to calculate changes in net photosynthesis in relation to the control plants because of the natural and diurnal variation in net photosynthesis (Pnet) in the absence of sulphur

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dioxide. Percent inhibition in Pnet was calculated by measuring the photosynthetic rates of both the control and the treated plants immediately prior to SO_2 fumigation (C₉,P₉), and following the 4 h exposure period (C₉,P₉).

$$\% \text{inhibition} = \left(\frac{C_{e} - P_{e}}{C_{e}}\right) - \left(\frac{C_{o} - P_{o}}{C_{o}}\right) \times 100$$

Figures 3.3 and 3.4 show percent inhibition of net photosynthesis against ambient sulphur dioxide concentrations for both Dylan and Aquadulce Claudia plants. Regression analysis of the data for both varieties showed that a polynomial regression gave a closer correlation than a straightforward linear regression. For Dylan plants (Fig. 3.3) the correlation was 0.8969 ($r^2 = 80.45\%$, p < 0.001), the linear correlation having an r^2 value of 74.45\% for a total of twenty five individual experiments. For Aquadulce Claudia plants (Fig. 3.4) the correlation was 0.7763 ($r^2 = 60.27\%$, p < 0.001) the linear correlation having an r^2 value of 55.5% for a total of twenty individual experiments. The regression lines are shown in the figures.

In order to test for significant differences between the varieties in response to sulphur dioxide an analysis of covariance using pooled regression was performed. A variance ratio was calculated for both varieties individually and for the pooled data. This analysis gave an F value of 2.40 but the significant value ($\alpha = 0.05$, DF 1,42) is 4.08 showing the calculated F value not to be statistically significant. There is, therefore, no significant difference, overall, between the two varieties in their photosynthetic responses to a range of SO₂ concentrations.

It can be seen that inhibition of photosynthesis was proportional to the supplied SO_2 concentration for both varieties.

at concentrations above 400 ppb there was a marked increase in inhibition. This suggested that there was a threshold concentration of sulphur dioxide above which photosynthetic activity was even more severely limited. The precise threshold concentration was unclear, appearing to be between 400 and 500 ppb in both varieties. A more detailed conclusion concerning threshold values may be drawn when sulphur dioxide fluxes into the plant

However,





Figure 3.3. Percent Inhibition of Net Photosynthesis (in relation to control plants) in <u>Vicia faba</u> CV. Dylan in Response to a Range of Sulphur Dioxide Concentrations (ppb).

Figure 3.4



Figure 3.4.

The Degree of Inhibition of Net Photosynthesis (in relation to control plants) in <u>Vicia faba</u> CV. Aquadulce Claudia in Response to a Range of Sulphur Dioxide Concentrations (ppb).

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are examined. Pollutant fluxes in relation to sulphur dioxide concentration are described in §3.9.

Whilst no significant differences in photosynthetic response to sulphur dioxide response between the varieties were proven statistically when the data were examined as a whole, it was apparent from the figures that differences in response did occur at the extremes of the pollutant range. At concentrations of 90 - 100 ppb, Aquadulce plants (Fig. 3.4) showed an overall enhancement in net photosynthesis in response to SO_2 , up to 6% above the control plants. Dylan plants, at these concentrations showed an inhibition in net photosynthetic rate between 8 and 17%. Aquadulce plants also appeared to exhibit greater photosynthetic inhibition at concentrations above 450 ppb when the regression lines were compared.

The fact that a pooled regression analysis showed no significant differences between the varieties may be attributed to the amount of scatter within the data. In particular, the variability in the observed responses of Aquadulce Claudia plants to SO_2 concentrations between 200 -500 ppb. A measure of the standard deviation (S.D.) about the regression lines for each variety giving a value of 5.96 for Dylan and 13.92 for Aquadulce Claudia plants. This relatively large S.D. for plants of the variety Aquadulce Claudia presented some difficulty in allowing comparison of the data obtained for both varieties. The difference in observed plant responses for any given SO_2 application was not unusual and may have been due to natural physiological variation between plants of the same variety.

3.5 DARK RESPIRATION

Dark respiration rates, in the two varieties of <u>Vicia faba</u> used in this study, were monitored for both control and treated plants throughout the dark periods prior to and following sulphur dioxide exposure. Measurements of carbon dioxide enrichment of the air flowing through each plant chamber were taken automatically every fifteen minutes, enabling rates of dark respiration to be calculated as described in §2.3. A mean rate was then calculated for each plant for each dark period, from a total of between 20 and 30 data points, and the data tabulated.

There was an amount of natural variation in the dark respiration rates of plants within each variety in the absence of sulphur dioxide treatment, values ranging from 0.02 to 0.40 g CO_2 m⁻² h⁻¹. This variation







between the varieties was increased by exposure to a range of sulphur dioxide concentrations. Figure 3.5 gives a plot of dark respiration rates for both treated and control plants during the two dark periods prior to and following SO_2 treatment, for plants of the variety Dylan. The extent of the natural variability is clearly shown. It can be seen that dark respiration rates appeared to increase following sulphur dioxide fumigation and that the magnitude of the response was independent of the applied SO_2 concentration. Due to the lack of fit of any of the four plots to a straight line, regression analysis was not considered to be a satisfactory method for data analysis.

In order to differentiate between natural variation and pollutant responses, the data were analysed using pooled and twosample 't' tests; the pooled 't' tests being used for data of similar variance and the twosample 't' test being applied to data with dissimilar variance.

It was possible that the length of time in the exposure chambers could affect dark respiration rates if the plants were at all chamber stressed in the absence of SO_2 . To determine if this was so, comparisons of the rates of dark respiration in the control plants on the first and second nights in the chambers were made. Differences between the varieties were also examined.

The mean rates of dark respiration for each plant were tabulated into four groups, the control plants during each dark period and the treated plants before and after SO_2 exposure. The 't' tests were then performed between all four groups of data, the results for the analyses being shown in Tables 3.3 - 3.6.

Table 3.3 shows the results for plants of the variety Dylan. Comparison of the dark respiration rates of the control plants during the first and second nights in the chamber produced a 't' value of 0.5%0. The tabulated 't' value is 4.015 (DF = 16, α = 0.001) showing there to be no significant difference in dark respiration rates due to chamber conditions. Dark respiration rates between the control and the treated plants prior to SO₂ were also compared and no significant differences found, the calculated 't' value being 1.518 and the significant 't' value being 3.646 (DF = 35, α = 0.001).

However, when the dark respiration rates of the treated plants were compared before and after the SO_2 treatments, the 't' value obtained was -4.219 (Table 3.3), the 't' value from the statistical tables was -3.646

TABLE 3.3

Rates of dark respiration (g $CO_2 m^{-2} h^{-1}$) in <u>Vicia faba</u> CV. Dylan plants. The data for control plants and SO_2 treated plants are shown for the first and second nights of the experimental period. 't' test results are also shown.

	Control		Polluted	
	day 1	day 2	day 1	day 2
Mean	0-156	0 • 140	0.131	0.224
Variance	0.004	0.003	0.002	0.007
Maximum	0.241	0.200	0.191	0.340
Minimum	0.021	0.074	0.028	0·126
t =	0.580	ns	-4.219	sig
tabulated t =	-4.015 to 4.015		-3.646 to	3.646
significance	$\alpha = 0.001 \text{ df} = 16$		$\alpha = 0.001$	df = 30
t =	C1, P1 = 1	-518 df = 35	not signif	licant
tabulated t =	3·646 a	= 0.001	0	

TABLE 3.4

Rates of dark respiration in <u>Vicia</u> <u>faba</u> CV. Aquadulce Claudia plants. The data for control plants and SO_2 treated plants are shown for the first and second nights of the experimental period. 't' test results are also shown.

	Control		Pollut	Polluted	
	day 1	day 2	day 1	day 2	
Mean	0+259	0.225	0.265	0.236	
Variance	0.007	0.003	0.011	0.006	
Maximum	0-396	0.337	0•468	0.410	
Minimum	0 • 154	0 • 143	0 • 126	0.128	
t =	1 • 129	ns	0.821	ns	
tabulated t =	-3·707 to	3:707	-3.707 to	3.707	
significance	$\alpha = 0.001 \text{ df} = 26$		$\alpha = 0.001$	$\alpha = 0.001 \text{ df} = 26$	
t = tabulated t =	$\begin{array}{rcl} C1, P1 &= -C \\ 3 \cdot 646 & \alpha \end{array}$	•187 df = = 0•001	34 not signi	ficant	

TABLE 3.5

Comparison of rates of dark respiration (g $CO_2 m^{-2} h^{-1}$) in both varieties of <u>Vicia faba</u> on the first night of the experimental period, prior to SO_2 exposure. ['t' test results are also shown.]

Dyl an	Aquadul ce
0 • 156	0.259
0.004	0.007
0.241	0.396
0.021	0•154
-4.(071
-3.6	346
$\alpha = 0.00$	01 df = 30
	Dylan 0.156 0.004 0.241 0.021 -4.0 -3.0 $\alpha = 0.00$

TABLE 3.6

Comparison of rates of dark respiration in both varieties of <u>Vicia faba</u> on the second night of the experimental period, following SO_2 exposure. ['t' test results are also shown.]

'lan	Aquadul ce	
224	0.236	
007	0.006	
340	0-410	
126	0.128	
-0.:	353	
-3.(350	
not sign	nificant	
	224 007 340 126 -0.: -3.t not sign	Aquadul ce 224 0.236 007 0.006 340 0.410 126 0.128 -0.353 -3.850 not significant

(DF = 30, α = 0.001) showing the data to be significantly different. When the means of each group were examined, the mean rate of dark respiration in Dylan plants prior to SO₂ exposure was 0.131 g CO₂ m⁻² h⁻¹, following SO₂ treatment the mean rate was 0.224, showing dark respiration rate to be almost doubled in response to SO₂.

Table 3.4 gives the results for dark respiration rates in plants of the variety Aquadulce Claudia. Comparison of all the data for both control and treated plants before and after SO_2 treatment, showed no significant changes in dark respiration rate either in response to SO_2 or in response to chamber conditions, all the calculated 't' values being less than the tabulated values.

Tables 3.5 & 3.6 show the results obtained when dark respiration rates between the varieties were compared prior to and following SO_2 treatment. It can be seen from Table 3.5 that, prior to SO_2 treatment, dark respiration rates between the varieties were significantly different. The mean respiration rate for Dylan being 0.156 and 0.259 g CO_2 m⁻² h⁻¹ for Aquadulce Claudia plants, showing the unpolluted dark respiration rate in Aquadulce to be nearly twice the rate observed in Dylan plants.

After exposure to sulphur dioxide, comparison of the data for each variety showed no significant difference in the dark respiration rates (Table 3.6) between the varieties.

In conclusion, plants of <u>Vicia faba</u> CV. Dylan exhibited increased rates of dark respiration in response to sulphur dioxide, irrespective of the concentration of pollutant supplied; the rates increasing to nearly twice prefumigation levels. Aquadulce Claudia plants showed no apparent dark respiration response to sulphur dioxide, however, the natural (unpolluted) rate of dark respiration in Aquadulce plants was significantly higher than that observed in the variety Dylan.

It must be noted that any changes in dark respiration rate would be reflected by changes in net photosynthetic rate although gross photosynthetic rate would remain unaltered.

3.6 STOMATAL RESISTANCE

3.6.1 Time-Response Data

Figures 3.6 and 3.7 show examples of typical time responses of both varieties of <u>Vicia faba</u> to either 'clean' air or air containing sulphur dioxide.

In Fig. 3.6 it can be seen that stomatal resistance (r_s) in Dylan increased in response to a sulphur dioxide treatment of 600 ppb (1716 µg m⁻³) for four hours. For the first hour following the onset of fumigation the stomatal resistance decreased in relation to the control plant. However, there was a steady increase in r_s for the following three hours of the fumigation period. There was a great deal of natural variation in stomatal resistance throughout the photoperiod even in the absence of SO_2 , thus, all changes in r_s were expressed as percentage change in relation to the control plant (Fig. 3.8). At the end of the exposure period stomatal resistance in the treated plant had risen by 73% in relation to the control plant.

Recovery was rapid, beginning in the first hour following fumigation and had reached pre-fumigation levels by the following morning.

The stomatal responses of Dylan plants to a range of sulphur dioxide concentrations from 92 - 620 ppb are described in §3.6.2. The responses were variable (Fig. 3.8) and increases in stomatal resistance in response to SO_2 appeared to occur at concentrations above 450 ppb. Below this concentration stomatal responses differed, proving to be negative or positive in direction, 9 out of 12 plants showing a decrease in stomatal resistance in response to sulphur dioxide.

Figure 3.7 shows an example of the time response data for stomatal resistance in Aquadulce Claudia plants to either 'clean' air or air containing 500 ppb sulphur dioxide (1430 μ g m⁻³). There was no observable effect of SO₂ on stomatal resistance for the first two and a half hours of the four hour fumigation period in relation to the control plant. Again, there was a significant amount of natural variation in r₅ without the SO₂ treatment, the control plant also showing a slight increase in r₅ over the four hour fumigation period.

The stomatal responses of Aquadulce Claudia plants to a range of sulphur dioxide concentrations are shown in Figure 3.9. At concentrations


Figure 3.6.

Typical Time Response Data for <u>Vicia faba</u> CV. Dylan showing stomatal resistance (s cm⁻¹) in two plants, one control (O unpolluted) and one exposed to 600 ppb SO₂ for 4 h (\bullet).



Figure 3.7.

Typical Time Response Data for <u>Vicia faba</u> CV. Aquadulce Claudia showing stomatal resistance (s cm⁻¹) in two plants, one control (O unpolluted) and one exposed to 500 ppb SO₂ for 4 h (•).

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above 200 ppb SO₂, 10 out of 11 plants showed an increase in r_3 in response to SO₂ up to 35% above the control plants at the higher SO₂ exposures.

Analysis of the data from all the experiments showed there to be large degree of natural variation in the stomatal resistances measured, between the varieties and with or without sulphur dioxide treatment throughout the experimental period. This variability may be due in part to differences in the environmental conditions at the time of each experiment and to differences in plant age. The plants used in each experiment were of uniform size but the ages of the plants may differ between 2-3 weeks following germination. As mentioned previously, it was not possible, with the apparatus used, to control precisely the temperature and humidity on any given day. It was possible only to maintain these parameters within certain boundaries of 50 - 75% humidity and 23 ± 3°C temperature. This caused problems in data analysis and comparison and it would not be correct to state that differences in r, between the varieties in the absence of SO_2 are absolute. However, because the control plants were carefully monitored under the same conditions as the treated plants, it was possible to compare SO₂ responses in stomatal resistance between the varieties.

The small increase in r_s in Aquadulce Claudia plants in response to SO₂ contrasts sharply with the responses of Dylan plants at this concentration and above. Dylan plants showed an increase of 85% in relation to the control at 600 ppb.

Comparison of all the data for stomatal response in both Dylan and Aquadulce Claudia plants (Figs. 3.8 & 3.9) showed that, in general, Dylan plants were more responsive to higher SO_2 concentrations than Aquadulce Claudia plants. The pattern of stomatal response was similar for both varieties but it appeared that the magnitude of response was greater in plants of the variety Dylan.

The data also showed that, whereas Dylan plants responded to SO_2 within the first hour of fumigation, Aquadulce plants were slower to respond, especially at the higher SO_2 concentrations above 400 ppb. These differences in stomatal responses to SO_2 between the varieties are discussed fully in the following section.

3.6.2 Responses of Stomatal Resistance to SO₂

As stated above, there was a great deal of natural and diurnal variation in stomatal resistance (r_s) in plants in the absence of SO_2 . As a result of this natural variation, changes in stomatal resistance due to sulphur dioxide could not be expressed in terms of absolute values. In order to allow meaningful comparison of the data for each variety over a range of sulphur dioxide concentrations, changes in stomatal resistance due to SO_2 , were expressed as per cent change in relation to the control plants monitored simultaneously. The changes in r_s due to SO_2 were calculated from:

 $\frac{\text{%change } \mathbf{r}_{s} = [\mathbf{r}_{s}\mathbf{p}_{0} - \mathbf{r}_{s}\mathbf{p}_{e}] - [\mathbf{r}_{s}\mathbf{c}_{0} - \mathbf{r}_{s}\mathbf{c}_{e}] \times 100}{\mathbf{r}_{s} \text{ control}}$

where, $r_s p_0$ and $r_s c_0$ are the stomatal resistances of the polluted and control plants prior to pollutant fumigation and $r_s p_e$ and $r_s c_e$ are the stomatal resistances following SO₂ fumigation.

Using this calculation, a positive value indicated decreased stomatal resistance in polluted plants in relation to the control plants ie. stomatal opening, and a negative value indicated increased stomatal resistance ie. stomatal closure in polluted plants in relation to control plants.

The dose/response relationships between changes in stomatal resistance, r:, and applied sulphur dioxide concentration for <u>Vicia faba</u> CV. Dylan and Aquadulce Claudia are shown in Figures 3.8 and 3.9. The regression lines through the data suggest that the magnitude and direction of observed stomatal responses were proportional to applied sulphur dioxide concentration.

Plants of the variety Dylan (Fig.3.8) exhibited a variable response to sulphur dioxide concentrations between 92 and 400 ppb. Nine out of thirteen plants showed enhanced stomatal opening in response to SO_2 up to 50% in relation to the control plants. The remainder showed enhanced stomatal closure at these concentrations, between 5 and 18% above the control plants. The overall response appeared to be enhanced stomatal opening in response to SO_2 concentrations up to 400 ppb, the largest degree of opening occurring at 200 ppb. At concentrations above 400 ppb SO_2 r_s in Dylan plants was increased significantly being up to 85% Figure 3.8



Figure 3.8.

Degree of Changes in Stomatal Resistance in <u>Vicia</u> <u>faba</u> CV. Dylan in Response to a Range of Sulphur Dioxide Concentrations [positive values indicate stomatal opening, negative values indicate stomatal closure]. Figure 3.9



Figure 3.9.

Degree of Changes in Stomatal Resistance in <u>V.faba</u> CV. Aquadulce Claudia in Response to a Range of Sulphur Dioxide Concentrations [positive values indicate stomatal opening, negative values indicate stomatal closure].

greater than the control plants.

Again, there was evidence for a threshold concentration above which stomatal closure was induced by exposure to SO_2 . A classical regression line could be fitted to the data, giving an r value of 0.5751 which, according to tables, was significant at the 99.0% level. However, it was immediately apparent from a visual inspection of the data, that there was a distinct lack of fit of the data to the regression line produced. The regression, although significant, did not correctly explain the observed responses in r_1 to SO_2 . This perhaps demonstrated the point that regression analyses are not always the best way to interpret plant pollution responses. Using the regression line, the threshold concentration, above which SO_2 induces stomatal closure, was 270 ppb but visual examination put the threshold more accurately between 400 - 450 ppb. At concentrations above this, enhanced stomatal closure occurred in all plants tested.

Figure 3.9 shows the dose response relationship for plants of the variety Aquadulce Claudia. A regression line through the data gave a much closer correlation than that obtained for Dylan plants. In this case r = 0.7577 and was significant at the 99.9% level. Again a threshold concentration was indicated above which SO₂ induced stomatal closure. The regression line showed this to be 180 ppb; ten out of 11 plants exhibited stomatal closure at SO₂ concentrations above this value. The greatest concentrations resulting in the greatest stomatal closure of 34% above that of the control plant. At concentrations below 180 ppb there was an indication that r_s decreased in response to SO₂ up to 6% below the control plants.

Comparison of the data for both varieties suggested that the magnitude of stomatal response to SO_2 was much greater in Dylan plants than in Aquadulce Claudia. Responses in Aquadulce plants ranged from 6 to -34% whilst for Dylan responses ranged from 51 to -85% over the same range of sulphur dioxide concentrations.

From the data presented in this section, it was clear that for both varieties, the relationship between stomatal response and ambient sulphur dioxide concentration was not straightforward. It was hoped that an examination of actual pollutant flux to the plant and the components of the resistance pathway to gas transfer would give a clearer picture of the nature of changes in resistance in response to SO_2 . These are discussed in §3.7. 3.7 INFLUENCE OF LEAF RESISTANCES ON GAS EXCHANGE.

3.7.1 Resistance Pathways

It has been shown that sulphur dioxide altered the rates of net photosynthesis (Pnet) in the two varieties of <u>Vicia</u> faba studied. However, both varieties also exhibited changes in stomatal resistance (r_s) in response to SO₂ exposure. It was therefore necessary to determine whether the observed effects of SO₂ on net photosynthesis were due solely to changes in this resistance, or resulted from a direct action of SO₂ on the photosynthetic process itself. Photosynthetic rates may be influenced by changes in any of the components of the resistance pathway to carbon dioxide transfer and changes in these resistances may also influence SO₂ flux into the plant thus altering photosynthetic responses to the pollutant.

In order to understand the effect of sulphur dioxide on net photosynthesis it was necessary to examine the pathway of carbon dioxide transfer from the atmosphere into the leaf.

Resistance analogues are commonly used to describe gas exchange between plants and the atmosphere, the flux of a gas being regarded as driven by a potential difference using an analogy to Ohm's law. In this case the potential difference is that of the gas concentration which is limited by certain resistances to its flow (Gaastra, 1959; Sestak <u>et al.</u>, 1971; Biscoe <u>et al.</u>, 1973; Unsworth, Biscoe & Black, 1976). Flux is given as:

Flux = potential difference (gas concentration)

(3.1)

resistance

The ease with which carbon dioxide moves into a leaf (in the process of photosynthesis) is important in determining the photosynthetic rate and in determining the photosynthetic response to environmental factors. The resistances encountered by molecules of carbon dioxide in moving into the leaf, from the source in ambient air to the sink at the carboxylation sites in the chloroplasts, may be used to describe quantitatively specific physiological responses to the environment which may limit the rate at which photosynthesis proceeds (Sestak <u>et al.</u>, 1971). Humidity, CO_2 concentration, irradiation and air pollutants are some of the environmental factors that may affect photosynthesis.

The transfer pathway can be divided into a number of discrete segments characterised by position or transfer mechanism (eg. turbulent diffusion, free molecular diffusion or mass transfer). Several segments of the resistance pathway are common to water vapour transfer and carbon dioxide and sulphur dioxide transfer. Namely, aerodynamic resistance (r_a) , stomatal resistance (r_s) and cuticular resistance (r_c) . However water vapour is not considered to experience the added internal resistance to gas transfer (r_{int}) present for CO₂ and SO₂. Since resistances to water vapour transfer (rH_2O) are relatively easily determined, these can be used to derive certain of the resistances to CO₂ (rCO_2) and SO₂ (rSO_2) .

For a pathway in which transfer is by free molecular diffusion, the relationship between rH_2O and rCO_2 is the ratio of the relative molecular diffusion coefficients whereby:

$$rCO_2 = rH_2O DH_2O / DCO_2$$
 (3.2)

and D is the effective molecular diffusivity of water or carbon dioxide in air (Sestak <u>et al</u>., 1971).

Considerable uncertainty exists as to the precise values of DH_2O and DCO_2 and the nature of their temperature dependence (Lee & Wilke, 1954; de Vries & Krueger, 1967; Sestak <u>et al.</u>, 1971). Values of DH_2O / DCO_2 range from 1.5 to 2.9, the majority of the values being around 1.8 (Sestak, 1971). However, Fuller, Schettier & Giddings (1966) gave a value of DH_2O / DCO_2 equal to 1.65 which is independent of temperature and pressure.

The resistances encountered in gas exchange between the atmosphere and the leaf are shown in Figures 3.10a and 3.10b Firstly, there is the aerodynamic resistance (r_{R}) , then the stomatal resistance (r_{s}) in parallel with the cuticular resistance (r_{c}) , followed by an internal resistance (r_{int}) to gas transfer. Each of these resistances is discussed individually.

3.7.1.1 Boundary Layer Resistance, ra

The velocity of air movement about a plant increases with distance away from the leaf surface from zero at the leaf-air interface until it is indistinguishable from the bulk air movement around the leaf. The leaf boundary layer thus consists of a thin layer of air, close to the surface of the leaf, in which movement of the air is by laminar flow, and a





KEY

A = Cuticle B = Stomatal Pore C = Stomatal Cavity D = Mesophyll Cell E = Guard Cell

(from Black, 1982)

Figure 10b Resistance analogue model of leaf resistances



Resistances

 r_a = aerodynamic r_s = stomatal r_{int} = internal r_c = cuticular

(from Unsworth, Biscoe & Black, 1976).

Figure 3.10.

Resistances Encountered in Gas Exchange Between the Atmosphere and the Leaf.

transition region to fully turbulent conditions in the ambient air (Sutton, 1953; Sestak <u>et al.</u>, 1971). The thickness of the boundary layer is defined as the effective thickness across which a uniform concentration gradient, equal to that at the leaf-air interface, would have to exist to give the same total drop in concentration (Slatyer, 1967).

In the field, the boundary layer resistance, r_A , is a function of windspeed, leaf dimension and anatomy and as such is not under physiological control. In leaf chambers, r_A is also a function of chamber design and air flow rates and movement must be sufficient to simulate field conditions and efficient mixing of the air surrounding the plant.

The boundary layer resistance is in series with other resistances which are under physiological control. It is therefore, important that in plant chamber studies of the physiological response to environmental conditions, r_a should be maintained small in relation to other leaf resistances. If r_a is too large in comparison with the other resistances in the transfer chain, it will become the dominant resistance in determining rates of photosynthesis, transpiration and pollutant uptake.

In the experimental system used for this study, the air was well mixed by an internal fan, and r_a for CO₂ transfer was between 83 and 100 s m⁻¹ over the range of experiments performed. However, ra was constant throughout the duration of each individual experiment, ra changing only when the internal fans were renewed. The aerodynamic resistance should not be altered by SO₂ treatment unless leaf surface features have been changed ie. suffered damage due to excessively high pollution concentrations. Typical values for r_a in the field range from 10 - 30 s m⁻¹ for carbon dioxide transfer in exposed leaves and windy conditions, although ra may be much higher in still conditions. In leaf chamber experiments, in which the air is well stirred, typical values of the boundary layer resistance to carbon dioxide transfer range from 50 - 200 s m⁻¹ (Holmgren, Jarvis & Jarvis, 1965). The difference between r_A values in the field and in plant chambers may be attributed to there being a greater degree of turbulence occurring under natural conditions than is attained in leaf chambers (Sestak et al., 1971).

The boundary layer resistance for water vapour transfer was determined by measuring the evaporation of water from a model leaf of green blotting paper, in which there are no additional resistances, under Results

the same environmental conditions in the chamber system used for <u>Vicia</u>. The boundary layer resistance was then calculated from:-

$$r_a = \frac{\chi_i - \chi_o}{E}$$
(3.3)

where

 χ_i = water vapour concentration at the leaf surface. χ_o = water vapour concentration in the chamber.

and

The boundary layer resistance to carbon dioxide transfer was then calculated from:-

$$r_a CO_2 = 1.39 r_a H_2 O$$
 (3.4)

In the boundary layer, gas transfer is not due solely to free molecular diffusion but grades from molecular diffusion through a transition region to fully turbulent diffusion (Sestak <u>et al.</u>, 1971). As a result of this, equation 3.2 is inaccurate for r_a , applying only to free molecular diffusion, and equation 3.5 has been shown to be more accurately applied to this resistance. The relationship between molecular diffusivity coefficients for the aerodynamic resistance is:

$$r_{a}CO_{2} = r_{a}H_{2}O \left(\frac{DH_{2}O}{DCO_{2}}\right)^{2/3}$$
(3.5)

(Thom, 1968).

3.7.1.2 Cuticular Resistance, rc

E = evaporation rate

The pathway of carbon dioxide transfer through the cuticle is thought to consist of a short distance in the gas phase followed by a relatively long, high resistance pathway in the liquid phase (Sestak <u>et al.</u>, 1971). The liquid phase is long because, apart from the guard cells, epidermal cells do not contain chloroplasts and CO_2 molecules must travel at least one cell width before entering the cell in which they will be assimilated. The cuticular resistance is large in comparison with the parallel stomatal resistance (Fig.3.10b) and is usually ignored when resistances to gas transfer are being calculated. Holmgren, Jarvis & Jarvis (1965) found cuticular resistances ranging from 10³ to 10⁵ s m⁻¹ and concluded that this pathway of CO_2 transfer could be discounted. However, there is some evidence for the dependence of r_c on temperature and irradiance (Holmgren <u>et al.</u>, 1965). This interpretation of cuticular resistance for carbon dioxide transfer may not apply to gaseous pollutants and must be treated cautiously when determining leaf resistances to pollutant uptake. Indeed, Lendzian (1984) suggested that the cuticle is much more permeable to SO_2 and O_3 than to CO_2 or H_2O . Lendzian studied the membrane solubility of SO_2 and found SO_2 to be much more soluble in the cuticle (× 688) than in water for <u>Citrus</u> spp. and concluded that SO_2 permeated the cuticle via the lipophilic phase.

3.7.1.3 Stomatal resistance, r.

The passage of carbon dioxide and water vapour through the stomata is generally considered to be by free molecular diffusion. As such, stomatal resistances are inversely proportional to molecular diffusion coefficients. Again,

$$rCO_2 = rH_2O DH_2O / DCO_2$$
 (3.6)

the ratio of DH₂O / DCO₂ taken as being 1.65 (Unsworth et al, 1976).

Stomatal resistance to water vapour transfer may be determined in a number of ways, but in this experimental procedure r_3 was determined by measurements of transpiration rate, ambient and leaf temperatures and the water vapour concentration of the air entering and leaving the plant chamber.

$$\frac{\mathbf{E}}{\mathbf{r}_{s}} = \frac{\chi_{i} - \chi_{o}}{\mathbf{r}_{s} + \mathbf{r}_{a}}$$
(3.7)

where

E = Evaporation rate

 χ_i = water vapour concentration in the stomatal cavity

 χ_0 = water vapour concentration in the chamber

and r_a is measured as described earlier (Unsworth et al., 1976).

Therefore:

$$\frac{r_s = \chi_i - \chi_o}{E} = r_a \qquad (3.8)$$

The stomatal resistance depends on many environmental and physiological properties including temperature, irradiance, CO_2 concentration and water vapour deficit (Heath, 1959; Meidner & Mansfield, 1968; Raschke, 1975). Stomatal resistance also responds to many pollutant gases (eg. Majernik & Mansfield, 1970 & 1971; Biscoe <u>et al.</u>, 1973; Black & Black, 1979a,b; Black & Unsworth, 1979a,b,c; Olszyk & Tibbitts, 1981a,b; Rao <u>et al.</u>, 1983; Omasa <u>et al.</u>, 1985). Under optimal environmental conditions of light and humidity minimum values of r_3 range from 60 to 200 s m⁻¹ depending on species.

3.7.1.4 Internal (or Residual) Resistance, rr

The walls of the substantial cavity are usually assumed to be saturated with water, hence r_a , r_s and r_c are the only resistances to water vapour transfer and there is no extra resistance to H_2O transfer (r_{int} , Figure 3.10b) (Unsworth <u>et al.</u>, 1976). However, carbon dioxide passes into solution at the liquid-air interface on the surface of the mesophyll cell walls and has a further pathway for liquid phase transfer to carboxylation sites in the chloroplasts of the mesophyll cells.

The internal (residual) resistance is comprised of a number of separate components relating to carbon dioxide absorption and diffusion and mass transfer. Strictly speaking, it is incorrect to express this internal resistance as a diffusion resistance but this is normally done to allow comparison with other resistances in the transfer pathway (Sestak <u>et</u> <u>al.</u>, 1971). The length of the pathway is variable and is under physiological control, it is frequently kept short by the arrangement of the chloroplasts around the periphery of the mesophyll cell. However, chloroplasts may realign themselves depending on irradiance.

Although this internal resistance may be split into a number of components, it is difficult to assess each component individually, and this added resistance to carbon dioxide transfer is usually taken as a whole and termed residual (r_r) or internal (r_{int}) or mesophyll (r_m) resistance (Unsworth <u>et al.</u>, 1976: Carlson, 1983a).

3.7.1.5 Influence of Resistances on Photosynthetic Rates

Unsworth, Biscoe & Black (1976) illustrated the relative importance of r_A , r_s and r_r in determining gas exchange. Assuming the stomata are open, if r_A increases then photosynthetic rate always decreases. In the experiments performed in this study with Vicia faba, ra was constant throughout the experimental period and as such, did not influence changes in net photosynthetic rate. Unsworth and his co-workers described an hypothetical situation whereby stomatal resistance was decreased by 20% in response to air pollution, if the residual resistance was unchanged, there should have been a corresponding increase in photosynthetic rate of only 6%. However, experimental evidence suggests that photosynthesis may decrease in response to sulphur dioxide (eg. Black & Unsworth, 1979b; Muller, Miller & Sprugel, 1979; Winner & Mooney, 1980 a,b,c; Noyes, 1980; Heath, 1980; Takemoto & Noble, 1982; Saxe, 1983a; Katase, Ushijima & Tazaki, 1983). This decrease in photosynthesis in response to sulphur dioxide would require an increase in resistance to carbon dioxide transfer that would outweigh decreases in stomatal resistance. Unsworth et al. suggest that photosynthetic rate is governed predominantly by changes in residual rather than stomatal resistances. Experimental data collected in this study for both varieties of <u>Vicia</u> faba, have been analysed to show whether this is the case.

The residual resistance to carbon dioxide transfer can be calculated from:

$$Pmax = \phi - 0$$

$$\overline{r_s + r_a + r_r}$$

where Pmax is the gross photosynthetic rate (net photosynthesis plus respiration), ϕ is the carbon dioxide concentration of the air in the chamber (g m⁻³), r_a is aerodynamic (boundary) resistance to CO₂ (s m⁻¹), r_s is the stomatal resistance to CO₂ and r_r is the residual resistance to carbon dioxide transfer (s m⁻¹).

In equation 3.9 the carbon dioxide concentration at the carboxylation site is assumed to be zero by definition (Sestak, 1971), cuticular resistance is infinitely large and light intensity is not limiting. From this:

 $\mathbf{r}_{\mathbf{r}} = \frac{\mathbf{\phi}}{\mathbf{P}_{\max}} - \mathbf{r}_{\mathbf{s}} - \mathbf{r}_{\mathbf{a}} \tag{3.10}$

Pmax (g CO₂ m⁻² s⁻¹) and ϕ are known and r_a and r_s can be determined from analogy to water vapour transfer as described earlier.

(3.9)

Results

The extent of the influence of changes in stomatal resistance on changes in photosynthesis can be calculated using equation 3.9, assuming r_r to be unchanged, and using the new r_s following exposure to the pollutant. A value for Pmax is obtained and may be compared to the actual value of Pmax observed following fumigation with sulphur dioxide.

The validity of the above statements can be shown by working through an actual example. <u>Vicia faba</u> CV. Aquadulce Claudia was exposed to 500 ppb SO₂ in the manner previously described. After the four hour fumigation period net photosynthesis had been inhibited by 77% and stomatal resistance increased by 35% in relation to the control plant. Fifteen minutes prior to the onset of SO₂ fumigation $r_4CO_2 = 139 \cdot 0$ s m⁻¹, $r_5CO_2 = 148 \cdot 7$ s m⁻¹, Pmax = $3 \cdot 22$ g CO₂ m⁻² h⁻¹ and $\phi = 0.5622$ g m⁻³. Using equation 3.10:

> $r_{r} = 0.5622 - 139 - 148.7$ $3.22 \div 3600$ $= 342.7 \text{ s m}^{-1}.$

Immediately following SO₂ fumigation $r_a = 139$, $r_s = 168.3$, $\phi = 0.5883$ and $r_r = 342.7$. From equation 3.9 the expected Pmax would be:-

 $P_{max} = 0.5883 \times 3600$ 139 + 168.3 + 342.7 $= 3.25 g CO_2 m^{-2} h^{-1}.$

However, the actual gross photosynthetic rate following SO₂ fumigation was $0.9 \text{ g } \text{CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, therefore the residual resistance, r_r , must have changed. The actual r_r following exposure to SO₂ can be calculated from equation 3.10, using the actual Pmax,

 $r_r = 0.5883 - 139 - 168.3$ $0.9 \div 3600$ $= 2045.9 \text{ s m}^{-1}.$

These results are summarised in the following table:-

	Гa	.r _s (s m ⁻¹)	rr	Pmax (g CO ₂ m ⁻² h ⁻¹)
Rates		·		
Before SO ₂	139	149	343	3.22
Expected if only				
rs changes	139	168	343	3.25
Actual	139	168	2046	0-90

The expected Pmax following SO₂ fumigation is higher than that prior to SO₂ fumigation because ϕ had changed during the course of the experiment. The carbon dioxide concentration in the chamber, before SO₂, was lowered due to depletion by normal photosynthetic activity. When Pmax decreased in response to SO₂, the depletion of CO₂ in the chamber was reduced. As a result ϕ was higher following SO₂ fumigation and as a consequence the expected Pmax was raised. This, however, does not hold true for all SO₂ fumigations and data are presented in §3.7.2 to show that at low SO₂ concentrations, r_s dominates plant responses in Dylan plants.

3.7.2 The Importance of the Residual Resistance (r_r) : Influence on Rates of Photosynthesis

The example outlined in §3.7.1.5 showed how changes in stomatal resistance are not fully responsible for changes in gross photosynthesis occurring in response to sulphur dioxide fumigation. It was shown in the example that in, order to achieve the observed rate of photosynthetic activity following SO_2 exposure, the residual resistance increased from 343 to 2046 s m⁻¹. This increase in r_r indicates that sulphur dioxide has substantially altered one or more of the components of this segment of the gas transfer pathway.

The resistance data obtained for both varieties of <u>Vicia faba</u> are shown in Figures 3.11 and 3.12. Figure 3.11a and 3.11c show the data for Dylan and Aquadulce Claudia plants prior to sulphur dioxide fumigation. Total leaf resistance minus the component for aerodynamic resistance and measured stomatal resistances have been plotted together against SO_2 concentration. The difference between the two plots of $r_t - r_a$ and r_s is the residual resistance r_r . Figures 3.11b and 3.11d show the two resistances, measured for both varieties, at the end of the four hour



Figures 3.11a 3.11b.

Leaf Resistances to Carbon Dioxide Transfer in <u>V.faba</u> CV. Dylan prior to (3.11a) and following (3.11b) Sulphur Dioxide Exposure. [Total leaf resistance minus the component for aerodynamic resistance (\bullet : $r_t - r_a$) have been plotted together with stomatal resistance (O: r_s)].



Figures 3.11c 3.11d.

Leaf Resistances to Carbon Dioxide Transfer in <u>V.faba</u> CV. Aquadulce Claudia prior to (3.11c) and following (3.11d) Sulphur Dioxide Exposure. [Total leaf resistance minus the component for aerodynamic resistance ($0: r_t - r_a$) have been plotted together with stomatal resistance ($0: r_s$)]. fumigation period plotted against supplied SO₂ concentration.

It can be seen that Aquadulce Claudia plants showed an increase in r_r in response to SO₂ fumigations between 100 and 500 ppb, 12 out of 13 plants showing substantial increases in r_r of between 70 and 500 s m⁻¹ greater than measured r_r prior to SO₂ fumigation. At 550 ppb, r_r increased markedly by 1703 s m⁻¹ above prefumigation values. However, at 99 ppb the r_r following SO₂ fumigation was relatively unchanged from that measured prior to pollutant exposure. In addition there was little difference between the observed and the estimated rates of gross photosynthesis following treatment with this concentration of sulphur dioxide (Table 3.7).

The responses in Dylan plants were a little more variable. Eight out of 11 plants showed significant increases in r_r in response to SO₂ fumigations below 500 ppb, between 20 and 400 s m⁻¹ above prefumigation values. At concentrations between 500 and 600 ppb the residual resistance in Dylan was increased by 800 - 1600 s m⁻¹ above prefumigation values. At concentrations below 295 ppb r_r increased only slightly in response to SO₂ and at 290 ppb, r_r actually decreased from 788 to 748 s m⁻¹. At these SO₂ concentrations the observed changes in gross photosynthesis can be attributed largely to changes in stomatal resistance in response to SO₂. The observed and the estimated values for gross photosynthesis being similar (Table 3.8).

For both varieties, it was found that changes in photosynthetic rate in response to low concentrations of sulphur dioxide may be due, in part, to changes in r_3 . However, the observed changes in Pmax, induced by higher concentrations of SO₂, appeared to be due largely to changes in the residual resistance to carbon dioxide transfer. Figures 3.12a and 3.12b show the observed gross photosynthetic rate following SO₂ fumigation, plotted against the same resistances given in Figure 3.11 ie. $r_t - r_a$ and r_s , for both Dylan and Aquadulce Claudia plants. It can be seen that for plants of both varieties there was a good correlation between total leaf resistance (minus aerodynamic resistance) and observed gross photosynthetic rates following SO₂ treatment. However, stomatal resistance was variable in both varieties and there was no significant relationship observed between rates of gross photosynthesis and stomatal resistances following SO₂ exposure.

The variability in the resistance data for Dylan plants following SO_2 fumigation, may also be explained in part, by changes in dark respiration rates induced by sulphur dioxide. At SO_2 concentrations below

TABLE 3.7

Changes in the residual resistance to carbon dioxide transfer and differences in Observed and Estimated rates of gross photosynthesis (Pmax) in response to a range of sulphur dioxide concentrations for <u>Vicia faba</u> CV. Aquadulce Claudia. [The percentage change in stomatal resistance and net photosynthetic rates are also shown.]

[S02]	% change		Pmax		Residual resistance	
	Pnet	TS	Observed	Estimated	Before	SO ₂ After
(ppb)			(g CO ₂	m ⁻² h ⁻¹)		(s m~1)
99	-1-3	3.6	2.34	2.32	729・4	721.9
128	-13.9	6 • 2	1.73	2.07	599-9	802·5
200	16+8	-2-3	2.09	2.23	504•4	567·8
215	11-1	16.3	2.10	2.75	402.6	66 0 · 3
283	9•1	-5.7	2.34	2.31	498+9	486 • 4
302	5.2	-10.8	2.51	2.85	256.5	354 • 4
320	-6-5	9.5	2.49	3.01	332-0	473·5
350	26 • 6	-20.3	1.51	1.72	493-9	664-6
400	35.6	-3.3	2.08	$2 \cdot 65$	-111-3	98.7
400	42 • 9	-17.6	1.70	2 • 99	$257 \cdot 7$	791·5
422	10-7	17-9	2.07	2.35	515.7	631·4
500	32 • 8	-34.0	1.97	1 · 48	584.6	247.5
500	31.7	-15.6	1.73	2.43	647.6	992·2
550	77 • 2	-34.9	0.90	3 · 25	342 6	2045 • 9

TABLE 3.8

Changes in the residual resistance to carbon dioxide transfer and differences in Observed and Estimated rates of gross photosynthesis (Pmax) in response to a range of sulphur dioxide concentrations for <u>Vicia faba</u> CV. Dylan. [The percentage change in stomatal resistance and net photosynthetic rates are also shown.]

[S02]	% change		Pmax		Residual	resistance	
	Pnet	rs	Observed	Estimated	Before	SO ₂ After	
(ррђ)			$(g CO_2 m^{-2} h^{-1})$		(s m ⁻¹)		
92	6-7	-16.5	1-97	1-99	818-9	829 • 4	
210	15-6	-4.6	1.78	1.79	863•9	874 • 1	
290	15.9	7.6	2.09	2.01	788 - 9	748 - 8	
295	19.2	8•7	1-69	2 · 17	838 • 1	1109 • 3	
380	18.6	11.2	1.65	1.26	835.8	45 2・ 0	
390	18.7	12.5	1-86	2.63	562 . 7	886 0	
400	31-4	13.0	1-53	2 · 16	621 • 1	1024 • 7	
460	30.5	-40.3	2 • 10	2.01	718 • 4	699•5	
510	32 • 2	-23.7	0-81	2 • 26	840-8	2521 • 7	
600	47 • 1	-67.7	1 47	2 • 18	704.5	1596 • 0	
600	46 • 1	-41.0	1 46	2 • 29	448.9	1202 • 5	





Figures 3.12a 3.12b

Observed Gross Photosynthetic Rates (Pmax) Following SO₂ Fumigation in Both Varieties of <u>Vicia faba</u> CV. Dylan (3.12a) and Aquadulce Claudia (3.12b) plotted against Leaf Resistances $r_t - r_a$ (•) and r_5 (O). [The figures show the dependence of Pmax on changes in $r_t - r_a$]. 295 ppb, there were no significant changes in residual resistance observed in response to SO_2 and observed and estimated rates of gross photosynthesis did not differ appreciably. However, it was shown in §3.4 that net photosynthesis was inhibited between 6 and 19% in relation to the control plants at these SO_2 concentrations. It was also shown that rates of dark respiration were increased in response to SO_2 , to almost double the prefumigation rate, irrespective of the SO_2 concentration supplied (§3.5). It would appear that the measured net photosynthetic inhibition in Dylan plants, at these SO_2 concentrations, may be due to both changes in stomatal resistance and increased dark respiration rates, rather than solely to changes in the residual resistance arising from a direct effect of sulphur dioxide on the photosynthetic mechanism.

3.7.3 The Influence of Stomatal Resistance, r_s, on Net Photosynthesis

As was described in §3.7.1, changes in stomatal resistance in response to sulphur dioxide will alter the rate of carbon dioxide transfer through the stomatal pore, and as such, will have an effect on net photosynthetic rates. The influence of changes in leaf resistances, due to sulphur dioxide, on gross photosynthesis have been described in §3.7.2.

The relationships between measured changes in stomatal resistance, in the two varieties of <u>Vicia faba</u> used in this study, and the degree of inhibition of net photosynthesis due to sulphur dioxide are shown in Figures 3.13 and 3.14.

The stomatal and photosynthetic responses of plants of the variety Dylan are shown in Figure 3.13. The variability shown in the data emphasises the point that changes in stomatal resistance may not fully account for the accompanying changes in net photosynthetic rate due to sulphur dioxide exposure. From the figure it can be seen that photosynthetic inhibition of up to 31% in relation to the control plant occurred in conjunction with either increased or decreased stomatal resistance in response to SO₂ fumigation. Only four out of twelve plants showed stomatal closure in response to SO₂ when photosynthetic inhibition up to 31% had occurred. However, regression analysis of the data gave a correlation coefficient of -0.701 which is significant at the 99.0% level (the regression line is shown in the figure) and for the highest







Figure 3.13.

To Show the Relationship Between Measured Changes in Stomatal Resistance and % Inhibition of Net Photosynthesis in Response to a Range of Sulphur Dioxide Concentrations for <u>Vicia</u> faba CV. Dylan.





Figure 3.14.

To Show the Relationship Between Measured Changes in Stomatal Resistance and % Inhibition of Net Photosynthesis in Response to a Range of Sulphur Dioxide Concentrations for <u>Vicia</u> <u>faba</u> CV. Aquadulce Claudia. measurement of stomatal closure due to SO_2 there was a corresponding high degree of photosynthetic inhibition. When stomatal resistance increased by 85% in response to SO_2 , net photosynthesis was inhibited by 50% in relation to the control plant.

Figure 3.14 shows the data for plants of the variety Aquadulce Claudia. It can be seen that there was a good correlation between changes in stomatal resistance in response to sulphur dioxide and the extent of photosynthetic inhibition. Linear regression gave a correlation coefficient of -0.783 which is significant at the 99.9% level. It would appear that increases in stomatal resistance in response to SO₂ (negative values on the figure) are accompanied by inhibition of net photosynthetic rates, the highest increases in r_s (-35%) being followed by highest measured photosynthetic inhibition (77%). It can also be seen from the figure that when stomatal resistance decreased in response to sulphur dioxide (positive values on the figure) there was an accompanying enhancement of net photosynthetic rates up to 13% above that of the control plants.

One might expect that if stomatal resistance decreased, there would be a corresponding increase in the observed rate of net photosynthesis (see equation 3.9, §3.7.1) especially as these changes occurred at low sulphur dioxide concentrations. This response was observed in Aquadulce Claudia plants but not in Dylan; when stomatal resistance in plants of the variety Dylan decreased by 50% there was an accompanying inhibition of net photosynthesis of 14%. This apparent anomaly has been shown to be due in part to the increases in respiration rate in Dylan in response to sulphur dioxide which were not seen to occur in Aquadulce Claudia plants.

Comparison of the data for both varieties suggested that plants of the variety Dylan exhibited greater stomatal sensitivity to sulphur dioxide than plants of the variety Aquadulce Claudia since the magnitude and direction of the stomatal response was much greater in Dylan plants. This stomatal sensitivity was not closely correlated to the degree of photosynthetic inhibition observed in Dylan plants at low SO₂ concentrations. However, there was a close correlation, in Dylan plants, between changes in stomatal resistance and changes in Pnet at highest degrees of photosynthetic inhibition (>32%). There was a closer relationship, in Aquadulce Claudia plants, between changes in stomatal resistance and the ensuing changes in net photosynthesis. However, both varieties exhibited enhanced stomatal closure at the higher SO_2 concentrations which corresponded with maximum measured photosynthetic inhibition.

In conclusion, examination of the relationship between changes in stomatal resistance and the degree of photosynthetic inhibition occurring in response to SO₂, concurred with the results given in §3.7.2. Although changes in stomatal resistance were seen to account, in part, for changes in net photosynthetic rates in response to SO₂, the major controlling factor appeared to be SO₂ induced changes in the residual resistance, r_r , to CO₂ transfer and, for Dylan plants, concomitant changes in dark respiration rates.

3.8 GAS EXCHANGE MECHANISMS AND POLLUTANT FLUX

3.8.1 Net photosynthesis

The results presented so far have all related plant responses to the concentration of sulphur dioxide in the air surrounding the leaf. However, it is well reported that the concentration of sulphur dioxide experienced by the plant is only one of several factors that determine the actual flux to the leaf (Unsworth, Biscoe & Black, 1976; Mclaughlin & Taylor, 1981; Garsed, 1985; Fowler, 1985; McLaughlin & Taylor, 1985). In short, pollutant flux to the leaf is governed by several resistances, as described in \$3.7.1, the aerodynamic resistance, cuticular resistance, stomatal resistance and an added internal (residual) resistance. It is also known that environmental factors such as light, temperature, humidity and carbon dioxide concentration may alter the magnitude of stomatal resistance and thus alter the pollutant flux to the plant at any given sulphur dioxide concentration. Surface water on the leaf also influences pollutant flux to the plant (Garsed, 1985). The presence of water on the leaf surface lowers the surface resistance to sulphur dioxide deposition, the gas dissolving in the water and it is possible that flux into the plant, through the leaf surface, in this dissolved form may occur (Hocking & Hocking, 1977).

Any injury sustained by plants exposed to sulphur dioxide is a direct result of the pollutant entering the leaf and affecting plant

physiological and biochemical mechanisms. Therefore, relating observed injury to ambient sulphur dioxide concentrations may not be wholly accurate. Taylor, McLaughlin & Shriner (1982) gave the term "effective pollutant dose" to describe the quantity of sulphur dioxide available to affect the physiology of the plant. The effective dose of sulphur dioxide is defined as the cumulative amount of SO_2 absorbed per unit leaf area during the exposure period and it is a more reliable criterion for evaluating threshold concentrations for physiological and yield effects (McLaughlin & Taylor, 1985). For short exposures to sulphur dioxide, as used in this study, a measure of total internal flux is a reasonable estimate of effective pollutant dose (Garsed, 1985). In addition, relating responses to pollutant flux rather than dose, removes the influence of different resistances to gas transfer and allows for more accurate comparisons of results from different experimental systems (Black & Unsworth, 1979c).

It has been described in chapter 2 how sulphur dioxide fluxes to the plant may be assessed in two ways. Firstly, they may be estimated indirectly by analogy to water vapour transfer, where flux is calculated from a knowledge of the magnitude of the limiting resistances to gas transfer. In this case, aerodynamic and stomatal resistances to water vapour transfer, these values are corrected for differing diffusivity coefficients and the assumption being made that there is no internal resistance to SO₂ uptake. This flux is termed 'calculated flux', P_{fcalc}. Secondly, sulphur dioxide flux to the plant may be determined directly from mass balance calculations. The flux determined in this way is termed 'measured flux', P_{meas}. Sulphur dioxide fluxes are measured in $\mu g m^{-2} s^{-1}$.

Figures 3.15 and 3.16 show inhibition of net photosynthetic rate, Pnet, plotted as a function of calculated and measured pollutant flux for plants of the variety Dylan. It is immediately apparent that there are differences in the relationships between the observed effects on net photosynthetic rate and the two measures of flux. There was a 99.0% correlation between calculated flux and the degree of photosynthetic inhibition (Figure 3.16); the highest flux (7 μ g m⁻² s⁻¹) resulting in the highest degree of photosynthetic inhibition in treated plants in relation to control plants. The regression line is shown in the figure. However, when measured flux was plotted against photosynthetic inhibition (Figure 3.15), there was a curvilinear relationship for SO₂ fluxes up to 3 μ g m⁻² s⁻¹ but above this flux value the data were variable. When the data were analysed Figure 3.15





there was no significant correlation between P_{Fmeas} and the resulting degree of photosynthetic inhibition, again demonstrating that it is not always correct to force a straight line relationship between two variables.

The relationship between the observed responses of net photosynthesis and pollutant flux in plants of the variety Aquadulce Claudia differ markedly when compared with those for Dylan plants. Figure 3.17 shows photosynthetic inhibition plotted against measured sulphur dioxide flux, regression analysis gave a correlation coefficient of 0.720 which was significant at the 99.0% level. There was a 90% correlation between calculated pollutant flux and inhibition of net photosynthesis in Aquadulce Claudia plants (Figure 3.18). However, the regression fit and most of the data were on a line virtually parallel to the y axis. McLaughlin & Taylor (1985) assumed the regression line intercept of the x axis to be the threshold pollutant concentration, above which, injury to the plant occurs. If this is so then the threshold calculated flux for Aquadulce Claudia would be 1.5 μ g m⁻² s⁻¹ but it can be seen from the figure that photosynthetic inhibition between 16 and 36% occurred when the calculated flux values were between 1.1 and 1.3 μ g m⁻² s⁻¹. The degree of photosynthetic inhibition in Aquadulce Claudia plants did not appear to be closely correlated to the calculated amount of SO_2 entering the leaf given that calculated fluxes between 1 and 3 μ g m⁻² s⁻¹ produced measured photosynthetic inhibition between -20 and 70% in relation to the control plants. However, it may be that there was a narrow 'sensitivity' threshold in Aquadulce in operation.

Comparing the responses of the two varieties, Aquadulce Claudia plants showed a close correlation between measured sulphur dioxide flux and the degree of photosynthetic inhibition, whereas in Dylan plants, there was no apparent relationship between measured flux and the resulting degree of photosynthetic inhibition when measured flux exceeded 3 μ g m⁻² s⁻¹. Below this flux measurement photosynthetic inhibition was related to measured pollutant flux in a curvilinear fashion. The threshold measured flux in Aquadulce Claudia plants was 1.8 μ g m⁻² s⁻¹, for Dylan it appeared to be 0.3 μ g m⁻² s⁻¹.

Figure 3.18 shows photosynthetic inhibition in relation to calculated flux for Aquadulce Claudia plants and the equivalent regression line for plants of the variety Dylan. It can be seen that for a given photosynthetic response above 10% inhibition, the calculated pollutant flux







Net Photosynthetic Inhibition as a Function of Measured (3.17) and Calculated (3.18) Sulphur Dioxide Flux ($\mu g \ m^{-2} \ s^{-1}$) to <u>V.faba</u> Plants of the Variety Aquadulce Claudia. [The regression lines obtained for the data for Dylan plants are also shown for ease of comparison]. into Dylan was more than double that for Aquadulce Claudia. A calculated flux of 6.3 μ g m⁻² s⁻¹ resulted in a 50% inhibition in net photosynthetic rate in Dylan plants, whereas a calculated flux of only 2.6 μ g m⁻² s⁻¹ induced the same degree of response in Aquadulce Claudia plants. However, Aquadulce Claudia plants appeared to have a higher natural photosynthetic rate in the absence of SO₂ than those of Dylan, thus a 50% reduction in Pnet in Aquadulce plants was, in absolute terms, a greater effect than the 50% reduction in Pnet in Dylan plants occurring at a much higher flux. The threshold values for calculated pollutant flux (from the regression fits) were 1.5 μ g m⁻² s⁻¹ for Aquadulce Claudia and 0.8 μ g m⁻² s⁻¹ for Dylan plants.

These data for calculated pollutant fluxes would seem to imply that Aquadulce Claudia plants had a higher tolerance to sulphur dioxide than Dylan, given the higher threshold concentration. However, once the threshold concentration had been exceeded, Aquadulce plants were much more sensitive to sulphur dioxide flux than Dylan, photosynthetic inhibition increasing sharply with increasing pollutant flux. Aquadulce Claudia plants also exhibited enhancement of photosynthetic activity at low pollutant fluxes (<1.7 μ g m⁻² s⁻¹ measured flux, <1 μ g m⁻² s⁻¹ calculated flux), in contrast to Dylan where fluxes below 1 μ g m⁻² s⁻¹ induced photosynthetic inhibition of 6 - 17%.

This explanation of these data does not give a complete picture in describing the variation in the pollutant responses of Aquadulce Claudia and Dylan plants. When the data for measured pollutant flux were compared between the varieties, Aquadulce plants, again, had a higher threshold flux of $1.8 \ \mu g \ m^{-2} \ s^{-1}$ as opposed to $0.3 \ \mu g \ m^{-2} \ s^{-1}$ for Dylan. For measured fluxes between 0.3 and 1.8 Dylan plants appeared to be more sensitive to pollutant flux than Aquadulce Claudia plants, showing up to 20% photosynthetic inhibition in comparison to the enhancement of Pnet occurring in Aquadulce plants at these pollutant fluxes.

Before threshold tolerances to sulphur dioxide pollution can be fully defined, other factors must be taken into account. An important factor to be considered when comparing responses to pollutant flux is the relationship between ambient sulphur dioxide concentration and pollutant flux to the plant. This is discussed in §3.9.

It was discussed earlier in this section how the entry of sulphur dioxide into the leaf is governed by the same resistances described in \$3.7.1 for carbon dioxide transfer. Stomatal resistance plays a large part, initially, in governing SO_2 fluxes and importance of this is discussed in the following section.

3.8.2 Stomatal Resistance and Pollutant Flux

The relationship between stomatal resistance and pollutant flux into the plant is complex, and a 'feed forward/feed back' mechanism operates whereby, when SO_2 is first present, its entry into the plant is governed by the stomatal resistance. However, once inside the leaf, SO_2 induces changes in this resistance which in turn alter the rate of SO_2 entry into the leaf. This interaction must be considered when changes in stomatal resistance in response to pollutants are examined.

Changes in stomatal resistance in Vicia faba, as per cent difference from that of the control plants in response to ambient sulphur dioxide concentration, have been described previously in §3.6.2. It was seen that there was a good correlation between stomatal response and ambient sulphur dioxide concentration for Aquadulce plants in comparison to the data for Dylan. Although the linear regression calculated for Dylan was significant at the 99.0% level, visual examination of the data showed the straight line produced to be an inaccurate representation of the data obtained. At applied sulphur dioxide concentrations up to 400 ppb the stomatal response in Dylan plants was variable and it was difficult to define clearly the relationship between sulphur dioxide concentration and the resulting changes in stomatal resistance. Over half the data exhibited a decrease in r_s in response to SO_2 below 400 ppb, the remainder showing increases in r_s . At higher SO₂ concentrations there were significant increases in stomatal resistance suggesting a critical or 'threshold' concentration above which enhanced stomatal closure will occur.

A clearer picture was obtained when changes in stomatal resistance as a function of pollutant flux were examined. Taylor <u>et al</u>. (1982) stated that the pollutant dose to which a plant is exposed (ie. ambient concentration) is not a direct quantitative measure of the dose that causes a physiological response (ie. effective dose), the effective dose being a function of the rate at which pollutant molecules arrive within the cells inside the leaf. This rate is controlled primarily by the leaf resistances to gas transfer previously outlined. These workers suggested that only a portion of the actual pollutant present in the air reaches the leaf interior and induces a response. Stomatal resistance plays a large part in governing pollutant flux to the plant thus the relationships between changes in stomatal resistance and pollutant flux are important.

Figures 3.19 and 3.20 show changes in stomatal resistance for plants of the variety Dylan plotted as a function of calculated and measured pollutant fluxes. Regression analysis gave a 99.0% correlation between changes in stomatal resistance and both measurements of flux. In both cases there appeared to be a critical value for flux beyond which stomatal closure occurred. The threshold value for calculated flux, taken from the regression line, was $2\cdot3 \ \mu g \ m^{-2} \ s^{-1}$ (Figure 3.19) but visual examination of the data showed that the regression line did not accurately explain the relationship. The threshold flux, was more accurately, $3 \ \mu g \ m^{-2} \ s^{-1}$, below this value there were decreases in stomatal resistance in response to sulphur dioxide flux, the magnitude of the response being unrelated to flux. Between 3 and 4 $\mu g \ m^{-2} \ s^{-1}$, there were marked increases in stomatal resistance in relation to control plants, up to an 84% increase when calculated flux was $3\cdot9 \ \mu g \ m^{-2} \ s^{-1}$.

When the data for measured flux were examined (Figure 3.20), there was a similar trend to that for calculated flux and stomatal response, the data fitted more closely to the regression line and the threshold value, beyond which stomatal closure occurs, was 4 μ g m⁻² s⁻¹. The increases in stomatal resistance beyond this threshold concentration were more gradual than those observed for calculated flux.

When the stomatal responses of plants of the variety Aquadulce Claudia were plotted as a function of ambient sulphur dioxide concentration (Figure 3.9), it was found that there was a good straight line correlation (99.9%) relating changes in r_3 to applied sulphur dioxide concentration, 92% of plants exhibiting increases in r_3 above 200 ppb SO₂. Figures 3.21 and 3.22 show stomatal responses in Aquadulce in relation to pollutant flux. There appeared to be no significant correlation between calculated sulphur dioxide flux and observed stomatal responses. For flux values between 1.0 and 3.8 μ g m⁻² s⁻¹, 70% of plants exhibited increases in stomatal resistance in response to SO₂ flux, the degree of change in r_3 being unrelated to flux; 30% of plants exhibited decreases in stomatal resistance over the same range of calculated flux values.

The data for measured flux (Figure 3.22) followed the same trends



Figures 3.19 and 3.20.

Changes in Stomatal Resistance in Plants of the Variety Dylan, Plotted as a Function of Calculated (3.19) and Measured (3.20) Sulphur Dioxide Fluxes to the Plant ($\mu g m^{-2} s^{-1}$).



Figures 3.21 and 3.22.

Changes in Stomatal Resistance in Plants of the Variety Aquadulce Claudia, Plotted as a Function of Calculated (3.21) and Measured (3.22) Sulphur Dioxide Fluxes to the Plant ($\mu g m^{-2} s^{-1}$). The regression line obt The regression line obtained for plants of the variety Dylan is also shown (-----).
Results

observed when stomatal responses were plotted against ambient SO_2 concentrations. There was a 98% correlation between measured flux and observed changes in stomatal resistance in Aquadulce Claudia plants. The threshold concentration, above which stomatal closure was induced, was 1.8 $\mu g m^{-2} s^{-1}$, when taken from the regression line. However, decreases in stomatal resistance were observed at measured flux values up to 3.2 $\mu g m^{-2}$ s^{-1} . At flux values above 3.2, stomatal resistance increased in response to SO_2 . At flux values below 3.2, the stomatal response was variable, half the data showing decreases in r_5 up to 11%, the other half of the data showing increases in r_5 between 6 and 36% in relation to control plants.

Comparison of the stomatal responses of both varieties in relation to pollutant flux revealed significant varietal differences. Firstly, looking at changes in r, in relation to calculated pollutant flux. In plants of the variety Dylan flux values below 3 μ g m⁻² s⁻¹ induced stomatal opening ie. stomatal resistance decreased by up to 51% in relation to control plants, whereas, Aquadulce Claudia plants exhibited changes in r, ranging from -35% to 11% over the same range of calculated flux values; the majority of the data showing increases in r, in response to SO₂ flux. Aquadulce plants, unlike Dylan, did not appear to have a clear threshold value for calculated flux beyond which stomatal closure was induced.

Secondly, comparison of changes in stomatal resistance as a function of measured flux showed similar trends for both Aquadulce Claudia and Dylan plants. Figure 3.22 gave the data for Aquadulce with the equivalent regression line for Dylan also shown. It was seen that, for a given stomatal response, the flux to Dylan plants was greater than that to Aquadulce Claudia plants. Both varieties showed evidence of a threshold flux beyond which stomatal closure occurred. In Dylan plants this value of measured flux was $4 \cdot 1 \ \mu g \ m^{-2} \ s^{-1}$, in Aquadulce the critical value was $3 \cdot 2 \ \mu g \ m^{-2} \ s^{-1}$.

It must be noted that the threshold values for pollutant flux in both varieties were the point at which a switch in stomatal response occurred, from opening to closure and were not the concentrations needed to initiate a stomatal response. This must be remembered when defining varietal differences in stomatal response to pollutant flux. The threshold values for both measurements of pollutant flux were higher in Dylan than in Aquadulce Claudia plants. Below these critical flux values, the magnitude of stomatal opening in response to SO_2 was greater in Dylan plants. Once the critical flux had been exceeded, Dylan plants exhibited a greater degree of stomatal closure than that observed in Aquadulce plants.

These differences in response to pollutant flux and the differing threshold values between the varieties may be explained, in part, if flux is examined in relation to ambient sulphur dioxide concentrations for each variety. Also, the dissimilarities between the varieties in measured pollutant flux and calculated flux have been highlighted by the differing relationships, in each variety, between stomatal responses and net photosynthetic responses and both measurements of pollutant flux. The relationships between both measures of flux and ambient SO₂ concentrations and the disparities in their estimation are described in §3.9. in order to determine which of these flux assessments is the more accurate.

3.9 POLLUTANT FLUXES & AMBIENT SO₂ CONCENTRATION

It has been described in chapter 2 and \$3.8.1 how sulphur dioxide fluxes to the plant may be assessed in two ways, either from mass balance calculations giving measured flux, P_{fmeas} or from analogy to water vapour transfer, giving calculated flux, P_{fcalc} .

Evidence from the literature suggests that exposure time, for short fumigation periods, does not have a pronounced or significant effect on pollutant flux to the plant (Taylor & Tingey, 1983). Examination of the data for both varieties of <u>Vicia faba</u> showed little difference in measured flux over the four hour fumigation period. However at highest SO_2 concentrations, above 400 ppb, the calculated flux altered in accordance with changes in stomatal resistance induced by the pollutant. Therefore, all flux values used in these analyses have been taken three and a half hours after the onset of the four hour sulphur dioxide exposure period as stomatal changes occurred shortly after the start of the SO_2 exposure. In this way, differences in calculated and measured fluxes occurring early in the exposure period were discounted.

The relationships between pollutant fluxes to the plant and ambient sulphur dioxide concentrations, for both varieties of <u>Vicia</u> <u>faba</u>, are shown in Figures 3.23 to 3.26. Figures 3.23 and 3.24 show measured and calculated pollutant fluxes for plants of the variety Dylan. Total leaf flux ranged from 0.48 to 8.18 μ g m⁻² s⁻¹. Regression analyses gave a correlation coefficient of 0.683 for measured sulphur dioxide flux and 0.782 for calculated flux and showed a good linear relationship between ambient sulphur dioxide concentration and pollutant flux, both correlation coefficients being significant at the 99.0% level.

Figures 3.25 and 3.26 show measured and calculated pollutant fluxes for the variety Aquadulce Claudia plotted against ambient sulphur dioxide concentration. Total leaf flux ranged from 0.38 to 6.43 μ g m⁻² s⁻¹ over the range of sulphur dioxide concentrations supplied. Regression analyses gave good linear correlations of 0.732 (p < 0.001) for measured flux, and 0.740 for calculated pollutant flux (p < 0.01).

Analysis of covariance between both measures of flux, for both varieties, showed there to be significant differences between measured and calculated flux. For Aquadulce Claudia plants, the calculated F value was $5\cdot094$ ($\alpha = 0.025$) and for Dylan F = $4\cdot27$ ($\alpha = 0.05$). In both varieties, measured sulphur dioxide flux was significantly higher than calculated flux for a given ambient sulphur dioxide concentration. When both measures of flux were compared between the varieties, again using analysis of covariance, Aquadulce Claudia was found to have significantly lower pollutant flux, at a given ambient SO₂ concentration, than that to the variety Dylan when ambient sulphur dioxide concentrations exceed 100 ppb. The calculated F value for calculated flux was $6\cdot915$ ($\alpha = 0.025$) and $4\cdot605$ ($\alpha = 0.05$) for measured flux. This difference in flux may be explained by the fact that Aquadulce Claudia appeared to have higher stomatal resistances to sulphur dioxide transfer than Dylan when r_sSO_2 was calculated by analogy to water vapour transfer (Figures 3.29a and 3.30a).

It was seen from the data given in Figures 3.23 to 3.26 that, in both varieties, the calculated sulphur dioxide flux into the plant was less than the measured flux. Figures 3.27 and 3.28 show calculated flux plotted against measured flux for both Dylan and Aquadulce Claudia. It may have been expected that both estimations of flux would give similar results. However, analysis of covariance showed that in both varieties, measured flux was significantly higher than calculated flux when flux measurements exceed 1.5 μ g m⁻² s⁻¹.

The amount of sulphur dioxide deposited on the leaf surface during fumigation must be considered when estimates of measured flux are Figure 3.23















Measured Sulphur Dioxide Fluxes ($\mu g m^{-2} s^{-1}$) Plotted Against Calculated Fluxes for <u>V.faba</u> CV. Dylan (3.27) and Aquadulce Claudia (3.28). [The expected relationship between both measures of flux, allowing for surface deposition, is also shown in the figures (-----) to highlight the actual differences observed between both estimates of SO₂ flux]. Results

made. Adsorption on to the leaf surface and cuticular diffusion can represent between 10 and 50% of the total flux depending on conditions and plant species (Black & Unsworth, 1979c; Taylor & Tingey, 1981 & 1983; Taylor, Mclaughlin, Shriner & Selvidge, 1983). Black & Unsworth calculated surface deposition in <u>Vicia faba</u> to be 10% of the total flux for measured flux values up to 2.5 μ g m⁻² s⁻¹. These data were extrapolated for the two varieties of Vicia used in this study. Therefore one might expect measured flux values to be slightly higher than calculated fluxes since assessing pollutant flux from analogy to water vapour transfer does not involve surface deposition. Figures 3.27 and 3.28 show the expected relationship between measured and calculated pollutant fluxes. Allowing for surface deposition, P_{fmeas} should be proportionally higher than P_{fcalc} . It can be seen that the observed differences in Primess and Private, for both varieties, cannot be due solely to surface deposition. This was corroborated by the fact that at low flux values (below 2 μ g m⁻² s⁻¹) the calculated flux was higher than the measured flux.

In order to explain these observed differences between measured and calculated flux, stomatal resistance to pollutant flux must be examined. Stomatal resistance to sulphur dioxide can, as with flux, be calculated in two ways. Firstly from analogy to water vapour transfer where $r_3SO_2 = 1.98$ r_3H_2O , allowing for differing molecular diffusivities between the two gases. This value was used to derive calculated pollutant flux. Secondly, stomatal resistance to sulphur dioxide transfer may be calculated using an analogue modelling approach, independent of water vapour transfer, whereby resistance is calculated from the concentration gradient of sulphur dioxide between the interior and the exterior of the leaf and the measured pollutant flux via mass balance (Taylor & Tingey, 1983). The second value for stomatal resistance is termed $r_3SO_2^1$. If flux is given as:

$$Flux = \frac{C_0 - C_1}{r_t}$$
(3.11)

then,

$$\mathbf{r}_{i} = \underbrace{\mathbf{C}_{0} - \mathbf{C}_{i}}_{\text{Flux}} \tag{3.12}$$

where $r_t = \text{total leaf resistance to sulphur dioxide transfer (<math>r_a + r_s + r_r$), C₀ is the sulphur dioxide concentration outside the leaf ($\mu g \ m^{-3}$), C₁ is the sulphur dioxide concentration in the leaf interior and Flux is P_{fmeas} ($\mu g \ m^{-2} \ s^{-1}$).

The concentration of sulphur dioxide in the leaf interior, C_i , is very difficult to measure. Some researchers have assumed C_i to be close to zero (Black & Unsworth, 1979a; Winner & Mooney, 1980a,b,c) as sulphur dioxide dissolves in the water surrounding the cells in the substomatal cavity. Black & Unsworth considered C_i to be negligible at low ambient sulphur dioxide concentrations. Other workers have tried to measure C_i by using the equation given above (3.11), when all other parameters were known. Both Taylor & Tingey (1983) and Winner <u>et al</u>. (1985) calculated negative values for C_i . However, for these analyses, C_i was assumed to be effectively zero. The residual resistance, r_r , was, by definition, also assumed to be effectively zero in comparison to r_a and r_s (Black & Unsworth, 1979c). Therefore, from equation 3.12:

$$r_{s}SO_{2}^{1} = \frac{C_{0}}{P_{fmeas}} - r_{a}SO_{2} \qquad (3.13)$$

The aerodynamic resistance to sulphur dioxide transfer (r_aSO_2) is calculated from that obtained for water vapour transfer, allowing for differing molecular diffusivities where, $r_aSO_2 = 1.57r_aH_2O$ (s cm⁻¹).

Gaastra (1959) used this technique to investigate factors controlling carbon dioxide assimilation. Taylor & Tingey suggest that any differences in the two estimates of stomatal resistance to sulphur dioxide transfer are evidence for a non-stomatal, residual resistance (r_rSO_2) to the diffusion of sulphur dioxide into the leaf interior.

$$r_{r}SO_{2} = r_{s}SO_{2}^{1} - r_{s}SO_{2}$$
(3.14)

Taylor & Tingey found this residual resistance to be always negative in their own study of <u>Geranium carolinianum</u> and in their interpretation of data from other workers.

This method was used on the data collected for Aquadulce Claudia and Dylan plants and the results are shown in Tables 3.9 & 3.10. The majority of the data showed r_3SO_2 ¹ to be less than r_3SO_2 and the resulting TABLE 3.9

Summary of Residual (r_rSO_2) and Stomatal Resistances (r_sSO_2) , calculated from water vapour transfer and $r_sSO_2^1$ using analogue modelling) to Sulphur Dioxide Transfer as Related to Ambient Sulphur Dioxide Concentrations and Pollutant Flux in <u>Vicia faba</u> CV. Aquadulce Claudia.

[S02] (ppb)	P _{Fmeas} (µg m ⁻²	Pcalc s ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r _s SO ₂ ¹ (s cm ⁻¹)	rrSO2 (s cm")
99	0.87	1•99	0 • 48	2 · 31	1.83
128	1 • 76	0.76	2.79	0.04	-2.75
215	4.53	1 • 45	3.32	0•41	-2.09
283	1•70	1.86	3•40	3 • 82	0•41
305	3 ⋅ 16	1-61	3.37	0.72	-2.64
320	3 • 24	2 • 29	1.94	0.78	-1.15
350	4·32	1 • 21	6.24	0 • 27	-5-96
422	2.65	3.04	3.03	3.61	0.58
500	3.02	1•73	7.34	3 • 79	-3.54
500	3 · 12	6-11	1.39	3.64	2-24
550	6 • 43	3-87	2.02	0.40	-1.61

TABLE 3.10

Summary of Residual and Stomatal Resistances to SO_2 Transfer in <u>Vicia</u> <u>faba</u> CV. Dylan.

[SO2] (ppb)	Prmeas (µg m ⁻²	P _{FCALC} S ⁻¹)	r _{\$} SO ₂ (s cm ⁻¹)	r _s SO ₂ ¹ (s cm ⁻¹)	r _r SO ₂ (s cm ⁻¹)
92	0.69	1.03	1•59	2.89	1.29
210	7·32	2·16	0.97	-0.24	-1-20
285	2·15	3.06	1.72	2.86	1 • 13
300	2 · 17	2 • 93	1.98	3.01	1.03
390	8·18	2.64	2 · 41	-0-44	-2.85
410	5.09	3.08	1 • 99	0•49	-1.49
460	7.32	4 • 25	1.28	-0.01	-1-29
510	7·12	5.74	0.97	0•47	-0.49

residual resistance to be negative. The existence of an additional resistance to sulphur dioxide transfer which has a negative value would explain the differences observed between measured and calculated pollutant fluxes. Given that calculated flux is derived from:

$$P_{\text{fcalc}} = \frac{C - C_0}{r_a + r_s + r_r}$$
(3.15)

(see chapter 2), where r_r is usually assumed to be negligible in comparison with the other leaf resistances. If this negative residual resistance is ignored when calculating pollutant flux then the values of P_{FCALC} obtained would be low in comparison to those for measured pollutant flux.

Figures 3.29 and 3.30 show r₅SO₂ from water vapour transfer, $r_{s}SO_{2}$ from P_{Fmgas} and $r_{r}SO_{2}$ for both varieties of <u>Vicia</u> faba plotted against measured pollutant flux. Although there was a certain amount of scatter, these data indicated the existence of an additional, residual resistance to sulphur dioxide transfer. Taylor & Tingey suggested that the calculated residual resistance in Geranium carolinianum was always negative irrespective of the sulphur dioxide concentration supplied. These workers used the data from Black & Unsworth (1979b) to calculate residual resistances to sulphur dioxide transfer in Vicia faba and found these resistances to range from -0.3 to -5.0 s cm⁻¹. From the figures, it can be seen that residual resistances in Dylan were positive for measured flux values less than 3 μ g m⁻² s⁻¹, above this flux value the calculated residual resistances to sulphur dioxide transfer were negative. In Aquadulce Claudia plants, the data were a little more variable but followed the same pattern observed in Dylan ie. residual resistances were negative at measured flux values above $3\cdot 2 \ \mu g \ m^{-2} \ s^{-1}$. Hällgren and co-workers (1982a), in a study of Pinus sylvestris, reported a residual resistance to SO₂ flux that varied in both magnitude and direction.

In using equation 3.14 to estimate r_rSO_2 , the SO₂ concentration in the leaf interior, C_i, was assumed to be zero. Although Black & Unsworth (1979b) concluded that C_i was zero in <u>Vicia faba</u> at low SO₂ concentrations, it may be that at higher SO₂ concentrations, C_i is greater than zero. The effect of this in <u>Vicia faba</u> would be to make r_rSO_2 more negative since the concentration gradient of SO₂ used in equations 3.12 and 3.13 would be reduced.



Figure 3.29.

Stomatal and Residual Leaf Resistances (s cm⁻¹) to Sulphur Dioxide Flux in <u>Vicia faba</u> CV. Dylan, plotted as a function of Measured Pollutant Flux (μg m⁻² s⁻¹). [3.29a & 3.29b show stomatal resistance, r_sSO_2 , as calculated from that for water vapour transfer and $r_sSO_2^1$, as derived from mass balance calculations: 3.29c shows residual resistance, r_rSO_2 , to SO_2 uptakel.





Figure 3.30.

Stomatal and Residual Leaf Resistances (s cm⁻¹) to Sulphur Dioxide Flux in <u>Vicia faba</u> CV. Aquadulce Claudia, plotted as a function of Measured Pollutant Flux (μ g m⁻² s⁻¹). I3.30a & 3.30b show stomatal resistance, r_sSO₂, as calculated from that for water vapour transfer and r_sSO₂¹, as derived from mass balance calculations: 3.30c shows residual resistance, r_rSO₂, to SO₂ uptakel. The implications of this additional negative resistance to sulphur dioxide flux are open to debate. Because diffusive resistance is proportional to pathlength (Meidner & Mansfield, 1968), Taylor & Tingey suggested that, for sulphur dioxide, the mean diffusive path length in the gas phase is less than that for effluxing water vapour. This proposal is the reverse of that for carbon dioxide in that CO_2 is thought to have a longer path length for diffusion than H_2O due to its passage into the mesophyll cells. These authors suggested that the shorter pathlength of SO_2 is due to the high solubility of the gas in water and its unique chemical reactivity in solution, and postulated that the predominant site for SO_2 deposition in the leaf interior is the substomatal chamber and not the mesophyll tissue. This idea of a shorter pathlength of diffusion for SO_2 is disputed by Winner <u>et al.</u> (1985) because the average pathlength of water vapour efflux is considered to be extremely short.

The differences between measured and calculated pollutant flux have been shown to be due to incorrect assumptions of r_r when calculated pollutant flux is estimated. When stomatal resistances to sulphur dioxide were calculated from analogy to water vapour transfer, Aquadulce plants were found to have higher resistances to sulphur dioxide flux than Dylan plants (Figs. 3.29a & 3.30a) and the relationship between flux and stomatal resistance was variable. However, when stomatal resistance was calculated from the concentration gradient between the interior and the exterior of the leaf (Figs. 3.29b & 3.30b), there was a much closer correlation between measured pollutant flux and stomatal resistance to SO₂ flux, resistances appearing to be much lower at higher SO₂ fluxes in both varieties. There were no apparent differences in stomatal resistances to pollutant flux between the varieties when resistance was calculated in this way as opposed to the resistances obtained from analogy water vapour transfer.

3.10 CONCLUSIONS & DISCUSSION

3.10.1 Net Photosynthesis and Ambient SO₂

Whilst plants of the variety Dylan have been studied on many occasions by Black & co-workers (Black & Black, 1979a,b; Black & Unsworth, 1979a,b,c, 1980; Black, Ormrod & Unsworth, 1982), little evidence has been found in the literature concerning intra-specific variation in pollutant response in <u>Vicia faba</u>. The results in this study provide evidence for a number of differences in response to SO_2 occurring between the two varieties Dylan and Aquadulce Claudia.

That sulphur dioxide induces photosynthetic inhibition in plants is undisputed. As outlined in the introduction to this chapter, numerous reports have shown inhibition in Pnet in response to SO_2 . The extent of the inhibition shown in Figures 3.1 and 3.2 for both varieties of <u>Vicia faba</u> was typical of that observed for all SO_2 fumigations above 400 ppb. Plants of the variety Dylan exhibited full recovery to pre-fumigation rates within 15 h of the end of the fumigation period. However, Aquadulce Claudia plants did not show full recovery within 24 h following SO_2 fumigations above 400 ppb.

The responses of both varieties to a range of sulphur dioxide concentrations were shown in Figures 3.3 and 3.4. A polynomial regression fit demonstrated close correlations between ambient SO₂ concentration and observed photosynthetic response. However, Black & Unsworth (1979b), working with <u>Vicia</u> <u>faba</u> CV. Dylan found a curvilinear relationship between depression of photosynthetic rates and SO₂ concentration opposed to that obtained for Dylan plants in this study. Figure 3.31 gives a comparison of the data from this study with that from Black & Unsworth. The differences in observed photosynthetic responses to sulphur dioxide in Dylan plants between the two studies may be attributed to a combination of factors including differences in exposure system design and differing environmental parameters at the time of the exposure leading to differing leaf resistances (Unsworth, 1982; Mansfield, 1983). The observed differences in photosynthetic response may also be due to the differing lengths of the sulphur dioxide exposure periods, being two hours for Black & Unsworth and 4 h in this study.

Figure 3.31



Figure 3.31.

Comparison of the data obtained in this study (•) and that of Black & Unsworth (1979b) (I) to show variation in the degree of net photosynthetic inhibition in <u>Vicia faba</u> CV. Dylan in response to a range of sulphur dioxide concentrations (ppb).

Black & Unsworth (1979b) working with <u>Vicia faba</u>, and Winner & Mooney (1980c) working with <u>Atriplex</u> spp. reported a temporary enhancement of photosynthetic activity early in the exposure period and Takemoto & Noble (1982), working with <u>Glycine max</u>., found 250 ppb SO₂ for 2 h to cause an enhancement in Pnet throughout the exposure period. This enhancement of photosynthetic rate was observed in plants of the variety Aquadulce Claudia when SO₂ concentrations were below 110 ppb (Fig. 3.4), but was not observed in the Dylan plants. Aquadulce plants also exhibited greater photosynthetic inhibition than was observed in Dylan plants at higher SO₂ concentrations (above 450 ppb).

Although no studies have been performed exclusively to examine intra-specific variation in pollutant response in <u>Vicia faba</u>, it is interesting to note that Darrall (1986) reviewed the lowest SO₂ concentrations reported to inhibit photosynthesis in a number of plant species. Darrall examined two varieties of <u>Vicia faba</u> CV. 'Three Fold White' and 'Blaze' and stated the minimum SO₂ concentration to inhibit Pnet in both varieties was 300 ppb for 2 h. Black & Unsworth (1979b) observed 15% inhibition in Dylan after 35 ppb for 2 h and in this study both Dylan and Aquadulce Claudia plants responded to SO₂ fumigations below 100 ppb for 4h. The wide disparity in the lowest SO₂ concentration to initiate a photosynthetic response in <u>Vicia faba</u> between these data and that of Darrall cannot easily be explained.

It is now accepted that, in general, photosynthetic inhibition is more severe in cultivars with higher rates of photosynthesis (TERG, 1988); however, in this study Aquadulce Claudia plants were shown to have significantly higher 'natural' photosynthetic rates than plants of the variety Dylan and it was also shown that at SO₂ concentrations below 100 ppb, Aquadulce plants showed photosynthetic enhancement whilst photosynthetic rates in Dylan plants were inhibited. As stated in the introduction to this chapter there are a number of proposed mechanisms for differential sensitivity within species, differential pollutant uptake playing a large part (eg. Klein <u>et al.</u>, 1978); this being discussed in more detail later in this section. Varying capacity to detoxify hydrogen peroxide (H_2O_2) accumulated as a result of SO₂ fumigation has been proposed as another factor contributing to differential sensitivity (Alscher <u>et al.</u>, 1987). Tanaka <u>et al.</u> (1982a,b) reported that H_2O_2 accumulated in chloroplasts during SO₂ fumigation and suggested that photosynthetic inhibition in SO₂ fumigated plants was caused by H_2O_2 . In their study of the different sensitivities of two pea cultivars to SO_2 , Alscher <u>et al.</u> reported differing abilities to detoxify H_2O_2 and suggested that the difference between cultivars in the degree of photosynthetic inhibition reflected the relative resistance to SO_2 of the respective H_2O_2 detoxification systems.

Black (1982) suggests that enhanced rates of photosynthesis can often be attributed to increases in stomatal conductance or to depressed rates of photorespiration. It may be assumed that depression of photosynthetic rates may be due to decreases in stomatal conductance and/or changes in respiration rates in response to SO_2 . However, when the stomatal responses to SO_2 of the two varieties used in this study are examined, it can be seen that the substantial reductions in Pnet observed cannot result entirely from changes in these factors.

3.10.2 Dark Respiration and Ambient SO2

The dark respiration (*Rd*) responses of both varieties were outlined in §3.5. Aquadulce Claudia plants showed no respiratory response to sulphur dioxide but were found to have double the dark respiration rate of Dylan in the absence of pollutant. In contrast, Dylan plants exhibited increases in dark respiration that were large and independent of the SO_2 concentration supplied. These observed responses in Dylan plants agree well with the results of Black & Unsworth (1979b) where dark respiration rates were effectively doubled in response to sulphur dioxide. It is of interest to note that if this increase in dark respiration is assumed to continue in the light period during SO_2 exposure then the observed inhibition in net photosynthesis at low sulphur dioxide concentrations may be due entirely to increases in *Rd*. This would explain the fact that photosynthetic inhibition is seen to occur at these low sulphur dioxide fumigation levels when stomatal resistance has decreased in response to the pollutant.

3.10.3 Stomatal Responses to Ambient SO₂

Typical examples of stomatal responses to high sulphur dioxide concentrations, throughout one photoperiod, were shown in Figures 3.6 and 3.7. it was seen that stomatal resistance increased in response to SO_2 , the magnitude of response being much greater in Dylan than in Aquadulce Discussion

Claudia plants. Figure 3.6 portrayed changes in stomatal resistance in Dylan plants during a four hour fumigation period with 600 ppb SO₂ and it can be seen, from comparison with the control plant, that enhanced stomatal opening occurred during the first 30 minutes of the exposure period followed by a rapid and steady increase in r_s throughout the remainder of the fumigation period. These data contrast with that presented by Majernik & Mansfield (1970 & 1971) who observed enhanced stomatal opening in <u>Vicia</u> <u>faba</u> CV. Windsor Harlington during SO₂ fumigations from 0.25 to09 ppm. Enhanced stomatal conductance, following the onset of fumigation, was not observed in Aquadulce Claudia plants at 500 ppb SO₂ (Figure 3.7) and although an overall increase in r_s occurred after a four hour fumigation period, the increase in r_s was only detectable during the 4th hour of the fumigation period.

The stomatal responses of both varieties of <u>Vicia faba</u> to a range of sulphur dioxide concentrations were shown in Figures 3.8 and 3.9. Variability in stomatal response in Dylan plants at concentrations up to 400 ppb SO₂ was shown, the predominant response being enhanced stomatal opening. Both varieties exhibited stomatal closure at SO₂ concentrations above 400 ppb, the magnitude of response being much greater in Dylan plants. These results contrast with those obtained by Biscoe, Unsworth & Pinckney (1972) who polluted <u>Vicia faba</u> CV. Great Green Longpod with SO₂ concentrations between 35 and 500 ppb for 0.5 - 12 hours, and found decreases in r_s at all SO₂ concentrations for all exposure periods. In all reported studies with <u>Vicia faba</u>, enhanced stomatal opening has been shown in response to a wide range of sulphur dioxide concentrations (Table 3.2).

The variability in observed stomatal responses in Dylan plants, to SO_2 concentrations below 400 ppb, may be due to a number of factors. Stomata are extremely sensitive to the environment in the absence of aerial pollution and virtually any change in one of the major components such as light, temperature, humidity or gaseous composition of the atmosphere, results in a change in stomatal aperture. The switch in stomatal response from opening at lower SO_2 levels to closure at higher SO_2 levels, in <u>Vicia faba</u> may be due to the mechanism of action of SO_2 on the stomata. A number of theories have been proposed to explain stomatal responses to SO_2 . Elkiey & Ormrod (1979) found significant changes in leaf water potential in <u>Vicia faba</u> at high SO_2 concentrations. This is important because stomatal aperture is determined by the turgor of the guard cells and the

surrounding epidermal cells thus any change in the turgor of the cells in response to SO₂ will alter the aperture of the stomatal pore. Black & Black (1979a,b) used light microscopy to investigate SO₂ effects on <u>Vicia faba</u> and found that enhanced stomatal opening was associated with extensive destruction of the adjacent epidermal cells rather than any action of SO_2 on the guard cells themselves. However, at higher SO₂ concentrations, where stomatal closure was observed, the response was found to be associated with cellular disorganisation and reduced guard cell viability. Changes in carbon dioxide concentration in the substomatal cavity, as SO_2 depresses photosynthesis, have been proposed as inducing a stomatal response (Koziol & Jordan, 1978) but, as Black (1982) comments, this hypothesis is unsatisfactory in explaining enhanced stomatal opening at low sulphur dioxide concentrations where photosynthetic inhibition also occurs. Stomatal responses to SO_2 have also been linked to leaf abscisic acid content (Kondo & Sugahara, 1978 as described in \$3.1) and ethylene production (Bressan et al., 1979).

It is evident from the data presented in Figures 3.8 and 3.9 that, unlike photosynthetic inhibition, the relationship between stomatal response and ambient sulphur dioxide concentrations below 400 ppb is weak, the correlation between the two variables being relatively low compared to the correlations obtained for photosynthetic activity and SO_2 . It is perhaps important to remember that the actual flux to the plant is, in all likelihood, a more accurate indication of the relationship between plant damage and sulphur dioxide fumigations. The importance of internal sulphur dioxide flux to <u>Vicia faba</u> in relation to ambient sulphur dioxide concentration and observed plant responses is discussed later in this section.

3.10.4 Leaf Resistances to CO_2 , H_2O and SO_2 Transfer

In §3.7.1 the pathways for the transfer of gases into and out of the leaf were discussed and the important resistances to gas exchange were detailed. Because of the variability in both stomatal and net photosynthetic response to SO_2 in both varieties, the leaf resistances before and after sulphur dioxide fumigation were partitioned. The influences of SO_2 were studied on each resistance in turn in an attempt to distinguish between stomatal and non-stomatal effects of SO_2 on

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photosynthetic rate. The data for both Aquadulce Claudia and Dylan plants demonstrated that SO_2 had an effect on Pnet that could not be accounted for by changes in stomatal resistance alone. Several workers have tried to distinguish between stomatal and non-stomatal effects of SO_2 (Black & Unsworth, 1979b; Furakawa <u>et al.</u>, 1980a,b; Winner & Mooney, 1980b,c; and Carlson, 1983b) and suggested that increased internal leaf resistances were responsible for a large portion of pollutant induced increases in total resistance to carbon dioxide exchange. Figures 3.10 and 3.11 showed that changes in stomatal resistance were not fully responsible for changes in net photosynthesis. When the data in Tables 3.7 and 3.8 were examined it was seen that the estimated gross photosynthetic rate (Pmax), assuming only r_1 had changed, was greater than the observed gross photosynthetic rate at higher SO_2 concentrations. This indicates that increases in other non-stomatal resistances in the carbon dioxide transfer chain had occurred.

The residual resistance to carbon dioxide transfer increased in both varieties of <u>Vicia faba</u> in response to SO₂. In Aquadulce Claudia plants, the residual resistance increased by 70 - 500 s m⁻¹ above prefunigation values in the majority of plants exposed to SO₂ greater than 100 ppb . Again, in Dylan plants, the response was a little more variable, r_r increasing when SO₂ exceeded 290 ppb. This increase in r_r correlates well with data obtained by Carlson (1983a,b) working with <u>Glycine max</u>. where r_r increased with increasing SO₂ concentrations from 0.2 - 1.0ppm. This increase in r_r in response to SO₂ was much more pronounced between concentrations of 0.4 and 0.5 ppm, suggesting the passing of a threshold concentration, above which, SO₂ has a pronounced effect on carbon dioxide metabolism. This large increase in r_r around 500 ppb SO₂ was observed in both varieties of <u>Vicia faba</u>.

Differences between cultivars of the same species in pollutant induced changes in residual and stomatal resistances were also found by Furakawa <u>et al.</u> (1983, 1984a) in three poplar species exposed to ozone. These workers found photosynthetic inhibition in one variety to be attributed solely to increases in mesophyll diffusive resistance, whilst in the other two varieties, stomatal closure was also a factor inducing the reduction of net photosynthetic rates.

In short, in Aquadulce Claudia plants, changes in rates of gross photosynthesis at all SO_2 concentrations appear to be due in part to changes in stomatal resistance but the predominant influencing factor on CO_2 exchange was the residual resistance, r_r , which increased in response to SO_2 . Dylan plants however, had a slightly different response, photosynthetic responses were attributed in part, to changes in the stomatal resistance and the residual resistance to carbon dioxide transfer, but the effects on CO_2 exchange were moderated by changes in dark respiration rates in response to SO_2 exposure. At low SO_2 concentrations, changes in gross photosynthesis in Dylan plants, appeared to be due entirely to changes in stomatal resistance. However, net photosynthetic rate was seen to be depressed by SO_2 at low concentrations because dark respiration was stimulated. Carlson (1983 a,b) attributed significant inhibition in Pnet in <u>Glycine max</u>. to increases in r_r and, to a lesser extent, increases in r_3 in response to SO_2 but no dark respiration response was detected.

3.10.5 Stomatal Resistance and Photosynthesis

Figures 3.13 and 3.14 showed the extent of photosynthetic inhibition in <u>Vicia faba</u> as a function of changes in stomatal resistance. For Dylan plants, photosynthetic inhibition was correlated with both enhanced stomatal opening and stomatal closure, this being explained by the reasons given above. Aquadulce Claudia plants, however, showed that when Pnet was enhanced by low SO₂ fumigations, it was as a result of enhanced stomatal opening, photosynthetic inhibition being associated with stomatal closure. These were results one would expect given that there was no stimulation of dark respiration in Aquadulce Claudia plants. In Dylan plants, small increases in Pnet, at low SO₂ concentrations due to enhanced stomatal opening may be negated by the doubling in the dark respiration rate.

3.10.6 Sulphur Dioxide Flux

The importance of relating plant responses to SO_2 to flux rather than ambient sulphur dioxide concentration has been discussed in §3.8.1. Whilst significant intra-specific differences in SO_2 response in <u>Vicia faba</u> have been determined with respect to ambient sulphur dioxide concentration, there is evidence in the literature to suggest that differential sensitivities of plants to SO_2 arise from different rates of pollutant

uptake from similar ambient SO₂ concentrations (Klein et al., 1978; Taylor, 1978; Butler & Tibbetts, 1979c; Winner & Mooney, 1980a; Kimmerer & Kozlowski, 1981). Thus the observed differences in response between Dylan and Aquadulce Claudia plants may arise from differences in pollutant uptake or, if the amount of SO_2 entering the plant is the same, from different sites/modes of action of SO_2 on the metabolic processes within each variety. In \$3.8, observed changes in net photosynthesis and stomatal resistance for each variety, were examined in relation to the actual pollutant flux to the plant. Figures 3.15 to 3.18 showed photosynthetic inhibition as a function of both measured and calculated pollutant flux. The threshold values for measured flux, above which photosynthetic inhibition was seen to occur, were 0.3 μ g SO₂ m⁻² s⁻¹ in plants of the variety Dylan and 1.8 μ g SO₂ m⁻² s^{-1} in Aquadulce Claudia plants. When calculated flux is considered, again, Aquadulce plants had a higher threshold flux of 1.5 µg m⁻² s⁻¹ compared to the 0.8 μ g m⁻² s⁻¹ found for Dylan plants. The data from calculated flux suggested that Aquadulce plants had a higher tolerance to SO_2 than Dylan, as indicated by a higher threshold concentration. However, once this threshold flux had been exceeded, Aquadulce plants were much more sensitive to SO₂ than those of the variety Dylan, Dylan plants exhibiting half the degree of photosynthetic inhibition found in Aquadulce for the same degree of flux. Similar results were observed by Winner & Mooney (1980a) when sulphur dioxide flux was plotted against photosynthetic inhibition in Diplacus aurantiacus and Heteromeles arbutifolia. Photosynthesis in D.aurantiacus was more sensitive than that of H.arbutifolia for a given sulphur dioxide flux but <u>D.aurantiacus</u> was seen to have lower rates of SO₂ absorption for a given ambient sulphur dioxide concentration. However, using the same two species, Winner & Mooney (1908b) state that D.aurantiacus has a higher absorption rate for SO₂ than <u>H.arbutifolia</u> at any given SO₂ concentration. When SO₂ flux exceeded 5 μ g cm⁻², D.aurantiacus showed more than double the depression in photosynthetic rate observed in <u>H.arbutifolia</u>.

There was a contradiction in relative sensitivities when measured flux was examined in Dylan and Aquadulce Claudia plants. Although Aquadulce plants had a higher threshold flux value, when the threshold value had been exceeded, Dylan plants appeared to be more sensitive to SO_2 than Aquadulce and showed up to 20% inhibition for flux values between 0.3 and 1.8 µg m⁻² s⁻¹ whereas enhancement of Pnet was observed in Aquadulce plants at these flux values.

Winner & Mooney (1980a) suggested that the responses of stomata during SO₂ exposure may have been one of the factors contributing to variation in SO₂ sensitivities between plants. Figures 3.19 to 3.22 showed the stomatal responses of Vicia faba plotted as a function of pollutant flux. For both varieties, there was a good linear correlation between measured flux and observed stomatal responses. In Dylan plants, flux values below 4 μ g m⁻² s⁻¹ were associated with stomatal opening, when SO₂ flux exceeded this value stomatal closure was seen to occur. In Aquadulce Claudia plants, the threshold concentration, where the switch from stomatal opening to closure occurred, was approximately 3.2 µg m⁻² s⁻¹. Winner & Mooney (1980a,b) correlated pollutant flux with stomatal closure in both D.aurantiacus and H.arbutifolia, stomatal conductance decreasing with increasing SO₂ flux. Similarly, Carlson (1983b) associated increasing pollutant flux in <u>Glycine max</u>. with increasing stomatal resistance. In another study, using five clones of Populus tremuloides, Kimmerer & Kozlowski (1981) associated stomatal closure with increasing pollutant flux, the extent of the stomatal closure observed at any given flux being dependent on the variety.

As stomatal conductance determines gas-phase sulphur dioxide flux into the substamatal cavity, many studies have focused upon differences in gas-phase conductance as the primary mechanism causing differences in plant responses to SO₂. Most workers assumed that stomatal conductance to water vapour was proportional to internal SO₂ flux. In a number of species, differences in stomatal conductance account for differences in pollutant sensitivity (Bonte et al., 1977; Amundsen & Weinstein, 1981). However, in other species, different plant responses to SO2 were not clearly associated with differences in stomatal conductance (Ayazloo, Garsed & Bell, 1982). In Vicia faba, Dylan and Aquadulce plants showed different relationships between changes in stomatal conductance and sulphur dioxide flux. When measured sulphur dioxide flux was 1 μ g m⁻² s⁻¹ Aquadulce plants exhibited enhanced stomatal opening of only 7%, whereas Dylan plants exhibited a 30% enhancement in stomatal opening. It was apparent that stomatal resistance/conductance were not the only factors determining pollutant flux in Vicia faba.

The observed differences between the varieties may be explained if the relative flux to each variety is examined in relation to ambient sulphur dioxide concentration and total leaf resistance to pollutant flux. Figures 3.23 to 3.26 demonstrated that, for both measures of flux, Aquadulce Claudia plants had significantly less flux into the leaves of the plant than Dylan plants at any given SO_2 concentration above 100 ppb. When stomatal resistance to sulphur dioxide flux was calculated from analogy to water vapour transfer, Aquadulce plants were found to have significantly higher resistance to flux during SO_2 fumigations than was observed in Dylan plants.

In both varieties, measured flux (from mass balance calculations) to the plant was significantly higher than calculated flux (from analogy to water vapour transfer). It might be expected that both determinations of flux would give similar results, and Carlson (1983b) found a 1:1 correspondence between calculated and measured flux to <u>Glycine max</u>. To ascertain which of the flux values was more accurate, stomatal resistances to pollutant flux were examined. Firstly, stomatal resistance to SO_2 , r_2SO_2 , was calculated from analogy to water vapour transfer and this value was used to derive calculated pollutant flux. Alternatively, stomatal resistance to SO_2 transfer, $r_sSO_2^1$, was calculated from SO_2 data using analogue modelling techniques (Taylor & Tingey, 1983). When both measurements of resistance were compared, the estimates of $r_{3}SO_{2}$ from the SO₂ data were consistently different to the simultaneous estimates of r_sSO₂ derived from analogy to water vapour transfer. Taylor & Tingey postulated that these differences were indicative of a residual resistance to SO₂ transfer, suggesting that the diffusive pathways for SO₂ and H₂O are not completely synonymous. In Geranium carolinianum, the resulting residual resistance was always negative, indicating that SO_2 has a shorter diffusive pathway than H₂O. However, the results for Dylan and Aquadulce Claudia showed the residual resistance to be positive at low sulphur dioxide flux values and negative when flux values exceeded a certain threshold value. For Dylan plants, this value was 2 μ g m⁻² s⁻¹, for Aquadulce it was 3 μ g m⁻² s⁻¹ (Figures 3.29a, 3.30a). These data show that measured pollutant flux is more accurate assessment of true SO₂ flux than calculated flux. Water vapour has no added resistance to transfer so that when calculated flux is determined from analogy to water vapour transfer incorrect assumptions of rr are made, r_r to SO₂ being assumed to be effectively zero.

The existence of a residual resistance to sulphur dioxide transfer, positive or negative in direction, influencing SO_2 flux is of

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importance in explaining differences in observed plant responses to SO_2 . The influence of residual factors have not been considered in most studies (eg. Winner & Mooney, 1980a; Kimmerer & Kozlowski, 1981; Carlson, 1983a), although the existence of a residual resistance to pollutant transfer has been suggested by many authors (Hällgren, 1978; Black & Unsworth, 1979b; Heath, 1980). Where residual factors have been considered, the data are still conflicting, some studies suggesting that pollutant flux is less than may be expected from stomatal conductance measurements (Taylor & Tingey, 1982 [ozone]; Hällgren <u>et al.</u>, 1982a). Other studies suggest that SO_2 flux is higher than that expected from gas-phase conductance values (Klein <u>et al.</u>, 1978; Taylor & Tingey, 1983).

The data for Aquadulce Claudia and Dylan plants suggested that at low SO₂ concentrations, when r_r is positive, less flux occurred than may have been expected from stomatal resistance measurements, rs decreasing in response to SO₂. It appeared that a switch occurred at higher SO₂ concentrations, the residual resistance became negative and as a result, flux was greater than expected given the observed increases in stomatal resistance in response to SO₂. These data correlated well with the data relating ambient sulphur dioxide concentration to pollutant flux. For both varieties, flux was shown to be proportional to SO_2 concentration, highest flux values being recorded at higher SO_2 concentrations. However, because stomatal closure has been shown to occur at high SO₂ concentrations, one might expect lower flux values. Taylor, McLaughlin & Shriner (1982) showed that for <u>Phaseolus</u> vulgaris the ratio of SO₂ flux to concentration decreased several fold as SO_2 concentration increased from 0.2 ppm to 0.8 ppm. This was not seen to occur in either variety of Vicia faba, even though stomatal resistance decreased at higher SO_2 concentrations. The ratio of flux to ambient SO₂ does not decrease in <u>Vicia</u> faba at higher SO₂ concentrations because the residual resistance to SO₂ transfer becomes increasingly negative with increasing SO_2 and thus facilitates SO_2 uptake.

Having said that sulphur dioxide flux to Aquadulce plants was significantly less than to Dylan, and Aquadulce plants had significantly higher stomatal resistance to SO_2 transfer for a given ambient SO_2 concentration, it must also be noted that when flux values were equal in each variety, Aquadulce plants exhibited significantly greater depression of net photosynthetic rates. This implied that the photosynthetic mechanism in Aquadulce Claudia plants was more sensitive to sulphur dioxide than

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Dylan although the threshold flux was higher in Aquadulce Claudia. Klein <u>et</u> <u>al</u>. (1978) found similar trends when they compared the relative sensitivities of <u>Pisum sativum</u> and <u>Zea mays</u> to sulphur dioxide. <u>Pisum</u> <u>sativum</u> accumulated more inorganic sulphur than <u>Zea mays</u> under identical fumigation conditions, consequently <u>Pisum</u> was said to be more sensitive to SO_2 than <u>Zea mays</u>. However, Klein associated the greater uptake in <u>Pisum</u> to be due not only to a lower diffusive resistance but to a greater 'internal sink' for SO_2 uptake. These authors were not able to determine if this 'sink' was based mainly on physical, chemical or physiological parameters.

Differential sensitivities in plants to sulphur dioxide can therefore be due to avoidance mechanisms, involving high diffusive resistances and/or a low internal sink, or to SO_2 tolerance. Experiments have been performed on differentially sensitive plants which take up comparable amounts of SO_2 under identical fumigation conditions. These data indicated that the mechanisms of resistance must be concerned with physiological processes within plant tissues (Klein <u>et al.</u>, 1978).

In this study, differential sensitivity to SO_2 between Aquadulce and Dylan plants appeared to be due to a combination of avoidance and tolerance depending on SO_2 concentration.

The table below gives representative examples of both varieties of <u>Vicia faba</u> in their response to low and high sulphur dioxide concentrations.

	External SO ₂ Conc (ppb)	Flux (µg m ⁻² s ⁻¹)		Photosynthetic Inhibition	Change in Stomatal resistance	residual resistance
<u>VARIETY</u>		PFmeas	PFCALC	(%)	(76)	r _r , to 30 ₂ (s cm ⁻¹)
AQUA	128	1.76	0.76	-13.9	6·1	-2.75
DYLAN	92	0-68	1.03	6 • 7	-16.4	1 • 29
AQUA	550	6·43	3-87	77 • 1	-34.9	-1-61
DYLAN	510	7.12	5.74	32 • 2	-40.2	-0•49

Summary of Responses of Both Varieties of Vicia faba to SO2.

CHAPTER FOUR

4.1 INTRODUCTION

In Chapter 3 the effects of sulphur dioxide on the gaseous exchange mechanisms of two varieties of Vicia faba were defined under optimum environmental conditions of light and temperature. However, environmental conditions are seldom 'ideal' or constant in the field. As is described below environmental conditions affect plant physiological processes and modify the absorption of pollutant into the leaf thus indirectly influencing plant pollutant responses. Secondly, prevailing environmental conditions may influence plant sensitivity to absorbed pollutants thus exerting a direct effect. Conversely, pollutant induced physiological changes may influence the plants sensitivity to adverse environmental conditions and as is outlined below, there is growing evidence in recent literature to substantiate such interáctions. Investigations into environmental/pollutant interactions are of paramount importance to the UK and other countries with temperate climates as most economically important crops are grown during autumn, winter & spring months when adverse environmental conditions such as low light and temperature prevail and most gaseous pollutant concentrations are at their highest. Thus, this section of the study deals with the modification of plant pollutant responses by added environmental stress prior to and during pollutant exposure.

In order to understand the combined effects of environmental stress and sulphur dioxide fumigations, it is first necessary to define the effects of such environmental stress on plants in the absence of air pollutants. There is much evidence in the literature documenting the effects of such environmental stresses on plant physiological processes.

4.1.1 The Effects of Low Light Intensities on Net Photosynthesis

Owing to the fundamental role of photosynthesis in plant metabolism, light is considered one of the most important environmental factors as solar radiation not only supplies the energy for plant metabolism but also plays an important regulatory role in the life of a plant, influencing plant temperature, growth and development. Photosynthetic activity is influenced by three properties of light; spectral quality, intensity and duration (Fitter & Hay, 1981).

Light intensity markedly affects the rate of oxygen evolution or carbon dioxide uptake in photosynthesis. A generalised plot of light intensity (irradiance) against photosynthesis (as measured by CO_2 exchange) is shown in Figure 4a. There is a linear relationship over a considerable range up to a saturating light intensity, at which point, other factors such as CO_2 concentration become rate limiting. It can be seen from the figure that reducing light intensity below saturating point has a profound effect on photosynthetic rate. At lowest light intensities, gross photosynthetic rate, Pmax (net photosynthesis + respiration), becomes less than the rate of respiration and net photosynthesis becomes negative. The point at which the rates of the two processes just balance is the compensation point.

A major problem for plants subject to low light intensities is that of maintaining a positive carbon balance and there are three ways of achieving this. Firstly, under low light intensities there is a reduction in respiratory rates, therefore, the compensation point is lowered. However, reduced respiration rates also result in much slower growth rates. Chippindale (1932) observed much reduced growth rates in Festuca pratensis under low light intensities but normal growth was resumed when the stress was removed. A similar case was reported by Cross (1975) for Rhododendron ponticum seedlings. Secondly, plants grown under low light intensity have been shown to have increased leaf area providing a greater surface for light adsorption, while severe shade stress has been shown to cause only slight changes in the ratio of leaf weight to plant weight (LWR) eg. in Impatiens parviflora (Evans, 1972) and in Veronica montana (Fitter & Ashmore, 1974). However, significant changes in specific leaf area, SLA, the ratio of leaf area to lcaf weight, have been found in response to low light stress. Newton (1963) showed the SLA of Cucumis sativa to be inversely proportional to total radiation and Evans & Hughes (1961) showed a threefold increase in SLA for Impatiens parviflora when grown in 7% full sunlight. The increased SLA observed under low light stress implies important anatomical changes in internal leaf structure (Fig. 4b). In general, under low light stress, leaves are larger and thinner than those of plants grown under higher light intensities. The palisade tissue is poorly



Figure 4a.

Representative photosynthetic response curve showing the compensation point, the light intensity at which net CO_2 exchange is zero, as the point at which gross photosynthesis equals respiration (from: Fitter & Hay, 1981).



Figure 4b.

Cross-sections of sun and shade leaves of silver maple (<u>Acer Saccharinum</u>). [From Wilson & Loomis, 1967] developed and often reduced to one cell thickness, there is less mesophyll tissue and intercellular spaces are large; also the cuticle is much thinner (Jackson, 1967 [deciduous trees]; Wilson & Loomis, 1967 [<u>Acer saccharinum</u>]; Hiesey, Nobs & Björkman, 1971 [<u>Mimulus</u> spp.]).

These changes in leaf morphology under low light stress have been found to affect CO_2 diffusion. The internal (mesophyll) resistance to CO_2 transfer is reduced by increased pore space in the mesophyll tissue (Holmgren, 1968). This effect has been demonstrated in the field by Fekete, Szujkó-Lacza & Horvath (1973) who found a high correlation between photosynthetic rate and mesophyll chamber size and the ratio of intercellular spaces to assimilating cells, whereas there was little correlation between photosynthetic rate and stomatal number.

The third strategy adopted by the plant to maintain a positive carbon balance under low light stress is an increase in photosynthetic activity per unit light energy and leaf area. It has been described above that, under low light intensities, leaves have higher CO_2 diffusion rates and therefore have improved access to substrate. Shaded leaves have also been shown to have enhanced chlorophyll concentrations per unit weight (Shirley, 1929) which confer increased light gathering capacity. Plants grown under low light intensities also exhibit increased activity of the photosynthetic apparatus ie. the initial slope of the rate/intensity curve shown in Figure 4a is increased.

4.1.2 Influence of Low Light Intensities on Stomata

Although morphological and photosynthetic changes have been shown to occur in plants in response to low light stress, the influence of this stress on stomatal movement is unclear. It is well documented that light is the stimulus for stomatal opening during the day, however, stomatal movement is also dependent on the CO_2 concentration inside the leaf. In leaves, well provided with water, an increase in light intensity causes stomatal opening (Gaastra, 1959). However Heath & Russell (1954) found that an increase in ambient carbon dioxide concentration induces stomatal closure. Evidence exists for both the direct stomatal response to light (Meidner & Mansfield, 1965, Mansfield & Meidner, 1966; Wong, Cowan & Farquhar, 1978, 1979; Mansfield, Travis & Jarvis, 1981; Sharkey & Raschke, 1981) and the indirect one which is mediated by changes in the intercellular CO_2 concentration (Raschke, 1975; Raschke, Haneburth & Farquhar, 1978; Sharkey & Raschke, 1981).

Sharkey & Raschke examined five plant species and determined the stomatal response to light to be mainly a direct response to light and, to a small extent only, a response to changes in intercellular CO_2 concentration. However, under low light intensities, the stomata of <u>Zea mays</u> responded primarily to the depletion of CO_2 from the intercellular spaces which was, in turn, caused by changes in the assimilation rate of CO_2 . Stomata of all five species responded to light even when net CO_2 exchange was reduced to zero through the application of cyanizine, an inhibitor of photosynthetic electron transport. The findings of Sharkey & Raschke (1981) concur with the work of Gaastra (1959) who reported that, in high light, stomata of turnip were less sensitive to CO_2 than in low light.

Under high light intensities, in the field, stomata open rather slowly during the early hours of sunlight before reaching a steady state maximum which is maintained during the greater part of the day. However, at low light intensities, stomatal opening is sluggish and produces a low steady state opening (Martin, Donkin & Stevens, 1983).

The minimum amount of light necessary to induce stomatal opening varies between species but Virgin (1956) showed an opening response in etiolated wheat seedlings at 0.01% full sunlight (10 lux). The most reliable work has been performed on cereals because they do not exhibit night opening of their stomata, and the stomata of wheat have been shown to open at a light intensity of 900 lux ie. 1 - 2% full sunlight (Heath & Russell, 1954).

Thus it has been shown that reduced light intensities have profound effects on rates of photosynthesis and respiration. Low light stress may also lead to severe morphological and anatomical alterations in leaf structure and may influence stomatal resistance both directly and indirectly via changes in intercellular CO_2 concentrations.

4.1.3 Effects of Low Temperature on Net Photosynthesis

Plants are unable to maintain their cells and tissues at a constant optimum temperature and, as a result, their leaves, stems and roots are normally within a few degrees of the temperature of the surrounding air or soil. Because of this, the growth and metabolism of plants are

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severely affected by changes in environmental temperature.

The primary effect of cooling plants below their optimum temperature is a reduction in rates of growth and metabolic processes due to a slowing down of enzyme controlled reactions (Fitter & Hay, 1981). Although there is a reduction in the rate of metabolic processes, chilling stress does not always result in visible injury symptoms. Levitt (1972) suggested that, in most cases, plants do not suffer chilling injury until the temperature drops below 10°C, however, there are many exceptions mainly in tropical or subtropical species, for example both rice and sugar cane suffer chilling injury at 15°C.

In general, chilling stress takes place at low, non-freezing temperatures (10 - 12°C). Chilling disrupts the entire physiology of sensitive plants and a number of mechanisms have been proposed to account for the effects. The major factor contributing to chilling injury is a change in membrane permeability. Cell membranes, in chilling sensitive plants, undergo a physical phase transition from normal flexible liquid crystal to a solid gel structure thus bringing about a contraction of the membrane components resulting in the formation of holes and increased membrane permeability (Lyons, 1973). This phase transition may increase the activation energy of membrane bound enzymes leading to interference with metabolic processes. Such changes lead to changes in both photosynthetic and respiratory rates (Levitt, 1972). At low temperatures, photosynthesis has a very high activation energy and, therefore, decreases more rapidly than respiration (Selwyn, 1966). As a result, chilling sensitive plants may be below the compensation point at low temperatures and starvation may result. Translocation has also been shown to be inhibited by low temperatures (Geiger, 1969) resulting in starvation of nonphotosynthesizing plant parts.

The length of the exposure period to chilling stress is very important; exposure must often be prolonged (several weeks) before visible injury occurs but some species have been shown to be damaged by very brief exposures to 10°C (Sutcliffe, 1977). The time spent under chilling stress before visible injury occurs is very much dependent on individual plant species.

Of importance also, is that, in some species, chilling stress effects have been shown to be reversible. In seedlings of <u>Zea mays</u> visible injury symptoms occur within 36 h of exposure to 3°C, but upon transfer to 21°C the leaves return to normal and injury symptoms disappear within 72 h (Sutcliffe, 1977).

Thus the severity of the effects of chilling temperatures on plants can be seen to depend on the sensitivity of the plant species used and the duration of the exposure period.

The effects of periods of cold stress on gaseous exchange, processes in plants, in the absence of visible injury, have been reported. There is considerable evidence in the literature indicating that photosynthetic rate is reduced by exposure to low, non-freezing temperatures (Hesketh, 1968; Taylor & Rowley, 1971; Austin & MacLean, 1972; Björkman, 1981). Oquist (1983) presented a review of current literature of both reversible and irreversible photosynthetic responses to low temperatures and the underlying physiological and biochemical mechanisms. Many workers have concluded that stomatal closure is the primary cause of chilling impairment of net photosynthesis (eg. Drake & Salisbury, 1972; Crookston, O'Toole, Lee, Ozbun & Wallace, 1974) due to the good correlations observed between decreases in Pnet and decreases in stomatal conductance in response to cold stress. However, recent results do not support this hypothesis and Martin, Ort & Boyer (1981) showed that photosynthetic inhibition in tomatoes, following chilling in darkness, could be attributed predominantly to impairment of chloroplast function although stomatal conductance was seen to decrease slightly.

It has also been shown that the most severe reduction in photosynthesis is brought about by chilling stress in strong light (Taylor & Rowley, 1971; Linderman, 1979; Powles, Berry & Björkman, 1980). It appears that chilling stress may result not only in a decrease in carbon dioxide uptake, but also photosynthetic impairment caused by photo-inhibition or photo-oxidation (Rowley & Taylor, 1972; Powles <u>et al.</u>, 1980) and changes in chloroplast ultra-structure (Taylor & Craig, 1971).

That exposure to cold temperatures for short periods may severely disrupt photosynthetic processes was shown by Crookston <u>et al.</u> (1974). These workers showed that <u>Phaseolus vulgaris</u> plants exposed to 5°C for one night exhibited severe reductions in photosynthesis the following day. Photosynthetic reductions were accompanied by a parallel drop in transpiration, a rise in both stomatal and mesophyll resistances to CO_2 uptake and a decrease in leaf water potential. Crookston <u>et al</u>. concluded that photosynthetic reduction following exposure to cold was due to changes in stomatal resistance brought on by temporary water stress. Several other workers have also shown reductions in net photosynthesis when plants are subjected to cold temperatures prior to measurement (Hesketh, 1968; Austin & Maclean, 1972; Drake & Salisbury, 1972). These reductions in Pnet were found to be reversible in most cases, <u>Phaseolus</u> <u>vulgaris</u> exhibiting a recovery to one third that of control plants 5 h after exposure (Austin & MacLean, 1972).

Musser, Thomas & Kramer (1983) exposed 16 - 17 day old plants of <u>Glycine max</u>, to 1 week at 10°C and then returned the plants to 25°C. Both net CO_2 uptake rate and stomatal conductance decreased and large changes in leaf water potential were observed. Most measured processes returned to the levels of the control plants two days after the end of the cold period, however, substantial changes were observed in vegetative morphology between control and treated plants when the plants had reached 90 days of age.

4.1.4 Stomatal Responses to Cold Temperatures

The reductions in photosynthetic rate described above have been attributed, in part, to changes in stomatal resistance (eg. Crookston <u>et al.</u>, 1974). However, a number of contrasting results have been obtained in investigations of the responses of stomatal aperture to low temperatures. This may arise from the fact that temperature may affect guard cell metabolism directly or indirectly through effects on the plant water balance and on the water vapour pressure difference between the leaf interior and ambient air (Oquist, 1983). Decreases in temperature usually result in decreased stomatal conductance (Drake & Salisbury, 1972; Crookston <u>et al.</u>, 1974).

Stomatal aperture is also influenced by temperature-induced changes in intercellular CO_2 concentration. Stomatal closure has been shown to occur in response to increases in internal CO_2 concentration resulting from reductions in Pnet at low temperatures (Drake & Raschke, 1974; Raschke, 1975; Hällgren, Sundbom & Strand, 1982b).

Changes in stomatal resistance in response to environmental stresses such as low light and temperature may significantly influence pollutant uptake by plants and thus alter observed pollutant responses. This is one of several hypotheses, as outlined below, that have been proposed to explain environmental modification of plant pollutant responses. However, few workers have tried to separate out direct as opposed to indirect interaction effects and exact mechanisms have not yet been elucidated.

4.1.5 Environmental Stress and Pollutant Interactions

Environmental stresses such as low light and cold temperature treatments have been shown to markedly influence gaseous exchange processes in plants in the absence of air pollutants. Both stresses have been shown to result in decreased rates of photosynthesis and stomatal conductance. Sulphur dioxide, at optimum environmental conditions, has also been shown to markedly influence net photosynthetic rates and stomatal conductance (Chapter 3). It may be expected that combinations of both environmental and pollution stress would have an additive or even synergistic effect on plants given the profound changes observed in response to either stress alone.

Heck et al. (1965 & 1986) published reviews concerning the interactions of environmental factors on plant pollutant sensitivity. Heck, Heagle & Shriner (1986) suggested that plants are generally more sensitive to SO₂ as light intensity, wind speed, temperature and humidity increase. However, although there have been a great many studies concerning sulphur dioxide and plants until recently, comparatively few workers had studied the combined effects of both environmental and pollutant stress or the modification of plant pollutant responses by added environmental stress. In the last four years there has been an increased awareness of the . implications of environmental/pollutant interactions on plant growth and yield and much more research has been concentrated on trying to identify the nature and causes of such actions. This has lead to the publication of a number of comprehensive reviews detailing current advances involving interactions between air pollutants and several environmental factors (CEC/COST Workshop, 1986; TERG, 1988; Environmental Pollution Special Issue, 1988). However, much more work is needed before definitive responses and mechanisms are elucidated.

In the studies performed, reported results are conflicting, some authors have found pollutant responses to be lessened in response to low light or temperatures (Taniyama, 1972; Heck & Dunning, 1978a,b; Rist & Davis, 1979). Other researchers have found plant pollutant responses to be increased by exposure to low light or cold temperatures prior to or during pollutant fumigation (Juhren, Noble & Went, 1957; Davies, 1980; Miller & Davis, 1981a,b; Jones & Mansfield, 1982; Freer-Smith, 1985; Mansfield & Jones, 1985; Mansfield, Davies & Whitmore, 1986).

These conflicting reports may arise from a number of factors including plant species and the range of temperatures or light intensities used. More importantly, the length of the exposure period to environmental stress and the timing of the exposure in relation to pollutant exposure is critical in determining the interactive response.

4.1.5.1 Light Intensity and Pollutant Response

As stated above, a major theory that has been proposed to explain light induced modification of plant pollutant responses is that light may influence stomatal behaviour and thus pollutant uptake by the plant. However, as described below, data from the literature are conflicting and more recent hypotheses suggest light induced modification of pollution responses may be attributed to respiratory, carbon fixation and allocation processes (TERG, 1988). Experiments on the influence of light intensity on plant pollutant susceptibility have shown that a positive correlation exists between injury and increasing light intensity, up to 38,000 Lux, during pollutant exposure (Guderian, 1977). This has been found to be true with SO, (Setterstrom & Zimmermann, 1939) and with various components of photochemical smog (Juhren et al., 1937; Heck, Dunning & Hindawi, 1965). Heck et al. (1965) found foliar injury in pinto beans in response to ozone increased as light intensity increased up to the maximum available ie. full sunlight. Both Juhren et al. and Heck et al. proposed the existence of a 'threshold' light intensity, below which, no pollutant injury occurs. Both groups correlated this with stomatal closure occurring at very low light intensities. Juhren and co-workers also postulated that small changes in light intensity should not affect the amount of pollutant damage because reductions in stomatal aperture due to low light stress should not necessarily lead to decreased diffusion. These workers suggested that the principles of diffusion of gases through small apertures allows for maximum diffusion of gases even when the stomata are not fully open. This theory may well be supported by the results obtained by Heagle & Letchworth (1982). These workers exposed four cultivars of <u>Glycine</u> max. to ozone either in full sunlight or with 6% or 12% reduction in light intensity. No significant differences in ozone sensitivity were observed between all light
treatments.

In direct contrast to the results described above, several workers have found pollutant sensitivity to be significantly enhanced under reduced light intensities. Menser, Heggestad, Street & Jeffrey (1963) examined the effects of ozone on <u>Nicotinia tabacum</u> plants grown under high or low light conditions. These workers found low light treated plants to be far more sensitive to ozone in that a greater degree of visible necrosis was observed. Ting & Dugger (1968) examined the effects of ozone on cotton plants. Two week old plants were transferred to a range of lower light intensities for 1 week prior to ozone fumigation. An inverse correlation between light intensity and ozone sensitivity was found when measured as % leaf damage. Neither Menser <u>et al</u>. nor Ting & Dugger found any correlation between increased ozone sensitivity under low light intensities and changes in stomatal resistance. Both groups measured higher resistances at lower light intensities even though more injury had occurred.

It can be seen that light modification of plant pollutant responses cannot be explained by purely short term changes in stomatal aperture and reduced pollutant flux. The stomata are assumed to govern pollutant entry into the leaf the and the cuticular pathway is often overlooked. However, Lendzian (1984) has shown the cuticle to be much more permeable to SO_2 , O_3 and NO_2 than H_2O or CO_2 ; sulphur dioxide was shown to be nearly 700 times more soluble in the cuticle than water and Lendzian concluded that SO_2 permeated the cuticle via the lipophilic phase. Thus stomatal closure in response to light may not preclude pollutant entry into the plant. In §4.1.1 it was described how low light stress alters leaf morphology, leaves being larger and thinner and, more importantly the cuticle is much thinner thus pollutant uptake via the cuticular pathway may be of greater significance when plants are grown under low light intensities.

Both Davies (1980) and Jones & Mansfield (1982) found sulphur dioxide sensitivity in <u>Phleum pratense</u> to be enhanced at low light intensities. Mean relative growth rates of leaf area were significantly lowered in response to 0.12 ppm SO₂ under low light stress. Jones & Mansfield suggested that increases in SLA at low light intensities may cause increases in SO₂ uptake per unit leaf dry weight, and this, in conjunction with a lack of energy for repair and detoxification mechanisms, would result in more visible injury being observed.

It can be seen that relatively few workers have correlated changes in pollutant sensitivity with light intensity despite the fact that low light conditions are common, especially in Britain during autumn and winter. There appears to be little data available for 'invisible' injury symptoms such as changes in net photosynthetic responses or dark respiration responses to pollutants under low light stress. Most workers have concentrated on visible injury symptoms or changes in dry matter accumulation and reported data are conflicting. However, Mansfield & Jones (1985) in continuation of their earlier work on Phleum pratense (Jones & Mansfield, 1982) studied the photosynthetic and respiratory characteristics and pollutant uptake of plants grown under high or low light intensities in an effort to identify the cause of enhanced SO₂-induced reductions in leaf dry weight under low light intensities. Important differences were observed in the light response curves of plants grown in the two photoenvironments. Plants grown in the high light environment showed no significant SO_2 response above the compensation point but below this, a stimulation in dark respiration in the polluted plants was evident. In contrast, plants grown under low light intensities showed no respiratory stimulation below the light compensation point but SO₂-induced photosynthetic inhibition was seen to increase with increasing light intensity. Analyses of pollutant flux showed flux to be 50% higher to plants grown under high light intensity thus the smaller effect of SO₂ on these plants could not be attributed to lower pollutant dose. These data correlate, in part, with the earlier observations of Black & Unsworth (1979b) of the light response curves of Vicia faba CV. Dylan. Black & Unsworth observed respiratory stimulation in response to SO_2 below the light compensation point, this increase being independent of SO_2 concentration. However, these authors found increasing SO₂-induced photosynthetic inhibition with increasing light intensity up to saturation point; also inhibition increased with increasing SO₂ concentration. Mansfield & Jones (1985) attributed differences between their data and that of Black & Unsworth to the length of the SO₂ exposure period (3 d for Vicia faba and 26 d for Phleum pratense) and suggested that the longer exposure period had allowed for metabolic adjustments for repair or detoxification. Differences in species studied would also have contributed to differences in data. Both Black & Unsworth and Mansfield & Jones suggested that the data were compatible with the view that SO₂ competes with CO₂ for binding sites in RuBP

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carboxylase since responses were readily reversible. Mansfield & Jones suggested that, under high light intensities, net photosynthesis is maintained in polluted plants at the cost of increased respiratory activity which must reduce the amount of carbohydrate available for use elsewhere in the plant. The increase in pollutant sensitivity under low light conditions was a result of not enough carbohydrate being available to support additional respiration thus repair processes did not take place and photosynthesis continued to be inhibited by SO₂.

It is clear that much more work is required to identify the mechanisms behind low light stress and pollutant interaction. Of significance also is that low light conditions commonly occur in winter when temperatures are also limiting thus the interaction of a combination of limiting environmental factors and plant pollutant responses requires investigation.

4.1.5.2 Chilling Temperatures and Pollutant Response

As stated previously, several studies have been made of the effects of temperature on responses of plants to pollutants, but few have separated the influence of temperature during fumigation episodes from that of temperature before or after exposure to the pollutant. Norby & Kozlowski (1981a) suggest that temperature during exposure is likely to affect plant responses to SO_2 primarily by affecting stomatal aperture and hence pollutant uptake whereas effects of temperature before or after exposure are more likely to be mediated through changes in plant metabolism. Therefore the timing of added temperature stress in relation to pollutant fumigation must be considered when results are compared.

One of the first papers concerning the importance of exposure temperature was that of Swain (1923) who concluded that plants were less sensitive to SO_2 at exposure temperatures of 5°C or less. Similarly, Setterstrom & Zimmermann (1939) exposed alfalfa and buckwheat to SO_2 at temperatures of 4°C or between 18 and 40°C. These workers found both species to be less sensitive to SO_2 at 4°C and equally sensitive at all temperatures between 18 and 40°C.

However, in 1965, Heck, Dunning & Hindawi exposed both pinto beans and tobacco plants to ozone at a range of exposure temperatures from 18 to 35°C. Results showed an inverse correlation between sensitivity to ozone and exposure temperature and this was the first report of this

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nature. Rist & Davis (1979) also investigated the effect of exposure temperature and subjected plants of <u>Phaseolus vulgaris</u> to sulphur dioxide at either 13, 21 or 32°C after plants had been grown at 23°C. These workers found greater visible injury due to SO_2 to occur at highest exposure temperatures and correlated this with increased stomatal conductance leading to increased pollutant uptake. In contrast, Miller & Davis (1981a) exposed <u>Phaseolus</u> to ozone and/or SO_2 at either 15, 24 or 32°C and found visible injury to be increased by exposure temperatures of either 15 or 32°C. The lesser degree of injury observed at 24°C was not correlated with decreases in stomatal conductance. In a further paper Miller & Davis (1981b) examined changes in stomatal conductance in <u>Phaseolus</u> over the same pollutant concentrations and range of exposure temperatures. Stomatal conductance was found to increase with increasing temperature therefore the enhanced pollutant effect at 15°C could not be explained by changes in stomatal conductance.

When the effects of temperature regimes prior to pollutant exposure are considered, results are again conflicting. Hull & Went (1952) subjected a variety of crop plants to periods of 1 to 8 days at either 30, 17 or 3°C prior to exposure to ozonated hexane at 26°C. These workers found injury to be directly related to increased temperature; also less injury was observed when plants had been subjected to eight days at lower temperatures.

Similarly, Menser <u>et al.</u> (1963) used two different growth temperatures (25 and 20°C) for four varieties of tobacco. Plants were maintained at these two temperatures for two weeks prior to exposure to ozone. The plants grown under cool conditions were less sensitive to ozone and showed significant growth variations ie. smaller, darker green leaves. In a second experiment these workers determined the effects of a 14 h period at either 25 or 5°C prior to ozone fumigations and found there to be much less ozone injury after the cold temperature pre-treatment. Heck <u>et al.</u> (1965) concluded that low temperatures during growth for one or more days prior to pollutant exposure are effective in reducing plant sensitivity. In later work, Heck & Dunning examined the responses of <u>Avena sativa</u> to SO₂ (1978a) and <u>Phaseolus vulgaris</u> to O₃ (1978b) following a range of temperature pre-treatments. Plants were subject to periods of 10 to 30 days at temperatures between 18 and 30°C. In all cases, plants were more sensitive to pollutant at higher temperatures. These authors again concluded, that in episodic pollutant exposures, cool periods during growth will favour plant resistance to pollutants, even if the cold periods are fairly short in duration.

In contrast to other reports on pollutant sensitivity following cool temperature pre-treatments Ormrod, Adedipe & Hofstra (1973) investigated the effects of ozone on <u>Raphanus sativus</u>. Plants were grown at either 20 or 30°C daytime temperatures and were exposed to ozone at 25°C. When plants were harvested, the authors reported greater dry weight reductions due to ozone at the lower temperature. However, these experiments were also combined with investigations into the influence of phosphorus and nitrogen nutrition so that all plants were subject to either high or low nitrogen treatments in conjunction with growth temperature and ozone treatments. As a result, temperature effects may have been influenced by the nutritional status of the plant.

It would appear that available data regarding the effects of temperature on plant pollutant responses are conflicting. Several reports show that exposure to low temperature, prior to pollutant fumigation, reduces plant sensitivity to the pollutant. In contrast, low temperatures during pollutant fumigation may enhance or inhibit plant pollutant responses and appears to be dependent on the plant species and pollutant used although mechanisms are not well understood.

There has been far less work directed toward investigating the effects of post-fumigation temperatures on plant pollutant responses. To a large degree, temperature effects during exposure appear to be mediated through changes in stomatal aperture, whereas effects of temperature following pollutant exposure are more likely to be related to effects on metabolism (Norby & Kozlowski, 1981b). In their study in 1952, Hull & Went not only investigated the effects of pre-fumigation temperature but also post-fumigation temperature on the degree of injury to several crop plants from artificial smog. These authors found a positive correlation between the degree of injury observed and post-fumigation temperature: however, the effects were much less than those observed for pre-fumigation temperatures. More recently, Norby & Kozlowski (1981a,b) have examined the effects of post-fumigation temperature on the sulphur dioxide responses of several woody plant species. Plants were grown and subjected to SO₂ at 25°C and were then transferred to 32, 22 or 12°C for 4 to 8 weeks. These workers found that post-fumigation temperature had little effect on the amount of

injury to leaf tissue but did influence SO_2 effects on relative growth rates (RGR). At higher temperatures, RGR was highest and the reduction in RGR due to SO_2 was greatest. However, for <u>Pinus resinosa</u> seedlings (1981b) subject to the same SO_2 and temperature treatments, there was no effect of temperature on the reduction of RGR by SO_2 but at 12°C SO_2 had a significantly greater effect on root growth.

It is apparent that results from short-term laboratory experiments involving changes in temperature before, during and after pollutant fumigations cannot in reality, be extrapolated to reflect plant responses at environmental conditions in the field; although such studies do serve to show the existence of temperature/pollutant interactions influencing plant growth. There is now a growing body of evidence to suggest that plants are more sensitive to pollution under winter conditions in comparison to summer conditions and that this enhanced sensitivity is correlated to slow growth in winter conditions (Bell, Rutter, Relton, 1979; Jones & Mansfield, 1982; Whitmore & Mansfield, 1983; Mansfield, Davies, Whitmore, 1986); although the mechanisms behind this enhanced pollutant sensitivity in winter have not been elucidated.

4.1.6 Low Temperature Effects on Gaseous Metabolism

All the reports cited above, concerning the effects of temperature before, during or after pollutant fumigations suggest caution in generalising about temperature effects on plant sensitivity to air pollution. Most studies have concentrated on visible injury symptoms or reductions in growth rates or plant dry matter accumulation. Therefore, there are very few reports concerning temperature effects on pollutant responses of the actual gaseous exchange mechanism including net photosynthesis and respiration and on the pathways of pollutant uptake. This is of importance since one explanation for variable temperaturedependent responses to air pollution stress is that a difference exists in pollutant flux to the foliage so that levels of toxic pollutant derivatives that accumulate at sensitive metabolic sites in the leaf interior differ among treatments (Taylor, Selvidge & Crumbly, 1985). Differences in SO₂ flux have been shown to be stomatally controlled (Rist & Davis, 1979) but Taylor et al. (1985) suggest that changes in stomatal conductance may explain only part of the differences in pollutant flux if temperature has a

significant effect on the conductivity of the diffusive media (gas and liquid phases) or the kinetic energy of SO_2 molecules.

One of the few studies of temperature effects on photosynthesis was reported by Taniyama (1972) who studied the mechanism of injury of crops exposed to SO_2 and the influence of environmental parameters on this process. Taniyama found increases in dark respiration in response to SO_2 to be depressed by low temperature (10°C). SO_2 -induced photosynthetic inhibition was also reduced at 10°C. These responses were correlated with decreased stomatal conductance at lower temperatures.

Taylor et al. (1985) determined the effects of exposure temperatures (from 21 - 35°C) on the gaseous exchange mechanisms of plants exposed to SO2. More specifically, these workers investigated the factors governing pollutant flux. Plants were grown under optimum environmental conditions and subject to different temperatures 16 h prior to pollutant fumigation. Measurements of transpiration, net photosynthesis and SO₂ flux were made at hourly intervals. In the three plant species used, SO₂ flux was highest at 35°C. This higher flux did not necessarily result in greater physiological responses in all species ie. greater photosynthetic inhibition or changes in transpiration rate. Increased flux was not always associated with higher stomatal conductance to water vapour. A direct effect of temperature on the rate of SO₂ diffusion was suggested as another explanation of increased flux. Guderian (1977) offered an explanation for the fact that increased pollutant flux may not always result in increased injury in proposing temperature to have a direct influence on the effects of pollutants adsorbed by mesophyll cells.

It can be seen that there is a lack of understanding of the modifying influences of environmental stresses on plant pollutant responses. Although many reports exist concerning changes in visible injury symptoms, the mechanisms of action of such stresses are poorly understood. Examinations of gas exchange processes directly, in combination with environmental and pollutant stress are needed to elucidate the site and nature of the mechanisms involved in such interactions.

4.1.7 Pollutant Effects on Plant Responses to Winter Stress

The effects of adverse environmental conditions in modifying plant pollutant responses have been outlined above but there is now also

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growing evidence to suggest that pollutant exposure can modify the sensitivity of plants to adverse environmental conditions such as drought (Wright, Lucas, Cottam & Mansfield, 1986) or winter stress (eg. Davison & Bailey, 1982; Davison & Barnes, 1986; Barnes <u>et al.</u>, 1988). The recent TERG report (1988) gives a comprehensive review of current data which indicate that pollutant exposure reduces resistance to winter stress. Fumigation with sulphur dioxide has been shown to predispose plants to freezing injury (Keller, 1978; Davison & Bailey, 1982; Baker, Unsworth & Greenwood, 1982). Davison & Barnes (1986) showed that freezing resistance in <u>Lolium perenne</u> was reduced by exposure to 94 ppb SO₂ for only two weeks during the hardening phase of the experiment and that one of the effects of SO₂ was to increase potassium leakage. Greater electrolyte leakage in response to SO₂, rendering the inability to regulate membrane permeability, was proposed by Feiler (1981; in Davison & Barnes, 1986) as a major factor contributing to enhanced frost sensitivity.

Although both SO₂ and NO₂ have been shown to reduce sensitivity to winter stress, ozone has proved to be of increasing concern as it now considered to play a central role in forest decline. The widespread die-back of conifers and some hardwoods in Europe and USA has been the subject of much discussion and has attracted much media attention. Die-back has coincided with drought years and harsh winters and it has been suggested that forest decline is a multiple-stress related syndrome and that air pollutants (particularly ozone) and physical stresses such as drought and frost are the main contributors. It is not proposed to provide a detailed review of current literature here as a number of recent publications serve this purpose adequately (Davison & Barnes, 1986; TERG, 1988; Barnes <u>et al.</u>, 1988). However, it is clear that pollutant/winter stress interactions may have profound implications, not only for forest growth but for agricultural crops and that much more information regarding such interactions is required.

4.2 AIMS

The aims of this section of the experimental work were to determine the modifying influences of environmental stress on the photosynthetic, respiratory and stomatal responses of two varieties of <u>Vicia faba</u> CV, Dylan and Aquadulce Claudia to a range of sulphur dioxide fumigations. The effects of reduced light intensity or periods of 24 h, 72 h or i week of chilling temperatures prior to pollutant fumigation were studied.

Varietal differences in pollutant responses following added environmental stress were investigated as differences had been found to occur under optimum environmental conditions.

The effects of both environmental and pollutant stress on pollutant flux and resistances to gaseous transfer were also studied. The processes examined in turn are:

(i) net photosynthesis

- (ii) dark respiration
- (iii) stomatal resistance
- (iv) resistance to CO_2 transfer
- (v) pollutant flux
- (vi) resistance to pollutant transfer.

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4.3 EXPERIMENTAL PROTOCOL

4.3.1 Low Light Stress

Seeds of both varieties were planted in the manner described in \$3.3.1. However, low light stress commenced immediately after sowing and thus the seed pots were placed in the bottom of an environmental growth cabinet set at a constant temperature of 22°C. The photoperiod was 14 h light/10 h dark and relative humidity was 70 \pm 5%. Light intensity reaching the pots was further reduced by shading with a neutral filter of muslin netting. Quantum flux density was measured with a Li-Cor Quantum Radiometer (Model LI-185B) and was 100 μ E m⁻² s⁻¹ (60 W m⁻²) at plant height.

When the plants had developed three fully expanded leaf pairs, two plants were selected for sulphur dioxide fumigation experiments as described in §3.3.1. The photoperiod in the exposure system was again 14 h light/10 h dark and chamber temperature was 23 ± 3 °C. Both the control and the pollutant chamber were shielded with muslin netting to give a quantum flux density of 100 μ E m⁻² s⁻¹ throughout the duration of the experimental period.

4.3.2 Cold Temperature Stress

Seedlings of <u>Vicia faba</u> CV. Dylan and Aquadulce Claudia were grown to the three leaf pair stage under optimum environmental conditions of light and temperature in the manner described in chapter 3, §3.3.1. Before transfer to the exposure system plants were transferred to a second growth cabinet maintained at a constant temperature of 10°C with a quantum flux density of 300 μ E m⁻² s⁻¹ (120 W m⁻²), the photoperiod being 16 h light and 8 h dark and relative humidity 70 ± 5%. Plants were subjected to periods of 24 h, 72 h or 1 week at the chilling temperature and were then transferred to the experimental system for pollutant fumigation. Sulphur dioxide fumigations were carried out under optimum conditions of light and temperature. For both low light-stressed and cold-stressed plants experiments were carried out as described in §3.3.3. For low light stressed plants environmental conditions in the exposure system were as described above (§4.3.1). For low temperature stressed plant environmental conditions in the exposure system were as described in §3.3.2.

Plants were left for 24 h to acclimatise prior to pollutant fumigation and were monitored for a further 24 h after the end of the SO_2 exposure period to permit recovery to be measured.

4.4 NET PHOTOSYNTHESIS (Pnet)

4.4.1 Time-Responses to SO₂ and Environmental Stress

The photosynthetic responses of both varieties of Vicia faba to a range of sulphur dioxide concentrations under optimum environmental conditions were described in \$3.4. This section of the work describes the photosynthetic responses of both varieties to SO₂ following additional pretreatments of environmental stress. Examples of time-response data under optimum environmental conditions were given in \$3.4.1. Similar patterns of response to SO₂ were also observed in plants which had been pre-treated with environmental stress prior to exposure: Time course data for pretreated plants, therefore, have not been included. However, it must be noted that when plants were grown and exposed to SO₂ under low light intensities, the natural rate of Pnet in, the absence of SO_2 , was half that observed under 'ideal' environmental conditions. No significant differences in photosynthetic rate were observed following 24 h at 10°C but after 72 h of low temperature stress Pnet was reduced by approximately 10% in relation to unstressed plants and after 1 week periods of cold stress Pnet was reduced by approximately 20%.

4.4.2 Responses to SO₂ and Low Light Stress

Figures 4.1 and 4.2 show percent inhibition of net photosynthesis (Pnet) against ambient sulphur dioxide concentration for both Dylan and Aquadulce Claudia plants under low light intensities. Changes in net photosynthetic rate were calculated in relation to the control plants in the manner described in §3.4.2. Regression analysis of the data for both varieties gave polynomial correlation coefficients of 0.7901 (p <0.02) for Dylan (Fig. 4.1) and 0.961 (p <0.001) for Aquadulce Claudia plants (Fig. 4.2). The regression lines for the data obtained under optimum environmental conditions are also shown in the figures. Analysis of covariance showed that, in Dylan plants, photosynthetic inhibition due to SO₂ under conditions of low light intensity was significantly different from that observed at high light intensities, the F value being 23.08 ($\alpha = 0.001$), there being







% Change in Net Photosynthetic Rates in Response to Sulphur Dioxide Fumigation Under Low Light Intensity (60 W m⁻¹) for Two Varieties of <u>Vicia</u> <u>faba</u>, Dylan (Fig. 4.1) and Aquadulce Claudia (Fig. 4.2). [The regression lines for the data obtained under optimum light conditions (-----) are also shown in the figures].

400

Sulphur Dioxide concentration (ppb)

200

600

800

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much less inhibition in low light when ambient SO_2 concentrations exceeded 200 ppb. However, in Aquadulce Claudia plants, there were no significant differences in photosynthetic response to SO_2 under high light or low light regimes. Analysis of covariance also showed there to be significantly less photosynthetic inhibition in Dylan plants under low light intensities than for plants of the variety Aquadulce Claudia under the same conditions (F = 10.61, $\alpha = 0.025$).

At optimum conditions of light and temperature both varieties exhibited marked increases in the extent of photosynthetic inhibition when ambient SO₂ concentrations exceeded 400 ppb, providing evidence for a threshold concentration, above which Pnet is even more severely limited. Whilst this still appeared to hold true for Dylan plants under low light conditions, the reverse was seen to occur in Aquadulce Claudia plants; no such threshold was observed at low light intensities and a plateau for maximum degree of photosynthetic inhibition occurred when ambient SO₂ concentrations exceeded 500 ppb.

The data for both varieties therefore, suggest that additional environmental stress may have had a significant effect on plant responses to sulphur dioxide, particularly at high SO₂ concentrations ie. above 400 ppb. At lower SO₂ levels the effect of added low light stress appears to depend on the variety of <u>Vicia</u> plants: Responses of Aquadulce Claudia plants to SO₂ concentrations below 400 ppb remained unchanged from those observed under optimum light conditions whilst the responses of Dylan plants were significantly lessened.

4.4.3 Pnet: Responses to SO_2 and Low Temperature Stress

Plants of both varieties were also subjected to treatments at 10°C for varying periods prior to exposure to SO_2 . The photosynthetic responses to SO_2 following either 24 h, 72 h or 1 week at 10°C, for both varieties of <u>Vicia faba</u> are shown in Figures 4.3 - 4.8.

Photosynthetic responses to SO_2 in plants of the variety Dylan were found to be significantly less for all SO_2 concentrations following a 24 h pre-treatment at 10°C than those observed under 'ideal' environmental conditions (Fig. 4.3). Enhancement of net photosynthetic rates in response to low SO_2 concentrations were observed in the cold-stressed Dylan plants and this had not been seen to occur in non cold-stressed plants. Regression







% Change in Net Photosynthetic Rates in Response to Sulphur Dioxide Fumigation for Two Varieties of <u>Vicia faba</u>, Dylan (Fig. 4.3) and Aquadulce Claudia (Fig. 4.4) subjected to a pre-treatment of 24 h at 10°C. [The regression lines for the data obtained in the absence of any cold pretreatment are also shown in the figures (-----)]. Results

analysis of the data gave a linear correlation coefficient of 0.7973 (p <0.001) and analysis of covariance gave an F value of 7.846 ($\alpha = 0.001$). The regression line for non cold-stressed plants is also shown in the figure.

Conversely, the photosynthetic responses of Aquadulce Claudia plants to SO₂ following a period of 24 h at 10°C were not found to be significantly different from those observed in non cold-stressed plants for the range of sulphur dioxide concentrations used (Fig. 4.4). Regression analysis gave a polynomial correlation coefficient of 0.5389 (p < 0.01) and analysis of covariance gave an F value of 1.73 (not significant). However, the stimulation in Pnet in response to \approx 100 ppb SO₂ observed in non coldstressed plants was not seen to occur in plants subjected to 24 h at 10°C prior to SO₂ fumigations and photosynthetic inhibition in response to SO₂ concentrations below 200 ppb was greater in cold-stressed plants in comparison to non-environmentally stressed plants.

When the data for both varieties following a 24 h pre-treatment at 10°C were compared, there was found to be no significant difference in the degree of photosynthetic inhibition observed in response to SO_2 treatments, analysis of covariance giving an F value of 0.346. This contrasts to the data presented in chapter 3 were, in the absence of cold temperature stress, varietal differences in response were noted.

In cold-stressed Dylan plants, photosynthetic inhibition was not significantly enhanced when SO_2 concentrations exceed 400 ppb, thus there was no evidence of a tolerance threshold concentration above which net photosynthetic rates were even more severely limited. The existence of a threshold concentration was demonstrated in §3.4 and was seen to occur in both varieties of <u>Vicia faba</u> in the absence of additional environmental stress. The threshold was still evident in Aquadulce Claudia plants following a period of 24 h at 10°C.

Varietal differences in photosynthetic response to SO_2 were also observed following pre-treatments of 72 h at 10°C. The responses of both varieties to SO_2 following a period of 72 h at 10°C are shown in Figures 4.5 and 4.6. In both varieties, the degree of SO_2 -induced photosynthetic inhibition was lessened when plants had been subjected to cold stress prior to SO_2 fumigation; again, this difference was noticeablely greater at higher SO_2 concentrations ie. above 300 ppb. For Dylan plants (Fig.4.5), regression analysis gave a linear regression coefficient of 0.595 (p <0.01). Analysis of covariance gave an F value of 13.81 ($\alpha = 0.001$) showing there







% Change in Net Photosynthetic Rates in Response to Sulphur Dioxide Fumigation for Two Varieties of <u>Vicia faba</u>, Dylan (Fig. 4.5) and Aquadulce Claudia (Fig. 4.6) subject to a pre-treatment of 72 h at 10°C. [The regression lines for the data obtained in the absence of any cold pretreatment are also shown in the figures (-----)]. to be a significant difference between the photosynthetic responses of plants with no chilling pre-treatment and those subject to 72 h at 10 °C prior to SO_2 fumigation, although from the Figure, it can be seen that at low SO_2 concentrations ie. 100 ppb, no significant differences were apparent and no enhancement in Pnet was observed. Again, there was no increase in response when SO_2 exceeded 400 ppb.

For plants of the variety Aquadulce Claudia (Fig. 4.6), regression analysis gave a polynomial regression coefficient of 0.4973 (p < 0.02), and it can be seen from the figure that, when SO₂ concentration exceeds 300 ppb, there is a very marked difference in response between unstressed and pre-stressed plants. Analysis of covariance gave an F value of 17.62 ($\alpha =$ 0.001) showing there to be much less photosynthetic inhibition in prestressed plants. However, it can also be seen from the Figure that lessening in degree of SO₂-induced photosynthetic inhibition occurring at higher SO₂ concentrations, was not apparent at lower SO₂ concentrations ie. below 300 ppb and the enhancement of Pnet at SO₂ concentrations below 100 ppb was still observed.

Of special interest here is a comparison of the data for both varieties following 72 h at 10°C. Analysis of covariance gave an F value of 8.30 ($\alpha = 0.001$) and showed there to be significant differences in response to SO₂ between the varieties. The majority of Aquadulce Claudia plants, after showing little modification in SO₂ response under low light conditions or following a period of 24 h at 10°C, exhibited significantly less SO₂-induced photosynthetic inhibition than Dylan plants at all SO₂ concentrations. Also, as was observed in Aquadulce Claudia plants under low light intensities, there was a maximum degree of photosynthetic inhibition occurring at 400 ppb; further increases in SO₂ concentration did not increase the extent of Pnet inhibition and in some plants, inhibition was less than at lower SO₂ concentrations.

The last environmental regime studied was the effects of a 1 week cold pre-treatment on the photosynthetic responses of both varieties of <u>Vicia faba</u> to sulphur dioxide. Figures 4.7 and 4.8 show inhibition of Pnet plotted against SO₂ concentration for both varieties. Again, plant responses are modified by the cold treatment. Regression analysis of the data for Dylan plants (Fig. 4.7) gave a polynomial correlation coefficient of 0.472 (p <0.05). Analysis of covariance gave an F value of 12.97 ($\alpha = 0.001$) showing there to be significantly less photosynthetic inhibition



Figs 4.7 & 4.8

% Change in Net Photosynthetic Rates in Response to Sulphur Dioxide Fumigation for Two Varieties of <u>Vicia faba</u>, Dylan (Fig. 4.7) and Aquadulce Claudia (Fig. 4.8) subject to a pre-treatment of 1 week at 10°C. [The regression lines for the data obtained in the absence of any cold pretreatment are also shown in the figures (-----)].

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after a treatment at 10°C. These differences were again, most marked at higher SO_2 concentrations; however, it can be seen from the Figure that at lowest SO_2 concentrations, there was also significantly less photosynthetic inhibition in cold-stressed plants in response to SO_2 and a small enhancement of net photosynthetic rate was observed.

Examination of the data for Aquadulce plants (Fig. 4.8) also showed there to be significantly less photosynthetic inhibition in response to SO₂ following a one week pre-treatment at 10°C. Regression analysis of the data obtained gave a polynomial correlation coefficient of 0.432 (p \langle 0.05) and analysis of covariance gave an F value of 20.59 ($\alpha = 0.001$) showing the data to be significantly different to that obtained under "ideal" environmental conditions. However, these differences were, again, more readily apparent when SO₂ concentrations exceeded 250 ppb.

As observed in the 72 h pre-treatment, analysis of covariance of the data for both varieties following 1 week at 10°C, showed Aquadulce Claudia plants to exhibit significantly less SO_2 -induced photosynthetic inhibition than Dylan plants, the F value being 3.62 ($\alpha = 0.01$). However, it can be seen from the figures that there was no varietal difference in response to SO_2 concentrations around 100 ppb.

Comparison of the data within the varieties for all cold treatments showed there to be significantly less SO_2 -induced photosynthetic inhibition following periods of 72 h or 1 week at 10°C in relation to the data for 24 h cold. There was no significant difference in either variety, in the degree of SO_2 -induced photosynthetic inhibition observed following the 72 h or 1 week cold pre-treatments. A summary of the analysis of covariance results for each variety for all pre-treatments is given in Table 4.1.

The data obtained for both varieties following a range of added environmental stresses prior to SO_2 fumigation produced a number of interesting points. Firstly, plants of the variety Dylan were responsive to all added stresses, photosynthetic inhibition due to SO_2 being significantly reduced following cold temperature or low light pre-treatments; although these responses were most marked at SO_2 concentrations above 100 ppb. However, Aquadulce Claudia plants showed no significant difference in response to SO_2 under optimum environmental conditions or low light intensities or following 24 h at 10°C; although following the 24 h cold pretreatment, no enhancement in Pnet was observed in response to 100 ppb SO_2

TABLE 4.1

Summary of Analysis of Covariance Results Comparing Environmental Pre-conditioning Effects on Net Photosynthetic Responses to SO_2 in two Varieties of <u>Vicia faba</u> CV. Dylan and Aquadulce Claudia.

	Analysis of F value	Covariance Significance α =
Dylan Optimum Environmental	conditions	
v Dylan low light	23.08	0.001
v Dylan 24 h cold	7•84	0-001
v Dylan 72 h cold	13.81	0.001
v Dylan 1 week cold	12.97	0.001
(%Pnet inhibition l environmental conditions in	ess than that observed almost all cases.)	under optimum
Aquadulce Optimum Environmen	tal Conditions	
v Aquadulce low light	0.72	ns
v Aquadulce 24 h cold	1 - 73	ns
v Aquadulce 72 h cold	17.62	0.001
v Aquadulce 1 week cold	20.59	0.001
conditions in the majority of Dylan Low Light v Aquadulce low light	of plants.) 7.15	0.025
	(%Pnet inhibition	less in Dylan)
Dylan 24 h Cold v Aquadulce 24 h cold	0.35	ns
Dylan 72 h Cold v Aquadulce 72 h cold	8+30	0.001
	(%Pnet inhibition less	in Aquadulce.)
Dylan 1 Week Cold v Aquadulce 1 week cold	d 3.62	0.01
	(%Pnet inhibition less	in Aquadulce.)

TABLE 4.1 (continued)

	Analysis of	covariance
	F value	Significance
		α =
Dylan 24 h Cold		
v Dvlan 72 h cold	2 • 83	0.02
v Dylan 1 week cold	2.77	0.05
(%Pnet	inhibition less in 72	h and 1 week.)
Dylan 72 h Cold		
v Dylan 1 week cold	0 • 18	ns

Aquadulce 24 h Cold		
v Aquadulce 72 h cold	6·45	0+001
v Aquadulce 1 week cold	7.02	0.001

(%Pnet inhibition less in 72 h and 1 week.)

ns

Aguadul	.ce 72 h Co	10	1	
· v	Aquadul ce	1	week cold	0.087

suggesting that the cold pre-treatment enhanced the sensitivity of Aquadulce Claudia plants to low SO_2 concentrations. In contrast, a period of 24 h at low temperatures prior to SO_2 fumigation was shown to significantly reduce the sensitivity of Dylan plants to low SO_2 concentrations. However, there was significantly less SO_2 -induced inhibition in net photosynthetic rates in Aquadulce plants after longer periods of chilling in comparison to Dylan plants. Following either 72 h or 1 week at 10°C, the majority of Aquadulce plants exhibited significantly less photosynthetic inhibition than was observed in Dylan plants under the same conditions. Again, the exception being at SO_2 concentrations below 100 ppb when no varietal difference in response was observed in plants previously subjected to 1 week cold temperature stress

Secondly both varieties, under optimum environmental conditions, showed there to be a tolerance threshold to SO_2 around 400 ppb, beyond which Pnet was even more severely limited. This 'threshold' was not the dividing line between no or some response to SO_2 but was the point at which the relationship between SO_2 concentration and observed photosynthetic inhibition was significantly altered, the degree of inhibition being much more serious. At low light intensities and following 72 h or 1 week at 10°C, Aquadulce plants did not show this enhancement in response, indeed, a plateau was reached between 300 - 400 ppb above which, the degree of inhibition in Pnet declined. In Dylan plants, at low light intensities, this threshold was still in evidence, but following 24 h or 72 h cold the response became linear and after 1 week at 10°C Dylan plants exhibited a maximum degree of inhibition in Pnet at 400 ppb; above this concentration Pnet inhibition declined.

Thirdly, in Dylan plants, photosynthetic inhibition was less than that observed under optimum environmental conditions for all pre-treatments and all SO_2 concentrations. Aquadulce Claudia plants however, demonstrated less photosynthetic inhibition only when SO_2 concentrations exceeded 250 ppb.

Lastly, it was observed, in both varieties, that the data obtained were increasingly scattered with increasing length of cold pretreatment. Although the regression lines shown in the figures were significant to the levels stated above, the standard deviation of the data about the regression lines increased. The causes of this variability in the data may be due to the mechanisms involved in the interactions between environmental and pollutant stress effects on net photosynthetic rates. It was hoped that such variability may be explained when the effects of added environmental stresses on other plant parameters were examined.

4.5 DARK RESPIRATION (Rd)

In §3.5 it was shown that dark respiration rates, (Rd), in plants of the variety Dylan were enhanced by exposure to sulphur dioxide, whereas there was no influence of SO₂ on dark respiration rates in plants of the variety Aquadulce Claudia. However, natural rates of Rd in Aquadulce plants were significantly higher than those measured for Dylan in the absence of SO₂. Changes in dark respiration rate in response to SO₂, if they persist during the light periods, are important when determining the influences of SO₂ on carbon assimilation rates because net photosynthesis is the net result of maximum photosynthesis (Pmax) minus respiration rates.

In this section, the influences of added environmental stresses on dark respiration rates and the modification of SO_2 responses by these added stresses are discussed. Because of the natural variability in the data obtained and also because increases in *Rd* in Dylan plants were found to be independent of the SO_2 concentration used, regression analyses of the data obtained were not appropriate. Therefore, as for the data obtained under 'ideal' conditions, the data presented in this section were analysed using 't' tests.

Table 4.2 shows the results for both varieties of <u>Vicia faba</u> in response to SO₂ under low light intensities or following periods of either 24 h, 72 h or 1 week at 10°C prior to SO₂ fumigation. C1 and P1 are measurements of dark respiration rates in the dark period prior to SO₂ fumigation for control (C1) and plants that were to be SO₂ treated (P1). C2 and P2 are measurements of *Rd* in control and treated plants during the dark period following SO₂ fumigation which had occurred in the intervening light period. Table 4.3 gives a comparison of the dark respiration data from the environmental pre-treatments with that obtained for each variety under optimum environmental conditions; comparison of the data for each variety is also shown.

When respiratory data prior to SO_2 fumigation were examined, no significant differences could be observed between the respiration rates of

Results

TABLE 4.2

Analysis of data, Using 't' tests for dark respiration rates (Rd, g $CO_2 m^{-2}$ h⁻¹), in two varieties of <u>Vicia faba</u> CV. Dylan & Aquadulce Claudia in response to SO₂ and a range of added environmental stresses. [High Light = saturating light intensity of 150 W m⁻² and optimum temperature 23 ± 3°C: Low Light = light intensity of 60 W m⁻² and optimum temperature: 24 h, 72 h and 1 week = high light conditions plus a pre-treatment at 10°C prior to SO₂ fumigation].

C1 and P1 are the control and polluted plants prior to SO_2 exposure. C2 and P2 are the control and treated plants on the second night of the experimental period, following SO_2 exposure.

Variety &	•					
Treatment	Mean	Maximum	Minimum	Variance	't'	α =
Dylan Low	Light					
C1	0.126	0 • 197	0.058	0.003	0·152	DS
C2	0.121	0.210	0.080	0.004		
P1	0+086	0.127	0.047	0.001	0.283	ns
P2	0.082	0.105	0.047	0.001		
C2 / P2					1.599	0•10
Aquadul ce	Low Ligh	it				
C1	0 • 197	0.348	0.064	0.011	0.437	ns
C2	0.174	0.287	0.036	0.008		
P1	0.181	0.293	0.049	0.011	0.987	ns
P2	0 • 136	0-207	0.038	0.004		
C2 / P2					0.948	DS
Dylan 24 h	Cold		• •			
C1	0.182	0.241	0.107	0.002	3.717	0.001
C2	0 • 117	0·165	0.051	0-001		
P1	0.175	0-242	0.097	0.001	2.707	0.01
P2	0.141	0.162	0.109	0.001		
C2 / P2					1.500	0.02

To allow comparison the data presented in \$3.5 showing the mean values of Rd obtained for each variety under optimum environmental conditions are given below:

			DILAN		AQUADU	LCE CLAUDIA
			Mean	Variance	Mean	Variance
Before	SO ₂	(P1)	0.131	0.002	0.265	0.011
After	S0z	(P2)	0.224	0.007	0.236	0.006

,

TABLE 4.2 (continued)

Variety &						
Treatment	Mean	Maximum	Minimum	Variance	' t'	α =
Aquadul ce	24 h Col	đ			·	
C1	0.252	0-372	0.284	0.003	0.550	ns
C2	0.241	0.355	0.171	0.003		
P1	0.274	0.462	0.193	0.003	0.820	ns
P2	0.259	0.354	0 • 190	0.002		
C2 / P2					0.745	ns
Dylan 72 h	Cold					
C1	0 • 181	0.309	0.090	0.003	2.093	0.025
C2	0 • 130	0.181	0.082	0.002		
P1	0.173	0.265	0.072	0.003	1.889	0.05
P2	0.132	0.201	0.081	0.001		
C2 / P2			•		0.097	ns
Aquadul ce	72 h Col	d				
Cl	0.305	0.447	0•153	0-006	0.571	ns
C2	0.283	0.434	0 • 159	0-01		
P1	0.298	0.390	0.232	0.003	1.712	0·10
P2	0.259	0.326	0-186	0.002		
C2 / P2					0.562	ns
Dylan 1 We	ek Cold	-			-	:
C1	0 • 183	0.307	0.128	0.003	0.699	ns
C2	0.164	0.211	0-090	0.002		
P1	0 • 180	0.346	0.097	0.004	0•960	ns
P2	0.155	0.200	0.106	0.002		
C2 / P2					0.362	ns
Aquadul ce	1 Week	Cold				
C1	0.281	0.476	0.114	0.009	1 251	ns
C2	0.231	0.371	0.135	0.008		
P1 .	0.278	0 • 437	0 • 146	0.002	1 472	0 • 10
P2	0.232	0.359	0 • 139	0.006		
C2 / P2					-0.024	ns

plants in the control chamber (C1) and plants in the treatment chamber (P1). This was true for the whole range of environmental treatments used.

4.5.1 Rd: Responses to SO₂ and Low Light Stress

Under low light intensities, examination of the data for both varieties showed there to be no significant differences in dark respiration rates of the control plants on the first (C1) and second (C2) nights of the experimental period showing the plants not to be stressed as a result of being in the exposure chambers. However, following SO₂ fumigation (P2), dark respiration rates were found to be significantly lowered in plants of the variety Dylan whereas there was no influence of SO₂ on the dark respiration rates of Aquadulce plants. Significant varietal differences in dark respiration rates were found both before and after SO₂ fumigation (Table 4.3). Plants of the variety Aquadulce Claudia were found to have significantly higher dark respiration rates than plants of the variety Dylan during the dark period prior to SO₂ fumigations (C2,P2) when rates of dark respiration were reduced in Dylan plants but were unchanged in Aquadulce plants (Table 4.2).

Table 4.3 also shows the results obtained when dark respiration data from plants grown under low light intensity are compared with data obtained from plants grown under high light intensity. In Dylan plants, dark respiration rates prior to and following SO_2 treatments were significantly lowered when plants were grown under low light intensities. Rather than the enhancement in *Rd* seen under high light conditions, exposure to SO_2 under low light conditions appeared to further reduce *Rd*. Dark respiration rates in Aquadulce plants were also significantly lower under low light conditions and as was observed under high light conditions, SO_2 had no significant effect on *Rd*.

4.5.2 Rd: Responses to SO₂ and Low Temperature Stress

Following a pre-treatment of 24 h at 10°C, there was again no observable effect of SO_2 on dark respiration rates of Aquadulce Claudia plants. There were no significant differences in *Rd* either in control or treated plants or during each dark period (Table 4.2). Similarly, there was

TABLE 4.3

Results of 't' test analyses of dark respiration data of two varieties of <u>Vicia faba</u> CV. Dylan & Aquadulce Claudia in response to SO_2 and a range of environmental conditions. [High Light = saturating light intensity 150 W m⁻² and optimum temperature 23 ± 3°C: Low Light = light intensity 60 W m⁻² and optimum temperature: 24 h, 72 h and 1 week = high light conditions plus a pre-treatment at 10°C prior to SO_2 fumigation]. C1 and P1 are the control and polluted plants prior to SO_2 exposure. C2 and P2 are the control and treated plants on the second night of the experimental period, following SO_2 exposure.

Variety &	1					
Treatment	C1 / C1	C2 / C2	P1 / P1	P2 / P2		
	level of significance, $\alpha =$					
Dylan High Light						
v DY Low light	1.605	0.603	3.392	5.254		
5	0 • 10	ns	0.005	0.0005		
v DY 24 h cold	-1.345	0.855	-3.282	3.097		
•	0-10	ns	0.05	0.01		
v DY 72 h cold	-1.113	0-393	-2.671	3.104		
	DS	ns	0.01	0.005		
v DY 1 week	-1.249	-0.774	-2.774	2 • 268		
	DS	ns	0.01	0.025		
Aquadulce High Li	ght					
v AQ Low light	1.389	1.305	1-773	3.016		
	0 • 10	ns	0.05	0.005		
v AQ 24 h cold	0.304	-0.667	-0-319	-0.854		
	ns	DS	ກຮ	ns		
v AQ 72 h cold	-1.636	-1.377	-1.198	-0.758		
	0.10	0 • 10	ns	ns		
v AO 1 week	-0.729	-0.165	-0.457	0·128		
	11S	DS	ns	ns		
Dylan v Aquadulce		·				
Low light	-1.826	-1-320	-2-320	-2.145		
Ŭ	0 • 10	ns	0.025	0.05		
24 h cold	-4.177	-6.083	-6.336	-8.227		
	0.0005	0·0005	0.0005	0.0005		
72 h cold	-4.940	-3.847	-6.853	-5.395		
	0.0005	0.005	0.0005	0.0005		
1 week	-3.682	-1.719	-4.125	-2 • 486		
	0.001	0.05	0.0005	0.025		

no observable effect of the 24 h cold temperature pre-treatments on dark respiration rates in Aquadulce Claudia plants there being no significant differences between all the dark respiration data obtained following 24 h cold and the data obtained under 'ideal' conditions (Table 4.3).

However, dark respiration rates in Dylan plants were altered in response to both SO₂ and the cold pre-treatment in comparison with the data obtained under optimum environmental conditions (Tables 4.2 and 4.3). Dark respiration rates were significantly higher on the first night of the experimental period as a result of the cold temperature pre-treatment (C1,P1); this proved to be a temporary enhancement as dark respiration rates had decreased in both the control and SO₂-treated plants by the second night of the experimental period. However, the SO₂ polluted plants did not show the full extent of the recovery from cold stress exhibited by the control plants during the exposure period and 't' tests showed the dark respiration rates of the polluted Dylan plants on the second night to be significantly higher than that of the control plants. This decline in Rd after the first dark period resulted in there being no significant difference between non cold-treated and cold treated plants during the second dark period, in the absence of SO₂. However, because SO₂ induced an enhancement in Rd under 'ideal' conditions and this was not observed following the 24 h cold pre-treatment, there were significant differences between cold stressed and unstressed plants following SO₂ fumigations; unstressed plants having higher dark respiration rates.

When the results for each variety following 24 h at 10°C were compared, Aquadulce Claudia plants again had significantly higher dark respiration rates than Dylan plants in both control and treated plants throughout the experimental period.

The results for both varieties following a period of 72 h at 10°C showed that the cold temperature stress induced increases in dark respiration rate (Table 4.2). Comparison of the data obtained during the first dark period (C1,P1) with that obtained for non cold-stressed plants showed these increases in Rd to be significant (Table 4.3). In Dylan plants, as observed following the 24 h cold period, the enhancement in Rd proved to be temporary and respiration rates were lower on the second night of the exposure period in both SO₂ treated and control plants. However, unlike the effects of the 24 h cold treatment, there was no significant effect of SO₂ on dark respiration rates in Dylan plants subjected to 72 h at 10°C prior

to SO₂ fumigation.

Conversely, in Aquadulce Claudia plants the significant increases in dark respiration observed during the first dark period were seen to persist in control plants throughout the experimental period. However, SO_2 fumigation resulted in a depression in dark respiration rates in Aquadulce plants so that during the second dark period there was no significant difference in *Rd* between non cold-stressed SO_2 treated plants and those subjected to 72 h at 10°C prior to SO_2 fumigation.

Comparison of the data for both varieties following the 72 h cold pre-treatment showed, again, that Aquadulce Claudia plants had significantly higher dark respiration rates than those of Dylan in both control and treated plants throughout the experimental period.

As for the 24 h and 72 h cold temperature treatments, following a period of 1 week at 10°C prior to SO_2 fumigations, the nature of changes in dark respiration rates were found to differ. In Dylan plants, dark respiration rates were again enhanced in response to cold stress as shown by comparison of the data obtained during the first dark period (C1,P1) with that for non cold-stressed plants (Table 4.3). This enhancement again declined throughout the exposure period indicating recovery from cold stress. However, the decreases in *Rd* observed during the second dark period (C2,P2) were not as pronounced as those observed following 24 h or 72 h cold pre-treatments suggesting that the plants were slower to recover from the prolonged cold treatment. As observed following the 72 h cold treatment, there was no significant effect of SO_2 on dark respiration rates of Dylan plants previously subjected to a 1 week cold temperature treatment (Tables 4.2 and 4.3).

In Aquadulce Claudia plants exposure to 10°C for 1 week did not result in enhanced rates of dark respiration, there being no significant differences in dark respiration rates during the first dark period (C1,P1) in comparison to rates observed in non cold-stressed plants (Table 4.3). This suggested that the increase in Rd following the 72 h cold treatment was temporary and that Aquadulce plants had the ability to recover during the 1 week cold temperature exposure. There was no significant effect of SO₂ fumigations on dark respiration rates of Aquadulce plants previously subjected to a 1 week cold temperature treatment. Aquadulce plants, again, were found to have significantly higher dark respiration rates than Dylan plants. In conclusion, for all environmental regimes, Aquadulce Claudia plants had significantly higher dark respiration rates than those of the variety Dylan.

Low light intensities induced lower dark respiration rates in both varieties in comparison to the data obtained under high light conditions. There was no response to SO_2 in Aquadulce plants under low light conditions but, in Dylan plants, dark respiration rates were further reduced by SO_2 fumigations.

Periods of time spent at 10°C induced increased dark respiration rates in plants of the variety Dylan but some recovery occurred as this increase in Rd declined throughout the exposure period and approached the rates observed under 'ideal' conditions for control plants by the second night of the exposure period. However, the rate of decline in this increased Rd appeared to be dependent on the length of the cold pre-treatment; comparison of the data for C1 and C2 presented in Table 4.2 showing that plants subject to 24 h cold were quicker to recover than plants subjected to 72 h or 1 week cold respectively. Although SO₂ did not induce the increases in dark respiration rates, observed under optimum environmental conditions, in Dylan plants following cold pre-treatments, after 24 h cold, the decline in Rd during the second dark period was inhibited in response to SO₂. This did not occur following 72 h or 1 week cold pre-treatments.

Dark respiration rates in Aquadulce plants were increased only in response to a cold pre-treatment of 72 h and showed no response to the 24 h or 1 week cold temperature pre-treatments. However, it may be assumed that dark respiration rates in Aquadulce plants were enhanced during the 1 week cold period, as shown by the data for the 72 h cold treatments, but the plants have the ability to recover dark respiration rates during prolonged cold temperature stress. Sulphur dioxide did not influence dark respiration rates following 24 h or 1 week at 10°C in Aquadulce Claudia plants. However, following a period of 72 h at 10°C, *Rd* was significantly reduced in Aquadulce plants in response to SO₂.

4.6 STOMATAL RESISTANCE (rs)

The stomatal responses, of both varieties of <u>Vicia</u> <u>faba</u>, to a range of sulphur dioxide concentrations under optimum environmental conditions were described in §3.6. Examination of the time-response data for each variety, under optimum conditions, showed there to be a great deal of natural and diurnal variation in stomatal resistance in the absence of SO_2 . This variation was also seen to occur following pre-treatments of cold temperature and under low light intensities. Because of this variation, changes in stomatal resistance in response to SO_2 have been expressed as percentage change in relation to control plants, rather than as absolute values (§3.6.2).

All time-response data have not been displayed due to the amount of data collected. However, the generalisation can be made that stomatal resistances prior to SO₂ fumigation were found to be slightly higher in plants pre-treated with environmental stress compared with more optimum conditions. In addition, as with photosynthesis, stomatal responses to SO₂ were modified in plants subjected to prior environmental stress.

4.6.1 rs: Responses to SO₂ and Low Light Stress

Figures 4.9 and 4.10 show changes in stomatal resistance of Dylan and Aquadulce Claudia plants in response to a range of sulphur dioxide concentrations, under low light intensities. The regression lines for the data for 'high light' are also shown in the figures. Under high light conditions, Dylan plants exhibited a threshold concentration of 400 ppb representing a switch in stomatal response from opening to closure; below 400 ppb the dominant response was enhanced stomatal opening whereas, enhanced stomatal closure occurred at all SO₂ treatments above this concentration. When the stomatal responses to SO₂ of Dylan plants, under low light intensities were examined (Fig. 4.9) it was seen that there was a distinct contrast between responses of plants to SO_2 in both environmental regimes. Under low light conditions, stomatal responses were reversed, the greatest degree of stomatal closure occurred at low SO₂ concentrations and enhanced stomatal opening occurred in response to higher SO₂ levels. Again, there was evidence of a threshold concentration around 400 ppb, but under low light intensities, this represented a switch in plant response from stomatal closure to opening. Regression analysis of the data gave a linear correlation coefficient of 0.85 (p < 0.01). The magnitude of stomatal responses to SO₂ was reduced under low light intensities, ranging from -44% to 35%, whereas, under high light conditions stomatal responses ranged from -85% to 51%.







Changes in stomatal resistance in two varieties of <u>Vicia faba</u>, Dylan (Fig. 4.9) and Aquadulce Claudia (Fig. 4.10) in response to a range of sulphur dioxide concentrations under conditions of low light intensity. [The regression lines for the data obtained under 'high light' conditions are also shown in the Figures to allow comparison (-----)].

Figure 4.10 shows the data for stomatal responses in Aquadulce plants obtained under low light intensities and the regression line obtained for Aquadulce Claudia plants under 'high light' conditions. In 'high light' conditions there was a close correlation between stomatal responses and ambient SO₂ concentration (p < 0.01) but when the data for low light were analysed there was no significant correlation between these two parameters, the regression coefficient being -0.246. The threshold concentration under 'high light' conditions, above which stomatal closure occurred was 180 ppb. Under low light conditions, stomatal closure occurred at all SO₂ concentrations above 300 ppb. The magnitude of the stomatal responses of Aquadulce plants to SO₂ appeared to be similar under both environmental regimes.

The reversal of the stomatal responses to SO_2 in Dylan plants when plants were grown under low light intensities resulted in there being statistically significant differences in comparison to the data obtained for plants grown under high light conditions.

4.6.2 rs: Responses to SO₂ and Low Temperature Stress

Figures 4.11 and 4.12 show the stomatal responses to SO_2 of Dylan and Aquadulce plants following a period of 24 h at 10°C prior to SO₂ fumigation (the regression lines for the data obtained for plants grown under optimum temperature conditions ie. 23 \pm 3°C and not subjected to cold stress, are also shown). Regression analyses, for both varieties, showed no significant correlations between ambient sulphur dioxide concentration and per cent change in stomatal resistance, the correlation coefficients being -70.388 for Dylan plants and 0.085 for Aquadulce plants. Unlike the trends observed in the absence of cold temperature stress, there was no apparent relationship between changes in stomatal resistance and ambient sulphur dioxide concentration for plants of either variety. Thus SO₂-induced changes in stomatal resistance appeared to be independent of SO_2 concentration. In Aquadulce plants (Fig. 4.12), the data for plants subjected to the 24 h cold pre-treatment were much more scattered than that obtained under optimum temperature conditions, 3 out of 10 plants exhibited enhanced stomatal opening in response to SO₂ concentrations below 500 ppb; the remaining plants showed increased stomatal closure. The degree of stomatal opening in Aquadulce plants was much greater than that observed





Figures 4.11 and 4.12 Supra divide concentration (ppp) Changes in stomatal resistance in two varieties of <u>Vicia faba</u>, Dylan (Fig. 4.11) and Aquadulce Claudia (Fig. 4.12) in response to a range of SO_2 concentrations following a cold temperature pre-treatment of 24 h at 10°C. [The regression lines for the data obtained under optimum temperature conditions are also shown in the figures to allow comparison (-----)]. under optimum temperature conditions, ranging from 24% to 31% above control plants at SO_2 concentrations between 200 and 400 ppb. In the absence of cold stress the maximum degree of enhanced stomatal opening found in Aquadulce plants was 9% at SO_2 concentrations below 300 ppb.

There was no evidence, in Dylan plants (Figure 4.11), of the threshold concentration at 400 ppb, found in non cold-stressed plants, which represents the switch from enhanced stomatal opening to enhanced stomatal closure. Similarly, the clear threshold, observed under optimum temperature conditions was not observed in Aquadulce Claudia plants following a pre-treatment of 24h at 10°C (Figure 4.12).

Figures 4.13 and 4.14 show the stomatal responses of Dylan and Aquadulce Claudia plants to SO_2 following a pre-treatment of 72 h at 10°C (the regression lines obtained from the data for non cold-stressed plants are also shown). Again, in Dylan plants (Fig. 4.13), there was no significant correlation between ambient sulphur dioxide concentrations and the degree of change in stomatal resistance, the calculated correlation coefficient being 0.037. There was, also, no evidence of a threshold concentration, representing a switch from stomatal opening to enhanced closure, and unlike the responses observed under optimum temperature conditions, the majority of Dylan plants exhibited enhanced stomatal closure in response to SO_2 , even at concentrations below 300 ppb.

There was a closer relationship between the stomatal responses of Aquadulce Claudia plants (Fig. 4.14) and ambient SO₂ concentrations following a 72 h cold pre-treatment. Regression analysis gave a linear correlation coefficient of 0.680 (p < 0.05) and showed that stomatal resistance increased at low SO₂ concentrations and decreased at SO₂ concentrations above 400 ppb. This was a reversal of the responses of Aquadulce plants not subjected to cold temperature pre-treatments as can be seen from comparison of the regression lines in figure 4.14. The magnitude of stomatal response to SO₂ was increased in Aquadulce plants following a period of 72 h at 10°C. Responses ranged from -66% to 33%, whilst under optimum temperature conditions responses ranged from -34% to 9% in relation to control plants.

The stomatal responses of both varieties of <u>Vicia faba</u> to SO_2 following a cold temperature pre-treatment of 1 week at 10°C are shown in figures 4.15 and 4.16 (the regression lines obtained from the data for non cold-stressed plants are also shown). Unlike the data obtained for plants




Figures 4.13 and 4.14 Supplier divide concentration (ppp) Changes in stomatal resistance in two varieties of <u>Vicia faba</u>, Dylan (Fig. 4.13) and Aquadulce Claudia (Fig. 4.14) in response to a range of SO_2 concentrations following a cold temperature pre-treatment of 72 h at 10°C. IThe regression lines for the data obtained under optimum temperature conditions are also shown in the figures to allow comparison (-----)].







Figures 4.15 and 4.16 Suprov dioxide concentration (ppp) Changes in stomatal resistance in two varieties of <u>Vicia faba</u>, Dylan (Fig. 4.15) and Aquadulce Claudia (Fig. 4.16) in response to a range of SO_2 concentrations following a cold temperature pre-treatment of 1 week at 10°C. [The regression lines for the data obtained under optimum temperature conditions are also shown in the figures to allow comparison (-----)]. in the absence of cold stress, there was no significant correlation between ambient sulphur dioxide concentrations and the degree of stomatal response in either variety following the 1 week cold pre-treatments. In Dylan plants (Fig. 4.15), the calculated correlation coefficient was 0.133 (ns). However, all plants exhibited stomatal closure at all SO_2 concentrations used (95 to 520 ppb). The magnitude of stomatal response in Dylan plants was much less following 1 week cold, ranging from -28% to -4% whereas in the absence of cold stress stomatal responses ranged from -85% to 51% in relation to control plants.

The responses of plants of the variety Aquadulce Claudia to SO_2 produced a correlation coefficient of 0.163 (ns). However, visual inspection of the data (Figure 4.16), in relation to that obtained under optimum temperature conditions, showed there to be significant differences in response. Stomatal resistance increased at all SO_2 concentrations used and the majority of the data showed that increases in stomatal resistance were proportional to the SO_2 concentration supplied. Increases in r_s were much greater than those observed under optimum temperature conditions for the same SO_2 concentrations, ranging from -16% at 99 ppb to -62% at 490 ppb. Under optimum temperature conditions stomatal responses were 4% at 99 ppb and -34% at 490 ppb.

In conclusion, the stomatal responses of both varieties to SO_2 were influenced by added environmental stress. The magnitude of changes in resistance appeared to decrease in response to increasing lengths of time spent at 10°C for plants of the variety Dylan. However, the magnitude of stomatal response in Aquadulce Claudia plants was increased with increasing length of cold pre-treatments. The threshold response exhibited by both varieties in the absence of cold stress, at which stomatal response switched from enhanced opening to closure, was not evident when plants have been pre-stressed with cold temperatures; following periods of 1 week cold prior to SO_2 fumigation no enhancement in stomatal opening in response to SO_2 treatments was observed in plants of either variety.

In both varieties, the relationship between ambient sulphur dioxide concentration and changes in stomatal resistance became much more variable in response to added environmental stress. It was hoped that analyses of actual SO_2 fluxes to the plants, would give a clearer indication of relationships between changes in stomatal resistance in response to SO_2 following added environmental stress, because, as was shown in the previous chapter, ambient SO_2 concentrations were not found to be an entirely accurate representation of actual SO_2 dosage to plants. Changes in stomatal resistances as a function of pollutant flux are described in §4.8.

4.7 RESISTANCE FACTORS INFLUENCING NET PHOTOSYNTHESIS

In S3.7.2 the influence of changes in residual resistance to CO_2 transfer due to SO_2 exposure was discussed. It was shown that the major factor governing SO_2 -induced changes in photosynthetic rate was changes in the residual resistance to CO_2 transfer, r_rCO_2 , a lesser part was played by changes in stomatal resistances, r_3CO_2 . Also, for plants of the variety Dylan, increases in respiration rates due to SO_2 contributed to changes in net photosynthetic rate in response to the pollutant.

4.7.1 rrCO₂ & rsCO₂: Effects of SO₂ & Low Light Stress

When the resistances to CO₂ transfer were examined following low light stress (Tables 4.4 and 4.5) both varieties showed much higher residual resistances to CO₂ diffusion, before SO₂ treatment in comparison to the resistance data obtained under high light intensities (Tables 3.7 and 3.8, previous chapter). Absolute values of r_rCO_2 in Dylan plants (Table 4.4) in the absence of SO_2 , under optimal environmental conditions ranged from 448 - 864 s m⁻¹ but under low light intensities values ranged from 1374 -2120 s m⁻¹. Similarly, in Aquadulce Claudia plants (Table 4.5) r_rCO_2 values under high light intensities in the absence of SO_2 , ranged from -111 - 729s m⁻¹ but under low light stress values range from 1018 s m⁻¹ to 1870 s m^{-1} . Stomatal resistances to CO₂ transfer in the absence of SO₂ were also raised in response to low light stress. In Dylan plants, under high light conditions values for r_3CO_2 ranged from 20 - 270 s m⁻¹ but under low light intensities values ranged from 300 - 550 s m⁻¹. In Aquadulce Claudia plants stomatal resistances are effectively doubled in response to low light stress, increasing from $100 - 400 \text{ sm}^{-1}$ to $200 - 800 \text{ sm}^{-1}$.

Gross photosynthetic rates, Pmax, were found to be significantly reduced when plants had been grown under low light intensities when rates of Pmax prior to SO_2 fumigation of both varieties were compared for both light regimes. Under high light conditions, values of Pmax were found to range from 1.26 to 3.25 g CO_2 m⁻² h⁻¹, whereas under low light conditions

TABLE 4.4

<u>Vicia faba</u> CV. Dylan. Stomatal (r_s) and Residual (r_r) Resistance Data for CO₂ Transfer Before and After Exposure to SO₂ Under Low Light Intensities. Per cent SO₂-induced inhibition in net photosynthetic rates and stomatal resistance in relation to control plants are also shown.

[S02]	% change) r _r (s m ⁻¹)	% change	r; (s	m ⁻¹)
(ppb)	in Pnet	Before SO ₂	After SO ₂	in rs	Before SO ₂	After SO ₂
180	12.3	1374 • 2	1567 • 1	-28.3	460•3	254·1
200	-2•9	1816•3	1625 • 3	-44.5	301.9	387•7
255	16.9	1688•6	1611.4	-21 · 1	334.9	417·4
390	6 • 1	1726•6	1960-6	13.7	523·1	460·3
400	11.6	2119•4	2701 · 1	-6.4	554.4	516.4
600	26 • 2	1740.5	2743.5	34.7	514.8	56·1
610	25.4	1767.5	2373 • 1	2.2	26•4	118.8

TABLE 4.5

<u>Vicia</u> <u>faba</u> CV. Aquadulce Claudia. Stomatal (r_s) and Residual (r_r) Resistance Data for CO₂ Transfer Before and After Exposure to SO₂ Under Low Light Intensities. Per cent SO₂-induced inhibition in net photosynthetic rates and stomatal resistance in relation to control plants are also shown.

[S02]	% change	; r,	(s m ⁻¹)	% change	r; (s	m~1)
(ppb)	in Pnet	Before SO ₂	e After SO ₂	in r _s	Before SO ₂	After SO2
220	8·1	1689-2	1427.8	-28 • 8	526-3	600•6
270	17.3	1748 • 4	1772 • 3	+3•3	592•3	892.6
285	20 • 2	1018-8	1694.0	+18-5	755•7	379.5
360	22 • 1	1870.6	2038 • 8	-22.9	374.5	476·8
440	30 • 1	1560.6	2130.0	-8-2	191•4	336.6
510	40·3	1481.7	2231.9	-19-7	485 • 1	503·2
615	34•4	1644·7	2039 • 2	-16 • 2	810 • 1	811-8

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values for Pmax ranged from 0.63 to $1.05 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$. As shown in chapter 3, gross photosynthesis can be calculated from:

$$Pmax = 0 - 0$$
$$r_a + r_s + r_1$$

where r_A is he aerodynamic resistance to CO₂ transfer, r_s the stomatal resistance, r_r is the residual resistance and Φ is the ambient carbon dioxide concentration. The relative contributions of changes in stomatal and residual resistance to CO₂ transfer in causing the observed reduction in Pmax in response to low light stress can be analysed. If photosynthetic rates are reduced between two thirds and one half that of unstressed plants the amount of inhibition caused by changes in both the stomatal and residual resistances under low light stress can be calculated as the aerodynamic resistance is unchanged.

For example:

Two plants of the variety Dylan were grown under low or high light intensities prior to exposure to 400 ppb SO₂. Under high light intensities Pmax, prior to SO₂ treatment, was 2.06 g CO₂ m⁻² h⁻¹, r_a was 181 s m⁻¹, r_s was 234 s m⁻¹, r_r was 621 s m⁻¹ and Φ was 0.5810 g m⁻³.

Under low light intensities Pmax, prior to SO_2 , was $0.75 \text{ g} CO_2$ m⁻² h⁻¹, r_a was 181 s m⁻¹, r_s was 554 s m⁻¹, r_r was 2119 s m⁻¹ and Φ was 0.5974 g m⁻³. It can be seen that Pmax was reduced by 64% from 2.06 to $0.75 \text{ g} CO_2 \text{ m}^{-2} \text{ h}^{-1}$ in response to low light stress.

If r_s is assumed to be unchanged by low light stress, Pmax would be calculated from:

 $P_{max} = \frac{0.5974}{181 + 234 + 2119} \times 3600$ $= 2.346 \times 10^{-4} (\times 3600)$ $= 0.844 \text{ g } \text{CO}_2 \text{ m}^{-2} \text{ h}^{-1}$

showing Pmax to still be reduced by 59% in comparison with the unstressed plant.

However, if \mathbf{r}_r is assumed to be unchanged by low light stress then Pmax becomes:

 $P_{\text{max}} = \frac{0.5974}{181 + 554 + 621} \times 3600$ $= 4.38 \times 10^{-4} \quad (\times 3600)$ $= 1.578 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$

showing Pmax to be reduced by 24% in comparison to the unstressed plant. In order to confirm the above results, Pmax can be calculated with the assumption that neither r_s nor r_r were influenced by low light stress. Pmax would then be:

 $\frac{P_{max} = 0.5974}{181 + 234 + 621} \times 3600$

 $= 2.066 \text{ g } \text{CO}_2 \text{ m}^{-2} \text{ h}^{-1}$.

From the example given above it can be estimated that 10% of the observed changes in Pmax were due to changes in the stomatal resistance under low light stress but 90% of the change in Pmax was due to increases in the residual resistance to CO_2 transfer. These results are summarised in the following table:-

	r _a	rs	° r _r	Pmax
		(sm)	-1)	(g CO ₂ m ⁻² h ⁻¹)
High Light	181	234	621	2.06
Low Light	181	554	2119	0.75
Low Light if rs				
unchanged.	181	234	2119	0.84
Low Light if rr				
unchanged	181	554	621	1.58

Total % reduction in Pmax is 64% 10% of this is due to rs 90% of this is due to rr

Having determined the major factor influencing reductions in photosynthetic rate due to low light stress, the effects of sulphur dioxide fumigations must be considered. In plants of the variety Aquadulce Claudia, there was no significant difference in the degree of SO_2 -induced photosynthetic inhibition under high or low light conditions (S4.4). However, photosynthetic inhibition in response to SO_2 was significantly reduced in plants of the variety Dylan under low light intensities.

For the Dylan plants cited in the above example, following exposure to 400 ppb SO₂, net photosynthesis was inhibited by 31% under high light intensities and by only 11% under low light conditions. Values for r_r , r_s and Pmax before and after SO₂ fumigation, for both light treatments, are summarised below:-

	High Light	Low Light	(Units)
r; before SO ₂	234	554	s m ⁻¹
after	199	516	
r _r before SO ₂	621	2119	5 m ⁻¹
after	1025	2701	
Pmax before SO ₂	2.06	0 • 75	$g CO_2 m^{-2} h^{-2}$
after	1.52	0.63	

In both cases, stomatal resistance to CO_2 transfer decreased slightly over the SO_2 fumigation period: therefore it might be concluded that differences in photosynthetic inhibition were due to changes in the residual resistance to CO_2 transfer which increased markedly in both cases.

However, in chapter 3 it was shown that, in Dylan plants, dark respiration rates, Rd, were enhanced in response to SO_2 under optimum environmental conditions. Thus if Pmax = Pnet + Rd, then increases in Rdwill result in decreased rates of net photosynthesis. In §4.5 it was shown that dark respiration rates were reduced in both varieties of <u>Vicia faba</u> in response to low light stress; also, in Dylan plants, SO_2 exposure resulted in a further reduction in Rd.

Therefore, it may be that reductions in SO_2 -induced net photosynthetic inhibition in Dylan plants under low light intensities could be accounted for by these changes in dark respiration rates. The relative rates of Pmax, Pnet and *Rd* before and after SO_2 fumigations under low and high light intensities, for the two Dylan plants considered in the above example, are summarised in the following table:

Results	5
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	High Light	Low Light	
Rd before SO ₂	0.15	0-11	(g CO ₂ m ⁻² h ⁻¹)
after	0.21	0-07	
Pnet before SO ₂	1-91	Q•64	(g CO ₂ m ⁻² h ⁻¹)
after .	1.31	0-56	
Pmax before SO ₂	2.06	0.75	(g CO ₂ m ⁻² h ⁻¹)
after	1.52	0+63	
Reduction in Pnet	0.60	0-08	
=	31-4%	12.5%	-
Reduction in Pmax	0.54	0 • 12	
=	26 • 2%	22 • 2%	

Examination of the data in the above table confirms that there are marked differences in the degree of net photosynthetic inhibition in response to 400 ppb SO_2 for each light treatment. However, if dark respiration rates are assumed to persist in the light, examination of the data for gross photosynthesis shows the difference in the degree of photosynthetic inhibition between the light treatments to be much reduced.

The data presented above considered just one example and all data must be examined before generalisations concerning the influence of changes in dark respiration rates can be made. Table 4.6 shows relative percentage inhibition of gross and net photosynthetic rates for Dylan plants under high and low light intensities over a range of sulphur dioxide concentrations. It can be seen that under high light intensities, Pmax is less inhibited, on a percent basis, than Pnet in response to SO₂ and under low light intensities Pmax is inhibited more than Pnet. These data show differences in dark respiration rates in response to SO₂ to be an important factor determining the degree of net photosynthetic inhibition in Dylan plants due to SO₂ fumigations, especially under low light intensities.

However, the data presented in Table 4.6 also show that there was still less photosynthetic inhibition in Dylan plants under low light intensities in response to SO_2 even when dark respiration rates have been taken into consideration. This may be explained if the data for both stomatal and residual resistances are examined. Values for both resistances before and after SO_2 fumigations under low light intensities were presented in Tables 4.4 and 4.5. For plants of the variety Dylan (Table 4.4) it can be seen that when SO_2 exceeded 390 ppb the greatest inhibitions in Pnet were

TABLE 4.6

Percent change in comparison to control plants of rates of gross photosynthesis (Pmax) and net photosynthesis (Pnet) in <u>Vicia faba</u> CV. Dylan plants grown and exposed to sulphur dioxide (4 h) under low or high light intensities.

	Hi	gh Light	Low Light		
SO ₂ Conc. (ppb)	Pnet	(%) Pmax	Pnet	(%) Pmax	
180	1•3	-2 - 9	-14.3	0	
255	2 • 9	3.6	-6-3	-4.3	
295	6•4	4 • 2	3.7	1 • 1	
390	17.6	14-3	6•3	6-8	
400	31.4	26 • 2	12.5	22 • 2	
600	45.9	36•6	20•9	26 • 2	
610	46·1	40.6	18•4	19.5	

observed, but in most cases r_s decreased in response to SO_2 and r_r was significantly increased. It was shown in S4.6 that, under low light intensities, stomatal responses in Dylan plants were reversed, stomatal closure in response to SO_2 decreasing with increasing SO_2 concentration. At lower SO_2 concentrations (below 390 ppb) r_s increased and r_r changed only slightly.

It may be concluded that, in Dylan plants grown under low light intensities, at lower SO₂ concentrations changes in stomatal resistance in conjunction with changes in rates of respiration, determined the degree of photosynthetic inhibition observed in response to SO₂. However, at higher SO₂ concentrations, r_rCO_2 was significantly increased, therefore photosynthetic inhibition was a result of changes in r_r and dark respiration rates due to SO₂ exposure, r_s exerting little influence.

In Aquadulce Claudia plants, SO_2 had no effect on rates of dark respiration at either low or high light intensities and there were no significant differences in the degree of photosynthetic inhibition due to SO_2 at either light intensity. In Aquadulce plants, both stomatal and residual resistances were doubled in response to low light stress in the absence of SO_2 . It may be concluded that as for high light intensities, photosynthetic inhibition in Aquadulce plants occurred due to increases in r_r when SO_2 exceeds 285 ppb (Table 4.5). Below this concentration, changes in Pnet appeared to be a result of increased stomatal resistance to CO_2 transfer in response to SO_2 fumigations.

4.7.2 rrCO₂ & rsCO₂: Effects of SO₂ & Low Temperature Stress

4.7.2.1 24 h Cold

When the resistances to CO_2 transfer in Aquadulce and Dylan plants were examined following a period of 24 h at 10°C prior to SO_2 fumigations the marked changes noted following low light stress were not observed, although the data were much more variable in comparison to that obtained under optimum environmental conditions. The data for stomatal and residual resistances to CO_2 transfer before and after SO_2 fumigations are presented in Tables 4.7 and 4.8. Absolute values for residual resistances to CO_2 transfer in Dylan plants, under optimum conditions in the absence of SO_2 , ranged from 448 to 864 s m⁻¹ (mean = 730; SD = 129) and following 24 h cold ranged from -83 to 1070 s m⁻¹ (mean = 543; SD = Results

TABLE 4.7

<u>Vicia faba</u> CV. Dylan. Stomatal (r_s) and Residual (r_r) Resistance Data for CO₂ Transfer Before and After Exposure to SO₂ Following a 24 h Pretreatment at 10°C. Per cent SO₂-induced inhibition in net photosynthetic rates and stomatal resistance in relation to control plants are also shown.

[S0,]	% change	r _r (s	m ⁻¹)	% change	r: (s	m ⁻¹)
(ppb)	in Pnet	Before SO ₂	After S02	in r _s	Before SO ₂	After SO ₂
101	3.3	533 • 4	644.9	-13.2	143.5	100-6
108	14-9	-83.7	469.3	22 · 1	902·5	469•1
160	0+6	330 • 1	399-1	14.6	818•4	782 • 1
285	13.6	826-8	961.5	-15.3	371.2	348 • 1
340	14•4	-72 • 4	559.0	-15 • 4	788·7	351•4
395	19•5	825.5	991-3	11.5	29•7	47.8
420	36•3	1070 • 1	1709-8	6 • 1	204.6	21 · 1
465	18.5	904 • 4	548 • 1	-75.0	62·7	496.6
480	49•3 [°]	554•6	1193 • 1	-3.6	445.5	503 • 2

TABLE 4.8

As for Table 4.7. Vicia faba CV. Aquadulce Claudia.

[S0 ₂]	% change	\mathbf{r}_r (s m ⁻¹)	% change	rs (s	5 m ⁻¹)
(ppb)	in Pnet	Before SO ₂	After SO ₂	in r _s	Before SO ₂	After SO ₂
101	5.2	760•9	709 - 8	-8.6	52.8	69·3
110	8 • 2	429-3	129•4	-27.6	471·9	488·4
200	· 6•9	536.6	602 • 7	31.6	201.3	179.9
210	5.8	515·2	473 • 8	-8.9	235 • 9	316.8
255	2.5	11-5	-378 • 7	. 27•5	767 • 2	1255 • 8
270	-5.6	445·5	709.3	-16.7	371.2	204.6
278	5.5	359•1	255.0	-21.5	493·3	615•4
285	45 • 1	122 • 4	561.7	-7•4	526·3	653·4
398	11.3	1043 • 2	993 • 7	24 • 2	80+8	41.2
560	55.9	191-2	698 - 5	-14.8	585.7	780•4

392). Similarly, in Aquadulce, r_r for unstressed plants ranged from -111 to 729 s m⁻¹ (mean = 432; SD = 203) and following a 24 h cold treatment ranged from 11 to 1043 s m⁻¹ (mean = 441; SD = 288). Thus, cold stress had no significant effect on r_r prior to SO₂ exposure even though the variability in the data was increased in response to added cold stress as can be seen from the increased standard deviations (SD).

However, stomatal resistances to CO_2 transfer were influenced by the imposition of cold stress. In Dylan plants, under optimum conditions, values for r_sCO_2 ranged from 20 to 270 s m⁻¹ but after the 24 h cold pretreatment ranged from 29 to 902 s m⁻¹. In Aquadulce Claudia plants values for r_sCO_2 in the absence of SO_2 or cold, ranged from 100 to 400 s m⁻¹ but following cold stress ranged from 52 to 767 s m⁻¹. The majority of plants showed increased stomatal resistance in response to cold stress when compared with resistance data for unstressed plants; the increases being higher for plants of the variety Dylan.

Cold periods for 24 h were found to have no significant effect on gross photosynthetic rates prior to SO_2 fumigations when the gross photosynthetic rates were compared with the data for non cold-stressed plants. However in S4.5, it was shown that dark respiration rates in the variety Dylan were enhanced in response to the 24 h cold period, dark respiration in Aquadulce plants being unaffected. This increase in *Rd* rate in Dylan plants resulted in slight reductions in net photosynthetic rates even though gross photosynthetic rates appeared to be unaffected. However, these enhanced rates of *Rd* were shown to decline to pre-treatment rates within 24 h of the end of the cold pre-treatment (S4.5).

Having defined the effects of cold temperatures for a period of 24 h on leaf resistances in the absence of SO_2 , the effects of cold stress on responses to SO_2 were examined. In Aquadulce plants there were no significant differences in the degree of SO_2 -induced net photosynthetic inhibition in cold-treated and non cold-treated plants. However, photosynthetic inhibition in response to SO_2 was lessened in plants of the variety Dylan as a result of the 24 h cold exposures. Unlike the responses observed under low light intensities, changes in dark respiration rates could not account for the observed reductions in SO_2 induced photosynthetic inhibition in Dylan plants.

The lessening of the degree of SO_2 -induced net photosynthetic inhibition due in Dylan plants following 24 h at cold temperatures may be

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explained if the data for both stomatal and residual resistances following SO_2 exposure are examined (Table 4.7). Stomatal resistance to CO_2 transfer in Dylan plants decreased in response to SO_2 concentrations up to 420 ppb and above this concentration r_s was seen to increase. At the same time, the residual resistance to CO_2 transfer was, in most cases, increased in response to SO_2 fumigations. However, these increases in r_r were not as great as those observed in non cold treated plants exposed to SO_2 .

It may be concluded that in Dylan plants subject to 24 h of cold stress prior to pollutant exposure, reduced net photosynthetic inhibition was a result of decreased stomatal resistances and smaller increases in residual resistances to CO_2 transfer in response to SO_2 . Another contributing factor was that exposure to SO_2 did not induce enhanced dark respiration rates in Dylan plants following the cold pre-treatment whereas increased respiration rates in Dylan plants under optimum environmental conditions were a contributing factor to reductions in net photosynthetic rates in response to SO_2 exposure.

Whilst no significant differences in photosynthetic or respiratory responses to SO_2 were observed in Aquadulce plants when the results for 24 h cold stressed and non cold stressed plants were compared, analysis of the resistance data for CO_2 transfer showed some significant changes due to cold stress. Under optimum environmental conditions, when SO_2 exceeded 285 ppb, residual resistances to CO_2 were increased in Aquadulce plants, the increase being very large when SO_2 concentrations exceeded 500 ppb. However, following the imposition of cold stress there were no large increases in r_rCO_2 in response to SO_2 fumigations and when SO_2 concentrations were below 285 ppb r_r was seen to decline in response to SO_2 . At the same time, when r_r declined, stomatal resistance was seen to increase so that the resulting photosynthetic inhibition was due to a combination of changes in both r_3CO_2 and r_rCO_2 .

When per cent changes in stomatal resistance were plotted against ambient sulphur dioxide concentrations (Figure 4.13), the magnitude of the stomatal closure response in Aquadulce plants was seen to increase following the imposition of cold stress. Together with the data presented in this section, this implied that changes in stomatal resistance in response to SO_2 played a larger part in determining changes in net photosynthetic rates in Aquadulce plants after a 24 h cold treatment.

It must be noted that in three plants of Aquadulce subject to 24

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h cold stress, photosynthetic inhibition due to SO_2 was significantly reduced (Table 4.8). This occurred in the plants subjected to 200, 270 and 398 ppb SO_2 and in all cases stomatal resistance was significantly reduced by SO_2 and residual resistances altered only slightly. These responses were also evident when % change in r_s was related to SO_2 concentration (Fig. 4.13) as these three plants were the only ones in which stomatal opening was seen to occur in Aquadulce after 24 h cold pre-treatment.

4.7.2.2 72 h Cold

The resistances to CO₂ exchange in Dylan and Aquadulce plants before and after SO₂ fumigations are presented in Tables 4.9 and 4.10. Both residual and stomatal resistances were very variable following the 72 h cold treatment in the absence of SO_2 and the data spanned a wide range of values when compared with data for non cold-stressed plants. In Dylan plants (Table 4.9) residual resistances to CO_2 exchange, before pollutant exposure, ranged from -38 to 1281 s m⁻¹ (mean = 589; SD = 448) as compared to 448 to 864 s m⁻¹ (mean = 730; SD = 129) without cold stress and stomatal resistances ranged from 135 to 892 s m⁻¹ as opposed to 20 to 270 s m⁻, measured under optimum environmental conditions. The data obtained for Aquadulce Claudia plants showed similar variations in stomatal resistances to CO₂ after the 72 h cold treatments, ranging from 89 to 1570 s m⁻¹ as opposed to 100 to 400 s m⁻¹ for optimum environmental conditions. Residual resistances to CO_2 exchange in Aquadulce did not differ greatly from the values obtained before the imposition of cold stress, values ranged from -384 to 804 s m⁻¹ (mean = 411; SD = 411) after 72 h at 10°C and were -111 to 729 s m^{-1} (mean = 432; SD = 203) in optimum conditions, although, as for 24 h cold data, the variance was substantially increased.

As a result of these changes in leaf resistances to CO_2 in coldstressed Dylan plants the majority of plants tested exhibited reductions in rates of gross photosynthesis in response to cold stress. Estimated rates of Pmax were lower than those observed under optimum conditions. Reductions in Pmax were also observed in Aquadulce plants in response to cold stress but the reductions in Pmax in both varieties were small in comparison with those observed in response to low light stress.

As observed in plants subject to 24 h at 10°C, dark respiration rates were enhanced in Dylan plants in response to cold temperatures and these increases in Rd declined gradually at the end of the cold treatment.

TABLE 4.9

<u>Vicia faba</u> CV. Dylan. Stomatal $\langle r_s \rangle$ and Residual $\langle r_r \rangle$ Resistance Data for CO₂ Transfer Before and After Exposure to SO₂ Following a 72 h Pretreatment at 10°C. Per cent SO₂-induced change in net photosynthetic rates and stomatal resistance in relation to control plants are also shown.

[S0 ₂]	% change	$\mathbf{r}_{\mathbf{r}}$	s m ⁻¹)	% change	rs (s m ⁻¹)
(ppb)	in Pnet	Before SO ₂	After SO ₂	in r _s	Before SO ₂	After SO₂
97	3.6	686+9	230.7	-9•1	282 · 1	166 • 9
119	11.2	120 • 4	-73 · 4	-1-8	821.7	1032 • 9
230	2.8	839-6	737•9	-2.7	306•9	354.7
310	28 • 4	1281•2	1706 • 7	-27 · 1	176-5	133-6
385	28+9	63•3	-204.8	-15.9	892.6	1176 • 4
460	30.2	890.3	1308-9	-51.2	181.1	310 · 2
469	8.8	-38 · 1	379.0	24 · 3	747•4	351•4
520	34.5	873 • 7	1345.8	-9.3	135-3	94 • 1

TABLE 4.10

<u>Vicia</u> <u>faba</u> CV. Aquadulce Claudia. Stomatal (r_s) and Residual (r_r) Resistance Data for CO₂ Transfer Before and After Exposure to SO₂ Following a 72 h Pre-treatment at 10°C. Per cent SO₂-induced change in net photosynthetic rates and stomatal resistance in relation to control plants are also shown.

[S02]	% chang	e r _r	(s m ⁻¹)	% change	r <u>s</u> (s	3 m ⁻¹)
(ppb)	Pnet	Befor	e After	in r _s	Before	After
• -		S02	SO2		S0₂	S02
101	4 • 9	-384.3	-2783 • 4	-66.9	1570.8	3910·5
198	15.6	747•7	732·1	-6-9	109.5	257·4
272	13•9	532 • 2	593 • 2	-12.5	381.1	308 • 2
285	13 • 1	804•0	940.9	-18-9	122 • 7	77.5
372	4•9	510.0	557 • 5	18•1	207•9	212.8
455	22 • 8	-19-8	215 - 8	32.6	815 • 1	859•6
505	20.3	691·4	910·B .	-17.5	89.9	43.8

Unlike the results for 24 h cold, dark respiration rates were also enhanced in Aquadulce Claudia plants in response to 72 h of cold temperatures and these enhanced rates persisted for more than 48 h after the end of the cold treatment.

In both varieties the net result of decreases in Pmax and increases in *Rd* were reductions in net photosynthetic rates in response to cold temperatures in the absence of sulphur dioxide. The average reduction in Pnet due to cold stress was calculated at 20% when compared with non cold-stressed plants on the first day of the experimental period. However, plants were exposed to sulphur dioxide on the second day of the experimental period by which time measured rates were approaching the levels for unstressed plants.

Both Dylan and Aquadulce plants showed decreases in the extent of SO₂-induced net photosynthetic inhibition following periods of 72 h at 10°C. In Dylan plants, inhibition was much less than that observed under optimum environmental conditions when SO₂ concentrations exceeded 200 ppb. Reduced inhibition in Aquadulce plants was significant only when ambient SO₂ concentrations exceeded 300 ppb. These reductions in inhibition could not be attributed to changes in dark respiration rates as SO₂ had no effect on rates of *Rd* in Dylan plants after cold treatments. Exposure to SO₂ caused reductions in *Rd* in Aquadulce plants but because respiration rates had been enhanced by the cold pre-treatments there were no significant differences in rates of dark respiration between cold-stressed and non coldstressed plants following SO₂ fumigations.

In Dylan plants, both stomatal and residual resistances to CO_2 showed very variable responses to SO_2 concentrations up to 385 ppb and photosynthetic inhibition was associated with either decreases or increases in the stomatal and residual resistances to CO_2 transfer. At higher SO_2 concentrations residual resistances to CO_2 increased in Dylan plants in response to SO_2 but these increases were not as great as those observed in the absence of cold stress. At the same time stomatal resistances to CO_2 were seen to decrease in response to SO_2 . It would appear that SO_2 -induced net photosynthetic inhibition in Dylan plants following the 72 h cold pretreatments was a result of changes in both r_r and r_s and at higher concentrations, reduced inhibition arose from much smaller increases in r_r in comparison with non cold-stressed plants. The degree of net photosynthetic inhibition in Dylan plants was also reduced because SO_2 did not cause enhanced rates of dark respiration after the cold pre-treatments.

Reduction in the degree of net photosynthetic inhibition caused by SO₂ following 72 h cold pre-treatments in Aquadulce Claudia plants appeared to be due solely to smaller increases in r_rCO_2 due to SO₂ than were observed under optimum environmental conditions. Changes in stomatal resistance were very small in cold-stressed Aquadulce plants in response to SO₂ concentrations above 200 ppb and could not account for the marked differences in percent net photosynthetic inhibition in response to SO₂ occurring between cold-stressed and non cold-stressed plants.

4.7.2.3 <u>1 Week Cold</u>

The CO_2 resistance data for periods of 1 week cold stress prior to SO_2 fumigations for both Dylan and Aquadulce Claudia plants are presented in Tables 4.11 and 4.12. The imposition of cold stress for 1 week lead to reductions in rates of gross and net photosynthesis in both varieties of <u>Vicia faba</u>. In Dylan plants, reductions in Pnet were due in part to increases in dark respiration rates in response to cold stress but this did not occur in Aquadulce plants. When plants were first transferred to the exposure system Pnet was found to be reduced by an average of 20% when rates for both varieties were compared with non cold stressed plants. However 24 h later, immediately before SO_2 treatments Pnet was down an average of only 10% in both varieties as plants recovered.

In both varieties, before SO₂ fumigations, residual resistances to carbon dioxide exchange following a 1 week cold pre-treatment were not significantly higher than those measured for non cold stressed plants and were in some cases much lower, particularly for Dylan plants. Residual resistances in Dylan ranged from -484 to 815 s m⁻¹ (mean = 305; SD = 401) and were 448 to 864 s m⁻¹ under optimum conditions (mean = 730; SD = 129). In Aquadulce plants residual resistances to CO₂ transfer ranged from 96 to 782 s m⁻¹ (mean = 470; SD = 219) after the 1 week cold pre-treatment and were -111 to 729 s m⁻¹ (mean = 432; SD = 203) without cold stress. Therefore reductions in photosynthetic rates due to cold stress did not result from increases in residual resistances to CO₂ exchange.

However, stomatal resistances to CO_2 transfer were found to be significantly higher in both varieties after cold stress. Stomatal resistances in Dylan plants ranged from 257 to 1928 s m⁻¹ as opposed to 27 to 270 s m⁻¹ in the absence of cold stress and values for Aquadulce plants Results

TABLE 4.11

<u>Vicia faba</u> CV. Dylan. Stomatal (r_s) and Residual (r_r) Resistance Data for CO₂ Transfer Before and After Exposure to SO₂ Following a 1 week Pretreatment at 10°C. Per cent SO₂-induced change in net photosynthetic rates and stomatal resistance in relation to control plants are also shown.

[S0 ₂]	% change	$\mathbf{r}_{\mathbf{r}}$ (a	5 m ⁻¹)	% change	r: (s	s m ⁻¹)
(ppb)	in Pnet	Before	After	in r _s	Before	After
		SO ₂	S0₂		S02	SO2
90	10 • 1	670·7	565.9	-24 • 4	257・4	320·2
105	9-9	135 • 2	432·1	-4.2	617 • 1	341.5
190	12 - 2	-89-3	29•3	-7.8	924.0	910.8
195	17.6	-484.6	-531.9	-28 · 1	1928 • 8	1902 • 4
288	17.0	815-6	338 • 9	-9.6	316.8	1064 • 1
368	38+1	442·7	989•1	-23 · 1	430·6	168+3
470	15 • 1	555·6	781·1	-14.9	549•4	371.2
520	38 - 7	401-1	1011-5	-19-2	354•7	193-0

TABLE 4.12

<u>Vicia faba</u> CV. Aquadulce Claudia. Stomatal (r_s) and Residual (r_r) Resistance Data for CO₂ Transfer Before and After Exposure to SO₂ Following a 1 week Pre-treatment at 10°C. Per cent SO₂-induced change in net photosynthetic rates and stomatal resistance in relation to control plants are also shown.

[S0 ₂]	% change	r_{r} (s m ⁻¹)		% change	r _s (s m ⁻¹)	
(ppb)	in Pnet	Before SO ₂	After S02	in r _s	Before SO ₂	After SO₂
99	14.1	96•6	467·1	-15.9	992-6	719•4
205	13.3	397•9	832 • 3	-26+9	921.7	848 9
208	-4.1	713.9	523•6	-29.2	89 • 1	114.0
282	5.8	783•7	937·7	-43-4	234 · 8	291-4
380	24.6	573•4	758+7	-53 • 8	41.2	28 • 1
465	2.5	431•4	562+6	-11.4	640.2	714.5
490	5.3	217.2	-367•9	-61-2	505.9	1154.8
492	9.6	557.6	993-5	-9•1	810 • 1	696 • 3

ranged from 41 to 992 s m⁻¹ after 1 week cold but were between 100 and 400 s m⁻¹ under optimum conditions. It would appear that cold temperature stress induced reductions in net photosynthetic rates via increased stomatal resistances to CO_2 exchange and in plants of the variety Dylan, increased rates of dark respiration.

When stomatal resistances to CO_2 transfer, following SO_2 treatments, were compared between cold-stressed and non cold-stressed plants resistances were again found to be much higher in the plants subject to the 1 week cold pre-treatments. Values for r, in cold-stressed Dylan plants following a range of SO_2 treatments ranged from 168 to 1902 s m⁻¹ (14 to 633 s m⁻¹ under optimum conditions) and in cold-stressed plants of Aquadulce Claudia r, values ranged from 28 to 1154 s m⁻¹ (39 to 520 s m⁻¹ without added cold stress). It may be expected that such high r, values would lead to greater photosynthetic inhibition in cold stressed plants however, this was not seen to occur. As was shown in §4.4, both varieties exhibited a reduction in net photosynthetic responses to SO_2 following the 1 week cold pre-treatment, this difference being most marked when SO_2 concentrations exceeded 200 ppb.

It can be seen in Table 4.11 that in the majority of plants of the variety Dylan, stomatal resistance decreased over the 4 h SO₂ fumigation period. Examination of the residual resistances to CO_2 exchange following SO₂ exposure in Dylan plants showed these resistances to be much lower than those for plants exposed to SO₂ under optimum environmental conditions; r_r ranged from -532 to 1011 s m⁻¹ in cold-stressed plants (432 to 2521 s m⁻¹ without cold stress). Similarly, residual resistances in Aquadulce plants (Table 4.12) following SO₂ exposure were much lower than under optimum environmental conditions ranging from -308 to 993 s m⁻¹ (98 to 2045 s m⁻¹ without added cold stress).

From the data presented in Tables 4.11 and 4.12 it may be concluded that reductions in photosynthetic response to SO_2 in both varieties of <u>Vicia faba</u> following cold temperature stress for 1 week were due predominantly to much smaller changes in the residual resistances to CO_2 exchange than were observed under optimum environmental conditions and were also due in part, to decreases in stomatal resistances to CO_2 in response to sulphur dioxide fumigations.

4.7.3 Relationship Between Changes in Stomatal Resistance and Net Photosynthesis.

Under optimum environmental conditions there were found to be significant linear correlations (p < 0.01) when SO₂-induced changes in net photosynthetic rates were plotted against SO₂-induced changes in stomatal resistance for both varieties of <u>Vicia</u>, although there was a degree of scatter in the data showing changes in Pnet to be not solely due to changes in r₃. In general, highest measures of stomatal closure were correlated with greatest measured photosynthetic inhibition (S3.7.3); also in Aquadulce Claudia plants decreases in r₃ were associated with enhanced photosynthetic rates in response to SO₂. Under optimum environmental conditions Dylan plants appeared to exhibit greater stomatal sensitivity to SO₂ because observed stomatal responses in Dylan plants were of greater magnitude and direction than those of Aquadulce plants (S3.6).

However, when plants were subjected to added environmental stresses such as low light intensities or periods of cold temperature stress prior to SO_2 fumigation significant changes in the relationship between % change in r_3 and % change in Pnet were found in both varieties. Unlike the responses observed under optimum environmental conditions, there were no significant correlations found between % change in r_3 and % change in Pnet for either variety in any of the added environmental stress regimes. The regression data are summarised in Table 4.13 and the data are presented in Figures 4.17 to 4.20, the regression lines obtained for the data obtained under optimum environmental conditions being also shown in the figures.

When plants were grown and subjected to SO_2 under low light intensities there appeared to be no significant difference in the data obtained for Aquadulce Claudia plants in comparison with the data obtained under high light conditions (Fig. 4.17b), although the data were much more variable following low light stress. However, the data for Dylan plants under low light conditions showed an inverse relationship in comparison with the data for high light conditions (Fig. 4.17a), the highest degree of photosynthetic inhibition being associated with enhanced stomatal opening. These data concur with the trend observed when % change in r_s was plotted against SO_2 concentration (ppb) (Figure 4.9) where stomatal opening was seen to occur in Dylan plants at highest SO_2 concentrations.

Following cold pre-treatments of 24 h, 72 h or 1 week prior to

TABLE 4.13

Summary of regression analyses to show the relationships between SO_2 induced changes in stomatal resistance and changes in net photosynthetic rates in two varieties of <u>Vicia faba</u> CV. Dylan & Aquadulce Claudia exposed to a range of sulphur dioxide concentrations following a range of environmental pre-treatments.

Variety & Environmental Pre-treatment	Correlation Co-efficient	Significance	
<u>No environmental stress</u>			
Dylan Aquadul ce	-0·761 -0·701	p < 0.001 p < 0.01	
Low Light			
Dylan Aquadul ce	0 · 647 -0 · 076	ns ns	
24 h Cold	•		
Dyl an Aquadul ce	-0·029 -0·164	ns ns	
<u>72 h_Cold</u>		• •	
Dylan Aquadul ce	-0·534 0·424	ns ns	
1 Week Cold			
Dylan Aquadul ce	-0·365 -0·210	ns ns	





Figures 4.17a and 4.17b.

Changes in photosynthetic inhibition (Pnet) in response to SO_2 as related to corresponding changes in stomatal resistance (r_s) for two varieties of <u>Vicia faba</u>, Dylan (4.17a) and Aquadulce Claudia (4.17b) under conditions of low light intensity. [The regression lines for the data obtained under high light conditions are also shown in the figures (-----)].

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SO₂ fumigations (Figs. 4.18a, 4.19a & 4.20a), the data for Dylan plants showed no significant differences from the data obtained for plants under optimum environmental conditions, although the increased variability of the data following the cold stress pre-treatments resulted in there being no significant linear correlations between changes in rs and Pnet. When the data presented in Figures 4.18a, 4.19a and 4.20a for Dylan plants were compared there was evidence of a 'shift' in the relationship between changes in rs and changes in Pnet in response to SO₂, this 'shift' progressing with increased duration of cold temperature stress. Following the 24 h cold treatment changes in Pnet were related to stomatal opening in almost half the treated plants (Fig. 4.18a), after the 72 h cold treatment only one plant exhibited stomatal opening correlated to Pnet inhibition (Fig. 4.19a) and finally, following the 1 week cold treatment none of the SO₂-treated Dylan plants exhibited stomatal opening in connection with SO₂-induced photosynthetic inhibition (Fig. 4.20a). This 'shift' in the responses of Dylan plants appeared to be related to a reduction in the magnitude and direction of stomatal responses to SO_2 with increasing duration of cold temperature stress.

In Aquadulce plants following periods of 24 h at 10°C there was no apparent relationship between changes in stomatal resistance in response to SO_2 and changes in net photosynthetic rates (Figs. 4.18b) although the majority of data showed enhanced stomatal closure to be correlated with net photosynthetic depression. However, following 72 h and 1 week at 10°C prior to SO_2 exposure the data obtained were increasingly significantly different from that obtained in the absence of cold pretreatment (Figs. 4.19b, 4.20b). The magnitude of stomatal response was much greater than that observed under optimum environmental conditions and this was correlated with much smaller measures of net photosynthetic inhibition for both cold stress regimes. As for Dylan plants (Fig. 4.20a), following the 1 week cold pre-treatments all changes in net photosynthetic rate were associated with stomatal closure in response to SO_2 (Fig. 4.20b).

In §3.7.3 it was concluded that changes in stomatal resistance contributed significantly to reductions in net photosynthetic rates in response to SO_2 in both varieties of <u>Vicia faba</u>. However, when the data following environmental pre-treatments were considered there were no





Figures 4.18a and 4.18b.

Changes in photosynthetic inhibition (Pnet) in response to SO_2 as related to corresponding changes in stomatal resistance (r_3) for two varieties of <u>Vicia faba</u>, Dylan (4.18a) and Aquadulce Claudia (4.18b) following a cold temperature pre-treatment for 24 h prior to SO_2 fumigation. [The regression lines for the data obtained under optimum temperature conditions are also shown in the figures (-----)].





Figures 4.19a and 4.19b.

Changes in photosynthetic inhibition (Pnet) in response to SO_2 as related to corresponding changes in stomatal resistance (r_5) for two varieties of <u>Vicia faba</u>, Dylan (4.19a) and Aquadulce Claudia (4.19b) following a cold temperature pre-treatment for 72 h prior to SO_2 fumigation. [The regression lines for the data obtained under optimum temperature conditions are also shown in the figures (-----)].





Figures 4.20a and 4.20b.

Changes in photosynthetic inhibition (Pnet) in response to SO_2 as related to corresponding changes in stomatal resistance (r_s) for two varieties of <u>Vicia faba</u>, Dylan (4.20a) and Aquadulce Claudia (4.20b) following a cold temperature pre-treatment for 1 week prior to SO_2 fumigation. [The regression lines for the data obtained under optimum temperature conditions are also shown in the figures (-----)]. significant correlations between changes in r_3 and changes in Pnet and it may be concluded that changes in net photosynthetic rates in response to SO_2 and environmental stress occurred in a seemingly independent manner of changes in r_3 and therefore, some other factor was exerting an influence. From the data presented in §4.7.1 changes in Pnet appeared to be due, predominantly, to changes in the residual resistance to CO_2 exchange.

4.8 POLLUTANT FLUXES & AMBIENT SO2 CONCENTRATION

The relationships between sulphur dioxide flux and ambient SO_2 for both varieties of <u>Vicia</u> faba under optimum environmental conditions were described in §3.9. In both cases, pollutant fluxes were proportional to applied SO_2 concentrations and estimates of measured pollutant flux were consistently higher that estimates of calculated pollutant flux.

Under optimum environmental conditions estimates of pollutant flux from analogy to water vapour transfer (P_{fcalc}) were found to differ from estimates of pollutant flux from mass balance calculations (P_{fmeas}). This difference between the two measures of flux was found to be due to incorrect assumptions about the magnitude of the residual resistance to SO₂ transfer when estimating flux. However, it could not be assumed that similar conclusions could be drawn for plants subjected to periods of environmental stress and, as a result, both measures of flux were still assessed in this section of the work.

The relationships between pollutant fluxes to the plant and ambient sulphur dioxide concentration following a range of environmental pre-treatments are shown in Figures 4.21 to 4.28; where appropriate the regression lines obtained for the data are shown in the figures. The regression lines for the data obtained under optimum environmental conditions are also shown in the figures. All data obtained for each environmental regime were compared with that obtained in the absence of environmental stress for each variety using the analysis of covariance test. A summary of the results obtained from the analyses are shown in Table 4.14.

TABLE 4.14

Summary of Analysis of Covariance Tests Comparing Regression Analyses of both Measured (from mass balance calculations) and Calculated (from analogy to water vapour transfer) Pollutant Flux versus Ambient sulphur Dioxide Concentration in two Varieties of <u>Vicia faba</u> CV. Dylan and Aquadulce Claudia under Optimum Environmental Conditions with Data Obtained Following Added Environmental Stress.

		Analysis of	Covariance
Measured Flu	UX.	F value	Significance
			α =
Dylan "High	Light/Optimum Temper	ature"	
v Dyla:	n low light	2.502	ns
v Dyla:	n 24 h cold	0.936	DS
v Dyla:	n 72 h cold	0-887	ns
v Dyla	n 1 week cold	10•437	0+01
Aquadul ce "	High Light/Optimum Te	mperature"	
v Aqua	dulce low light	0 • 968	ns
v Aqua	dulce 24 h cold	1 • 795	ns
v Aqua	dulce 72 h cold	4.347	0.05
v Aqua	dulce 1 week cold	11.877	0-01
<u>Calculated</u>	Flux		
Dylan "High	Light/Optimum Temper	ature"	
v Dyla	n low light	1.956	ns
v Dyla	n 24 h cold	0.563	ns
v Dyla	n 72 h cold	0 • 150	ns
v Dyla	n 1 week cold	1.035	ns
Aquadul ce "	High Light/Optimum Te	mperature"	
v Amia	dulce low light	5.980	0.025
v Ama	dulce 24 h cold	0.290	ns
v Amia	dulce 72 h cold	7.158	0.025
v Aqua	dulce 1 week cold	0.046	ns

4.8.1 PFCALC & PFmeas v SO2: Low Light Conditions

The data presented in Figures 4.21a and 4.21b show both measured and calculated pollutant fluxes to plants of the variety Dylan under low light intensities. For measured flux (Fig. 4.21a) linear regression gave an r value of 0.223 which was not significant, however, regression analysis of the data for calculated flux (Fig. 4.21b) produced an r value of 0.904 (p <0.001). Although the data were scattered there appeared to be no significant difference in the degree of flux to the plant under high or low light conditions. This was confirmed when the the F values obtained from a statistical analysis of the data were not significant for either measure of flux (Table 4.14).

Figures 4.22a and 4.22b show the data obtained for plants of the variety Aquadulce Claudia under low light conditions. As for Dylan plants subject to low light stress, regression analysis did not produce a significant correlation between measured pollutant flux and ambient SO₂ concentrations (r = -0.112) (Fig. 4.22a) and analysis of covariance also showed there to be no significant differences in measured flux to Aquadulce plants under high or low light intensities (Tab. 4.14). However, regression analysis showed there to be a significant correlation between calculated pollutant flux values and ambient SO₂ concentrations (r = 0.715; p < 0.005) (Fig. 4.22b) and estimates of calculated SO₂ flux were found to be significantly lower than those obtained under high light intensity (F = 5.980, $\alpha = 0.025$ [Tab. 4.14]).

4.8.2 PFCALC & PFmeas v SO2: Low Temperature Treatments

Figures 4.23 and 4.24 show the data obtained for both Dylan and Aquadulce plants following cold pre-treatments of 24 h at 10°C prior to SO_2 fumigation under optimum environmental conditions. Again no significant differences in either measure of pollutant flux were found when results were compared with data for non cold-stressed plants. Regression analyses gave significant correlations for both varieties when measured pollutant flux was plotted against ambient SO_2 concentrations. For Dylan plants (Fig. 4.23a) the calculated correlation coefficient for the regression line shown in the figure, following 24 h cold stress treatments was 0.908 (p < 0.001). For Aquadulce plants (Fig. 4.24a) the correlation coefficient was 0.794 (p <

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Figures 4.21a & 4.21b.

Measured and calculated pollutant fluxes ($\mu g \ m^{-2} \ s^{-1}$) to Vicia faba plants of the variety Dylan as related to ambient sulphur dioxide concentrations (ppb) under conditions of low light intensity. [The regression lines obtained for plants under high light conditions are also shown in the figures (----)].



Figures 4.22a & 4.22b.

Measured and calculated pollutant fluxes ($\mu g m^{-2} s^{-1}$) to Vicia faba plants of the variety Aquadulce Claudia as related to ambient sulphur dioxide concentrations (ppb) under conditions of low light intensity. [The regression lines obtained for plants under high light conditions are also shown in the figures (----)].



Figures 4.23a & 4.23b. Measured and calculated pollutant fluxes ($\mu g \ m^{-2} \ s^{-1}$) to Vicia faba plants of the variety Dylan as related to ambient sulphur dioxide concentrations (ppb) following cold temperature pre-treatments of 24 h prior to SO₂ fumigation. [The regression lines obtained for non cold-stressed plants are also shown in the figures (-----)].







Figures 4.24a & 4.24b. Measured and calculated pollutant fluxes ($\mu g \ m^{-2} \ s^{-1}$) to Vicia faba plants of the variety Aquadulce Claudia as related to ambient sulphur dioxide concentrations (ppb) following cold temperature pre-treatments of 24 h prior to SO₂ fumigation. [The regression lines obtained for non coldstressed plants are also shown in the figures (-----)]. 0.001). The regression lines for both sets of data for plants subject to 24 h cold stress were very similar to those obtained under optimum environmental conditions. When calculated pollutant fluxes were plotted against ambient SO_2 for plants of both varieties subject to 24 h cold stress (Figs. 4.23b & 4.24b) no significant correlations were found due to increased variability of the data obtained. For Dylan plants (Fig. 4.23b) r = 0.507 and for Aquadulce plants (Fig. 4.24b) r = 0.371, neither value being statistically significant. However, analysis of covariance (Tab. 4.14) showed calculated pollutant flux in relation to ambient sulphur dioxide concentrations to be unchanged by the 24 h cold pre-treatments.

The data obtained for both varieties following periods of 72 h cold stress prior to SO₂ treatments are shown in Figures 4.25 and 4.26. For Dylan plants (Fig. 4.25) both measured and calculated pollutant fluxes were significantly correlated to ambient SO_2 concentration and as can be seen in the figures, the regression lines obtained were very close to those obtained for data obtained in the absence of the cold pre-treatments: For measured flux r = 0.736 (p < 0.001) and for calculated flux r = 0.702 (p < 0.001). Analysis of covariance again showed SO₂ flux to Dylan plants to be unaffected by the imposition of 72 h cold temperature stress prior to SO_2 fumigations. However, it can be seen in Figure 4.26, that following periods of 72 h at 10°C pollutant fluxes to Aquadulce Claudia plants were influenced by the cold pre-treatments. Measured SO_2 flux (Fig. 4.26a) was significantly reduced following a 72 h cold pre-treatment and regression analysis gave a correlation coefficient of 0.795 (p < 0.001). Analysis of covariance showed the data for cold-stressed plants to be significantly different to that obtained under optimum temperature conditions (Tab. 4.14). In contrast, calculated pollutant flux to Aquadulce plants (Fig. 4.26b) appeared to be increased when ambient SO₂ concentrations exceed 200 ppb. Analysis of covariance produced a significant F value when compared with the data for unstressed plants, however, this value was treated with caution because of the high degree of scatter in the data obtained in the 72 h treatments (r = 0.668, ns).

When pollutant fluxes were determined following a 1 week pretreatment at 10°C prior to SO_2 fumigations, the results for both varieties differed markedly from those obtained in the absence of added environmental stress. In both Dylan and Aquadulce plants measured flux values were consistently less than the values obtained in the absence of







Measured and calculated pollutant fluxes ($\mu g \ m^{-2} \ s^{-1}$) to Vicia faba plants of the variety Dylan as related to ambient sulphur dioxide concentrations (ppb) following cold temperature pre-treatments for 72 h prior to SO₂ fumigation. [The regression lines obtained for non cold-stressed plants are also shown in the figures (-----)].


Measured and calculated pollutant fluxes ($\mu g m^{-2} s^{-1}$) to Vicia faba plants of the variety Aquadulce Claudia as related to ambient sulphur dioxide concentrations (ppb) following cold temperature pre-treatments for 72 h prior to SO₂ fumigation. [The regression lines obtained for non coldstressed plants are also shown in the figures (----)]. cold stress when ambient SO_2 concentrations exceeded 100 ppb for 4 h (Fig. 4.27a, 4.28a). Significant correlations were produced from regression analysis of the data for measured flux for both varieties; for Dylan plants r = 0.891 and for Aquadulce plants r = 0.636, both values being significant at the 99.9% level. The calculated F values from analysis of covariance for both varieties were also significant (Table 4.14).

When calculated pollutant fluxes were considered the data for Dylan plants showed there to be significantly less flux in comparison with that to non cold-stressed plants, at SO_2 concentrations below 300 ppb (Fig. 4.27b) as can be seen from the data shown in the figure. The correlation coefficient for cold-stressed plants was 0.866 (p < 0.001). However, analysis of covariance did not produce a significant F value showing there to be no significant differences in the fluxes to cold-stressed and non cold-stressed plants. The data for obtained for Aquadulce plants were very variable (Fig. 4.28b), regression analysis gave an r value of 0.189 which was not significant and analysis of covariance did not reveal any significant differences in calculated pollutant fluxes to Aquadulce plants between plants subject to cold temperature pre-treatments and non cold-stressed plants.

Under optimum environmental conditions there were good linear correlations between both measures of flux and ambient SO_2 concentrations for both varieties. However, it can be seen from the figures presented above that environmental stress influenced the relationships between flux and ambient SO_2 concentration in plants of both varieties such that increased variability in data occurred. This was especially true in calculated flux data for Aquadulce Claudia plants when no significant linear correlations were found following periods of cold temperature stress.

4.8.3 Differences Between Measured and Calculated Flux

It was stated above that under optimum environmental conditions, values for measured pollutant flux were consistently greater than values for calculated flux in both varieties indicating the existence of a residual resistance to SO_2 uptake which was underestimated in estimations of calculated pollutant flux. However, comparisons of both measures of flux for each variety following added environmental stresses showed that this difference occurred only under low light intensities. Periods of cold stress



Figures 4.27a & 4.27b.

Measured and calculated pollutant fluxes ($\mu g \ m^{-2} \ s^{-1}$) to Vicia faba plants of the variety Dylan as related to ambient sulphur dioxide concentrations (ppb) following cold temperature pre-treatments for 1 week prior to SO₂ fumigation. [The regression lines obtained for non cold-stressed plants are also shown in the figures (-----)].





Measured and calculated pollutant fluxes ($\mu g \ m^{-2} \ s^{-1}$) to Vicia faba plants of the variety Aquadulce Claudia as related to ambient sulphur dioxide concentrations (ppb) following cold temperature pre-treatments for 1 week prior to SO₂ fumigation. [The regression lines obtained for non coldstressed plants are also shown in the figures (----)]. for 24 h, 72 h or 1 week prior to SO₂ fumigation resulted in there being no significant difference between measured and calculated pollutant fluxes in either variety indicating that added environmental stress had influenced the residual resistance to pollutant transfer. The effects of added environmental stress on resistance to pollutant uptake are considered below in §4.8.4. The results obtained for the analysis of covariance tests comparing both measured and calculated pollutant fluxes to plants of both varieties following added environmental stress are summarised in Table 4.15. The data for plants of the variety Aquadulce Claudia following cold pretreatments for 72 h could not be analysed in this way because residual variation (calculated from mean sum of square values) was too great.

4.8.4 Varietal Differences in Pollutant Flux

When both measures of SO_2 flux were compared between the varieties under optimum conditions Aquadulce plants were found to have significantly less flux than plants of the variety Dylan for any given ambient SO_2 concentration above 100 ppb. In order to test whether this also occurred in pre-stressed plants flux to both varieties following periods of environmental stress were compared using analysis of covariance and the results obtained are summarised in Table 4.16.

Under low light intensities there were no significant differences in measured SO_2 flux to either variety and calculated pollutant fluxes were only less in Aquadulce Claudia than in Dylan plants when ambient SO_2 concentrations exceeded 250 ppb.

Following periods of 24 h or 72 h at 10°C prior to SO_2 fumigations, measured sulphur dioxide flux to plants of the variety Dylan was still significantly higher than that to Aquadulce Claudia plants when ambient SO_2 concentrations exceeded 100 ppb. After 24 h at 10°C neither variety exhibited changes in flux in response to the cold pre-treatment. However, flux to Aquadulce plants was significantly reduced following periods of 72 h at 10°C and because this did not occur in the variety Dylan, differences in measured flux between the varieties were amplified. In contrast, there were no significant differences in calculated flux to either variety following periods of 24 h or 72 h at 10°C but this may be a result of the high degree of variability in the data obtained.

It can be seen from Table 4.16 that there were no significant

TABLE 4.15

Summary of Analysis of Covariance Tests Comparing Measured and Calculated Pollutant Flux to two Varieties of <u>Vicia</u> <u>faba</u> Following Added Environmental Stress.

Measured Flux v Calculated Flux	Analysis o F value	f Covariance Significance α =
Dylan low light	7 • 16	0.025
Aquadulce low light	7.63	0.025
Dylan 24 h cold	0 • 45	ns
Aquadulce 24 h cold	0.50	ns
Dylan 72 h cold	0 · 17	ns
Aquadulce 72 h cold	-	-
Dylan 1 week cold	3.82	ns
Aquadulce 1 week cold	0+59	ns

TABLE 4.16

Summary of Analysis of Covariance Tests for Varietal Differences in either Measured or Calculated Pollutant flux Following Added Environmental Stress.

•		
Measured Flux F value	Significance	
	α =	
Dylan v Aquadulce	•	
low light 3.56	ns	
24 h cold 4.55	0.02	
72 h cold 5.29	0.02	
1 week cold 2.02	ns	
Calculated Flux	·	
Dylan v Aquadulce		
low light 16.22	0.01	
24 h cold 0.04	ns	
72 h cold 1.17	ns	
1 week cold 0.68	ns	

differences between measured and calculated pollutant fluxes to either variety following a 1 week cold pre-treatment prior to SO_2 fumigations. Measured SO_2 flux was reduced in both varieties in response to the cold pre-treatment and the similarity in flux measurements was a result of the degree of change being greater in plants of the variety Dylan. Again the data for Aquadulce Claudia plants for calculated flux were very variable and therefore the conclusion that there were no significant differences in calculated flux between the varieties cannot be made with confidence.

4.8.5 Stomatal and Residual Resistances to SO₂ Transfer Following Environmental Stress

Although in §4.8.3 it was shown that no significant differences between measured and calculated pollutant fluxes were found in either variety following periods of cold stress. Differences were found to occur when stomatal and residual resistances to SO_2 transfer were considered. It was described in §3.9 how stomatal resistances to SO_2 uptake were calculated in two ways, either from analogy to water vapour transfer (r_sSO_2) or from mass balance calculations giving a theoretical value of stomatal resistance ($r_sSO_2^1$). The difference in the two estimates of r_s being indicative of a non-stomatal, residual resistance to SO_2 transfer (r_rSO_2) where:

$$r_{r}SO_{2} = r_{s}SO_{2}^{\dagger} - r_{s}SO_{2}^{\dagger}$$

and $r_{5}SO_{2}$ is actually a measure of $r_{5}SO_{2} + r_{r}SO_{2}$ or total leaf resistance to SO_{2} transfer ($r_{t}SO_{2}$) minus the aerodynamic resistance component ($r_{a}SO_{2}$). so that:

$$\mathbf{r}_t SO_2 = \mathbf{r}_a SO_2 + \mathbf{r}_s SO_2 + \mathbf{r}_r SO_2$$

ог

$$r_t SO_2 = r_a SO_2 + r_s SO_2^1$$

In these experiments aerodynamic resistances remained unchanged thus changes in total leaf resistance to SO_2 could be inferred from changes in $r_3SO_2^{1}$.

The methods described in \$3.9 were used on the data obtained for both varieties following periods of added environmental stress and the results are summarised in Tables 4.17 to 4.24 and significant data are presented in Figures 4.29 to 4.38.

4.8.5.1 Low Light Stress.

Under optimum environmental conditions, the residual resistance to SO₂ transfer became increasingly negative with increasing SO₂ concentration and increasing pollutant flux in both varieties of <u>Vicia</u>. This resulted in greater SO₂ uptake by the plant than was indicated from measures of stomatal resistance (Figures 3.29 and 3.30).

In plants of the variety Dylan stomatal resistance to SO_{22} as calculated from analogy to water vapour transfer (r_5SO_2), showed no significant correlation with ambient SO_2 concentration or measured pollutant flux under optimum environmental conditions. As a result, it was concluded that actual flux was determined predominantly by the residual resistance to pollutant uptake, r_rSO_2 . However, under low light conditions r_3SO_2 was found to decrease with increasing sulphur dioxide concentration (Figure 4.29a). In contrast r_rSO_2 was found to become less negative as SO_2 concentration increased (Figure 4.29b). These results are presented in Table 4.17. It would appear that changes in both stomatal and residual resistances together govern flux to Dylan plants under low light intensities.

This difference in response between high and low light treated plants was not shown when the theoretical measure of stomatal resistance to SO₂ transfer, r_sSO_2 ¹ was plotted as a function of measured flux (Figure 4.30) or ambient SO₂ concentration. The plot obtained was the same as that obtained under high light intensities ie. resistance to SO₂ decreased as flux/SO₂ concentration increased; also, as stated previously, no significant differences were observed between high and low light treated plants for measured pollutant flux at any SO₂ concentration supplied. This is of importance because $r_sSO_2^1$ is the sum of r_sSO_2 and r_rSO_2 . If $r_sSO_2^1$ alone was considered no significant differences in resistances governing SO₂ entry between low light-stressed and non-stressed plants would be detected. However it was shown above that both stomatal and residual resistances to SO₂ transfer were altered by the imposition of low light stress.

From the results presented here it may be concluded that although actual flux values were not altered in response to low light stress in Dylan plants, resistance factors governing the entry of SO_2 into the

TABLE 4.17

Summary of Residual (r_rSO_2) and Stomatal Resistances $(r_sSO_2, from analogy to water vapour transfer and <math>r_sSO_2$ ¹ from mass balance calculations) to SO_2 Transfer in <u>Vicia faba</u> CV. Dylan Under Low Light Intensities. Measured pollutant flux (from mass balance calculations) and Calculated flux (from analogy to water vapour transfer) are also shown.

[S02] (ppb)	Prmeas <µg m ⁼²	P _{FCALC} s ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r _s SO ₂ ¹ (s cm ⁻¹)	$r_{r}SO_{2}$ (s cm ⁻¹)
180	4 • 14	1.01	3.05	0-79	-3.84
200	1.59	0•85	4 · 6 5	1.54	-3.11
255	4·16	1 • 13	4 • 39	-0-29	-4.68
390	1-86	1 • 47	5.52	3.95	-1-56
400	4 • 42	1.38	6•19	0.55	-5.64
600	2.57	6.32	0.67	4.63	3 • 96
610	5.27	5.03	1 • 42	1.27	-0 • 15

TABLE 4.18

Summary of Residual (r_rSO_2) and Stomatal Resistances $(r_sSO_2, from analogy to water vapour transfer and <math>r_sSO_2^i$ from mass balance calculations) to SO_2 Transfer in <u>Vicia faba</u> CV. Aquadulce Claudia Under Low Light Intensities. Measured pollutant flux (from mass balance calculations) and Calculated flux (from analogy to water vapour transfer) are also shown.

[S02] (ppd)	PFmeas (µg m ⁻²	PFCALC S ⁻¹)	$r_{s}SO_{2}$ (s cm ⁻¹)	r _s SO ₂ 1 (s cm ⁻¹)	r _r SO ₂ (s cm ⁻¹)
220	1.28	0.69	7.21	2.87	-4.33
270	3.61	0.65	10-72	0.57	-10-15
285	2.26	1.23	4 - 55	1-56	-2.98
360	1.90	1.54	5.72	3.38	-2.34
440	2 • 93	2.07	4.04	2.25	-1.78
510	1 • 85	1.91	6.04	6-31	0.27
615	1-98	1•49	9.74	6.84	-2-89

Figure 4.29a

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Stomatal and Residual Resistances to SO_2 transfer in Dylan plants under low light conditions in relation to ambient SO_2 concentrations/measured pollutant flux. 4.29a shows stomatal resistance as derived from data for water vapour transfer. 4.29b shows residual resistance to SO_2 transfer and 4.30 shows theoretical values for stomatal resistance to SO_2 transfer as calculated from mass balance calculations. plant were influenced by the added environmental stress. In short, under high light intensities flux is governed largely by residual resistance to SO_2 transfer but under low light conditions stomatal and residual resistances are both important.

In Aquadulce Claudia plants subject to low light stress there were no significant differences in the influence of residual or stomatal resistances on SO₂ transfer in comparison with data from high light conditions. However stomatal resistances to SO₂ transfer as estimated from resistance to water vapour transfer, under low light conditions appeared to be higher than under high light intensities whilst the residual resistance appeared unchanged (Table 4.18). This resulted in the theoretical stomatal resistance, $r_sSO_2^1$, being raised in response to low light stress and this may account for the lower flux values observed at highest SO₂ concentrations that were presented earlier (S4.8.1).

4.8.5.2 24 h Cold Stress

When plants of the variety Dylan were subjected to cold temperature stress for 24 h prior to SO_2 fumigations both stomatal and residual resistances to SO_2 transfer were found to be very variable (Table 4.19) and a greater range of values was obtained in comparison with resistances measured in non cold-stressed plants. Stomatal resistance (r_3SO_2) ranged from 0.2 to 9.4 s cm⁻¹ after the cold pre-treatment but was 1 to 2.4 s cm⁻¹ under optimum conditions. Similarly, residual resistances to SO_2 transfer ranged from 1.8 to -4.6 s cm⁻¹ after the 24 h pre-treatment and from 1.3 to -2.9 in the absence of cold stress.

No significant relationships were found between either of the two resistances and increasing SO₂ concentration or pollutant flux following the 24 h cold pre-treatments, the data are presented in Table 4.19. However, when both resistances are summed ie. to give the theoretical values for $r_sSO_2^1$, and plotted against SO₂ or flux (Fig. 4.31) then the relationship between flux and resistance was unchanged. $r_sSO_2^1$ declined with increasing SO₂ concentration in the same manner as described for non cold-stressed plants (Fig. 3.29b) although values were higher in the cold treated plants (Fig. 4.31).

In plants of Aquadulce Claudia subject to 24 h cold stress prior to SO_2 fumigation differences in both stomatal and residual resistances to SO_2 transfer (Table 4.20) due to cold stress were similar to those described

TABLE 4.19

Summary of Residual (r_rSO_2) and Stomatal Resistances $(r_sSO_2, from analogy to water vapour transfer and <math>r_sSO_2^1$ from mass balance calculations) to SO_2 Transfer in <u>Vicia</u> <u>faba</u> CV. Dylan Following Cold Temperature Pre-treatments for 24 h. Measured pollutant flux (from mass balance calculations) and Calculated flux (from analogy to water vapour transfer) are also shown.

[SO ₂] (ppb)	Prmeas (µg m ⁻²	PFCALC S ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r _s SO ₂ 1 (s cm ⁻¹)	r _r SO _z (s cm ⁻¹)
101	0.77	1.34	1 • 21	2.81	1.60
108	0.46	0•43	6 • 22	5.77	-0.45
160	0.79	0.44	9•36	4 • 78	-4.58
285	3.60	1.59	4 • 18	1•32	-2.85
340	3.02	1.88	4 • 22	2+28	-1.94
395	3.42	7•45	0.57	2.36	1.78
420	4 • 11	10.05	0 • 25	1•98	1.73
465	3.69	1•93	5•96	2.66	-3.29
480	5-63	1.92	6.04	1.45	-4.59

TABLE 4.20

Summary of Residual and Stomatal Resistances to SO_2 Transfer in <u>Vicia faba</u> CV. Aquadulce Claudia Following a Cold Temperature Pre-treatment for 24h.

[S02] (ppb)	P _{Fmeas} (µg m ⁻²	Pfcalc s ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r _s SO ₂ 1 (s cm ⁻¹)	r _r SO ₂ (s cm ⁻¹)
101	0•42	1.63	0+83	5.93	5.10
110	0•39	0•46	5-86	7·12	1.26
200	1-58	1-85	2 • 16	2.68	0.52
210	1.01	1 • 27	3.80	5-01	1.21
255	1.08	0•46	14.46	5.80	-8.65
270	3•43	2.75	2 • 45	1.31	-1.14
278	3.15	0•95	7.38	1.58	-5-80
285	1.92	0 • 93	7•84	3-29	-4.55
398	1-67	7•89	0.50	5•87	5.37
560	5-65	1 • 55	9•36	1•89	-7.65



Figures 4.31 to 4.34.

Stomatal resistance to SO_2 transfer (as derived from mass balance calculations) in relation to measured pollutant flux for two varieties of <u>Vicia faba</u> CV. Dylan & Aquadulce Claudia subject to cold temperature stress for 24 h or 72 h prior to SO_2 fumigation.

above for the variety Dylan. Stomatal resistance, r_3SO_2 was found to be very variable and larger values were obtained in comparison with non coldstressed plants. Similarly, a much larger range of values for r_rSO_2 was found (5.3 to -8.6 s cm⁻¹) after the 24 h cold treatment, resistance values ranging from 2.2 to -5.8 s cm⁻¹ in the absence of cold stress. Values of $r_sSO_2^1$ (equal to the sum of the stomatal and residual resistance to SO_2 transfer) when plotted against measured flux (Figure 4.32) followed the same trend observed in non cold-stressed plants ie. resistance decreased with increasing flux/SO₂ concentration. However, values were higher than those observed under optimum environmental conditions.

Measured pollutant flux to plants of both varieties following 24 h cold temperature pre-treatments has been shown not to be significantly different from that to non cold-stressed plants (S4.8.1), however, from the results presented above it can be seen that both stomatal and residual resistances to SO_2 transfer were altered as a result of the cold temperature stress.

4.8.5.3 72 h Cold Stress

Following pre-treatments of 72 h at 10°C resistances to pollutant flux in plants of the variety Dylan were again found to be influenced by the imposition of cold stress (Table 4.21). Stomatal resistances to SO_2 transfer (r_2SO_2) were very variable but were mostly much greater than resistances in unstressed plants. Residual resistances to SO_2 transfer were also variable but were largely negative with increasing SO_2 concentration and flux, values again being much greater than those for unstressed plants. However, although changes in both resistances occurred in response to cold stress, values of r_1SO_2 ¹ were not significantly different from those obtained for Dylan under optimum environmental conditions (Figure 4.33). These data concur with results presented in §4.8.1 which showed pollutant fluxes to Dylan plants to be unaltered in response to 72 h cold temperature pre-treatments.

In the variety Aquadulce Claudia cold periods of 72 h prior to SO_2 fumigation also influenced both stomatal and residual resistances to SO_2 uptake. Both resistances showed no correlation with increasing SO_2 concentration or pollutant flux (Table 4.22) and when ambient SO_2 concentration was 101 ppb both resistances were extremely large. However, these variations in resistance were negated when the sum of the two

TABLE 4.21

Summary of Residual (r_rSO_2) and Stomatal Resistances $(r_sSO_2, from analogy to water vapour transfer and <math>r_sSO_2^+$ from mass balance calculations) to SO_2 Transfer in <u>Vicia</u> faba CV. Dylan Following Cold Temperature Pre-treatments for 72 h. Measured pollutant flux (from mass balance calculations) and Calculated flux (from analogy to water vapour transfer) are also shown.

[S02] (ppb)	P _{Fmeas} (µg n	Prcalc a ⁻² s ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r _s SO ₂ 1 (s cm ⁻¹)	r _r SO ₂ (s cm ⁻¹)
97	0.63	0.94	2.00	3•46	1-46
119	0.69	0.26	12.39	3 99	-8-39
230	2.54	1.26	4 • 25	1.64	-2.61
310	2.27	3 • 47	1.61	2+96	1•36
385	5-67	0.73	14•12	0 • 99	-13.12
460	2.85	2.82	3.72	3.67	-0.04
469	4.20	2.60	4 • 22	2 • 25	-1.96
520	7.62	7 • 18	1 - 13	1.01	-0.12

TABLE 4.22

As for Table 4.21. Summary of Residual and Stomatal Resistances to SO_z Transfer in <u>Vicia faba</u> CV. Aquadulce Claudia Following a Cold Temperature Pre-treatment for 72 h.

[SO ₂] (ppb)	Prmeas (µg m ⁻²	P _{fcalc} s ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r _s SO ₂ 1 (s cm ⁻¹)	$r_{r}SO_{2}$ (s cm ⁻¹)
101	0.68	0.06	46.93	3.31	-43.62
198	2 • 13	1-41	3.08	1.72	-1.36
272	1.69	1.67	3.69	3.66	-0.04
285	2.61	4•35	0.94	2 • 18	1.25
372	1.59	3.04	2.55	5.72	3 · 17
455	3.81	1 · 15	10.31	2.47	-7.84
505	2.02	10•39	0•48	6 • 22	5.77

resistances was plotted (as $r_sSO_2^1$) against measured flux. Resistance to SO_2 uptake, as under optimum conditions, decreased with increasing flux (Figure 4.34) and values were significantly higher than those obtained in the absence of added environmental stress. This correlated well with the reductions in SO_2 flux in Aquadulce after 72h at 10°C described earlier in this section.

4.8.5.4 <u>1 Week Cold</u>

A 1 week period of cold temperature prior to SO_2 fumigations was seen to have marked effects on measured plant responses. Resistance data for SO_2 uptake in plants of the variety Dylan are shown in Table 4.23. It can be seen that stomatal resistances to SO_2 transfer were increased in response to the cold pre-treatment particularly at the lower range of SO_2 concentrations used. Residual resistances to SO_2 transfer were also altered in response to cold stress and were largely negative at low SO_2 concentrations (Figure 4.35). These changes in both resistances resulted in changes in the theoretical values of $r_sSO_2^1$ and the relationship between $r_sSO_2^1$ and measured flux (Figure 4.36). Unlike the trends observed in all other environmental treatments, this resistance did not decrease with increasing SO_2 concentration or flux and was very variable. Values of r_sSO_2 plus r_rSO_2 ie. $r_sSO_2^1$ were more than double those observed in non coldstressed plants and were correlated with the reductions in measured flux to Dylan described earlier in section \$4.8.1 (Figure 4.27a).

Stomatal and residual resistances to SO_2 uptake in Aquadulee plants were not as severely affected by the 1 week cold pre-treatments as were those of Dylan plants. Stomatal resistances to SO_2 uptake as derived from resistance to water vapour transfer, were increased in comparison with those for unstressed plants and appeared to increase with increasing SO_2 flux (Figure 4.37). The data for residual resistance to SO_2 transfer in Aquadulce plants following 1 week at 10°C were variable in relation to SO_2 concentration or flux but values were larger than those for unstressed plants (Table 4.24). The net results of these changes in resistance, were changes in the relationship between $r_5SO_2^1$ and measured flux. In all other environmental treatments $r_3SO_2^1$ was seen to decrease in response to increasing flux or SO_2 concentration but after 1 week at 10°C prior to pollutant fumigation this resistance appeared to increase with increasing SO_2 flux (Figure 4.38) and values were much higher than those for

TABLE 4.23

Summary of Residual (r_rSO_2) and Stomatal Resistances $(r_3SO_2, from analogy to water vapour transfer and <math>r_3SO_2$ [†] from mass balance calculations) to SO_2 Transfer in <u>Vicia faba</u> CV. Dylan Following Cold Temperature Pre-treatments for 1 Week. Measured pollutant flux (from mass balance calculations) and Calculated flux (from analogy to water vapour transfer) are also shown.

[S02] (ppb)	P _{Fmeas} (µg m ⁻²	Preale s ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r _s SO ₂ ¹ (s cm ⁻¹)	rrSO ₂ (s cm ⁻¹
90	0•80	0.54	3 • 85	2 • 28	-1.57
105	0.51	0.59	4 • 10	4-97	0-86
190	1.01	0•46	10.94	4•42	-6.55
195	1.09	0.23	22.83	5.09	-17.7
288	0•95	0.59	12.87	7.70	-5-16
368	1•72	3.54	2.03	5.18	3 · 15
470	3 • 15	2.52	4•45	3.33	-1.12
520	1.95	4.54	2•33	6•69	4.36
			•	· · · ·	

TABLE 4.24

As for Table 4.23. Summary of Residual and Stomatal Resistances to SO_2 Transfer in <u>Vicia</u> <u>faba</u> CV. Aquadulce Claudia Following a Cold Temperature Pre-treatment for 1 Week.

[SO ₂] (ppb)	Prmeas (µg m ⁻²	PFCALC S ⁻¹)	r _s SO ₂ (s cm ⁻¹)	r ₃ SO ₂ 1 (s cm ⁻¹)	r _r SO ₂ (s cm ⁻¹)
99	1-08	0 • 29	8.63	1.68	-6-95
205	1-99	0-53	10•19	2.01	-8.18
208	1.08	2.57	1.37	4.58	3.21
282	2 • 27	1 • 82	3 • 49	2.61	-0-88
380	1.66	8 • 46	0.34	5.60	5.26
465	1.86	1-39	8-79	6 • 21	-2.35
490	3-81	1-01	13-86	2 • 73	-11-12
492	1-68	1-51	8.35	7 • 41	-0.93

Results





Residual and Stomatal Resistances (from mass balance calculations) to SO_2 transfer in <u>Vicia faba</u> CV. Dylan subject to 1 week of cold temperature stress prior to SO_2 fumigation.



Figures 4.37 and 4.38

Stomatal Resistances (from analogy to water vapour transfer [4.37] and from mass balance calculations [4.38]) to SO_2 transfer in <u>Vicia</u> <u>faba</u> CV. Aquadulce Claudia subject to 1 week of cold temperature stress prior to SO_2 fumigation.

unstressed plants. This correlated well with the data presented earlier showing both measured and calculated flux to be reduced in Aquadulce plants following 1 week at 10°C (Figure 4.28).

In conclusion it would appear that although environmental stresses such as low light intensities and periods of 24 h or 72 h at 10°C prior to SO₂ fumigations did not influence sulphur dioxide fluxes to <u>Vicia</u> faba directly, the individual components of the resistance pathway governing pollutant entry into the plant were substantially affected by added environmental stress. Both stomatal and residual resistances to SO₂ transfer were increased in magnitude by the imposition of added environmental stress. However, since the magnitude of these changes was virtually equal but opposite in direction, total leaf resistance to SO₂ remained unchanged.

In contrast, periods of 1 week at cold temperatures prior to SO_2 fumigations induced changes in both stomatal and residual resistances to SO_2 transfer in both varieties of <u>Vicia</u> which resulted in increased total leaf resistance to SO_2 transfer and reduced SO_2 flux.

4.9 GAS EXCHANGE MECHANISMS IN RELATION TO POLLUTANT FLUX

4.9.1 Net Photosynthesis

In \$3.8 the importance of relating pollutant responses to actual flux rather than ambient SO_2 concentrations was shown. Both measured and calculated pollutant fluxes were shown to be proportional to ambient sulphur dioxide concentrations for both varieties of <u>Vicia</u> under optimum environmental conditions (\$3.9); however, this did not invariably occur following periods of added environmental stress (\$4.8).

Figures 4.39 to 4.48 show % changes in net photosynthesis as related to sulphur dioxide fluxes to both varieties of <u>Vicia faba</u> subjected to added environmental stress. Only the data which were significantly different to the responses measured under optimum environmental conditions are shown.

4.9.1.1 Dylan Plants

Under optimum environmental conditions inhibition of net photosynthesis in Dylan plants was not significantly correlated with measured pollutant flux although there was a good curvilinear relationship for SO₂ fluxes up to 3 μ g m⁻² s⁻¹ (Figure 3.15). However, photosynthetic responses were found to be significantly correlated with calculated pollutant flux to Dylan plants (Figure 3.16).

With the addition of low light stress, the relationship between net photosynthetic inhibition and measured SO_2 flux was altered in Dylan plants such that less photosynthetic inhibition was seen for the same range of flux measurements (Figure 4.39). The regression line obtained from statistical analysis of the data (r = 0.697, p < 0.05) is also shown in the figure together with the relationship between flux and photosynthetic inhibition for Dylan plants under high light intensities for measured flux values up to 3 µg m⁻² s⁻¹. The data presented in Figure 4.21 showed there to be no significant difference in flux to Dylan plants under high or low light intensities. Therefore the reduced inhibition in net photosynthesis in response to SO_2 under low light intensities did not arise from reductions in flux and may be assumed to be the result of a reduction in sensitivity to the pollutant.

Following cold stress pre-treatments for 24 h and 72 h significant linear correlations were found in Dylan plants between measured pollutant fluxes and the degree of photosynthetic inhibition, the correlation coefficient, r, was 0.870 (p < 0.001) after 24 h (Figure 4.40) and was 0.601 (p < 0.01) after 72 h (Figure 4.41). As for low light stress, the reductions in photosynthetic inhibition in Dylan plants due to SO₂ after cold temperature pre-treatments of 24 h or 72 h were not a result of reduced fluxes to the plant as no significant differences in SO₂ flux to cold-stressed and non cold-stressed plants were found (\$4.8). Lessening of net photosynthetic inhibition in response to SO_2 appeared to be due to reduced sensitivity to the amount of pollutant entering the plant ie. plants appeared to be more tolerant of SO₂ after the cold temperature pretreatments. After 24 h at 10°C prior to SO₂ fumigation photosynthetic responses to SO_2 in Dylan plants were shown to be reduced at all SO_2 concentrations (Figure 4.3) and this was also shown when photosynthetic responses were plotted against measured flux (Figure 4.40).

After a cold treatment of 72 h photosynthetic responses were shown to be reduced when ambient SO_2 concentrations exceeded 200 ppb (Figure 4.5) and again this was reflected in the flux data as differences in the degree of photosynthetic response were only significant when







Sulphur dioxide-induced changes in net photosynthetic rates in relation to measured pollutant flux ($\mu g \ m^{-2} \ s^{-1}$) in <u>Vicia</u> faba CV. Dylan. Figure 4.39 shows the responses under low light intensities and Figure 4.40 gives the responses following cold temperature stress for a period of 24 h prior to pollutant fumigation. [The relationship between measured flux up to 3 $\mu g \ m^{-2} \ s^{-1}$ and % changes in Pnet in non cold-stressed Dylan plants is also shown (-----)].

measured flux exceeded 1.5 µg m⁻² s⁻¹ (Figure 4.41)

Examination of the data for % net photosynthetic inhibition as related to measured pollutant flux to Dylan plants following a 1 week cold pre-treatment showed there to be significant differences in comparison with the previous cold treatments. After 1 week at 10°C prior to SO_2 fumigations, photosynthetic inhibition in Dylan plants was shown to be significantly reduced only when SO_2 concentrations exceeded 300 ppb (Figure 4.7). However, when responses were plotted against measured pollutant flux (Figure 4.42) the same relationship as that for plants under optimum environmental conditions (Fig. 3.15) was seen although maximum values for measured SO_2 flux were much reduced to $3\cdot 2 \ \mu g \ m^{-2} \ s^{-1}$ in cold-stressed plants when the highest value for measured flux under optimum conditions was $8\cdot 1 \ \mu g \ m^{-2} \ s^{-1}$. It would appear that the reductions in photosynthetic inhibition in Dylan plants in response to SO_2 following a 1 week cold pretreatment were not due to reduced pollutant sensitivity but arose from reductions in pollutant flux to the plant.

When the net photosynthetic responses of Dylan plants were considered in relation to calculated pollutant flux for low light stress and for 24 h or 72 h cold pre-treatments the same relationships as were observed for measured pollutant flux as described above, occurred ie. the range of flux values were unchanged indicating that the observed reductions in net photosynthetic inhibition arose from reduced pollutant sensitivity.

However, when data for Dylan plants following 1 week cold temperature pre-treatments were examined it was found that as for measured flux, the range of calculated values flux was reduced although ambient SO_2 concentrations were as for optimum environmental conditions. The maximum flux under optimum conditions was 7.7 µg m⁻² s⁻¹ and following cold stress was 4.6 µg m⁻² s⁻¹. However, a greater photosynthetic response was observed in comparison with unstressed plants for all measures of flux. These data are shown in Figure 4.43, regression analysis of the data for cold-stressed plants produced a correlation coefficient of 0.878 (p < 0.001) and the regression line obtained for plants in the absence of added cold temperature stress are also shown.

It can be seen that, particularly at lower fluxes, Dylan plants were far more sensitive to SO_2 flux following 1 week periods of low temperature stress than under optimum temperature conditions. Reduced





 SO_2 -induced inhibition in net photosynthetic rates of Dylan plants as related to pollutant flux. Fig. 4.41 shows data in relation to measured pollutant flux for plants subjected to 72 h of cold temperature stress prior to SO_2 fumigation. Figs. 4.42 & 4.43 show data for plants subject to 1 week cold stress in relation to measured and calculated pollutant flux respectively. I(-----) relationship for non cold-stressed plants]. Results

inhibition in Pnet following 1 week at 10°C in plants of the variety Dylan appeared to result from much reduced fluxes to the plant, and plants being far more sensitive to SO_2 than was observed in the absence of environmental stress.

4.9.1.2 Aquadulce Claudia Plants

When the degree of net photosynthetic inhibition in Aquadulce Claudia plants was plotted against pollutant fluxes for each of the environmental pre-treatments studied, significant differences in response in comparison to unstressed plants were observed. The data for % inhibition in Pnet in Aquadulce plants in relation to measured pollutant flux under low light conditions are shown in Figure 4.44. The regression lines obtained for data under optimum environmental conditions are shown in all the figures presented. There was no significant linear correlation obtained between photosynthetic inhibition and measured flux in Aquadulce under low light conditions but it can be seen in the figure that the majority of data obtained lie to the left of the regression line for unstressed plants indicating greater photosynthetic inhibition for each flux measurement. This was also seen to occur when responses were plotted against calculated pollutant flux (Figure 4.45). All the data and the regression line obtained (r = 0.830, p < 0.01) again lie to the left of the regression line for plants under high light intensities showing there to be greater photosynthetic inhibition for each flux value. This is very important because as described earlier (54.4), SO_2 -induced net photosynthetic inhibition of Aquadulce plants was not found to be influenced by low light stress when data were expressed in relation to ambient SO_2 concentrations. However, calculated flux as related to ambient SO₂ concentrations were significantly reduced under low light intensities. Thus the data for both measures of flux (Figs. 4.44 & 4.45) suggested that following the imposition of low light stress, the photosynthetic mechanism of Aquadulce plants was far more sensitive to actual SO₂ entering the plant. However, this enhanced sensitivity was masked when results were expressed in relation to ambient SO_2 concentrations (\$4.4) because for any given ambient SO₂ concentration pollutant flux to plants grown under low light intensities was reduced.

Following cold pre-treatments of 24 h there were no significant differences in photosynthetic responses of Aquadulce plants to ambient SO_2 concentrations between cold-stressed and non cold-stressed plants (Figure







Sulphur dioxide induced changes in net photosynthetic rates in relation to both measured pollutant flux ($\mu g m^{-2} s^{-1}$) (Fig.4.44) and calculated pollutant flux (Fig.4.45) in <u>Vicia</u> <u>faba</u> plants of the variety Aquadulce Claudia under low light intensities. [The relationship between flux and % changes in Pnet in Aquadulce plants under high light conditions are also shown (-----)]. 4.4). However when responses were plotted against measured pollutant flux (Fig. 4.46) it can be seen that for flux values between 0.2 and 3 μ g m⁻² s⁻¹ photosynthetic responses were greater than those observed in the absence of environmental stress. These data again suggest that the imposition of added environmental stress enhanced the sensitivity of the photosynthetic mechanism in Aquadulce plants to SO₂ entering the leaf. However, unlike the data obtained under low light intensities, the results presented in \$4.8.1 did not show that pollutant flux in relation to ambient SO₂ concentration was reduced in response to a 24 h cold temperature period. Thus the fact that no significant differences were observed between non cold-stressed and 24 h cold-stressed plants in \$4.4 cannot be explained by the fact that this enhanced pollutant sensitivity was masked when results were expressed in relation to ambient SO_2 concentration because of reduced pollutant flux. It may be that the increased variability in data following cold temperature treatments did not allow reductions in flux to be detected, this being supported by the fact that stomatal resistance to SO_2 uptake in Aquadulce plants was shown to be increased following the 24 h cold treatment (§4.8.4).

In direct contrast to the above, when SO_2 fluxes exceeded 3 µg $m^{-2} s^{-1}$ photosynthetic responses appeared to be much less than those observed in non cold-stressed plants suggesting reduced pollutant sensitivity to higher flux levels.

After 72 h cold treatment prior to SO₂ fumigations, photosynthetic inhibition of Aquadulce plants in response to SO₂ was seen to be much reduced when ambient SO₂ concentrations exceeded 300 ppb (Figure 4.6). When responses were plotted as a function of measured pollutant flux (Figure 4.47), as observed in the 24 h cold treatments, photosynthetic responses were much lower than those for unstressed plants when flux exceeded 2 μ g m⁻² s⁻¹. However, when measured flux was below 2 μ g m⁻² s⁻¹ photosynthetic responses were seen to be greater than those of unstressed plants. It would appear that in Aquadulce Claudia plants, at SO₂ concentrations up to 300 ppb, photosynthetic responses were similar in both reduced pollutant flux to the plant and increased pollutant sensitivity. The reductions in photosynthetic inhibition at SO₂ concentrations above 300 ppb after the imposition of cold stress appeared to arise from both reductions in actual flux and reduced plant sensitivity to SO₂.



Figures 4.46 to 4.48.

Sulphur dioxide induced changes in net photosynthetic rates in relation to measured pollutant flux ($\mu g m^{-2} s^{-1}$) in <u>Vicia faba</u> plants of the variety Aquadulce Claudia subject to cold temperature stress for periods of either 24 h (Fig. 4.46), 72 h (Fig. 4.47) or 1 week (Fig. 4.48) prior to SO₂ fumigation. [The relationship between flux and % changes in Pnet in non cold-stressed Aquadulce plants are also shown (-----)].

In Aquadulce Claudia plants subjected to 1 week at 10°C prior to SO₂ fumigations, photosynthetic inhibition in response to SO₂ was seen to be reduced when ambient SO_2 concentrations exceeded 250 ppb (Figure 4.8). When photosynthetic responses were plotted as a function of measured pollutant flux there was no significant linear correlation obtained (Figure 4.48) but the data for pollutant flux below 2 μ g m⁻² s⁻¹ showed a much greater photosynthetic response than was observed in the absence of added environmental stress indicating plant pollutant sensitivity to be increased. In contrast, as observed in the previous cold treatments, when measured flux values exceeded 2 μ g m⁻² s⁻¹ photosynthetic responses were less than those observed under optimum environmental conditions. When ambient SO_2 concentrations exceeded 250 ppb measured pollutant flux to Aquadulce plants was shown to be significantly reduced (Fig. 4.28a). Therefore the reductions in photosynthetic inhibition observed at higher pollutant concentrations, following 1 week periods of cold temperature stress may be assumed to result from both reduced pollutant flux and reduced plant sensitivity.

In §3.8 the idea of threshold SO_2 flux values was introduced, this being the level of pollutant flux at which photosynthetic inhibition was first seen to occur. Aquadulce plants were seen to have a higher threshold flux value for both measured and calculated pollutant fluxes and when measured flux values were considered, Dylan plants were found to be more sensitive to increasing SO_2 flux below 2 µg m⁻² s⁻¹ ie. substantially more photosynthetic inhibition was observed in Dylan plants than Aquadulce plants for measured fluxes between 0.3 and 2 µg m⁻² s⁻¹. However, when calculated pollutant fluxes were considered Aquadulce plants were found to be more sensitive to SO_2 when the threshold flux had been exceeded although the threshold flux was greater for Aquadulce plants.

In Dylan plants, following periods of added environmental stress, the threshold measured SO₂ flux was unchanged but this value was only 0.3 μ g m⁻² s⁻¹ in the absence of added environmental stress. However, for all environmental pre-treatments the threshold value for calculated pollutant flux is decreased from 0.8 μ g m⁻² s⁻¹ to 0.2 μ g m⁻² s⁻¹

In Aquadulce Claudia plants, the imposition of environmental stress resulted in substantial reductions in the threshold values for both measures of flux. Under optimum conditions the threshold measured flux, above which photosynthetic inhibition was seen to occur in Aquadulce plants was $1.8 \ \mu g \ m^{-2} \ s^{-1}$; enhancement in Pnet was seen to occur at flux Results

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values below this. Under low light conditions the threshold flux was reduced to $1.2 \ \mu g \ m^{-2} \ s^{-1}$ and no enhancement in photosynthetic rate was observed at lower flux measurements. For all the cold pre-treatments this threshold value was reduced to $0.25 \ \mu g \ m^{-2} \ s^{-1}$. Similarly, the threshold value for calculated pollutant flux was reduced from 1.5 to 0.6 $\ \mu g \ m^{-2} \ s^{-1}$ by the imposition of low light stress and was 0.15 $\ \mu g \ m^{-2} \ s^{-1}$ after cold pre-treatments.

It would appear from the data presented in this section, that periods of environmental stress prior to SO_2 fumigations resulted in increased plant pollutant sensitivity to low sulphur dioxide fluxes, particularly that of Aquadulce Claudia plants, However, reductions in pollutant flux to the plants in relation to ambient SO_2 concentrations and reduced plant sensitivity to higher flux values combined, resulted in decreased photosynthetic responses to sulphur dioxide treatments after periods of added environmental stress.

4.9.2 Stomatal Resistance (r_s)

The relationships between changes in r_s in response to SO₂ and both measured and calculated pollutant flux were described in §3.8.2 for both varieties of <u>Vicia</u>. Significant correlations were found for both varieties for measured flux and for calculated flux in Dylan plants. In both varieties there was found to be a critical flux value beyond which stomatal closure occurred, this threshold flux was the point at which stomatal responses to SO₂ switched from enhanced opening to enhanced stomatal closure. For Dylan plants the threshold value for measured flux was 4 µg m⁻² s⁻¹ and for calculated pollutant flux the threshold value was 3 µg m⁻² s⁻¹. For Aquadulce Claudia plants measured flux threshold was 1.8 µg m⁻² s⁻¹, however, there was no significant correlation between calculated pollutant flux and changes in stomatal resistance in Aquadulce plants in response to SO₂. When calculated SO₂ fluxes were between 1 and 3.8 µg m⁻² s⁻¹ 70% of plants exhibited stomatal closure.

Following the imposition of added environmental stress the relationships between changes in r_s and pollutant flux were substantially altered. There were no correlations between changes in r_s and either measure of flux in both Aquadulce Claudia and Dylan plants subjected to low light stress or 24 h or 72 h of cold temperature stress prior to pollutant funigation. Similarly, there were no significant relationships between calculated or measured pollutant flux and observed stomatal responses in Dylan plants following a period of 1 week at 10°C prior to SO_2 fumigations. For all the data obtained the one significant relationship between changes in r_s and pollutant flux was that to Aquadulce Claudia plants following 1 week of cold temperature stress. These data are shown in Figures 4.49 and 4.50. It can be seen that stomatal closure resulted from all SO_2 treatments. For measured flux (Fig. 4.49) stomatal closure was seen to increase with increasing pollutant flux. If the data are compared with that obtained in the absence of added environmental stress (Figures 3.21 & 3.22) it can be seen that the relationships between stomatal response and both measures of flux are similar but threshold values have been reduced and the magnitude of stomatal responses are much greater in the plants subject to cold temperature stress.

It would appear that the 'feed forward/feed back' system that operates in the absence of environmental stress, whereby r_s governs the entry of SO₂ into the plant but is then altered by the presence of SO₂ inside the plant, did not occur following environmental stress. In Dylan plants changes in r_s seemingly became independent of pollutant flux following the imposition of added environmental stress. There was no evidence in any of the environmental treatments to suggest a threshold flux above which enhanced stomatal closure occurs. In Aquadulce Claudia plants subject to low light stress or 24 h or 72 h of cold temperature stress prior to pollutant fumigation there was also no evidence of the threshold pollutant fluxes, above which enhanced stomatal closure occurred, which were observed under optimum environmental conditions. However, following a 1 week cold pre-treatment, these threshold pollutant flux values did occur but were much reduced so that any flux value above 0.1 µg m⁻² s⁻¹ resulted in stomatal closure (Figs. 4.49 & 4.50).

The implications of these data for changes in r, suggested that the imposition of environmental stress removed a degree of stomatal control over pollutant entry into the plant. Changes in stomatal resistance were seen to occur independently of pollutant concentration and it may be concluded that the added environmental stress prior to pollutant fumigation had profound effects on that part of the stomatal control mechanism that is sensitive to sulphur dioxide pollution.





Figures 4.49 and 4.50.

Sulphur dioxide induced changes in stomatal resistance of <u>Vicia</u> faba CV. Aquadulce plants, subjected to 1 week of cold temperature stress prior to pollutant fumigation, in relation to both measured (4.49) and calculated (4.50) pollutant flux. [(----)] regression line obtained for non cold-stressed plants].

4.10 CONCLUSIONS & DISCUSSION

4.10.1 Environmental Factors Influencing Net Photosynthetic Rates.

4.10.1.1 Low Light Stress

When plants of both varieties of <u>Vicia faba</u> were grown under low light intensities (60 W m⁻²) net photosynthetic rates were, as expected, found to be much reduced. The mean value for Pnet was 0.9 g CO_2 m⁻² s⁻¹ in comparison to the mean value of 2.0 g CO_2 m⁻² s⁻¹ found in plants grown under high light conditions (150 W m⁻²). These results correlated well with the widely accepted, and much published, view of the influence of light intensity on Pnet as presented in the introduction to this chapter and the generalised plot of the relationship given in figure 4a.

Plants of both varieties also showed visible signs of low light stress, being much smaller and 'weaker' than plants of comparable age grown under higher light intensities. Low light stressed plants were also much more 'pale' in colour, indicating a reduction in total leaf chlorophyll content.

4.10.1.2 Cold Temperature Stress

The duration of the chilling period which is required to injure a plant has been shown to be dependent on both species and temperature (Taylor & Rowley, 1971; Graham & Patterson, 1982). Similarly, the extent of injury increases with the degree of chilling eg. Minchin & Simon (1973) found cucumber leaves survived 1 week at 10°C but showed injury after 3 d at 8°C and at 5°C within a few hours. Given this variability in plant responses to low temperatures it was not surprising to find that the imposition of low temperature stress (ie. 10°C) for periods of 24 h prior to S0₂ fumigation did not appear influence net photosynthetic rates in either Dylan or Aquadulce Claudia plants. However, periods of 72 h or 1 week at 10°C were shown to inhibit net photosynthetic rates in plants of both varieties by up to 20%; but photosynthetic rates in plants subjected to 72 h cold treatments were found to recover on return to optimum temperatures during the 24 h period prior to SO_2 fumigation. The mean value for Pnet for plants subjected to 1 week at 10°C was found to be 1.6 \pm 0.2 g CO₂ m⁻² h⁻¹. However, rates were found to increase during the 3 d experimental period

when plants were returned to optimum temperatures although recovery to rates comparable to non cold-stressed plants was not seen to occur in the majority of plants. Similarly, Taylor & Rowley (1971) monitored photosynthetic rates of three plant species at 25°C following various periods at 10°C and found recovery to be dependent on species but most plants had not recovered to pre-stress rates up to 3 d after exposure.

4.10.2 Interaction of Environmental and Pollutant Stress on Rates of Net Photosynthesis

The influence of sulphur dioxide on net photosynthetic rates of both varieties of <u>V.faba</u> was discussed in chapter 3 and it can be seen from the results presented in this chapter (§4.4) that the effects of SO_2 were moderated by added environmental stress.

4.10.2.1 Low Light Intensities

It was shown in §4.4 that the degree of net photosynthetic inhibition (%Pnet) in response to a range of SO₂ concentrations, in Dylan plants was significantly reduced when plants were grown under low light intensities as opposed to high light conditions. Significant varietal differences were also found to occur, as analysis of covariance showed Aquadulce plants to exhibit no significant difference in SO₂ response under high or low light conditions. Similarly, analysis of covariance showed Dylan plants to exhibit significantly less photosynthetic inhibition than Aquadulce plants when exposed to SO₂ under low light conditions. However it was shown that under high light conditions, there was a 'threshold' SO_2 concentration (≅ 400 ppb) in both varieties, above which photosynthetic rates were even more severely limited. When plants of both varieties were exposed to SO_2 under low light conditions this 'threshold' was still apparent for Dylan plants but did not occur in Aquadulce plants where a 'plateau' was reached such that increasing SO_2 concentrations above 400 ppb did not increase the extent of net photosynthetic inhibition.

It is of importance to note that at lower SO_2 concentrations (below 250 ppb) in Dylan plants, although % inhibition of Pnet is the same in both low light and high light treated plants, in absolute terms net photosynthetic depression is much less. This is because net photosynthetic rates are much reduced in response to the imposition of low light stress and results for SO_2 -induced inhibition are expressed as per cent change in comparison to control plants. Therefore, a 10% change in Pnet under high light intensities would be equal to a reduction in Pnet of $\cong 0.2$ g CO_2 m⁻² h⁻¹, whereas a 10% change in Pnet under low light conditions would be equal to a reduction of $\cong 0.1$ g CO_2 m⁻² h⁻¹ in absolute terms. This also held true for Aquadulce plants especially at higher sulphur dioxide concentrations (>400 ppb) where in absolute terms, there was much less depression of net photosynthetic rates when plants are exposed to SO_2 under low light intensities.

These results add to the contention amongst current published results which were outlined in the introduction to this chapter (\$4.1.5.1) where light intensity has been shown to both decrease and increase plant sensitivity to pollutants, although relatively few workers have examined the effects of pollutant/environmental stress interactions on 'invisible injury' symptoms such as Pnet depression. However, Kropff (1987) examined the effects of short fumigations (2 h) of SO₂ (400 μ g m⁻³) on the photosynthetic light response curves of individual leaves of Vicia faba CV. Minica and found the CO_2 -assimilation light-response curve to be significantly affected by SO_2 fumigations. Examination of the data presented in Kropff's paper showed much less Pnet inhibition due to SO₂ at lower light intensities. The effects of SO₂ on the photosynthetic lightresponse curve of individual leaves was very similar to that found for whole plants of Vicia faba CV. Dylan by Black & Unsworth (1979b). These authors found that at low light intensities (60 W m⁻²) there was significantly less SO_2 -induced Pnet inhibition than was observed at 150 W m⁻² but at small irradiances Black & Unsworth found inhibition of Pnet to be independent of SO₂ concentration. Both Kropff and Black & Unsworth examined the light response curves of plants grown under high light intensities. However, Mansfield & Jones (1985) compared the light response curves of Phleum pratense grown under low or high light intensities. In contrast to both Black & Unsworth and Kropff, these authors found no significant SO₂ response above the light compensation point in plants grown under high light intensities but in plants grown under low light intensities SO₂induced net photosynthetic inhibition was seen to increase with increasing light intensity. This enhanced sensitivity could not be attributed to increased pollutant flux and Mansfield & Jones proposed that enhanced sensitivity to SO₂ under low light intensities may result from low

photosynthetic rates, a reduced store of carbohydrate and thus a reduction in repair capacity. SO_2 -induced respiratory stimulation was observed in plants under high light conditions but not under low light conditions thus these authors suggested that higher mitochondrial activity may be necessary for repair or detoxification mechanisms but these can operate only when given sufficient respiratory substrate. However, as is discussed later in this section (S4.10.3), this hypothesis does not serve to explain the observed effects on <u>Vicia faba</u> CV. Dylan and Aquadulce examined in this study.

Another of the main theories concerning the influence of light intensity on plant pollutant susceptibility is that a positive correlation exists between injury and increasing light intensity (Guderian, 1977; Halbwachs, 1984), and that this increase in injury is related to decreasing stomatal resistance with increasing light intensity thus leading to increased pollutant uptake. However, this was not found to explain the changes in net photosynthetic sensitivity to SO_2 under low light conditions in the plants examined in this study, as will be discussed later in this section when changes in stomatal resistance and pollutant flux are considered.

Reinert, Heggestad & Heck (1982) suggested that environmental factors influence the overall sensitivity of plants to air pollutants but generally do not alter the relative sensitivity of cultivars. However, the results for Dylan & Aquadulce plants in this study do not appear to agree with this theory. Under optimum environmental conditions, at highest SO_2 concentrations (>500 ppb), Aquadulce plants were found to exhibit greater photosynthetic sensitivity to SO_2 than Dylan plants; however, under low light intensities the relative sensitivities were reversed. These changes in relative sensitivity are also seen to occur when the results for the imposition of cold temperature stress are examined, as will be discussed later in this chapter.

4.10.2.2 Low Temperature Stress

As net photosynthetic rates were unchanged by the imposition of 24 h of cold stress alone, changes in the degree of SO_2 -induced Pnet inhibition could be directly compared between non cold- and 24 h coldstressed plants. The relatively short cold stress period prior to SO_2 fumigation was seen to alter the photosynthetic responses to SO_2 in plants of the variety Dylan but not in Aquadulce plants (Figs. 4.3 & 4.4). Dylan plants previously subjected to 24 h cold stress showed much less Pnet inhibition at all SO_2 concentrations used, in comparison to non coldstressed plants. Following the 24 h cold temperature period the relationship between % change in Pnet and SO_2 concentration was seen to become linear and not polynomial as was observed under optimum environmental conditions (Fig. 3.4) where highest SO_2 concentrations lead to almost complete Pnet inhibition. In cold treated Dylan plants, there was a progressive increase in the degree of photosynthetic inhibition with increasing SO_2 concentration and enhanced rates of Pnet were observed at low SO_2 concentrations which did not occur in non cold-stressed plants.

Conversely, although 24 h at 10°C did not significantly alter the photosynthetic responses to SO_2 of Aquadulce plants, there were a number of important factors to consider. Following the cold temperature treatment, no enhancement in Pnet was seen at lowest SO_2 concentrations although this had been shown to occur in non cold-stressed plants. At SO₂ concentrations between 100 and 480 ppb, 14 out of 19 plants exhibited reductions in Pnet of below 15% in comparison to control plants and although there was still seen to be a marked increase in Pnet inhibition at SO₂ concentrations above 500 ppb, this was not seen to occur in all plants. It may be that a 'switch' occurs at a certain point because some plants show small inhibitions in Pnet in response to SO_2 and others show a much greater degree of inhibition. This may perhaps best be explained using the analogy, "..the straw that broke the camels back.". The photosynthetic mechanism in Aquadulce plants appears to be finely balanced, there is a broad tolerance band to SO₂ exposure in Aquadulce plants where very little photosynthetic response is observed in response to a range of SO_2 concentrations up to 500 ppb but there is a point where the balance tips and Pnet inhibition is very severe. This was also seen to occur to a lesser degree in non coldstressed plants and it is thought that the addition of cold temperature stress exacerbated the problem. This 'tipping of the balance' may be related to actual pollutant flux and enhanced pollutant sensitivity rather than to ambient SO_2 concentrations and this will be considered later in this section.

Analysis of covariance tests showed there to be no significant varietal differences in photosynthetic responses to SO_2 in plants previously subjected to 24 h at 10°C when results were analysed as a whole but it is
apparent from the results described above that significant differences do occur.

The results presented in figures 4.5 and 4.6 showed that the increasing length of the cold period prior to SO_2 fumigation lead to further changes in net photosynthetic responses to SO_2 for both varieties of <u>Vicia faba</u>. The data became increasingly more variable with increasing length of the cold period.

For Dylan plants exposure to 72 h cold did not alter the degree of photosynthetic response to SO_2 concentrations below 100 ppb. At higher SO_2 levels analysis of covariance showed there to be less photosynthetic inhibition in response to SO_2 in cold-stressed plants but it could be seen from figure 4.5 that, especially at SO_2 levels above 400 ppb, the responses were variable. Following the 72 h cold stress the degree of photosynthetic inhibition ranged from 8 to 35% in response to SO_2 concentrations between 450 and 500 ppb but for the same SO_2 range, inhibition in non coldstressed plants ranged from 30 to 50%. It would appear that the imposition of 72 h of cold temperature stress produced, for the first time, evidence for the fine balance point in photosynthetic response, representing enhanced pollutant sensitivity, in Dylan plants that was described above for Aquadulce plants.

In Aquadulce plants there were no significant differences in net photosynthetic responses to SO_2 concentrations up to 250 ppb between coldstressed and non cold-stressed plants. However, at higher SO_2 concentrations there was significantly less photosynthetic inhibition in cold-stressed plants. For non cold-stressed plants SO_2 treatments between 400 and 500 ppb resulted in changes in net photosynthetic rates of between 8 and 50% in relation to control plants but in plants subject to the 72 h cold treatments values where -5 to 22% over the same range of SO_2 concentrations. It can be seen from figure 4.6 that the variation in response described above for 24 h cold-stressed plants was still seen to occur in plants subject to 72 h cold temperature stress.

The effects of a 1 week cold temperature period prior to SO_2 fumigation also resulted in the modification of net photosynthetic responses to SO_2 and, as could be seen from the data presented in figures 4.7 and 4.8 and Table 4.1; these modifications were very similar to those described for the 72 h cold treatments and were most marked when SO_2 concentrations exceeded 400 ppb.

It is difficult to discuss environmental modification of net photosynthetic responses to SO_2 in relation to other published work because as previously stated, most workers have concentrated on visible injury symptoms and although there are a number of papers concerning cold temperature modification of plant pollutant response, the timing and duration of the cold stress periods invariably differ and there have been few studies made of the effects of cold temperature stress prior to SO_2 exposure. However, as stated earlier there is now a growing body of evidence to suggest that plants are more sensitive to pollutants under winter conditions of low light and low temperature and that this enhanced sensitivity is correlated to slow growth in winter conditions (see TERG, 1988). In contrast, a number of authors have concluded that plants are more resistant to SO₂ at lower temperatures (Menser et al., 1963; Heck & Dunning, 1978; Rist & Davis, 1979; Norby & Kozlowski, 1981b; Shanklin & Kozlowski, 1984 and Taylor, Selvidge & Crumbly, 1985); although Heck et al. (1965) found an inverse relationship between exposure temperature and sensitivity. A popular proposed theory for a reduction in plant sensitivity is that of reduced SO₂ flux following low temperature preconditioning (Shanklin & Kozlowski, 1984). These authors also showed that preconditioning environmental regimes greatly modified plant responses to a given dosage of SO_2 . The mechanism behind these modifications is thought to be induced stomatal closure as a result of low temperatures thus decreasing uptake of the pollutant, this in turn may be related to the temperature characteristics of enzymes involved in stomatal control (Rogers et al., 1979). Rist & Davis (1979) suggested that the influence of temperature on the rate of oxidation of sulphite ions to sulphate within the leaves, together with reduced SO₂ adsorption could explain temperature mediated differences in sensitivity. Taylor <u>et</u> <u>al</u>. (1985) also found increased SO₂ flux at higher exposure temperatures and concluded that the effects of temperature and air pollution stress are specific as the three plant species used in their study responded to added low temperature stress in no comparable manner. However, these authors were not convinced that reduced SO₂ flux was entirely related to changes in stomatal conductance and, again, proposed the presence of the non-stomatal residual factor influencing SO_2 flux which was affected by the imposition of added temperature stress. Taylor et al. also concluded that although plants took up more SO_2 at higher temperatures, the increase did not necessarily result

in greater physiological responses in all species and suggested that plant responses were 'uncoupled' from SO_2 flux, this being related to species specific characteristics of plant temperature tolerance limits.

In this study, SO_2 -induced photosynthetic inhibition was not seen to be enhanced by the imposition of low temperature or low light stress. Environmental modifications in the responses of <u>Vicia faba</u> to SO_2 were seen to be cultivar specific and stomatal and respiratory responses were considered along with SO_2 fluxes, as are discussed below, to try to identify the nature of this environmental modification.

4.10.3 Interaction of Environmental & Pollutant Stress on Rates of Dark Respiration

4.10.3.1 Low Light Stress

The results presented in §4.5. showed dark respiration rates (Rd) in plants of both varieties of <u>Vicia faba</u> to be significantly reduced when plants were grown under low light intensities in comparison to data obtained for plants under high light conditions; this being a typical and much reported plant response (Fitter & Hay, 1981).

Under high light conditions, sulphur dioxide was found to enhance dark respiration rates in plants of the variety Dylan but to have no effect on Rd of Aquadulce plants. However, under low light conditions the influence of SO₂ on Rd of Dylan plants was found to be altered such that exposure to SO₂ resulted in further reductions in dark respiration rates. Varietal differences were again found as SO₂ fumigations under low light intensities did not induce changes in dark respiration rates of Aquadulce plants, and, as for the data obtained under high light conditions Aquadulce plants were still found to have significantly higher Rd values than Dylan plants.

4, 10. 3. 2 Low Temperature Stress

Enhanced rates of dark respiration were observed in Dylan plants in response to all cold pre-treatments ie. 24 h, 72 h and 1 week but this respiratory stimulation was found to decline during the 3 day experimental period when plants were returned to 22°C. However, the rate of decline in *Rd* to pre-stress rates in Dylan plants was found to be dependent on and inversely proportional to the length of the cold pre-treatment. Conversely, Aquadulce plants exhibited enhanced dark respiration rates only in plants subjected to 72 h cold stress periods, no response being observed in plants subject to either 24 h or 1 week at 10°C. This enhancement in *Rd* was found to persist throughout the experimental period.

The effects of SO₂ on Rd were again found to modified by the imposition of environmental stress. No respiratory stimulation was observed in Dylan plants previously subjected to cold temperature stress although the rate of decline in Rd described above was found to be slowed in Dylan plants subjected to 24 h cold stress. However, for the first time, sulphur dioxide fumigations were found to influence rates of Rd in Aquadulce plants. No effects of SO₂ or cold stress were seen in Aquadulce plants subjected to 24 h or 1 week at 10°C prior to SO₂ fumigation but SO₂ exposure was found to decrease the enhanced rates of Rd observed in Aquadulce plants in response to the 72 h cold pre-treatments. These increases in rates of dark respiration in response to chilling stress are again typical of responses reported in current literature (Levitt, 1980). Levitt proposed these increases in Rd to be evidence of the onset of chilling injury in sensitive plants but as these responses have been shown to be reversible in <u>Vicia</u> faba it may be assumed that these plants are relatively chilling resistant, at least for short chilling stress periods.

For Dylan plants, enhanced respiratory rates could explain in part, the decreased rates of Pnet observed in plants subjected to 1 week cold stress but the same decreases in Pnet were observed in Aquadulce plants also subject to 1 week cold stress which did not show enhanced rates of dark respiration.

Black (1984) outlined the importance of relating pollutantinduced changes in respiration to the photosynthetic performance of the plant. When photosynthetic rates are high, a small change in respiratory rate would not have a significant effect on the carbon balance of the plant. However, when environmental conditions such as light and temperature are limiting for photosynthesis a change in respiration rates could alter the carbon balance of the plant significantly and negative rates of carbon fixation could result. Both Davies (1980) and Jones & Mansfield (1982) have reported that plants exposed to pollutants under low light conditions showed greater reductions than for plants exposed under high light conditions. However these authors studied the grass <u>Phleum pratense</u> and it may be concluded that these effects are dependent on species as the reverse was found to occur in the two varieties of Vicia faba used in this study. As described above, Mansfield & Jones (1985) suggested that higher mitochondrial activity ie. enhanced respiratory rates may be necessary for repair or detoxification mechanisms in SO₂ polluted plants, but these can only operate when given sufficient respiratory substrate. The observed resistance of photosynthesis to SO_2 when there is substrate for additional respiration in high light intensity plants and the inhibitory effect of SO, with no detectable effect on respiration in low light intensity plants, supported this interpretation. However, it can be seen from the data presented in this chapter that these effects were not observed in the two varieties of Vicia faba used in this study. Exposure to SO2 under low light intensities reduced the photosynthetic sensitivity to SO₂ of Dylan plants when dark respiration rates were seen to be reduced in response to SO₂. This reduced sensitivity was not correlated to reduced SO_2 flux (§4.9.1.1). Under high light conditions, dark respiration rates of Dylan plants were enhanced on exposure to SO_2 and greater photosynthetic inhibition was observed in comparison to low light conditions. Conversely, enhanced photosynthetic sensitivity to SO_2 of Aquadulce plants under low light intensities was seen (§4.9.1.2) although no SO_2 -induced changes in dark respiration rates were observed. Aquadulce plants were more sensitive to SO₂ than Dylan plants under low or high light intensities and dark respiration rates were consistently higher in Aquadulce plants than Dylan plants under both light regimes thus precluding the suggestion that enhanced respiratory rates are indicative of the operation of repair or detoxification mechanisms. The discrepancy in these results and those of Mansfield & Jones may be due to several factors including differences in species used and the length of the SO₂ exposure period.

The modification of SO_2 effects on Rd by added environmental stress in Dylan plants are important in explaining changes in SO_2 -induced net photosynthetic inhibition. Under low light intensities, the significant decrease in SO_2 -induced photosynthetic inhibition in comparison to the effects observed under high light intensities can be correlated with further decreases in Rd in response to SO_2 under low light conditions whereas enhanced rates of Rd were observed in response to SO_2 under high light conditions. Similarly, the absence of SO_2 -induced enhancement of Rdin Dylan plants subjected to low temperature stress prior to SO_2 fumigation can in part, account for the reductions in the degree of Pnet inhibition in comparison to that observed for non cold-stressed plants. However, the modification of net photosynthetic responses to SO_2 in environmentally stressed Aquadulce plants can not be attributed to changes in SO_2 effects on dark respiration rates.

4.10.4 Stomatal Responses to Combined Environmental and Pollutant Stress

4.10.4.1 Environmental Stress

The stomatal responses of both varieties of Vicia faba to combined environmental and pollutant stress were described in \$4.6 and \$4.7.1 and stomatal resistances of both varieties were found to be influenced by the imposition of environmental stress alone. When plants were grown under low light intensities stomatal resistance was effectively doubled in both Dylan & Aquadulce plants in comparison to that of plants grown under high light conditions. These increases in r, may be'due to a direct response to reduced light intensity (Jarvis & Morison, 1981) or increased internal CO₂ concentrations resulting from reduced rates of net photosynthesis at low light intensities or increased r: may result from a combination of both factors. Under high light intensities the stomatal sensitivity to CO_2 is considered to be too small to contribute substantially to the total stomatal response and light intensity is then the major controlling factor determining stomatal resistance, resistance decreasing with increasing light intensity. (Sharkey & Raschke, 1981). However, these authors concluded that at low light intensities, the intercellular CO2 concentration was the major controlling factor in determining rs. This problem will be discussed further when resistances to CO_2 transfer are considered.

Periods of cold temperature stress were also seen to alter stomatal resistances in comparison to non-stressed plants in both varieties of <u>Vicia faba</u>. Resistances were found to be much more variable in plants subjected to cold temperature stress but in general, stomatal resistances were substantially increased in response to all three cold temperature treatments ie. in the absence of cold stress values for r_s ranged from 20 to 400 s m⁻¹ in both varieties but following the 1 week cold treatments r_s values ranged from 28 to 1928 s m⁻¹. This effect of low temperature on stomatal resistance is widely reported in the literature eg. Crookston <u>et</u> <u>al.</u>, 1974; Sutcliffe, 1977; Jarvis & Morison, 1981; Badger <u>et al.</u>, 1982 and Oquist, 1983. Crookston <u>et al.</u> (1974) attributed increased stomatal resistance in <u>Phaseolus vulgaris</u> exposed to 5°C for 1 night to temporary water stress as a result of the low temperature stress.

The increases in r_s in response to environmental stress may contribute to the observed decreases in net photosynthetic rates but as stated earlier, decreased rates of Pnet result in increased intercellular CO₂ concentrations, Ci, which induce enhanced stomatal closure and it is unclear which event occurs first. Oquist in 1983 reviewed the then current literature concerning the stomatal control of CO₂ uptake at low temperatures and concluded that apart from a few examples in which root chilling decreased water uptake leading to stomatal closure (eg. Crookston et al., 1974), there were no clear examples of temperature-induced stomatal closure being the primary cause of low temperature inhibition of photosynthesis although this was frequently assumed. However, there are a number of reports which observed increased internal CO₂ concentration as net photosynthetic rates are reduced by low temperature stress and this increase in C_i may cause the stomata to close (Drake & Raschke, 1974; Raschke, 1975; Hällgren et al., 1982b). Musser et al. in 1983 studied the effects chilling on Glycine max. L. CV. Ransom and observed decreased stomatal conductance when plants had been subjected to 1 week at 5°C, recovery was observed within two days of rewarming. Net photosynthetic rates were also shown to decline over the cold stress period but this occurred more slowly than the observed decreases in stomatal conductance and these authors concluded that there was some other limitation to Pnet in addition to greater stomatal resistance which developed over the chilling period.

4.10.4.2 <u>Modified Stomatal Responses to SO₂</u>(a) Low Light

The results presented in §4.6 showed that stomatal responses to SO_2 were altered as a result of added low light stress and that these effects were most marked in plants of the variety Dylan where stomatal responses were the reverse of those observed under high light conditions. Enhanced stomatal closure was seen to occur at low SO_2 concentrations and SO_2 concentrations above 400 ppb induced enhanced stomatal opening. There was still evidence of the threshold SO_2 concentration of 400 ppb

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representing a switch from stomatal opening to closure but under low light stress conditions, this threshold represented the switch from enhanced closure to enhanced opening. In plants of the variety Aquadulce Claudia, unlike the relationship observed under high light conditions, there was found to be no significant correlation between stomatal response and ambient SO₂ concentrations under low light conditions although enhanced stomatal closure did occur at all SO₂ concentrations above 300 ppb.

It might be expected that enhanced stomatal opening at high SO_2 concentrations would lead to greater pollutant uptake and as a consequence, greater injury ie. more photosynthetic inhibition. However, although the data obtained for Dylan plants showed a positive relationship between changes in stomatal resistance and the degree of net photosynthetic inhibition in response to SO_2 (Fig. 4.17a), there was no correlation between these two factors in plants of the variety Aquadulce Claudia in low light conditions (Fig. 4.17b). Both Juhren et al. (1957) and Heck et al. (1965) studied the effects of low light intensities on plant pollutant responses and concluded that injury was reduced as a consequence of high stomatal resistances under low light conditions. However, Heck et al. also suggested that the stomata are only a pathway for entrance to the pollutant and were not the primary mechanism controlling injury. Menser et al. (1963), in a study of the response to ozone of Nicotinia tabacum L. plants preconditioned by light and temperature, found no evidence to implicate stomatal behaviour as a cause of differences in ozone sensitivity amongst varieties and pre-conditioning schemes; no correlation was found between transpiration rates and the degree of plant injury. In contradiction to the earlier published data, Ting & Dugger (1968) found an inverse correlation between light intensity and ozone injury in cotton plants but there was also an inverse correlation between light intensity and stomatal resistance. Therefore, these authors concluded that there was no possibility that stomatal opening influenced leaf sensitivity to ozone in cotton plants.

(b) Low Temperature Stress

Exposure to cold temperature stress for 24 h prior to SO_2 fumigation was shown to significantly alter the stomatal responses of both varieties of <u>V.faba</u> to SO_2 (Figs. 4.11 & 4.12). Unlike the responses observed in non cold-stressed plants, there was no significant correlation between ambient SO_2 concentration and the resulting change in stomatal resistance in either Dylan or Aquadulce plants. Similarly, there was no clear evidence of the 'threshold' concentration of SO_2 above which stomatal responses switched from enhanced opening to enhanced closure. Increasing the length of the cold temperature pre-treatment appeared to exacerbate changes in stomatal responses to SO_2 in comparison to non cold-stressed plants. Following the 72 h cold pre-treatments, unlike the responses observed under optimum conditions, the majority of Dylan plants exhibited enhanced stomatal closure in response to SO_2 , even at concentrations below 300 ppb. Conversely, following the 72 h cold stress periods, Aquadulce plants showed a reversal in stomatal response such that low SO_2 concentrations induced stomatal closure whilst higher SO_2 concentrations induced enhanced stomatal opening. One of the most significant effects of cold temperature stress was that a period of 1 week at 10°C prior to SO_2 fumigation was seen to result in enhanced stomatal closure in response to all $SO_{2_{\mu}}$ in every plant.

Varietal differences in cold-stress/pollutant responses were highlighted markedly when these data were examined and significant changes in stomatal sensitivity to SO_2 were apparent in both varieties. Increasing the length of the cold period was seen to result in a marked decrease in the magnitude of stomatal response to SO_2 in Dylan plants, whereas in Aquadulce plants, the magnitude of stomatal response was seen to increase with increasing length of the cold stress periods (S4.6). Thus the imposition of cold stress enhanced the sensitivity to SO_2 of the stomata of Aquadulce plants but reduced the stomatal sensitivity to SO_2 in Dylan plants. No evidence of a threshold SO_2 concentration, marking a switch in stomatal response, was found in either variety following the imposition of cold temperature stress.

The changes in stomatal resistance in response to SO_2 described above may be expected to significantly alter net photosynthetic rates in both varieties of <u>V.faba</u> given the correlation between increased stomatal resistance and increased inhibition in Pnet observed in non cold-stressed plants (S3.7.3). However, the data presented in S4.7.2 showed there to be no significant correlation between changes in stomatal resistance and the extent of SO_2 -induced photosynthetic inhibition in either Dylan or Aquadulce plants subjected to cold temperature stress prior to SO_2 fumigation. Thus, it was concluded that changes in Pnet in response to SO_2 and environmental stress occurred seemingly independently of changes in stomatal resistance and some other factor was exerting an influence.

Heck, Dunning & Hindawi in 1965 were one of the first groups to question the hypothesis that environmental effects on the sensitivity of plants to various phytotoxic pollutants must be mediated through stomatal control. These authors found plant sensitivity to O_3 to be reduced when growth temperature was lowered for one to several days prior to exposure but found no correlation with changes in stomatal conductance. As a consequence Heck et al. suggested the existence of a biochemical control of plant pollutant sensitivity apart from that controlling the stomatal apparatus. In a similar study with Avena sativa, Heck & Dunning (1978a) found sensitivity to SO₂ to be reduced when growth temperatures were lowered for a given period of time and concluded this to be a physiological rather than a stomatal response. Rist & Davis (1979) found there to be less foliar injury to Phaseolus vulgaris plants at 13°C and 21°C as opposed to 32°C and attributed this in part, to reduced stomatal resistance at higher temperatures leading to increased pollutant uptake; however SO₂-induced stomatal closure was not entirely co-incident with visible injury. Increases in leaf sulphur content were found to be the same at both 21 and 32°C and increases in stomatal conductance at high temperatures were found to be related to increased VPD and not to significant increases in stomatal aperture. Therefore these authors concluded that increased sensitivity at 32°C was more likely caused by a delay in stomatal closure in response to SO₂ at higher temperatures. In another study of the influence of temperature and stomatal conductance on pollutant induced foliar injury in Phaseolus vulgaris, Miller & Davis (1981b) found the stomatal conductance rate during O_3 exposure to be un-related to the severity of ozone injury over a range of exposure temperatures. Conductance was seen to increase with increasing temperature but more injury was observed at 15°C and 32°C. than at 24°C thus indicating that physiological factors other than decreased pollutant uptake were responsible for decreased foliar injury at lower temperatures. More recently, Taylor, Selvidge & Crumbly (1985) examined temperature effects on plant pollutant responses and, as outlined earlier in this discussion (§4.10.2.2), although SO₂ flux was seen to increase with increasing temperature, this did not necessarily result in greater physiological responses. These authors suggested that changes in stomatal conductance to water vapour might not effect an equivalent change in SO₂ flux, thus necessitating the presence of a non-stomatal or residual factor influencing SO₂ flux.

Thus, if stomatal resistance is not the major controlling factor in determining the modification of plant pollutant responses by added environmental stress and some other factor is exerting an influence, the next step was to try to elucidate the nature of this other contributing factor. Under optimum environmental conditions, the importance of changes in the residual resistance to CO_2 exchange in governing the responses of both varieties of <u>Vicia faba</u> to SO_2 was discussed. It may be that significant changes occur in response to added environmental stress which determine changes in plant pollutant response, therefore leaf resistances were examined in plants subject to both environmental and pollutant stress.

4.10.5 Environmental & Pollutant Effects on Leaf Resistances to Carbon Dioxide Transfer

4.10.5.1 Environmental Effects

From the data presented in §4.7.1 it was found that low light stress induced large increases in the residual resistance to CO_2 transfer in both varieties of <u>Vicia faba</u>. The reductions in photosynthetic rate observed in response to low light stress could be attributed to increases in both stomatal and residual resistances to CO_2 transfer. In Dylan plants, 10% of the reduction was found to be due to increases in r_5 and 90% due to increases in r_r , and for Aquadulce plants photosynthetic depression was associated with the combined effects of increases in both resistances rather than r_r being the dominant influencing factor.

The imposition of cold temperature stress was seen to result in much increased variability in measured residual resistances to CO_2 transfer in plants of both varieties. Following the 24 h and 72 h cold treatments, although resistances were variable they were not found to be significantly different from r_r values for non cold-stressed plants. Gross photosynthetic rates were unaffected by the cold pre-treatments but net photosynthetic rates were reduced as a result of enhanced dark respiration rates although recovery to pre cold-stress rates was seen to occur within 24 h of the end of the cold periods. However, following the 1 week cold pre-treatments, when net photosynthetic rates were found to be reduced by up to 20%, residual resistances were unchanged in Aquadulce plants. In Dylan plants, residual resistances were not significantly higher than those of non cold-stressed plants and were, in some plants much lower. It was concluded that reduced photosynthetic rates in response to 1 week at 10°C occurred as a result of increases in the stomatal resistance to CO_2 transfer and, in Dylan plants, increased rates of dark respiration.

4.10.5.2 Interaction of Environmental & Pollutant Stress

Under optimum environmental conditions, SO₂-induced net photosynthetic inhibition in Dylan plants was seen to result from changes in stomatal resistance and rates of dark respiration; increases in the residual resistance to CO₂ transfer contributed to photosynthetic reduction only at highest SO₂ concentrations ie. above 400 ppb. However, increased residual resistances in response to SO_2 were seen to be the major factor governing decreased photosynthetic rates in Aquadulce plants (§3.10). Following the imposition of environmental stress these varietal differences were still seen to occur. When Dylan plants were exposed to SO, under low light intensities the reductions in net photosynthetic inhibition were attributed to the same controlling factors (\$4.7.1.1). At low SO₂ concentrations (below 400 ppb) changes in r_s and Rd determined the degree of net photosynthetic inhibition in Dylan plants under both low and high light conditions, changes in r_r exerting little influence. In this case, reductions in photosynthetic inhibition under low light conditions arose from a combination of reduced respiratory rates and a reversal in stomatal response which was decreased stomatal resistance to CO_2 in response to SO_2 fumigations. At higher SO_2 concentrations the degree of photosynthetic inhibition in response to SO_2 was determined by increases in the residual resistance to CO₂ transfer, these increases being much less in plants subject to low light stress as opposed to plants treated in high light conditions. Similarly, in Aquadulce plants exposed to SO_2 under low light conditions, changes in the residual resistance to CO_2 transfer were still found to govern photosynthetic response.

In plants subjected to cold temperature stress prior to SO_2 fumigations significant differences in SO_2 -induced changes in resistances to CO_2 transfer were found. In Dylan plants, previously subjected to either 24 h or 72 h at 10°C, reduced photosynthetic inhibition was found to result from a combination of decreased stomatal resistances and much smaller increases in the residual resistance to CO_2 transfer; at the same time, SO_2 was not seen to enhance rates of dark respiration in cold-stressed Dylan plants. Of significance here is that although 24 h cold temperature stress

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did not alter the degree of net photosynthetic inhibition in response to SO₂ in Aquadulce Claudia plants when compared to non cold-stressed plants, analysis of the resistance data to CO_2 transfer showed some significant differences between the environmental regimes. As previously stated, under optimum environmental conditions changes in rr were the predominant factor governing photosynthetic responses to SO₂; however, in plants subjected to 24 h at chilling temperatures there were no large increases in r_r when photosynthetic inhibition occurred and, at lower SO₂ concentrations (below 285 ppb), r_r was seen to decrease with a concomitant increase in r_s . As the magnitude of stomatal response to SO₂ was seen to increase in cold-stressed Aquadulce plants, it was concluded that changes in stomatal resistance played a much larger part in determining photosynthetic response to SO_2 in 24 h cold-stressed Aquadulce plants in comparison to non cold-stressed plants. This increase in the stomatal control of Pnet in Aquadulce plants was not seen to occur in plants subjected to 72 h at 10 C prior to SO_2 fumigation when reduced photosynthetic inhibition in comparison to non cold-stressed plants was seen. Reductions in the degree of Pnet inhibition were found to result from much smaller changes in the residual resistance to CO_2 transfer in cold-stressed plants, changes in r_s were very small in cold-stressed plants and could not have accounted for the marked difference in photosynthetic inhibition in comparison to non cold-stressed plants.

The imposition of a 1 week cold temperature period was seen to result in much greater stomatal resistances in plants of both varieties of <u>Vicia faba</u> in comparison to non cold-stressed plants; at the same time the degree of SO_2 -induced net photosynthetic inhibition was seen to be much reduced. Comparison of the data for residual resistances to CO_2 transfer following SO_2 fumigations between cold-stressed and non cold-stressed plants revealed there to be significantly lower r_r values in cold-stressed plants of both varieties and it may be concluded that reduced photosynthetic inhibition occurred as a result of these much reduced changed in r_r and in Dylan plants, decreased stomatal resistance in response to SO_2 fumigations.

It can be seen from the results discussed above that the relative contributions of stomatal and residual (or mesophyll) resistances to photosynthetic inhibition differ according to variety, SO_2 concentration and environmental regime. A number of recent studies have tried to separate SO_2 -induced effects on photosynthesis into stomatal and non-stomatal

components and a number of authors have concluded that non-stomatal factors (ie. an increase in mesophyll resistance) appear to be primarily responsible for reductions in photosynthesis (Barton, McLaughlin & McConathy, 1980; Winner & Mooney, 1980b; Kropff, 1987). Indeed, Kropff (1987) analysed the role of stomatal resistances in the observed reduction of the rate of CO₂ uptake in <u>Vicia</u> faba CV. Minica and suggested that SO₂ induces an increase in rr which results in lowered Pnet rates; the stomata closing later as a result of a feedback loop between internal CO₂ concentration, net photosynthesis and stomatal resistance. This author concluded that stomatal behaviour was not influenced by sulphur dioxide and further analysed the results of Carlson's (1983a,b) data to support this conclusion. However, Mansfield & Freer-Smith (1981 & 1984) discussed the contradictory results of many authors concerning stomatal responses to pollutants and concluded that apart from at high SO₂ fumigations, changes in mesophyll resistance were not solely responsible for determining photosynthetic inhibition. These authors examined the responses of silver birch to SO_2 and concluded that the main effect of SO_2 was on the stomatal component with little influence on internal leaf resistances and demonstrated also that stomatal control of SO_2 uptake is of major significance in silver birch. The data obtained in this study do not support the conclusions of Kropff, especially that for Dylan plants where stomatal resistances in conjunction with dark respiration rates were found to be the major contributing factors to photosynthetic inhibition at SO_2 concentrations below 400 ppb. Although the data for Aquadulce plants under optimum environmental conditions appear to concur with Kropff's results in that changes in r_r determine net photosynthetic inhibition, it was shown that stomatal resistance becomes increasingly more important in determining SO₂-induced photosynthetic inhibition in Aquadulce plants subjected to environmental stress.

The significance of direct stomatal responses to SO_2 rather than effects mediated through changes in internal carbon dioxide concentration may be important in plant pollutant avoidance. This is because sulphur dioxide flux is closely correlated with the stomatal resistance to water vapour diffusion (Mansfield & Freer-smith, 1984) and the responses of stomata to SO_2 may be of importance in determining pollutant dose. Pollutant flux to both varieties of <u>Vicia faba</u> and leaf resistances to this flux were discussed in chapter 3 for plants exposed to SO_2 under optimum environmental conditions. The effects of added environmental stress are discussed below.

4.10.6 Influence of Environmental Stress on Pollutant Flux as Related to Ambient SO₂ Concentrations

Under optimum environmental conditions pollutant fluxes as estimated from both analogy to water vapour transfer (PFCALC) and from mass balance calculations (P_{fmeas}) were found to be directly proportional to ambient SO₂ concentration. Also, P_{Fmgas} was found to be greater than P_{fcalc} , this difference being attributed to incorrect assumptions of the magnitude of the residual or internal resistance to SO_2 uptake. When plants were subjected to either cold temperature or low light stress, the relationship between flux and ambient SO_2 concentrations were in some cases, found to be altered (\$4.8.1). Under low light conditions, measured pollutant flux to plants of the variety Dylan was not seen to be significantly different from the data obtained under high light intensities although no regression line . could be fitted through the data. However, calculated pollutant flux was found to be less in Dylan plants subjected to low light stress when ambient SO₂ concentrations were below 400 ppb and calculated pollutant flux was less to Aquadulce plants at all SO₂ concentrations. These data correlate well with the data for stomatal resistance which was shown to be markedly increased in response to low light stress in both varieties. At SO₂ concentrations above 400 ppb, stomatal resistance was found to decrease in response to SO_2 in Dylan plants and this correlates with high values for Pfcalc shown in figure 4.21b. Pfmcas was also found to be much reduced in Aquadulce plants in response to low light stress and this result was surprising because stomatal resistance is not a factor used in the estimation of this measure of flux. Changes in Prmeas in response to low temperature stress may be expected given that there would be a direct physical effect of temperature on SO_2 diffusion through a change in the conductivity of the diffusive media (gas and liquid phase) (Taylor et al., 1985) but it is unlikely that rates of diffusion could be directly affected by low light intensities. This point will be considered further when residual resistances to SO₂ transfer are discussed.

The effects of low temperature on the relation between pollutant fluxes and ambient SO_2 concentrations were found to depend very much on

the length of the cold treatment period. The imposition of cold temperature stress for 24 h prior to SO_2 fumigation was not seen to alter the relationship between P_{Fmgas} and ambient SO_2 in comparison to non coldstressed plants nor did analysis of covariance show estimates of P_{Fcalc} to be significantly different. However no significant correlation between P_{Fcalc} and ambient SO_2 concentrations were found for either variety following the 24 h cold periods and one would assume this to be related to the increasing variability in stomatal resistance data with increasing length of the cold stress periods as described earlier.

Varietal differences were observed in the effects of the 72 h cold temperature treatments on the relation between ambient SO_2 concentrations and pollutant flux. In Dylan plants, both measures of flux were significantly correlated to ambient SO_2 concentrations and the data were not significantly different from those obtained under optimum environmental conditions. However, measured pollutant fluxes to Aquadulce plants were shown to be much reduced in the cold treated plants on comparison with data for non cold-stressed plants. In contrast, calculated pollutant fluxes to cold stressed Aquadulce plants were found to be much increased in comparison to non cold-stressed plants, when SO_2 concentrations exceeded 200 ppb. This apparent anomaly can be correlated to the reversal in stomatal responses to SO_2 of Aquadulce plants subject to 72 h cold temperature stress prior to SO_2 treatment as described earlier (S4.10.4.2.[b]).

The imposition of 1 week of cold temperature stress prior to SO_2 fumigation resulted much reduced flux values to both varieties of <u>Vicia</u> <u>faba</u> when P_{Fmeas} in relation to ambient SO_2 concentrations was considered (Figs. 4.27a & 4.28a). However, calculated pollutant fluxes were not found to be statistically significantly different between cold-stressed and non coldstressed plants of either variety. It may have been expected that estimations of P_{fcalc} would be reduced in plants subject to 1 week cold stress as enhanced stomatal closure had been seen to result from all SO_2 treatments. However, as this was not seen to occur it may be concluded that stomatal resistance was not the major controlling factor determining pollutant entry into cold-stressed plants. This point is discussed further below when residual resistances to SO_2 transfer are considered.

4.10.7 Influence of Environmental Factors on Leaf Resistances to Sulphur Dioxide Flux

4.10.7.1 Low Light Stress

The results presented in S4.8 showed that, for plants of the variety Dylan, there was no significant difference in pollutant fluxes to the plants under high or low light conditions. However, when the components of the resistance pathway for SO_2 uptake were analysed individually (S4.8.4.1) it was shown that the imposition of low light stress reversed the relationship between both the stomatal and residual resistances to SO_2 and ambient SO_2 concentrations observed under high light conditions. It was thus concluded that although actual fluxes were not altered in response to low light stress in Dylan plants, resistance factors governing SO_2 entry were affected. As a result, pollutant fluxes were governed equally by both stomatal and residual resistances in low light stress in Dylan plants, fluxes were seen to be governed largely by residual resistances to SO_2 transfer.

Varietal differences were significant under low light intensities. In contrast to Dylan plants, in plants of the variety Aquadulce Claudia there were no apparent effects of added stress on residual resistances to SO_2 transfer although stomatal resistances were found to be higher than those observed under high light conditions. This resulted in the reduced flux to Aquadulce plants observed under low light conditions.

4.10.7.2 Low Temperature Stress

One of the most unexpected aspects of considering pollutant fluxes to plants following the imposition of low temperature stress was the absence of differences between estimates of measured and calculated pollutant fluxes. In chapter 3 the significance of the disparities in both measures of flux was shown to provide evidence for the residual resistance to SO₂ transfer which under optimum environmental conditions, was shown to be increasingly negative with increasing SO₂ concentration thereby producing higher flux values than would be expected from measures of stomatal resistance alone. However, analysis of the resistance data for pollutant uptake in cold-stressed plants produced some interesting and surprising results (S4.8.4). A residual resistance to SO₂ uptake was still very much in evidence in cold-stressed plants even though both estimates of flux were not significantly different.

All three cold treatments resulted in much increased variability in both stomatal and residual resistances to SO_2 uptake. Plants subjected to a 24 h cold treatment showed increased stomatal resistance in response to cold temperature stress but these changes appeared to be matched by reduced residual resistances (these being increasingly negative with increasing SO_2 concentration), the net result being that pollutant flux was unaffected by the imposition of cold stress. These changes in resistance to pollutant flux were also seen to occur in Dylan plants subjected to 72 h cold treatments. However, although similar changes in both residual and stomatal resistance were observed in Aquadulce plants subjected to 72 h cold treatments the significant reductions in flux described earlier were found to be attributed to smaller reductions in residual resistances to SO_2 transfer when stomatal resistances were increased.

The results presented in \$4.8.4.4 showed that the imposition of a period of 1 week of cold temperature stress had marked effects on resistance to sulphur dioxide flux in both varieties of <u>Vicia faba</u>. However, significant varietal differences were found to occur. For plants of the variety Dylan, stomatal resistance to sulphur dioxide transfer was increased in response to cold stress but these increases were most marked at lower $\$0_2$ concentrations ie. below 300 ppb. Concomitantly, residual resistances to $\$0_2$ flux were largely negative at low $\$0_2$ concentrations (the reverse of the responses observed in the absence of cold stress) and the net result of these changes were the reductions in flux as described earlier. In plants of the variety Aquadulce Claudia, stomatal resistances to $\$0_2$ transfer were also increased in response to cold stress but unlike for Dylan plants, were also seen to increase with increasing $\$0_2$ concentration. Residual resistances were very variable but were greater than those observed in the absence of cold stress; again, these changes resulted in much reduced flux.

4.10.8 Gas Exchange Mechanisms and Pollutant Fluxes Following Environmental Stress

There is little information in current literature regarding the influence of environmental stress on pollutant fluxes to plants, however a number of points have been considered. Jones & Mansfield (1982) stated that the imposition of low light stress, resulting in an increase in leaf area

per unit leaf weight to achieve more light capture, lead to a greater uptake of SO₂ per unit dry weight. As detoxification requires metabolism and metabolic capacity is related to dry weight then variations in SO_2 uptake would likely lead to variability in the threshold atmospheric SO_2 concentration required for injury. The results presented in \$4.9.1 would appear to uphold this theory as threshold levels of flux inducing net photosynthetic inhibition were much reduced as a result of all environmental pre-treatments. Taylor <u>et al</u>. (1985) found SO_2 flux to increase with increasing temperature although the mechanism behind this relationship was not resolved. In earlier studies both Rist & Davis (1979) and Norby & Kozlowski (1981a,b) found flux to increase with increasing temperature when ambient SO_2 concentrations were unchanged and correlated this to increased stomatal conductance to H_2O vapour. However, Taylor <u>et al</u>. pointed to the differences between H_2O transfer and SO_2 uptake (McLaughlin & Taylor, 1981) indicating that changes in stomatal conductance to H_2O vapour might not effect similar changes in SO₂ flux, thus re-introducing the existence of residual factors influencing SO_2 flux. The importance of this residual resistance in determining flux to both varieties of Vicia faba has been underlined by the results discussed above. Taylor suggested that one factor that could influence flux independently of stomatal conductance would be a direct effect of temperature on SO_2 diffusion through a change in either the kinetic energy of SO₂ molecules or the conductivity of the diffusive media (gas and liquid phases). However, it is unlikely that this could have effected the reductions in SO_2 flux observed in this study as cold temperature stress was applied prior to SO_2 fumigation, pollutant exposure occurring more than 24 h after the plants had been returned to optimum temperatures (23 \pm 3°C).

Taylor et al. also reported that increased flux with increasing temperature did not always result in greater physiological responses in all species. These authors suggested that this uncoupling of SO₂ flux and plant response could be related to species specific characteristics of their temperature tolerance limits. The results presented in this study would seem to indicate that not only species specific but cultivar specific characteristics are of importance in determining the effects of environmental stress on plant pollutant responses. In chapter 3 (\$3.10) the idea of differential sensitivity to SO₂ being related to avoidance or tolerance mechanisms was introduced. Aquadulce plants were found to be more sensitive to sulphur dioxide than Dylan plants although there was significantly less flux to Aquadulce plants when ambient SO₂ concentrations were similar, thus this variety relied more on avoidance mechanisms whereas Dylan plants appeared to show more tolerance to SO₂. The imposition of either low light or cold temperature stress was found to significantly alter the relative sensitivities of both varieties to SO₂ (S4.9). Under low light conditions SO₂ flux as related to ambient SO₂ concentration, to Dylan plants was unaltered in comparison to high light conditions but net photosynthetic inhibition was found to be markedly reduced, this was attributed to a reduction in pollutant sensitivity. Conversely, flux to Aquadulce Claudia plants was seen to be much reduced in response to low light stress although percent net photosynthetic inhibition was the same as for plants under high light conditions suggesting that the photosynthetic mechanism in Aquadulce plants was far more sensitive to SO₂ under low light conditions; but this was masked by reductions in SO₂ flux.

The effects of low temperature stress were very much dependent on the length of the cold treatment. For plants of the variety Dylan exposure to periods of either 24 h or 72 h did not alter pollutant flux and much reduced net photosynthetic inhibition was attributed once again, to reduced plant sensitivity to the pollutant ie. increased pollutant tolerance. Flux was not found to be reduced to Aquadulce plants following periods of 24 h at 10°C, nor were net photosynthetic responses to SO_2 found to be significantly different from those of non cold-stressed plants; however, plants were found to be more sensitive to low SO_2 flux values and, surprisingly, less sensitive to highest flux values (\$4.9.1). In Aquadulce plants subjected to either 72 h or 1 week cold temperature stress prior to SO₂ funigation, reduced photosynthetic inhibition was found to result from both reduced flux and a reduced sensitivity to SO₂. However, reduced SO₂induced photosynthetic inhibition in Dylan plants subject to 1 week cold stress was found to be a result of reduced flux and not a reduction in plant pollutant sensitivity.

It may be concluded that generalisations as to the mechanisms employed by plants to combat the combined effects of environmental and pollutant stress cannot be made. In the two varieties of <u>Vicia</u> used in this study both were seen to respond differently, Dylan plants appeared to rely on increased pollutant tolerance mechanisms to combat the effects of added environmental stress, although this may be thought to be a short-term solution as reduced pollutant sensitivity did not occur following the longer periods of exposure to cold temperature stress. Conversely, Aquadulce plants were seen to depend on avoidance mechanisms to deal with combined environmental/pollutant stress, again a short-term measure as reduced pollutant sensitivity was observed in plants subjected to longer cold stress periods. Of importance here is the influence of added environmental stress on the stomatal control over pollutant entry into the plant. Under optimum environmental conditions avoidance mechanisms were related to high diffusive resistances. However, the avoidance mechanisms described above can not be directly related to changes in stomatal conductance to SO_2 as changes in stomatal resistance were seen to occur independently of pollutant concentration in plants subject to environmental stress indicating a profound effect of environmental stress on that part of the stomatal mechanism sensitive to SO_2 .

Thus it may also be concluded that the environmental modification of the responses of both varieties of <u>Vicia faba</u> to SO_2 observed in this study are not explained purely by changes in stomatal aperture. Other physiological and metabolic factors must contribute to the observed stress interaction. Possible contributing mechanisms are considered in the following chapter.

However, the nature of the interactions of environmental and pollutant stress effects observed in this chapter have been shown to be very complex. Net photosynthetic and dark respiration rates together with changes in stomatal resistance, resistances to CO_2 and SO_2 transfer and pollutant flux have all been shown to be influenced by added environmental stress. This complexity being compounded by marked varietal differences in response. Thus it was decided to concentrate on one environmental stress only when possible mechanisms were considered. Low temperature stress was selected because it was hoped that the increasing length of the cold period would provide data regarding the progression of cold temperature stress interaction with sulphur dioxide fumigations and would make the identification of possible mechanisms a little easier. Nevertheless, the action of low light stress in modifying plant pollutant responses is certainly worthy of further study. Investigations as to the interactions of both low light and cold temperature stress in modifying plant pollutant, are also necessary as these stresses commonly occur together in the field in winter.

5.1 INTRODUCTION

In chapters 3 and 4 the effects of both sulphur dioxide and added environmental stress on the gaseous exchange processes of two varieties of Vicia faba were determined in an effort to gain insight into the modifying influences of environmental stresses on plant pollutant responses. It was shown (\$4.1) that although a number of studies have been reported, the majority of these were concerned with the assessment of visible injury symptoms and that the mechanisms of such actions are poorly understood. Having defined the modifying effects of low temperature and low light stresses on the responses of Vicia faba to SO₂ and determining that interactions could not be explained purely by stomatal factors, the next logical step was to try to determine the site and nature of the mechanisms involved in such interactions by investigating some of the physiological and metabolic effects of SO₂ and environmental stress, separately and/or in combination. However, as stated in the previous chapter, the nature of the observed interactions of environmental and pollutant stress effects were found to be very complex: this complexity being compounded by marked varietal differences in response. Thus it was decided to concentrate on one environmental stress only when possible mechanisms were considered. Low temperature stress was selected because it was hoped that the increasing length of the cold period would provide data regarding the progression of cold temperature stress interaction modifying responses to SO₂ and would aid the identification of possible mechanisms.

The effects of environmental stress on plant metabolic processes have been well documented; however, until the 1970's little research into the physiological effects of sulphur dioxide on plants had been undertaken. At this time, rapidly increasing interest and activity in environmental research lead to important advances on our knowledge of the metabolic effects of SO_2 and a number of reviews being published (Ziegler, 1973, 1975; Malhotra & Hocking, 1976; Horsman & Wellburn, 1976; Hällgren, 1978; Heath, 1980). Since this time research interest in enzymic and metabolic effects of SO_2 on plants has continued. However, the combined effects of environmental and pollutant stresses have not been widely studied to date. Some of the reported metabolic effects of both environmental and pollutant stress individually are outlined briefly below and provide a starting point for the elucidation of interactive effects.

5.1.1 Physiological and Metabolic Effects of Low Temperature Stress

Lowering temperature induces reduced net photosynthetic rates, reduced respiration and may induce some visible injury symptoms such as wilting due to changes in plant water relations, and chlorosis as described in the introduction to the previous chapter. In normal air, the photosynthetic rate is dependent on the intracellular CO_2 concentration and, as the stomata control the resistance to diffusive transfer of CO_2 and thus affect the intracellular CO_2 concentration of photosynthesising leaves, stomata may exert a strong influence on both the rate and temperature dependence of photosynthesis. However, the numerous reports on the responses of stomata to temperature provide widely conflicting data. Some reports show stomata to open with increasing temperature (eg. Hofstra & Hesketh, 1969b; Drake & Salisbury, 1972; Crookston et al., 1974), others indicate stomatal closure with increasing temperature (eg. Heath & Meidner, 1957; Downes, 1970), whilst there are many reports indicating maximum stomatal aperture at intermediate temperatures (Raschke, 1970; Neilson & Jarvis, 1975; Lösch, 1977). These conflicting data arise because it is now widely recognised that the stomatal response to temperature is strongly influenced by other interacting factors, particularly internal plant water status (Berry & Björkman, 1980; Oquist, 1983). It is clear from the reports cited above and a number of studies where measurements of stomatal conductance have accompanied determinations of the temperature response of photosynthesis (eg. Björkman & Mooney, 1975; Pearcy, 1977; Slatyer, 1977; Bauer, 1978; Björkman & Badger, 1979) that temperature-induced differences in photosynthetic rates can not be explained solely by stomatal responses; therefore some other mechanism must be considered, particularly a direct effect on the photosynthetic mechanism.

In very simplistic terms, photosynthesis involves a light reaction and a dark reaction; the light reactions involve the trapping of radiant energy by the plant pigments which is transformed by photophosphorylation into chemical energy in the form of ATP and NADPH₂. These are essential components of the dark reactions of photosynthesis which involve reductive CO_2 fixation. The two important photophosphorylation processes involved in the light reaction of photosynthesis are (i) cyclic photophosphorylation in which light energy is converted in to ATP energy but no NADPH₂ is generated and (ii) non-cyclic photophosphorylation involving the photolysis of water and resulting in the production of both ATP and NADPH₂. The dark reaction of photosynthesis involves the reduction of carbon dioxide via the Calvin Cycle to produce carbohydrate. One of the first and most important Calvin Cycle reactions is the carboxylation of ribulose-1,5-bisphosphate (RuBP) to form 3-phosphoglyceric acid (PGA). This reaction is catalysed by the enzyme RuBP carboxylase thus making this one of the key photosynthetic enzymes.

It is unlikely that the rates of the purely photochemical steps of photosynthesis are affected by temperature unless this induces structural changes which interfere with the organization of pigments and reaction centres (Oquist, 1983). The enzymatic steps of the electron transport chain in the chloroplast thylakoids, the coupling to photophosphorylation, the enzymes in the carbon reduction cycle in the stroma and the transport mechanisms of photosynthetic products from the chloroplast have all been shown to be affected by temperature. Low temperature stress leads to an irreversible or slowly reversible loss of photosynthetic activity which is usually preceded by a period of fully reversible inhibition (Berry & Björkman, 1980). These changes might be predicted to result from changes in the activity of enzymes which become rate limiting at low temperatures, a theory which has received considerable support. Pearcy, (1977) correlated temperature-induced changes in photosynthetic activity in Atriplex lentiformis with changes in the activity of ribulose bis-phosphate carboxylase (RuBPCase). Similarly, Björkman & Badger (1977) found that on measurement of 14 enzymes of photosynthetic metabolism, total leaf dry matter, chlorophyll and protein per unit leaf area, only the activity of RuBPCase differed in proportion to differences in photosynthetic capacity of Atriplex sabulosa and Tidestromia oblongifolia. However, these studies were of C_4 plants and for C_3 plants temperature-induced changes in photosynthetic activity were not shown to correlate exactly to RuBPCase activity. Alternatively, several authors have correlated the activity of the chloroplast fructose-1,6-bisphosphate phosphatase (Fru- P_2 phosphatase) with differences in Pnet at suboptimal temperatures (Portis, Chan, Mosbach & Heldt, 1977; Björkman & Badger, 1979;

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Björkman, Badger & Armond, 1980). These authors considered the reaction catalysed by $Fru-P_2$ phosphatase to be one of the key steps for regulation of the photosynthetic carbon reduction pathway and hypothesised that changes in the level of this enzyme are necessary to maintain effective control of this pathway as temperature is changed.

Some authors have reported a low-temperature induced reduction of the activation energy (E_a) for reaction steps in photosynthesis. Both Albrecht (1972) and Muhkin & Gins (1974) found a lower temperature optimum for the ferricyanide-Hill reaction in low temperature stressed plants but these changes did not parallel temperature effects on photosynthesis. Results are conflicting and appear to depend on species and on whether plants are chilling sensitive or resistant. Feher & Devay (1975) studied the temperature dependence of the activation energy of the DCPIP-Hill reaction in chloroplasts of frost-resistant and frost-sensitive wheat cultivars and found only the chloroplasts of the resistant cultivar to exhibit much reduced activation energy when temperature was lowered. However, Thomas, Stoddart & Potter (1980) were not able to show any significant differences in E_a of the DCPIP-Hill reaction between spring and winter oats acclimated to 20°C or 5°C. The significance of changes in E_a in relation to lowtemperature dependence of net photosynthesis has not been evaluated.

The length of the exposure period to cold stress has been shown to be important as low temperature damage is the result of cumulative and often indirect effects of temperature over time. Exposure to chilling periods (ie. 0 to 10°C) for a short time has often not been shown to be damaging (Berry & Björkman, 1980). Chlorophyll synthesis has been shown to be severely impaired at low temperatures (eg. McWilliam & Naylor, 1967; Slack, Roughan & Bassett, 1974; Smillie, Critchley, Bain & Nott, 1978) and Slack <u>et al</u>. (1974) proposed there to be a failure to synthesize chloroplast ribosomes at chilling temperatures.

Taylor & Rowley (1971) noted a stimulation of the severity of low temperature injury by exposure at high light intensity; this photoinhibition was attributed to the damaging effects of light absorption in excess of that which can be used for normal photochemical reactions. Since a reduction in temperature caused a general decline in the rate of the dark reactions of photosynthesis, the light required to saturate this capacity falls as temperature decreases and the threshold of sensitivity to photoinhibition increases. In a series of follow up papers Taylor & Craig (1971), Taylor, Jesper & Christeller (1972) and Taylor, Slack & McPherson (1974) found this sensitisation to become especially acute in species which have other low temperature-induced restrictions on their photosynthetic capacity. These authors found low temperature/high light stress to alter chloroplast ultrastructure and to affect both photosynthetic enzymes and products.

Total soluble leaf protein, carbohydrate and chlorophyll content have also shown to be influenced by low temperature stress (Levitt, 1980). Musser et al. (1983) detected reduced chlorophyll content in chilled soybean leaves but these reductions could not be correlated exactly to concomitant reductions in net photosynthetic rates. Badger et al. (1982) found leaf chlorophyll content to increase in response to low temperature stress but no alteration in soluble leaf protein content. However, several authors have noted increased soluble leaf protein in response to chilling stress (ref. Levitt, 1980) and Levitt has suggested this as a mechanism of chilling stress tolerance since increased amounts of protein may compensate for lowered enzyme activity. However, Graham & Patterson (1982) suggested that this general increase in soluble proteins at low temperatures could result from temperature-induced reductions in both protein synthesis and degradation but with rates of synthesis being less affected than protein degradation. This would lead to a greater pool of soluble protein in the tissue even though synthesis was actually being reduced by the imposition of low temperature stress. These authors also noted that sugars frequently accumulate in plants when they are chilled, more especially if the temperature to which they are exposed is not low enough to kill them. Hilliard & West (1970: see Levitt, 1980) proposed the inhibition of starch translocation out of the chloroplasts by low night temperature to account for decreased photosynthesis and growth. However, although Crookston et al. (1974) found increased leaf carbohydrate content in response to cold stress in <u>Phaseolus</u> vulgaris as a result of reduced translocation, this was not found to inhibit net photosynthesis.

Structural changes in response to chilling stress have been observed by a number of authors, these changes include increased leaf thickness, mesophyll cell size and reductions in the number of stomata (see Graham & Patterson, 1982). Characteristic crimping and reduced intrathylakoid space has been noted in barley grown at 2°C or 5°C (Smillie <u>et</u> <u>al.</u>, 1978) and Levitt (1980) attributed reduced net photosynthesis to the damage and/or inactivation of chloroplast thylakoids in response to low temperature stress. In addition, increased membrane permeability has been shown in response to cold stress and is thought to be due to changes in the physical state of membranes leading to an increased leakage of cell electrolytes (see: Lyons, 1973; Markhart <u>et al.</u>, 1980; Levitt, 1980; Paull, 1981 and Graham & Patterson, 1982).

It can be seen that cold temperature stress alone induces many physiological and metabolic changes in plants. Cold effects have been described briefly here but there a number of comprehensive reviews detailing plants responses to low temperatures eg. Levitt, 1980; Berry & Björkman, 1980; Björkman, 1981; Graham & Patterson, 1982 and Oquist, 1983.

5.1.2 Physiological and Metabolic Effects of Sulphur Dioxide

As stated earlier in this section, there is a wealth of information in current literature concerning the effects of sulphur dioxide on plant metabolism and is is not intended to consider these metabolic effects in detail here. Comprehensive reviews provide a guide to the data available (Ziegler, 1973, 1975; Mudd, 1975; Malhotra & Hocking, 1976; Hällgren, 1978; Heath, 1980; Black, 1982; Reinert, 1984; Malhotra & Khan, 1984; Treshow (Ed.), 1984; Koziol & Whatley (Eds.), 1984; Winner, Mooney & Goldstein (Eds.), 1985; Wellburn, 1982, 1987; TERG,1988) but a brief introduction to major effects is given below.

It is well known that cellular and biochemical effects of sulphur dioxide (and other gaseous pollutants) are determined not only by their atmospheric concentration but by their solubilities in water and their reactivities within plants. (Last, Fowler & Freer-Smith, 1985). Sulphur dioxide is extremely soluble in water, establishing equilibria with its dissociation products, bisulphite (HSO_3^{-}) and sulphite ($SO_3^{2^{-}}$) ions. Sulphite ions may be oxidised to sulphate ($SO_4^{2^{-}}$) and metabolised by the sulphate reduction pathway (Ziegler, 1975). Damage due to SO_2 is thought to occur when large accumulations of the intermediate oxidation products accumulate in tissues ie. when their rates of production exceed the ability of plants to incorporate sulphur by the sulphate reduction pathway (Last <u>et al.</u>, 1985).

Photosynthesis within chloroplasts is considered to be one of the initial and major process affected by the products of SO_2 in solution

(Ziegler, 1975; Hällgren, 1978). Because the pH of the aqueous stromal phase of chloroplasts is pH 8 - 9, sulphite ions are the major product and as a result, many <u>in vitro</u> studies of SO_2 at likely sites of action have been carried out using sulphite (Wellburn, 1987). However, these methods of using isolated chloroplasts, membranes or enzyme systems are seldom comparable with true physiological conditions and are often combined with the use of unrealistically high SO_2 concentrations thus providing results which bear no relationship to the 'subtle' responses that occur within the cell (Black, 1982). Nevertheless, use of these techniques in conjunction with low pollutant concentrations can (and have) provided useful insights into a number of SO_2 effects on the individual components of the photosynthetic mechanism.

Ribulose bisphosphate (RuBP) carboxylase, which effects the first step in CO_2 fixation in the C_3 pathway of photosynthesis, is an enzyme associated with the chloroplast membrane and most studies on the effects of pollutants have concentrated on this major enzyme concerned with carboxylation. Ziegler (1972) observed that $SO_3^{2^-}$ inhibited RuBP carboxylase in Spinacea oleracea chloroplasts. The kinetics of sulphite inhibition indicated competition between bicarbonate (HCO_3^-) and $SO_3^2^-$ at the CO_2 binding sites of the enzyme suggesting that the concentration of CO_2 (or bicarbonate ions) at the site of carboxylation influences the degree of sulphite-induced inhibition. A similar type of competitive inhibition by sulphite ions in respect to bicarbonate ions has been observed with isolated preparations of RuBP carboxylase from a lichen, <u>Pseudevernia</u> furfuracea (Ziegler, 1977). However, at higher SO₂ concentrations this inhibition was non competitive and Black (1982) suggested that such a mechanism would explain the rapid inhibition of photosynthesis on SO_2 exposure, the concentration-dependence of the magnitude of the response and the rapid recovery of pretreatment photosynthetic rates on removal of the pollutant. In contrast, Gezelius & Hällgren (1980) showed the SO_3^{2-} inhibition of RuBP carboxylase from Pinus sylvestris and Spinacia oleracea to be non competitive with respect to HCO_3^- and the nature of the inhibition was not affected by the presence of sulphite ions during the activation. Alternatively, both Miszalski (1983) and Khan & Malhotra (1982) found a competitive type of inhibition by SO_3^2 . Such discrepancies may arise from the differing experimental techniques used in chloroplast and enzyme extraction or may result from different plant species being used, in

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any event, the question of whether the action of SO_2 on photosynthesis is by competitive inhibition has not been completely resolved. However, new data from Parry & Gutteridge (1983) showed that sulphite has a variable effect on the affinity of RuBP carboxylase to CO_2 and their data suggested that the potential effects of SO_2 on this enzyme may have been seriously under-estimated given the progressive inactivation at very low concentrations (1 mM $SO_3^{2^-}$).

In addition to influencing the carboxylation reactions, SO_2 has been shown to affect photosynthesis by attacking photosynthetic electron transport and photophosphorylation reactions (Malhotra & Khan, 1984); however there is again controversy in the literature as to the nature of SO₂ effects. Malhotra (1976) isolated chloroplasts from needles of Pinus contorta and, using various concentrations of aqueous SO_{2} , showed that at a low concentration (50 ppm) SO_2 stimulated Hill reaction activity but this activity was completely inhibited at high concentrations (500 - 1000 ppm). A decrease in Hill reaction activity was accompanied by swelling and disintegration of chloroplast membranes and such alterations in the membranes could cause disorganisation of the two photosystems. Shimazaki & Sugahara (1980a) studied in detail the effects of gaseous SO_2 on chloroplast photosystems of Spinacea oleracea and found fumigation with SO_2 at 1 and 2 ppm for 1 hour produced no effect on Hill reaction activity; however there was rapid inhibition at longer exposures. These authors investigated the site of SO_2 attack in the electron transport systems by studying both photosystems. Electron transport of both the whole chain and Photosystem II was inhibited to the same magnitude by SO_2 but electron flow from reduced DCIP to NADP under uncoupled conditions was not inhibited suggesting that the site of SO₂ action was associated with Photosystem II and not Photosystem I. A similar effect was observed in photosystems of Lactuca sativa (Shimazaki & Sugahara, 1980b). These results differed from those reported in earlier studies using solutions of SO₃²⁻. Asada, Kitoh, Deura & Kasai (1965) observed inhibition of both cyclic and non-cyclic photophosphorylation but no overall effect on electron flow and Libera et al. (1973) found aqueous SO_2 to stimulate non-cyclic electron transfer. Silvius et al. (1975) also found that treatment of isolated chloroplasts with solutions of SO_3^2 , HSO_3^- and SO_2 to inhibit both cyclic and non-cyclic photophosphorylations.

In addition to damaging effects on plant metabolism, SO_2 has

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been shown to have marked effects on plant composition and plant structure. Several changes in the amount of plant constituents have been noted. Chlorophyll and other plant pigments are necessary in harnessing light energy by Photosystems I and II thus any effect of SO_2 on these pigments could greatly influence the photosynthetic ability of plants (Malhotra & Khan, 1984). Rao & LeBlanc (1965) found that destruction of chlorophyll occurred in lichens following exposure to high concentrations of SO_2 (5 ppm; 24 h) and chlorophyll molecules were degraded to phaeophytin and magnesium ions. Malhotra (1977) showed chlorophyll degradation in Pinus contorta needles treated with aqueous SO₂ and Malhotra & Khan (1984) found fumigation with low levels of gaseous SO_2 to cause a gradual decline in chlorophyll content in Evernia mesomorpha which was accompanied by a decrease in photosynthesis. Similarly, Hällgren & Gezelius (1982) found chlorophyll concentration to decrease in response to increasing SO_2 concentrations in Pinus sylvestris seedlings. Shimazaki et al. (1980) proposed that SO_2 fumigation of leaves increased the formation of superoxide radicals (0_2^-) in chloroplasts that in turn destroys chlorophylls and it was suggested that SO₂ destroys chlorophyll mainly by free-radical oxidation. However, Black (1982) noted that although prolonged exposures to low concentrations of SO_2 have been shown to result in injury at the molecular level by affecting enzymes such as chlorophyllase, increased activity of this enzyme which converts chlorophylls to chlorophyllides by removal of the phytol group has been shown (Malhotra, 1977), these effects are too slow and insufficient to account for substantial reductions in photosynthesis. Moreover, short term fumigations with SO₂, as used in this study, induce photosynthetic inhibition within minutes of the onset of exposure and it is unlikely that chlorophyll degradation would contribute to this initial photosynthetic decline.

Ziegler (1975) reported that as a consequence of SO₂ uptake, sulphate and sulphur containing amino acids increase during fumigation. However, reports on changes in the amount of protein or amino acids are conflicting; some authors reporting an increase in total amino acids (eg. Malhotra & Sarkar, 1979) and others a decrease (eg. Cowling & Bristow, 1979). Malhotra & Khan (1984) showed SO₂ to decrease the soluble cytoplasmic and chloroplast protein of pine-needles, and that this decrease was higher in the chloroplast than in the soluble cytoplasmic fraction. Similarly, Rabe & Kreeb (1979) studied a number of plant species and found total soluble protein content to decrease in response to SO₂ and Murray (1984) also found a decrease in soluble leaf protein in response to SO_2 in Lolium perenne and Trifolium subterraneum. However, both Beckerson & Hofstra (1979) and Murray (1985) reported no changes in soluble leaf protein in response to SO₂. In contrast, Horsman & Wellburn (1977), Sárdi (1981) and Saxe (1983b) reported increased soluble leaf protein in response to SO₂. Rabe & Kreeb (1979) suggested that decreased soluble protein content was a result of both reduced synthesis and increased decomposition to amino acids; this would concur with the results of Godzik & Linskens (1974) and Saxe (1983b) who found total concentration of free amino acids to increase on exposure to SO₂. More recently, Rowland, Borland & Lea (1989) reviewed the topic of changes in amino acids and proteins in response to air pollutants and concluded that much more detailed investigations in to the effects of pollutants are needed as observations solely on changes in leaf content fail to answer many fundamental questions, specifically whether these changes indicate damage to a usually balanced metabolism or are indicating a plants response to mitigate any potential damage.

As with soluble protein content, reported data concerning changes in carbohydrate content in response to sulphur dioxide are incomplete. It is generally accepted that plants exposed to SO₂ exhibit increasing amount of soluble sugars (Khan & Malhotra, 1977; Koziol & Jordan, 1978; Koziol & Cowling, 1980; Saxe, 1983b). However, high SO₂ concentrations may lead to decreased carbohydrate levels which can be correlated with decreased photosynthesis and visible injury symptoms (Koziol & Jordan, 1978). These authors suggested that this evidence of a correlation between leaf carbohydrate levels and pollutant damage required further investigation both for obtaining an understanding of functional changes in response to SO_2 exposure, and for providing information concerning the possible mechanisms of pollutant-metabolism interactions. However, little specific attention has been paid to elucidating pollutant effects on carbohydrate metabolism although many authors measure carbohydrate content as part of investigations into other pollutant effects. Saxe (1983b) measured carbohydrate content of Phaseolus vulgaris plants exposed to varying SO_2 concentrations because carbohydrates are important indicators of energy status and growth potential of plants and found similar results to Koziol & Jordan ie. high SO₂ concentrations resulted in reduced carbohydrate levels. These decreases were not accompanied by

increased dark respiration and Saxe concluded that SO_2 inhibited carbohydrate production.

Attention has recently been focused on changes in assimilate distribution and transport in response to pollutants. Several studies have demonstrated pollutant effects on the allocation of dry matter in plants (eg. Jones & Mansfield, 1982; Mansfield & Jones, 1985; Mansfield et al., 1986; Wright et al., 1986). These authors found root growth to be reduced whilst shoot growth was stimulated in response to SO_2 and SO_2/NO_2 mixtures; this increase in leaf area compensating for reductions in net assimilation rates. Thus allocation of photosynthates between shoots and roots had been changed. Proportionally more of newly fixed carbon was found to be retained in the shoot and used for leaf expansion at the expense of root development. Whilst this may not appear to be damaging in itself, reductions in root development may have severe implications to the plant if water is limiting and may render the plant more susceptible to drought conditions (Wright et al., 1986). Some evidence suggests that the primary effect may be on the translocation process itself eg. on phloem loading. Teh & Swanson (1977) found translocation in <u>Phaseolus</u> vulgaris to be more sensitive to SO_2 than photosynthesis and similar results were found by Noyes (1980). Noyes found that labelled $14CO_2$ was fixed by leaves and accumulated near or in minor veins suggesting that phloem loading or axial transport in sieve tubes was inhibited by SO₂. More recently, Marie & Ormrod (1988) have examined the effects of air pollutants on dry matter partitioning in developing two brassica species, rutabaga and cabbage. These authors found leaf tissue of both species to become less hydrated on exposure to SO_2 and/or O_3 whilst leaves of cabbage became more dense which suggested a reduction in carbohydrate export from the cabbage leaves. Cabbage plants seemed to allocate less dry matter to fibrous roots in comparison to rutabaga in response to O_3 . These authors also demonstrated some similarity between patterns of reducing sugar allocation and dry weight partitioning.

Reduced transport of carbohydrate to developing fruits has also been reported which may affect fruit and seed yield (TERG, 1988). Both ozone and sulphur dioxide have been shown to reduce the percentage of dry matter of potato tubers (Pell, Pearson & Vinten-Johansen, 1988). These authors also observed SO_2 to influence sucrose content of tubers whilst ozone reduced the fructose and glucose content but had no effect on sucrose content. Pell <u>et al</u>. suggested that, since sucrose is the precursor to starch that comprises 60 to 80% of the dry matter in the tuber and dry weight was found to be reduced by exposure to O_3 , it was possible that with diminished photosynthate available, the partitioning of sucrose to starch was reduced in order to maintain a relatively stable pool of sucrose.

In addition to changes in metabolites a number of structural and ultrastructural changes in response to SO_2 have been reported (see: Ziegler, 1975; Black, 1982; Wellburn, 1987). Black & Black (1979a) have observed chloroplast swelling in the guard cells of stomata in plants exposed to SO₂ and prolonged exposure to SO_2 has been found to result in cellular plasmolysis and mesophyll collapse (Ziegler, 1972). Mansfield, Davies & Whitmore (1986) noted structural changes in leaves of Phleum pratense in response to a mixture of SO₂ and NO₂: Leaf thickness was increased in polluted plants and this was found to be due to individual mesophyll cells becoming much larger and less tightly packed than those of control plants. Changes in chloroplast ultrastructure may be caused by swelling of the lumen within the chloroplast thylakoids (Wellburn, 1987); this being reversible at low SO_2 concentrations. A number of authors have proposed disruption of cellular membranes ie. changes in structure and permeability (see Black, 1982); such changes may result in alterations of a number of biochemical processes within the cell as many enzymes are associated with cell membranes.

From the brief review of existing data given above, it can be seen that both cold temperature and pollutant stress individually result in many biochemical and physiological changes within plants. It would appear from the data outlined above that the sites of action of each stress are similar, although resulting effects may differ. It is surprising therefore, that relatively little work has been done to elucidate the combined effects of environmental and pollutant stress on the major plant metabolic processes given current advances in experimental techniques. A number of studies are currently being undertaken to identify the relative contributions of pollutants and adverse environmental conditions to forest decline (see TERG, 1988) but it is clear that many more detailed investigations are necessary to provide much needed insight into the mechanisms of combined pollutant/environmental stresses on plants.

5.2 AIMS

The aim of this section of the experimental work was to provide indications as to how and at what site cold stress and pollutant interactions determined the responses outlined in chapters 3 and 4; specifically the effects on net photosynthetic rates in <u>Vicia faba</u> plants. Figure 5a gives a schematic of the photosynthetic mechanism (adapted from Whittingham, 1977) and shows the stages at which the data presented in this chapter are relevant. Firstly, measurement of chlorophyll content gives an indication of the energy trapping capability of the plant, light absorption being the first step in the photosynthetic process. Secondly, by monitoring Hill Reaction activity it was possible to gain an indication of the plants ability to transfer the light energy absorbed by chlorophyll molecules leading to the formation of ATP and NADPH₂ ie. non cyclicphotophosphorylation. Both ATP and NADPH₂ are the driving force for the Calvin cycle ie. photosynthetic carbon reduction.

The first step and one of the most important reactions in the Calvin cycle is the carboxylation of ribulose-1,5-bisphosphate (RuBP) to form 3-phosphoglyceraldehyde (PGA). The enzyme responsible for this incorporation of CO_2 is ribulose-1,5-bisphosphate carboxylase (RuBP carboxylase) and this enzyme is by far the largest component of leaf protein and can account for up to 60% of the soluble protein in C_3 leaves (Khan & Malhotra, 1982). Thus, although it was not possible to measure RuBP carboxylase activity directly, a measure of total soluble leaf protein gave a good indication of the plants capacity to incorporate CO_2 in to the Calvin cycle.

Lastly, the overall product of the photosynthetic mechanism is the formation of carbohydrates, the majority of which are re-incorporated into the carbon reduction cycle. However, one in every six molecules of carbohydrate produced is fed into the metabolic pool, sucrose being the most abundant product of photosynthesis within leaves. Therefore, measurement of leaf carbohydrate content was a measure of the end products of photosynthesis and an indicator of photosynthetic efficiency.

The effects of both cold and pollutant stress alone and in combination on each of the above parameters are examined in turn in this chapter.



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5.3. EXPERIMENTAL PROTOCOL

5.3.1 Determination of Chlorophyll Content in Leaves of <u>Vicia</u> <u>faba</u>

Chlorophyll determinations were made of the first three fully expanded leaf pairs from cold temperature stressed and non-stressed plants of both varieties of <u>Vicia faba</u>. Plants were subject to a cold treatment of 0, 24 h, 72 h or 1 week at 10°C prior to a 4 h fumigation with either 0, 100, 300 or 500 ppb sulphur dioxide. Leaf chlorophyll was extracted within one hour of the end of the SO_2 fumigation period.

5.3.1.1 Chlorophyll Extraction

The leaves were weighed and the leaf area measured. The leaves where then chopped into fine pieces and placed in a cold mortar which had been previously kept on ice. A little silver sand was added along with 8 ml 80% cold acetone. The material was thoroughly and quickly ground to a fine paste and filtered through Whatman No.1 filter paper into a cold centrifuge tube. The mortar was washed with a little more 80% acetone to give a final volume of 10 ml. The extract was then centrifuged for two minutes at 2000 rpm using a bench centrifuge and the resulting supernatant decanted into a cold clean test-tube.

The supernatant was diluted 1 : 5 with 80% acetone and the absorbance of the solution measured against an 80% acetone blank. The absorbance of the chlorophyll extract was measured from 350 - 750 nm using a dual wavelength scanning spectrophotometer connected to a chart recorder. The absorbance peaks at 663 and 645 nm were also measured.

5.3.1.2 Chlorophyll Estimation

The absorbance values at 663 and 645 nm (A663; A645) were fitted into McKinney/Arnon equations to give chlorophyll a, chlorophyll b and total chlorophyll content in mg per litre chlorophyll extract where:

> Chl a = 12.72 (A663) - 2.58 (A645) Chl b = 22.87 (A645) - 4.67 (A663) Chla+b = 8.05 (A663) + 20.29 (A645)

(Arnon, 1949)
Chlorophyll content was then expressed as mg Chl g⁻¹ leaf tissue (fresh weight) and mg Chl cm⁻² leaf area.

5.3.2 Hill Reaction Activity in Isolated Chloroplasts

The Hill Reaction activity was measured in chloroplasts isolated from both cold stressed and non-stressed plants of both varieties of <u>Vicia</u> <u>faba</u>. Plants were subject to 0, 24 h, 72 h or 1 week at 10°C prior to fumigation with either 100 or 500 ppb SO_2 . For each experiment for each cold regime, plants subject to a cold pre-treatment only were used as control. Chloroplasts were extracted from plants within one hour of the end of the SO_2 treatments.

Hill reaction activity was measured using the dye DCPIP (2,6dichloro phenol indo-phenol) as an artificial hydrogen acceptor. DCPIP is blue when oxidised but is colourless when reduced, therefore the rate of Hill reaction activity could be determined from measurements of the decrease in absorption of the reaction medium with respect to time.

5.3.2.1 Chloroplast Extraction

Chloroplasts were isolated from the plants following the method of Nakatani & Barber (1977). The extraction medium consisted of:

0.33 M Sorbitol

0.2 mM Magnesium chloride

20 mM MES (2 (N-morpholino) ethane sulphonic acid) brought to pH 6.5 with Tris (hydroxymethyl) aminomethane. Chloroplasts were resuspended in a cation free medium of 0.33 M Sorbitol brought to pH 7.5 with Tris base (0.5 mM).

A known fresh weight $(\cong 3.5 \text{ g})$ and area of leaf material was taken and placed in a Waring blender and homogenised for 20 seconds with 10 ml ice cold extraction medium. The extract was then filtered through ten layers of muslin, the first two layers being separated by cotton wool.

The filtrate was then centrifuged at 2200 g (17000 rpm) for 30 s using a refrigerated centrifuge. The supernatant was discarded along with most of the soft pellet. The remaining pellet was resuspended in 5 ml cation free medium and centrifuged again at 2200 g for 20 s; the total time including braking being less than 90 s.

The supernatant was again discarded and the remaining pellet

resuspended carefully in 5 ml of the resuspension medium. The suspension was kept cold throughout the extraction process and was stored on ice prior to determination of Hill reaction activity.

5.3.2.2 Chlorophyll Content of Chloroplast Suspension

It was necessary to determine the chlorophyll content of the chloroplast suspension as Hill reaction activity was expressed as rate per mg Chl. The procedure followed was that of Plummer (1978). One ml of chloroplast suspension was added to 10 ml cold 80% acetone in water and shaken. The extract was filtered through Whatman No.1 filter paper into a cold 25 ml volumetric flask. The test-tube was rinsed twice with 5 ml aliquots of 80% acetone and the filter paper washed. Finally the chlorophyll solution was made up to 25 ml with 80% acetone. The absorbance of the chlorophyll solution was read at 645 and 663 nm against an 80% acetone blank using a dual wavelength spectrophotometer. Total chlorophyll was calculated from:

Chla+b (mg 1^{-1}) = 8.05 (A663) + 20.29 (A645) (Arnon, 1949).

5.3.2.3 Hill Reaction Activity

A reaction medium of phosphate buffer (0.03 M) pH 6.5 containing 0.01 M potassium chloride was prepared. The chloroplast suspension was diluted with the reaction medium to give a chlorophyll concentration of approximately 5 mg ml⁻¹. The spectrophotometer was set to zero using a blank containing 9 ml reaction medium with the addition of 1 ml chloroplast solution.

The reaction tube contained 5 ml reaction medium, 4 ml DCPIP solution and 1 ml chloroplast suspension. This amount of DCPIP gave an initial absorbance of 0.8 units, the absorbance being read at 520 nm. The absorbance of the reaction tube was measured immediately following the addition of the chloroplast suspension. The test tube was then illuminated using a standard projection lamp giving a saturating light intensity (\cong 400 µE m⁻² s⁻¹) allowing maximum photosynthetic activity of the chloroplasts. The reduction of the dye was followed by reading the absorbance of the extract against the blank at 60 s intervals for periods up to 30 min. A plot of absorbance v time was produced and the rate of reaction calculated from this. The reaction rate was expressed in three ways:

i) absorbance units (au) per minute per mg Chlorophyll

ii) au min⁻¹ g⁻¹ leaf tissue (fresh weight)

iii) au min⁻¹ cm⁻² leaf area.

Four replicates of each treatment were performed for both varieties of <u>Vicia faba</u>.

5.3.3 Determination of Total Soluble Leaf Protein

The total soluble leaf protein content (giving a measure of RuBP carboxylase content) of cold stressed and non-stressed plants of both varieties of <u>Vicia faba</u> was determined following the method of Lowry (Lowry, Rosebrough, Farr & Randall, 1951). Plants were subject to either 0, 24 h, 72 h or 1 week at 10°C prior to fumigation with 500 ppb sulphur dioxide. Control plants were subjected to the cold treatment but were not fumigated with SO₂.

5.3.3.1 Principle

Lowry's method of protein determination is a colorimetric technique, protein reacts with the Folin-Ciocalteu reagent to give a blue coloured complex. The colour so formed is due to the reaction of the alkaline copper with the protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of colour is dependent on the amount of these aromatic amino acids present and thus varies for different proteins.

5.3.3.2 Calibration Curve

It was necessary to produce a standard curve to relate protein content to absorbance readings.

REAGENTS:

I. 50 ml sodium carbonate solution (20g l^{-1}] in 0.1 M NaOH); 1 ml copper sulphate solution (5 g l^{-1} in 10 g l^{-1} sodium/potassium tartrate).

II. Folin-Ciocalteu Reagent (sodium tungstate and sodium molybdate in phosphoric and hydrochloric acid), standard reagent obtained from Fisons U.K. Ltd. A series of test tubes were prepared containing $0 - 250 \ \mu g$ case in in distilled water taken from a stock solution of 250 μg case in. Three ml of reagent I were added to each tube and the solution was shaken then left to stand for fifteen minutes, 0.3 ml of reagent II was then added, the tubes shaken again, and left for a further 45 min for the colour to develop. The absorbance of the solutions was then read at 600 nm against a blank containing deionised water in place of protein solution.

A curve of absorbance versus protein content was plotted and used in the calculation of the protein content of the leaf extracts. This experiment was repeated three times to ensure the accuracy of the resulting calibration curve (Figure 5.1).

5.3.3.3 Protein Content of Leaves

EXTRACTION MEDIUM: 0.15 M Tris-HCl buffer (pH 7.5) 0.01M EDTA (pH 7.5) 0.01 M KCl 0.001 M MgCl₂ 10 mM dithiothreitol

The midribs were removed from the plant leaves and then 1 g of leaf material was weighed out and the leaf area determined. The leaf material was placed in a Waring blender that had been previously stored on ice and the material was homogenised for 30 s with 10 ml ice cold extraction medium. The extract was filtered through four layers of cheesecloth and centrifuged for five minutes at 5000 g. The clear extract was stored on ice. 0.05 ml and 0.1 ml aliquots of extract were placed into clean cold test tubes using micropipettes and the solution made up to 10 ml with deionised water. One ml units of the protein solutions were then taken and the protein content determined as described in the preparation of the calibration curve.

Six determinations were made for each plant. Protein content was expressed as mg g^{-1} leaf tissue (fresh weight) and mg cm⁻² leaf area.





Figure 5.1. Standard Curve for Protein Content ($\mu g \ ml^{-1}$): obtained using Lowry's method of protein determination on standard solutions of casein.

5.3.4 Determination of Carbohydrate Content

The carbohydrate content (products of photosynthetic activity) of both varieties of <u>Vicia faba</u> was determined using the phenol method of Dubois, Gilles, Hamilton, Rebers & Smith (1956). The phenol method is a colorimetric determination of sugars, methylated sugars and polysaccharides and is highly sensitive. The colour produced is very stable and the assay is largely unaffected by the presence of proteins. Plants were subjected to either 0, 24 h, 72 h or 1 week at 10°C prior to fumigation with 100 ppb sulphur dioxide for 4 h. Control plants were subjected to cold treatments only but were not exposed to SO_2 .

5.3.4.1 Calibration Curve

The following reagents, both Analytical Reagent grade, were used.

I. 5% (w/v) solution Phenol in water

II. Conc. Sulphuric Acid (sp.gr.1.84)

In order to generate a standard curve a stock solution of 1000 μ g ml⁻¹ glucose in distilled water was diluted to give 100 μ g ml⁻¹. A series of thick walled test tubes were then prepared containing a 1 ml sample of 0 to 100 μ g ml⁻¹ glucose solution. One ml of 5% phenol solution was added to each tube and the tubes well shaken. Five ml of concentrated sulphuric acid were then added to each tube from a fast flowing pipette with care being taken to direct the acid stream onto the surface of the liquid whilst the tube was being gently shaken to effect fast and complete mixing. The tubes were then allowed to stand for 10 minutes, shaken and placed in a water bath set at 25°C for a further 20 min before readings were taken. The absorbance of the solutions was then read at 488 nm against a blank of acid, phenol and deionised water. A curve of absorbance v carbohydrate content was plotted and used in the calculation of the carbohydrate content of the leaf extracts (Figure 5.2).

5.3.4.2 Carbohydrate Content of Leaves

Chloroplasts were extracted using the method of Nakatani & Barber as described in \$5.3.2.1. Using a micropipette, 0.1 ml of chloroplast extract was added to a test tube containing 9.9 ml deionised water and the



Figure 5.2

Figure 5.2.

Standard Curve for Carbohydrate Content ($\mu g \ ml^{-1}$): obtained using the method of Dubois <u>et al</u>, on standard glucose solutions.

tube was well shaken. One ml of this solution was then taken and the carbohydrate content determined as described above with reference to the calibration curve. Four determinations were made for each plant and carbohydrate content was expressed as mg g^{-1} and mg cm⁻².

5.3.5 Isolated Chloroplasts and Oxygen Electrode Studies

It was intended to examine the effects of bisulphite solutions on the photosynthetic rates of chloroplasts isolated from cold-stressed and non cold-stressed plants by monitoring CO_2 -dependent oxygen evolution under illumination using a Clark type oxygen electrode. However, despite the use of varying extraction, resuspension and reaction media together with a number of different isolation techniques, all attempts to obtain <u>Vicia</u> chloroplasts that would evolve oxygen were unsuccessful. The methods used were those of: Jensen & Bassham, 1966; Cockburn, Walker & Baldry, 1968; Nakatani & Barber, 1977: Edwards, Robinson, Tyler & Walker, 1978; Leegood & Walker, 1978; Plesničar & Kalezič, 1980; Shimazaki & Sugahara, 1980b; Ballantyne & Glover, 1981; de Kok, Thompson & Kuiper, 1983.

It was thought that the inability to evolve oxygen may have been the result of a low percentage of chloroplast intactness since optimum rates of CO_2 -dependent O_2 evolution are found in preparations with a high degree of structural intactness (Nakatani & Barber, 1977). However, ferricyanide assay of the preparations (Lilley, Fitzgerald, Rientis & Walker, 1975) showed intactness above 50%, which should have been enough to produce some chloroplast activity in the illuminated electrode. Isolation of chloroplasts from spinach or lettuce leaves using the above mentioned techniques resulted in good rates of oxygen evolution. Similarly, algal suspensions (Chlorella spp.) and the freshwater macrophyte, Keratophyllum happily evolved oxygen when suspended in a variety of the extraction media. Therefore the apparent failure of Vicia to produce results was baffling. It was found, on enquiry, that other researchers had experienced the same problems with <u>Vicia</u> (R.C. Leegood pers. comm.). Researchers at Lancaster University have also experienced difficulties with <u>Vicia faba</u> in their use of isolated plastids to investigate pollutant effects on plant enzymes (A.R. Wellburn pers. comm.). Although attempts were unsuccessful, it was felt that some mention must be made of the difficulties encountered which may be of aid to other researchers.

RESULTS

5.4 CHANGES IN LEAF AREA AND THICKNESS IN RESPONSE TO COLD TEMPERATURE STRESS

The results presented in Chapter 4 showed that the responses of plant gaseous exchange mechanisms to sulphur dioxide were modified in response to added environmental stress such as cold temperature pretreatments. In addition, cold stress alone was found to induce significant morphological and anatomical changes in the leaves of both varieties of Vicia faba. In a small number of plants of both varieties subjected to 1 week at 10°C, visible injury symptoms appeared upon rewarming. Plants appeared perfectly healthy on removal from the cold temperature growth cabinet, however, within 24 h of being placed in the exposure chambers at optimum temperatures (23 ± 3°C), black necrotic lesions were evident at the leaf margins as shown in Plate 5.1. As outlined in the previous chapter, secondary water-stress is known to occur in response to chilling stress and the visible injury symptoms observed in <u>Vicia</u> faba were characteristic of water stress. It must be emphasised that this damage occurred prior to SO_2 exposure and was observed in a small number of plants only; the majority of plants of both varieties being apparently undamaged by the 1 week cold temperature treatments.

However, plants of both Dylan and Aquadulce Claudia subjected to 1 week cold stress prior to sulphur dioxide fumigation were visibly different from non cold-stressed plants in that the leaves appeared to be smaller and thicker following the cold treatments. In order to determine if this was so, the ratio of leaf fresh weight to leaf area (LW/LA) was calculated for both varieties for cold-stressed and non cold-stressed plants. At the same time, measurements of leaf thickness were made using a micrometer screw gauge. The leaf weights and leaf areas of 21 plants of each variety were determined for both temperature regimes giving a total of 84 data points. The leaf weight was divided by leaf area and the ratios obtained were analysed using 't' tests. The results of this analysis are presented in Table 5.1 and from the table it can be seen that there were significant varietal differences in the ratio of leaf area to leaf weight in the absence of cold stress: Leaves of the variety Aquadulce Claudia were found to have significantly more weight per unit leaf area than leaves of



Plate 5.1

Visible injury symptoms in <u>Vicia</u> <u>faba</u> CV. Dylan exposed to 1 week at 10° C. Damage did not appear during the cold treatment but appeared 24 h after the onset of rewarming and was not seen to occur in the majority of plants. Such injury was also observed in a small number of plants of the variety Aquadulce Claudia.

1:

Table 5.1.

Analysis of data for the ratio of leaf weight to leaf area for two varieties of <u>Vicia</u> <u>faba</u> and the effects of a 1 week cold temperature treatment.

	Mean Value LW / LA (g cm ⁻² ×100)	Variance
Aquadulce (no cold)	2.63	0•0837
Dylan (no cold)	2.20	0.0469
Aquadulce (cold)	2.90	0·1259
Dylan (cold)	2.51	0.0293

't' Test Results

	't' =	DF =	significance
Aquadulce (no cold)	······································		· · · · · · · · · · · · · · · · ·
v Dylan	$5 \cdot 46$	40	p < 0.001
v Aquadulce (cold)	2.68	40	p < 0.01
Dylan (no cold) v Dylan (cold)	5.04	40	p < 0∙001
Aquadulc e (cold) v Dylan (cold)	4·59	40	p < 0·001

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the variety Dylan suggesting that Aquadulce leaves were thicker. The mean value for Aquadulce plants was 0.026 g cm^{-2} and that for Dylan plants was 0.022 g cm^{-2} (t = 5.457, p < 0.001).

The imposition of cold stress was seen to result in increased leaf weight per unit leaf area in both varieties of <u>Vicia</u>. For Dylan plants the mean value for LW/LA following the 1 week cold treatment rose to 0.025 g cm⁻² and, when compared with the data for non cold-stressed plants, produced a significant t value of 5.042 (p < 0.001). For Aquadulce plants, mean values for LA/LW rose to 0.029 g cm⁻² (t = 2.685, p < 0.01). Plants of the variety Aquadulce were still found to have significantly higher values for LW/LA than Dylan plants when both had been subject to cold temperature stress (t = 4.951, p < 0.001).

These results were confirmed when actual measurements of leaf thickness were compared between the varieties with and without cold stress. A total of 144 measurements were made for each variety for each cold treatment giving a total of 576 data points. A summary of the 't' test analysis is shown in Table 5.2. As was implied from the results presented above, plants of the variety Aquadulce were found to have significantly thicker leaves than plants of the variety Dylan in both treated and non treated plants. Cold temperature stress again was shown to result in significant increases in the leaf thicknesses of both varieties.

These differences in leaf thickness and in the ratio of leaf weight to leaf area are important to the results discussed later in this chapter. Results for chlorophyll, protein and carbohydrate content may be expressed per unit leaf weight or per unit leaf area. However, in the light of the results presented above it was thought that significant differences may be overlooked when expressing results in one way only; for this reason, the data presented in subsequent sections are presented both per unit leaf weight and per unit leaf area.

5.5 CHLOROPHYLL CONTENT OF WHOLE LEAVES

The experimental design consisted of four cold treatments (0, 24 h, 72 h & 1 week) in combination with four SO_z treatments (0, 100, 300 & 500 ppb) for two varieties of <u>Vicia faba</u>. Three determinations of chlorophyll content were made for each treatment. This produced a three

Table 5.2.

Analysis of data for leaf thicknesses of two varieties of <u>Vicia</u> <u>faba</u> and the effects of a 1 week cold temperature (10°C) treatment.

-	Mean Value (mm)	Variance
Aquadulce (no cold)	0•40	0.0121
Dylan (no cold)	0-37	0.0025
Aquadulce (cold)	0•48	0.0036
Dylan (cold)	0 • 45	0.0032

't' Test Results

<u></u>	't' =	DF =	significance
Aquadulce (no cold)			•
v Dylan (")	3.243	286	p < 0.002
v Aquadulce (cold	l) 8·104	286	p < 0.001
Dylan (no cold)			
v Dylan (cold)	13.070	286	p < 0.001
Aquadulce (cold)			
v Dylan (cold)	$5 \cdot 142$	286	p < 0·001

factor factorial design with two levels in the first factor (variety), four levels in the second factor (sulphur dioxide) and four levels in the third factor (cold treatments) giving a total of 144 data points. The data obtained were analysed using Multivariate Analysis of Variance on the Honeywell Multics mainframe computer system using the programme 'GENSTAT' (GENSTAT V Mark 4.C3 © 1980 Lawes Agricultural Trust [Rothampstead Experimental Station]).

The analysis was performed on the data obtained for chlorophyll a per unit leaf weight and per unit leaf area, chlorophyll b per unit leaf weight and per unit leaf area and for total chlorophyll per unit leaf weight and per unit leaf area. However, no significant differences were detected in the influences of either cold or SO₂ on chlorophyll a and b content individually nor in the ratio of chlorophyll a to chlorophyll b and as a result data for total chlorophyll only are presented here. The results for both chlorophyll content per gram fresh weight and per cm² leaf area are presented because changes in the ratio of leaf weight to leaf area were observed in response to cold stress (S5.4). The summaries of the output from the ANOVA programme including F values and levels of significance are presented in Tables 5.3 and 5.4. and it can be seen that the results obtained varied depending on the units used. Therefore data are discussed separately below, initially on a fresh weight basis and, secondly, on a leaf area basis.

5.5.1 Chlorophyll per Unit Leaf Weight

Table 5.3 shows the analysis of variance for chlorophyll content per gram fresh leaf weight. It can be seen that there were significant varietal differences in chlorophyll content and that both cold and pollutant stress influenced chlorophyll content both singly and in combination. Figures 5.3 and 5.4 show mean values for chlorophyll content plotted against sulphur dioxide concentration for both Dylan and Aquadulce plants for each cold treatment, the standard errors of the mean (SEM) are also shown in the figures. It can be seen from these figures that plants of the variety Dylan had a significantly higher chlorophyll content than those of the variety Aquadulce Claudia in the absence of either cold or pollutant stress.

The imposition of cold stress alone resulted in reductions in

TABLE 5.3

Summary of Multivariate Analysis of Variance of Data for Chlorophyll Content of Two Varieties of <u>Vicia</u> <u>faba</u> Subject to both Environmental and Pollutant Stress where Chlorophyll Content is Expressed per Unit Leaf Fresh Weight.

Source of Variation	DF	SS	MS	VR	Sig.
Variety	1	0 • 197	0 · 197	4·75	0.02
Cold	3	7.023	2.341	56·44	0.001
Sulphur Dioxide	. 3	1 • 141	0.380	9·17	0.001
Variety * cold	3	0·175	0.028	1.40	ns
Variety * SO ₂	3	0.377	0-125	3.03	0.02
Cold * SO2	9	1.842	0.204	4-94	0.001
Variety * cold * SO ₂	9	$2 \cdot 905$	0.322	7.78	0.001
RESIDUAL	112	4.645	0.041		
TOTAL	143	18.309	0 · 128		
Grand Mean	= 0	• 87			
Total No. Observation	ns = 1	44			

TABLE 5.4

As for Table 5.3 but Data is Summary of Multivariate Analysis of Chlorophyll a + b Content per Unit Leaf Area.

(× 10 ³)							
Source of Variation	DF	SS	MS	VR	Sig.		
Variety	1	0.042	0.042	2.51	ns		
Cold	3	3 • 184	1.061	62·58	0.001		
Sulphur Dioxide	3	0.571	0•190	11.23	0.001		
Variety * cold	3	0.065	0.021	1.29	ns		
Variety $*$ SO ₂	3	0.136	0.045	2.67	ns		
Cold * SO2	9	0.662	0.073	4.34	0.001		
Variety # cold # SO ₂	9	1.200	0·133	7.86	0.001		
RESIDUAL	112	1.899	0.016				
TOTAL	143	7.762	0.024				
Grand Mean	= 0.	023					
Total No. Observation	s = 14	4					

Figure 5.3



Figure 5.3.

Chlorophyll a + b content (mg g⁻¹ fresh weight) in <u>Vicia</u> faba plants of the variety Dylan subject to either 0, 24 h, 72 h or 1 week at 10°C prior to exposure to either 0, 100, 300 or 500 ppb SO₂ for 4 h.



Figure 5.4



Figure 5.4.

Chlorophyll a + b content (mg g⁻¹ fresh weight) in <u>Vicia</u> <u>faba</u> plants of the variety Aquadulce Claudia subjected to either 0, 24 h, 72 h or 1 week at 10°C prior to exposure to either 0, 100, 300 or 500 ppb SO₂ for 4 h. chlorophyll content in both varieties of <u>Vicia</u>, the reduction appearing to be most severe in Dylan plants (Fig. 5.3). For plants of the variety Dylan, cold periods of 72 h or 1 week resulted in a similar reduction in chlorophyll content, this being greater than the reductions due to 24 h at 10°C prior to SO_2 fumigations. For Aquadulce Claudia plants (Fig. 5.4) reductions in chlorophyll content were similar in response to both the 24 h and 72 h cold treatments but much less chlorophyll was found to be present in plants subject to 1 week of cold stress.

Sulphur dioxide fumigations were found to have significant effects on the chlorophyll content of both varieties in the absence of cold stress. For Dylan plants (Fig. 5.3), 100 ppb for four hours had no significant effect on chlorophyll content per unit fresh weight but concentrations of 300 ppb resulted in significant increases in chlorophyll content whilst 500 ppb SO₂ resulted in significant decreases in chlorophyll content (Fig. 5.3). In plants of Aquadulce Claudia (Fig. 5.4), sulphur dioxide concentrations of 300 ppb had no significant effect on chlorophyll content, however, both 100 and 500 ppb SO₂ resulted in significant increases in chlorophyll content in the absence of cold stress.

From Table 5.3 it can be seen that significant interactions between cold and pollutant stress occurred in influencing chlorophyll content and that significant varietal differences in chlorophyll content in response to both cold and/or pollutant were observed.

In Dylan plants, the effects of cold treatments prior to SO_2 fumigations were to modify the effects of SO_2 (Fig. 5.3). The increases in chlorophyll content in response to 300 ppb SO_2 were not seen to occur when plants had been subject to cold temperature stress prior to SO_2 fumigation. Similarly, the significant decreases in chlorophyll content observed in response to 500 ppb SO_2 under optimum environmental conditions were not seen to occur following periods of cold stress. After periods of 24 h or 72 h cold stress chlorophyll content was significantly increased upon exposure to 500 ppb but periods of 1 week cold stress resulted in no significant changes in chlorophyll content in response to 500 ppb SO_2 .

The effects of sulphur dioxide on chlorophyll content in Aquadulce Claudia plants were also altered as a result of cold temperature pre-treatments (Fig. 5.4). The increases in chlorophyll content observed in response to 100 ppb SO_2 were also observed following cold pre-treatments of 24 h; however, the extent of the increase due to SO_2 was reduced in the Results

cold-stressed plants. This increase in response to 100 ppb SO_2 was not seen to occur after cold stress periods of 72 h or 1 week. Although 300 ppb SO_2 had no significant effect on chlorophyll content in Aquadulce plants in the absence of cold stress, periods of 24 h cold temperature stress resulted in significant increases in chlorophyll content in response to 300 ppb SO_2 . There were no significant effects of 300 ppb SO_2 on chlorophyll content in Aquadulce plants following 72 h or 1 week cold temperature pre-treatments. Similarly, the marked increase in chlorophyll content in response to 500 ppb SO_2 observed in the absence of cold stress was not seen to occur following cold stress pre-treatments of either 72 h or 1 week prior to SO_2 fumigations.

5.5.2 Chlorophyll per Unit Leaf Area

The data for chlorophyll content expressed per unit leaf area are shown in Figures 5.5 and 5.6 for Dylan and Aquadulce Claudia plants respectively and the multivariate analysis of variance was summarised in Table 5.4. Changes in chlorophyll content in response to cold and/or SO_2 showed significant differences from the data described above for chlorophyll content per gram fresh weight.

Firstly, no significant varietal differences in chlorophyll content were found in the absence of either cold or pollutant stress although these were seen to occur when chlorophyll content was expressed per unit leaf weight. The imposition of cold stress alone was still seen to result in reductions in chlorophyll content in both varieties of <u>Vicia faba</u> and the data followed the same patterns described above.

The effects of sulphur dioxide on chlorophyll content per unit leaf area varied from the responses described above. In Dylan plants, the reduction in chlorophyll content per gram in response to 500 ppb SO_{2} , without added cold stress, was not found to be significant when results were expressed per cm² leaf area (Fig. 5.5). Similarly, the increases in chlorophyll content in response to 500 ppb SO_2 following 24 h cold stress described above were not significant when results were calculated per unit leaf area. However, this increase was still seen to occur in response to 500 ppb in Dylan plants subject to 72 h cold stress.

When results for the variety Dylan were expressed per unit leaf weight there were significant differences in the chlorophyll content of Figure 5.5



Figure 5.5. Chlorophyll a + b content (mg cm⁻² leaf area) in <u>Vicia</u> <u>faba</u> plants of the variety Dylan subject to a range of cold temperature treatments, prior to exposure to either 0, 100, 300 or 500 ppb SO₂ for 4 h.



Figure 5.6.

Chlorophyll a + b content (mg cm⁻² leaf area) in <u>Vicia</u> <u>faba</u> plants of the variety Aquadulce Claudia subject to a range of cold temperature treatments, prior to exposure to either 0, 100, 300 or 500 ppb SO₂ for 4 h.

Results

plants subjected to 72 h or 1 week cold stress for all sulphur dioxide fumigations; however, these differences were not observed when chlorophyll content was expressed per unit leaf area with the exception of the 500 ppb SO_2 treatment.

When the results for Aquadulce Claudia plants were expressed per unit leaf area (Fig. 5.6), the data followed the same trends as described previously for chlorophyll content per g fresh weight with the exception of a significant increase in chlorophyll content in response to 100 ppb SO_2 following a 1 week cold pre-treatment.

The results for chlorophyll content of whole leaves of both varieties of <u>Vicia faba</u> are summarised in Table 5.5.

5.6 HILL REACTION ACTIVITY

The experimental design consisted of four cold treatments (0, 24 h, 72 h & 1 week) in combination with sulphur dioxide treatments or control treatments (no SO_2) for both varieties of <u>Vicia faba</u>. Each treatment was replicated four times and two determinations of Hill reaction activity were made for each treatment. This series of experiments was run twice, firstly using 500 ppb SO_2 and secondly using 100 ppb SO_2 in the design described above (hereafter experimental design will be referred to as the 500 or 100 series for case of identification). The data were analysed using the 'Genstat' programme for multivariate analysis of variance, the analyses being performed on data for:

(i) chlorophyll content per unit leaf weight
(ii) chlorophyll content per unit leaf area
(iii) Hill reaction activity per unit chlorophyll
(iv) Hill reaction activity per unit leaf weight and
(v) Hill reaction activity per unit leaf area.

The data for both the 100 and 500 series were not combined as significant differences were found in the Hill reaction activity of control plants (no cold, no SO_2) from both experimental sets. These differences were thought to be due to the use of a fresh stock of the buffer MES in the extraction medium for isolated chloroplasts for the second experimental set

Table 5.5

A summary of combined cold temperature (10°C; 0, 24 h, 72 h or 1 week) and sulphur dioxide (4 h; 0, 100, 300 or 500 ppb) stress effects on total leaf chlorophyll content of two varieties of <u>Vicia faba</u> when results are expressed per unit leaf weight and per unit leaf area.

DYLAN		AQUADULCE CLAUDIA
No stress	Higher than AQ	
SOz	No change at 100 ppb. Increase due to 300 ppb. Decrease due to 500 ppb.	Increase at 100 & 500 ppb. No effect at 300.
Cold	Decrease at all cold periods, greatest at 72 h & 1 week.	Decrease at all cold periods, greatest at 1 week.
SO ₂ × Cold	No effect of 100 or 300 ppb on cold decrease. 500 ppb increases Chl. content after 24 h and 72 h cold.	24 h same as no cold No effect of SO ₂ after 72 h or 1 week.

(i) mg Chl $a + b g^{-1}$ leaf tissue (fresh weight)

(ii) mg Chl $a + b \text{ cm}^{-2}$ leaf area

DYLAN		AQUADULCE CLAUDIA		
No stress	No significant varietal d	lifferences.		
50 ₂	Increase at 300 ppb. No effects of 100 & 500 ppb.	Increase at 100 & 500 ppb. No change at 300 ppb.		
Cold	Decrease at all cold periods, most at 72 h & 1 week.	Decrease at all cold periods, most at 1 week.		
SO ₂ * Cold	Increase at 100 ppb after 24 h cold. No increase at 300 ppb for any cold period. Increase after 500 ppb and 72 h cold.	24 h, increase at 100 & 500. 72 h, decrease at 100. 1 week, increase at 100 ppb only.		

(100 series), although the experimental procedure was unchanged. As a result of this, it was not thought advisable to pool the data obtained and each experimental set is considered separately although trends in the influence of cold and pollutant stress are compared.

5.6.1 Chlorophyll Content of Isolated Chloroplast Suspension

It was necessary to determine the chlorophyll content of the chloroplast suspension to enable Hill reaction activity to be expressed per unit chlorophyll. Chlorophyll content was again determined both per unit leaf weight and per unit leaf area for the reasons outlined in §5.4.

5.6.1.1 Chlorophyll per unit leaf weight

Table 5.6 shows a summary of the analysis of variance of chlorophyll content per gram leaf tissue for both the 500 and the 100 series. It can be seen that there are significant differences between both sets of results and the results presented in Table 5.3 for chlorophyll content of whole leaves (the levels of significance from Table 5.3 are represented in Table 5.6 for ease of comparison). However, a significant amount of chlorophyll was lost due to the nature of the chloroplast extraction procedure and it was not considered appropriate to compare results for isolated chloroplasts with that for whole plants.

It can be seen from Table 5.6 that there were differences in the analysis of variance results for each experimental run, varietal differences are seen to be significant in the 100 series but not in the 500 series. Similarly, significant effects due to cold alone are seen in the results for the 500 series but not the 100 series. These anomalies can be explained more fully when mean values of chlorophyll content are examined. The chlorophyll data per unit leaf weight for both Dylan and Aquadulce Claudia for the 500 series are presented in Figures 5.7 and 5.8, the data for the 100 series being presented in Figures 5.9 and 5.10.

From Figures 5.7 and 5.8 it can be seen that plants of the variety Dylan had a significantly higher chlorophyll content per gram leaf weight than plants of the variety Aquadulce Claudia in the absence of either cold or pollutant stress. The imposition of cold stress alone resulted in significant reductions in the chlorophyll content in Dylan plants (Fig. 5.7), the greatest reduction occurring in response to the 1 week cold

TABLE 5.6

Summary of Multivariate Analysis of Variance of Data for Chlorophyll Content of Isolated Chloroplast Suspensions of Two Varieties of <u>Vicia</u> faba – subject to a range of cold temperature treatments prior to exposure to either 0, 100 or 500 ppb sulphur dioxide (4 h): where chlorophyll content is expressed per unit Leaf Fresh Weight (mg g^{-1}) (A summary of data for chlorophyll content of whole plants is also shown for comparison).

	500 ppb S0 ₂		100 ppb SO2		Whole
	F	α =	F	α =	(α =)
Variety	0.869	ns	15.090	0+001	0.05
50 ₂	0.000	ns	0•935	ns	0.001
Cold	15 • 188	0.001	0•411	ns	0+001
Variety * SO ₂	0.003	ns	0.561	ns	0.05
Variety * Cold	16.350	0.001	2.335	ns	ns
SO ₂ * Cold	2.991	0.05	2.356	ns	0.001
Variety * SO ₂ * Cold	2 • 247	ns	3.030	0.05	0.001

(ns: not significant)

Chlorophyll content – Dylan



Figures 5.7 and 5.8. Chlorophyll content of isolated chloroplast suspensions (mg g⁻¹ leaf fresh weight) of two varieties of <u>Vicia</u> faba subjected to a range of cold temperature treatments prior to exposure to 0 or 500 SO₂ ppb for 4 h. Results

treatment. These reductions in chlorophyll content due to cold were not seen to occur in plants of the variety Aquadulce (Fig. 5.8); indeed, increases in chlorophyll content occurred in response to both the 24 h and the 72 h cold treatments.

The effects of a 4 h fumigation with 500 ppb SO_2 in the absence of cold stress did not significantly alter chlorophyll content in Dylan plants; however, significant increases in chlorophyll content were observed in plants of Aquadulce Claudia in response to the SO_2 treatment.

The imposition of cold stress prior to sulphur dioxide fumigation did not result in changes in the effects of SO_2 on chlorophyll content in plants of the variety Dylan (Fig. 5.7); sulphur dioxide exposure was not seen to significantly alter chlorophyll content in any plants subject to cold pre-treatments. However, cold pre-treatments were seen to alter the effects of SO_2 on the chlorophyll content of plants of Aquadulce Claudia (Fig. 5.8). The significant increase in chlorophyll content, observed in response to SO_2 in the absence of cold stress, did not occur following any of the cold pre-treatments. In plants of Aquadulce subject to 24 h at 10°C prior to fumigation with 500 ppb SO_2 , chlorophyll content was significantly decreased in response to the sulphur dioxide treatment although there was no significant change in chlorophyll content due to SO_2 in plants subject to a 1 week period of cold stress.

The data for both varieties for the 100 ppb SO₂ series are presented in Figures 5.9 and 5.10. It can be seen from the figures that, in the absence of either cold or pollutant stress, there were no significant varietal differences observed in chlorophyll content calculated per gram leaf tissue. The imposition of cold stress alone resulted in decreased chlorophyll content in plants of the variety Dylan thus re-affirming the results obtained in the 500 series. However, plants of the variety Aquadulce Claudia also exhibited reductions in chlorophyll content; these changes not being observed in the 500 series. Exposure to 100 ppb SO₂ without cold pretreatments was seen to result in reductions in chlorophyll content in both varieties of V.faba, the greatest reduction being observed in Dylan plants (Fig. 5.9). For Aquadulce plants, the reduction in chlorophyll content due to SO_2 was also seen to occur following cold pre-treatments of either 72 h or 1 week but the extent of this reduction was lessened (Fig. 5.10). SO_2 was not seen to influence chlorophyll content in Aquadulce plants after a 24 h cold pre-treatment. In plants of the variety Dylan, cold periods of 24 h or

Chlorophyll content – Dylan



Figures 5.9 and 5.10. Chlorophyll content of isolated chloroplast suspensions (mg g⁻¹ leaf fresh weight) of two varieties of <u>Vicia</u> faba subjected to a range of cold temperature treatments prior to exposure to 0 or 100 ppb SO_2 for 4 h. 72 h prior to SO_2 fumigations resulted in there being an increase in chlorophyll content per gram leaf tissue in response to 100 ppb SO_2 . There were no significant effects of 100 ppb SO_2 on chlorophyll content in Dylan plants subject to 1 week cold pre-treatments.

5.6.1.2 Chlorophyll content per unit leaf area

When the chlorophyll content of isolated chloroplast suspensions from both varieties of <u>Vicia</u> were calculated per unit leaf area there were a number of significant differences in the effects of cold and/or SO_2 in comparison with the data for chlorophyll content per unit leaf weight (\$5.6.1.1). These differences were more readily apparent when mean values for chlorophyll content were considered as opposed to the analysis of variance data. Table 5.7 is a summary of the multivariate analysis of variance data for chlorophyll per unit leaf area.

It can be seen from comparison of Tables 5.6 and 5.7 that the data for the 100 series produced the same results for both calculations of chlorophyll content. However, in the 500 series when results were expressed per unit leaf weight, no significant differences in chlorophyll content were observed arising from variety alone but when the results were expressed per unit leaf area, this factor was found to be significant.

The mean values for chlorophyll content of isolated chloroplast suspensions per cm² leaf area for the 500 series of experiments are depicted in Figures 5.11 and 5.12. Results for plants of the variety Dylan are presented in Figure 5.11 and upon comparison with Figure 5.7 it can be seen that the effects of 500 ppb SO_2 on plants not subject to cold stress were significant. Sulphur dioxide was seen to significantly reduce chlorophyll content per unit leaf area in Dylan plants, this reduction occurring in both non cold-stressed and 24 h cold-stressed plants but not after the longer cold treatments; exposure to 500 ppb SO_2 after a 1 week cold treatment was seen to result in a significant increase in chlorophyll content per unit leaf area. Sulphur dioxide effects were not significant at any of the cold treatments when results were expressed per unit leaf weight (Fig.5.7).

The data for chlorophyll content of Aquadulce Claudia plants per unit leaf area for the 500 series, are presented in Figure 5.12 and upon comparison with Figure 5.8 it can be seen that there were no significant differences in the effects of SO_2 and/or cold when chlorophyll content was

TABLE 5.7

Summary of Multivariate Analysis of Variance of Data for Chlorophyll Content of Isolated Chloroplast Suspensions of Two Varieties of <u>Vicia faba</u> subject to a range of cold temperature treatments prior to exposure to either 0, 100 or 500 ppb sulphur dioxide (4h): where chlorophyll content is expressed per unit Leaf Area (A summary of data for chlorophyll content of whole plants is also shown for comparison).

· · ·	500 pp]	50 ₂	100 ppb	S0₂	Whol e Leaf
	F	α =	F	α =	(α =)
Variety	5.509	0.025	41.170	0-001	ns
SO2	0.067	ns	0.212	ns	0.001
Cold	6•083	0.01	0 • 190	ns	0.001
Variety * SO ₂	0.009	ns	0.006	ns	ns
Variety * Cold	9.345	0.001	0.790	ns	ns
SO ₂ * Cold	3.322	0.02	1-837	ns	0.001
Variety $*$ SO ₂ $*$ Cold	1.683	ns	3.661	0.01	0.001



Figures 5.11 and 5.12.

Chlorophyll content of isolated chloroplast suspensions (mg cm⁻² leaf area) of two varieties of <u>Vicia faba</u> subjected to a range of cold temperature treatments prior to exposure to 0 or 500 ppb for 4 h.

calculated per unit leaf weight or area; changes in chlorophyll content due to either stress being the same as those described in §5.6.1.1.

The results for chlorophyll content per unit leaf area of isolated chloroplast suspensions from plants of the 100 series are presented in Figures 5.13 and 5.14. There were no significant differences in the nature of changes in chlorophyll content in plants of the variety Dylan for the 100 series when results were expressed per unit leaf weight (Fig. 5.9) or per unit leaf area (Fig. 5.13). Changes due to cold and/or SO₂ being described in S5.6.1.1. Figure 5.14 shows the data for the variety Aquadulce and upon comparison with Figure 5.10 significant differences are observed. The reduction in chlorophyll content due to SO₂ alone, in the absence of cold stress, was not seen to occur when results were expressed per unit leaf area although reductions due to cold stress alone were still noted. Sulphur dioxide fumigations did not alter the chlorophyll content in Aquadulce plants subject to either 24 h or 72 h cold temperature stress, however, the decrease due to SO₂ following a 1 week cold pre-treatment observed per unit leaf weight was enhanced when results were expressed per unit leaf area.

The results presented in this section can be summarised as follows:

(i) The imposition of cold temperature stress alone resulted in decreases in chlorophyll content of both varieties of <u>Vicia faba</u>. However, these decreases appeared to be significantly greater in plants of the variety Dylan.

(ii) Exposure to 500 ppb sulphur dioxide in the absence of cold stress resulted in decreased chlorophyll content in Dylan plants but chlorophyll content was increased in response to 500 ppb SO_2 in plants of the variety Aquadulce Claudia.

(iii) Exposure to 100 ppb SO_2 in the absence of cold stress induced a reduction in the chlorophyll of both varieties, the reduction being greatest in plants of the variety Dylan.

(iv) The imposition of cold temperature stress prior to SO_2 fumigations resulted in changes in the effects of SO_2 on chlorophyll content of both varieties. SO_2 fumigations of 500 ppb did not alter chlorophyll content in Dylan plants previously subjected to 24 h or 72 h





Figures 5.13 and 5.14. Chlorophyll content of isolated chloroplast suspensions (mg cm⁻² leaf area) of two varieties of <u>Vicia faba</u> subjected to a range of cold temperature treatments prior to exposure to 0 or 100 ppb for 4 h. cold stress; however, exposure to 100 ppb SO_2 resulted in increases in chlorophyll content. In Dylan plants subject to 1 week cold stress 500 ppb SO_2 induced small increases in chlorophyll content; exposure to 100 ppb did not result in any changes in chlorophyll content.

In plants of Aquadulce Claudia, exposure to 100 ppb SO_2 did not alter chlorophyll content after 24 h and 72 h cold temperature treatments but after the 1 week treatments small decreases in chlorophyll content were observed. However, 500 ppb SO_2 decreased chlorophyll content in plants subject to 24 h or 72 h cold stress prior to SO_2 fumigation and did not affect plants subject to 1 week cold stress.

5.6.2 Hill Reaction Activity Per Unit Chlorophyll

The significance of the effects of variety, cold and sulphur dioxide on rates of Hill Reaction activity are shown in Table 5.8 in a summary of the GENSTAT multivariate analysis of variance for both the 100 and 500 experimental series.

TABLE 5.8.

Summary of Multivariate Analysis of Variance of Data for Hill Reaction Activity per Unit Chlorophyll of Isolated Chloroplast Suspensions of Two Varieties of <u>Vicia faba</u> - subject to a range of cold temperature treatments prior to exposure to either 0, 100 or 500 ppb sulphur dioxide (4 h).

	500 ppb SO _z		100 pp	b SO ₂
	F	α =	F	α =
Variety	6•633	0.025	1 • 454	ns
SO ₂	0•225	ns	0-782	ns
Cold	1-257	ns	4.952	0.01
Variety * SO ₂	0•006	ns	2 · 182	ns
Variety * Cold	2.669	ns	5.204	0.01
SO ₂ * Cold	2 • 963	0.02	2.796	0.05
Variety * SO ₂ * Cold	3.089	0.02	0.141	ns

It can be seen from the table that there were a number of significant differences in the results obtained for the two sets of experiments. Varietal differences in reaction rate were seen to occur in the 500 series but were not significant in the 100 series. However, cold stress alone was not a significant influencing factor in the 500 series but was found to be important in the 100 series. Although SO₂ alone was not seen to be a significant variable, in both experimental sets interactions between cold and SO₂ had significant effects on Hill reaction activity. These anomalies can be discussed in relation to the mean values for the Hill reaction activity of both varieties in each of the experimental runs. Figures 5.15 - 5.18 present the data obtained for both varieties for Hill reaction activity per unit chlorophyll in both the 100 and 500 series.

5.6.2.1 <u>500 Series</u>

The data for Dylan plants exposed to 0 or 500 ppb SO_2 are shown in Figure 5.15 and that for Aquadulce plants in Figure 5.17. It can be seen that in the absence of either cold or pollutant stress the variety Aquadulce had a far greater reaction activity per unit chlorophyll than plants of the variety Dylan.

The imposition of cold stress alone resulted in significant increases in Hill reaction activity in plants of the variety Dylan subject to 72 h or 1 week cold stress; the reaction rate being more than doubled in response to the 1 week cold treatments. In Aquadulce plants the reverse was seen to occur, Hill reaction activity being reduced in response to cold stress and the slowest rates being measured in plants subject to the 72 h and 1 week cold treatments.

The response of both varieties to SO_2 in the absence of cold stress were, again, significantly different. In Dylan plants 500 ppb SO_2 did not substantially alter reaction rates but in plants of Aquadulce Claudia reaction rates were seen to be reduced by two thirds.

Cold temperature pre-treatments were seen to significantly alter the effects of sulphur dioxide on Hill reaction rates in both varieties of <u>Vicia</u>. In Dylan plants subject to 72 h at 10 °C prior to SO₂ fumigation, SO₂ induced a large increase in activity but following the 1 week cold treatments, SO₂ was seen to halve the reaction rate so that plants subject to both 500 ppb SO₂ and 1 week cold stress did not have significantly different reaction rates to those of un-stressed plants. In plants of

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chloroplast suspensions of two varieties of Vicia faba subjected to a range of cold temperature treatments prior to exposure to either 0, 100 ppb or 500 ppb SO_2 for 4 h. CONTROL

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Aquadulce Claudia, the cold pre-treatments halted the marked decreases in activity due to SO_2 which were seen to occur in non cold-stressed plants. At all cold treatments, SO_2 was seen to enhance Hill reaction activity, the decreases in activity due to cold alone being virtually negated following SO_2 exposure.

5.6.2.2 <u>100 Series</u>

The data for Hill reaction activity of isolated chloroplasts of both varieties for the 100 series are shown in Figures 5.16 and 5.18. Upon comparison with Figures 5.15 and 5.17 it can be seen that the results differed from those from the 500 series. Aquadulce plants were still seen to have significantly greater reaction rates than Dylan plants in the absence of either cold or pollutant stress but these differences were much less marked than those observed in the 500 series. For Dylan plants (Fig. 5.16) the increases in activity due to longer cold stress periods alone were not as marked as those observed in the 500 series although trends were the same. The effect of 100 ppb SO₂ in the absence of cold stress, was to reduce Hill reaction rates in Dylan plants although 500 ppb was not seen to influence activity. This decrease in activity due to 100 ppb SO₂ was also seen to occur after the 72 h cold pre-treatments however, in Dylan plants subject to 1 week cold stress prior to SO₂ funigation, the imposition of SO₂ lead to a marked increase in Hill reaction activity per unit chlorophyll.

The data for plants of Aquadulce Claudia for Hill reaction activity per unit chlorophyll for the 100 series are shown in Figure 5.18. The imposition of cold stress alone was not seen to influence rates of activity in the shorter cold treatments; however, rates were significantly reduced in plants subject to 1 week cold stress. Exposure to 100 ppb SO_2 lead to decreased activity in the absence of cold stress although this decrease was not as great as that observed in response to 500 ppb SO_2 (Fig. 5.17). Cold temperature pre-treatments prior to SO_2 fumigation resulted in a lessening in the inhibitory effect of SO_2 ; SO_2 had no observable significant effect on Hill reaction activity following all cold pretreatments.

Whilst the above section has discussed changes in Hill reaction activity per unit chlorophyll, it was shown earlier (\$5.5) that the chlorophyll content of both varieties of <u>Vicia faba</u> was markedly influenced by the imposition of either cold or pollutant stress. For this reason, rates of Hill reaction activity were also expressed per unit leaf weight and per unit leaf area.

5.6.3 Hill reaction activity per Unit Leaf Fresh Weight

5.6.3.1 500 ppb SO, Series

The data for Dylan and Aquadulce Claudia plants for the 500 series are shown in Figures 5.19 and 5.21. Prior to the imposition of either cold or pollutant stress significant varietal differences in reaction rate were observed in both experimental series, chloroplasts extracted from plants of the variety Aquadulce Claudia having significantly greater reaction rates than those from the variety Dylan.

For isolated chloroplasts from the variety Dylan (Fig. 5.19) the imposition of cold stress alone was seen to reduce Hill reaction activity, the reduction being inversely proportional to the length of the cold treatment. Exposure to 500 ppb SO₂ in the absence of cold stress led to increased activity in Dylan plants, however, following cold treatments the effects of SO₂ were significantly altered. There was no effect of SO₂ following the 24 h cold pre-treatments and cold temperature pre-treatments of 72 h resulted in there being greatly enhanced rates of activity in response to the SO₂ treatments. Conversely, the imposition of cold temperature stress for 1 week prior to SO₂ fumigation resulted in severe reductions in Hill reaction activity in Dylan chloroplasts in response to the SO₂ treatments.

For the variety Aquadulce Claudia (Fig. 5.21) the imposition of cold stress alone was seen to alter reaction rates, the nature of the change depending on the length of the cold stress periods. After 24 h at 10°C Hill reaction activity was enhanced, there was no change in activity in response to the 72 h cold treatments and 1 week cold periods resulted in large reductions in Hill reaction activity of Aquadulce chloroplasts. The imposition of 500 ppb SO₂ for 4 h in the absence of cold stress resulted in severe reductions in activity to one-third the rate of unstressed plants. However, cold temperature stress prior to SO₂ exposure was seen to inhibit the effects of SO₂ on Hill reaction activity. There were no changes in reaction rates in response to SO₂ following the 24 h cold pre-treatments Results



isolated chloroplast suspensions of two varieties of <u>Vicia faba</u> subjected to a range of cold temperature treatments prior to exposure to either 0, 500 ppb or 100 ppb SO_2 for 4 h.

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and SO_2 was seen to enhance Hill reaction activity in plants subject to either 72 h or 1 week at 10°C prior to SO_2 fumigation.

5.6.3.2 100 ppb SO₂ Series

As for the data presented in \$5.6.2 the data for the 100 ppb $\$0_2$ series differed from that for the 500 series and chloroplasts from plants of the variety Aquadulce were not seen to have slightly higher rates of activity than those for the variety Dylan. The data for chloroplasts extracted from plants of the variety Dylan are shown in Figure 5.20 and it can be seen that the imposition of 100 ppb $\$0_2$ in the absence of cold stress resulted in much reduced rates of Hill reaction activity. Cold temperature stress alone was seen to reduce activity only in the 24 h treatments, there being no significant change in activity in response to the 72 h cold treatments or the 1 week cold treatments.

The imposition of cold stress resulted in changes in the subsequent effects of SO_2 such that no decreases in activity due to SO_2 exposure were observed in any plant subjected to cold temperature stress prior to SO_2 fumigation. SO_2 was seen to enhance Hill reaction activity of isolated chloroplasts of Dylan plants following the 24 h and 1 week cold treatments and had no observable effect on Dylan plants subject to the 72 h cold pre-treatments.

The data for chloroplasts isolated from plants of the variety Aquadulce Claudia are presented in Figure 5.22 and it can be seen that cold temperature stress alone resulted in decreased rates of Hill reaction activity per unit leaf area. Similarly, the effect of 100 ppb SO₂ in the absence of cold stress was to inhibit Hill reaction activity. However, the effects of SO₂ were seen to alter when plants had been previously subject to cold temperature stress such that there were no observable effects of SO₂ on Hill reaction activity of chloroplasts of plants subject to either 24 h or or 72 h or 1 week at 10°C prior to SO₂ fumigations.

5.6.4 Hill Reaction Activity per Unit Leaf Area

The data for Hill reaction activity of isolated chloroplast suspensions per unit leaf area of both varieties of <u>Vicia</u> faba are shown in Figures 5.23 to 5.26 for both the 100 ppb and the 500 ppb SO_2 experimental series. In the absence of either cold or pollutant stress chloroplasts Results

extracted from plants of the variety Aquadulce were still seen to have significantly higher reaction rates than those taken from plants of the variety Dylan.

5.6.4.1 500 ppb SO, Series

The data for Dylan plants are shown in Figure 5.23 and it can be seen that when results are expressed per unit leaf area SO_2 did not alter Hill reaction rates in the absence of cold stress. Similarly, the imposition of either 72 h or 1 week periods of cold stress alone did not alter reaction rates in Dylan plants; however, a cold stress period of only 24 h resulted in severe reductions in Hill reaction activity. Sulphur dioxide did not further reduce activity following the 24 h cold pre-treatments but after the longer cold periods the effects of SO_2 were significantly altered. In Dylan plants subject to 72 h at 10°C prior to SO_2 fumigations there were marked increases in activity in response to the pollutant. Conversely, in plants subject to 1 week at 10°C sulphur dioxide was seen to significantly reduce Hill reaction activity of the isolated chloroplast suspensions.

The data for Aquadulce plants are shown in Figure 5.25 and it can be seen that in the absence of cold stress SO_2 severely inhibited Hill reaction activity. Cold stress alone was seen to result in significant changes in activity but the nature of these changes was dependent on the length of the cold treatment. Exposure to cold temperature stress for 24 h resulted in enhanced Hill reaction activity in Aquadulce Claudia plants, there being no change in response to the 72 h cold treatments and a marked reduction in activity occurring in response to the 1 week cold treatments. Again, cold temperature pre-treatments influenced the effects of the SO_2 treatments such that there was no change in activity in response to SO_2 following a 24 h cold pre-treatment and there were substantial enhancements in activity in response to SO_2 fumigations following either 72 h or 1 week cold stress periods.

5.6.4.2 100 ppb SO, Series

The data for Dylan plants are shown in Figure 5.24, unlike the response to 500 ppb SO₂ shown in Figure 5.23, the imposition of 100 ppb SO₂ resulted in inhibitions in Hill reaction activity in the absence of cold stress. However, the reductions in rates of activity in response to 24 h cold temperature stress observed in the 500 series, were still seen to occur

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ppb or 100 ppb SO₂ for 4 h.

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in the 100 series, there being no observable effects of either 72 h or 1 week cold stress periods on reaction rates. Cold temperature stress again altered the sensitivity of plants to SO_2 such that there were no significant changes in Hill reaction activity in response to SO_2 following the 24 h and 1 week cold treatments, although SO_2 was still seen to inhibit activity in plants subject to 72 h cold pre-treatments.

The data for plants of the variety Aquadulce are presented in Figure 5.26, as for the 500 series SO_2 was still seen to severely inhibit reaction rates in the absence of cold stress. The effects of cold stress alone were only significant in the 24 h treatments which resulted in a small decrease in activity, there being no significant changes in activity in response to either 72 h or 1 week cold stress periods. As for the 500 series, cold temperature pre-treatments were seen to alter the effects of subsequent SO_2 treatments such that no inhibition due to SO_2 was seen to occur in cold-stressed plants and SO_2 enhanced Hill reaction activity in Aquadulce plants previously subject to 24 h or 1 week at 10°C.

5.7 LEAF PROTEIN CONTENT

The experimental design consisted of four cold treatments (0, 24 h, 72 h & 1 week) in combination with two sulphur dioxide treatments (0 & 500 ppb) for both varieties of <u>Vicia faba</u>. Each treatment was duplicated and six determinations of protein content were made for each treatment giving a total of 192 data points. The data was analysed using both Multivariate Analysis of Variance using the 'GENSTAT' programme described in \$5.5 for chlorophyll content and two sample 't' tests. The analysis was performed on data for both protein content per unit leaf weight and per unit leaf area and the data discussed separately below.

5.7.1 Protein Content per Unit Leaf Fresh Weight

The summaries of the output from the ANOVA programme are shown in Tables 5.9 and 5.10. Table 5.9 shows the analysis of variance for protein content per gram leaf tissue. It can be seen that there were significant varietal differences in protein content and that both cold and pollutant stress resulted in changes in leaf protein content both singly and in

TABLE 5.9

Summary of Multivariate Analysis of Variance of Data for Protein Content of Two Varieties of <u>Vicia faba</u> Subject to both Low Temperature (10°C: 0, 24 h, 72 h or 1 week) and/or Sulphur dioxide (4 h: 0, 500 ppb) Stress where Protein Content is Expressed per Unit Leaf Fresh Weight (mg g⁻¹).

Source of Variation	DF	SS	MS	VR -	Sig.
Sulphur dioxide	1	2815.6	2815.6	3.856	0.05
Variety	1	4850 • 1	4850 - 1	6.642	0.01
Cold	3	17247.3	5749·1	7.873	0.001
SO ₂ * variety	1	911-8	911·8	1.249	ns
SO ₂ * cold	3	9842·9	3281-0	4.493	0.001
Variety * cold	3	11675 • 4	3891.8	5.329	0.001
SO ₂ * Variety * cold	3	354.3	118.1	0.162	ns
RESIDUAL 17	2 (4)	125604.3	730-3		
TOTAL	187	173301.8	926•7		
Grand Mean	= 11	1-1			
Total No. Observations	3 = 19	2			
Missing Values	= 4				

TABLE 5.10

As for Table 5.9 but Data is Summary of Multivariate Analysis of Protein Content per Unit Leaf Area (mg cm⁻²).

Source of Variation	DF	SS	MS	VR	Sig.
Sulphur dioxide	1	1-926	1.926	4.687	0.02
Variety	1	13.558	13.558	32.987	0.001
Cold	3	15.982	5-327	12.961	0.001
SO ₂ * variety	1	$1 \cdot 276$	1.276	3.107	ns
SO ₂ * cold	3	6.093	2.031	4.942	0.01
Variety * cold	3	4.942	1.647	4.008	0.01
SO ₂ * Variety * cold	3	$1 \cdot 435$	0 • 478	1.164	ns
RESIDUAL 17	2 (4)	70.694	0.411		
TOTAL	187	115.913	0-619	4	
Grand Mean	= 2	• 54			
Total No. Observation	s = 1	92			
Missing Values	= 4				

combination. However, a combination of all three factors of variety, cold and sulphur dioxide did not show significant interactions. These results are explained more fully when plots of protein content against the length of the cold pre-treatment for both SO_2 stressed and unpolluted plants are examined. These are presented in Figures 5.27 to 5.30.

Figures 5.27 and 5.28 show the data for both Dylan and Aquadulce Claudia plants for protein content per gram leaf tissue. Each data point is a mean of twelve values and the standard errors of the mean (SEM) are also shown in the figures. It can be seen that in the absence of either cold or pollutant stress, plants of the variety Dylan had a significantly higher protein content than plants of the variety Aquadulce Claudia; the mean value for Dylan was 96.7 mg g⁻¹ and 86.0 mg g⁻¹ for Aquadulce plants. A twosample 't' test gave a value of 2.083 which was significant at the 97.5% level.

The effect of a four hour fumigation with 500 ppb SO₂ in the absence of added cold stress, was to markedly increase the total soluble protein content in the leaves of plants of both varieties of <u>Vicia</u> up to 123.2 mg g⁻¹ in Dylan and 112.2 mg g⁻¹ in Aquadulce Claudia plants. Again, protein content was significantly higher in Dylan plants than in Aquadulce Claudia (t = 1.883, p < 0.05).

The imposition of cold stress in the absence of sulphur dioxide fumigations resulted in changes in protein content in both Dylan and Aquadulce Claudia plants. In plants of the variety Dylan (Fig. 5.27) a period of 24 h at 10°C resulted in small but significant reductions in total soluble protein content. However, a period of 72 h cold stress resulted in a marked increase in protein content from a mean value of 96.7 mg g⁻¹ to 115.8 mg g⁻¹, this representing an increase of almost 20%. Periods of 1 week at 10°C did not result in any significant changes in soluble protein content in Dylan plants although the variance of the data increased markedly as can be seen in Figure 5.27.

Varietal differences were found to be most marked when changes in protein content due to cold stress alone were considered. Unlike the responses described above for Dylan plants, the effects of cold stress on plants of the variety Aquadulce (Fig. 5.28) were to induce significant increases in total soluble leaf protein content. The extent of this increase was independent of the length of the cold period; for all three cold treatments there was an increase in protein content between 42.5 and 44.2%.

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Protein content – Dylan



Figures 5.27 and 5.28.

Total soluble protein content of leaves of two varieties of <u>Vicia faba</u>, Dylan (Fig.5.27) and Aquadulce Claudia (Fig.5.28) exposed to SO_2 (0, 500 ppb:4 h) and/or a range of cold temperature (10°C) pre-treatments. Results are expressed per unit leaf fresh weight (mg protein g^{-1}) leach point is a mean of twelve values and standard errors of the means are also shown].

These changes in protein content in response to cold stress in both varieties resulted in Aquadulce Claudia plants having significantly higher leaf protein content than the variety Dylan following periods of 24 h and 1 week at 10°C (t = 3.176, p < 0.005 for 24 h and t = 1.583, p < 0.01for 1 week). After the 72 h cold treatment there were no significant differences in protein content between the varieties.

The imposition of cold temperature stress prior to sulphur dioxide funigation resulted in changes in the effects of SO₂ on protein content in both varieties of <u>Vicia</u>. In the variety Dylan (Fig. 5.27) the marked increase in total soluble protein content in response to 500 ppb SO₂ observed in the absence of cold stress was not seen to occur following 24 h or 1 week cold temperature stress prior to SO₂ funigation. SO₂ did not affect protein content in Dylan plants previously subjected to 24 h or 1 week treatments. A significant increase in protein content due to SO₂ was observed in Dylan plants subject to 72 h cold stress prior to SO₂ funigations. This was an increase of 17% in comparison with the 27% increase observed in the absence of cold stress. However, because the 72 h cold treatment also increased leaf protein content in the absence of SO₂, the total increase in protein content after both SO₂ and 72 h cold stress was 40% rising from 96.7 mg g⁻¹ to 135.5 mg g⁻¹ (mean values).

In plants of the variety Aquadulce Claudia (Fig. 5.28) the marked increase in protein content due to SO_2 in the absence of cold stress was not seen to occur following periods of cold stress prior to SO_2 fumigations. Sulphur dioxide had no significant effect on protein content following cold stress periods of 24 h or 72 h. However, SO_2 fumigation resulted in reductions in leaf protein content in plants previously subjected to 1 week cold temperature stress, although cold stress alone increased leaf protein content such that the combined effect of both SO_2 and 1 week cold still resulted in a significantly higher protein content than was observed in the absence of either stress. The mean protein content of un-stressed plants was $86\cdot1$ mg g⁻¹ and was $105\cdot8$ mg g⁻¹ following both the 1 week cold pre-treatment and 500 ppb SO_2 .

In the absence of either stress Dylan plants were shown to have significantly higher total soluble protein per gram leaf tissue than Aquadulce plants. However, the influence of cold temperature stress on the effects of sulphur dioxide resulted in the variety Aquadulce Claudia having a significantly higher leaf protein content than Dylan plants following a Results

24 h cold pre-treatment in combination with a 4 h fumigation with 500 ppb SO_z (t = 3.208, p < 0.005). There were no significant differences between the varieties in protein content following 72 h or 1 week cold stress and sulphur dioxide fumigations.

5.7.2 Protein Content per Unit Leaf Area

When protein content was calculated per cm^2 leaf area multivariate analysis of variance produced the same trends (Table 5.10) as were found for protein per gram leaf fresh weight (Table 5.9). Figures 5.29 and 5.30 show this measure of protein content for each cold pre-treatment for both varieties of <u>Vicia</u>. It can be seen that responses to cold temperature stress of Aquadulce Claudia plants (Fig. 5.30) were the same as described above (§5.7.1) ie. an increase in protein content in response to cold stress. However, in plants of the variety Dylan, the reduction in protein content observed per gram fresh weight following the 24 h cold pre-treatment was not found to be significant when results were analysed for protein content per cm^2 leaf area (Fig. 5.29). There was also a significant increase in leaf protein content in Dylan plants in response to the 1 week cold pre-treatment although this increase was not found to be significant when results were expressed per unit leaf weight.

Responses to sulphur dioxide in plants of the variety Dylan were unchanged when results were expressed per unit leaf weight or leaf area but the decrease in protein content in Aquadulce Claudia plants in response to SO_2 after the 1 week cold pre-treatment was not found to be significant when results were expressed per unit leaf area.

An important point is that varietal differences in protein content in the absence of either cold or pollutant stress are not seen to occur when results are expressed per unit leaf area although Dylan plants were shown to have a greater leaf protein content than Aquadulce plants when results were expressed per unit leaf weight. However, plants of the variety Aquadulce were found to have significantly higher total leaf soluble protein content than Dylan plants after all cold pre-treatments. These varietal differences were not seen to occur following sulphur dioxide fumigations in plants previously subjected to the 72 h and 1 week cold treatments.

Protein content – Dylan



Figures 5.29 and 5.30.

Total soluble protein content of leaves of two varieties of <u>Vicia faba</u>, Dylan (Fig. 5.27) and Aquadulce Claudia (Fig. 5.28) exposed to SO_2 (0, 500 ppb:4 h) and/or a range of low temperature (10°C) pre-treatments. Results are expressed per unit leaf area (mg protein cm⁻²) leach point is a mean of twelve values and standard errors of the means are also shown].

In short, both sulphur dioxide and cold stress influenced total soluble leaf protein content in both varieties of <u>Vicia</u> but the effects of sulphur dioxide were reduced by the imposition of cold stress prior to SO_2 fumigation. The interactive effects of both cold and SO_2 were dependent on variety as both varieties responded differently to the combined stresses.

5.8 CARBOHYDRATE CONTENT

The experimental design consisted of four cold treatments (0, 24 h, 72 h or 1 week at 10°C) in combination with two sulphur dioxide treatments (0 or 100 ppb SO₂: 4 h) for both varieties of <u>Vicia faba</u>. Each treatment was duplicated and four replicate determinations of carbohydrate content were made for each treatment giving a total of 128 data points. The summaries of the 'GENSTAT' multivariate analysis of variance are shown in Tables 5.11 and 5.12 for data for carbohydrate content per unit leaf weight and per unit leaf area. It can be seen from the tables that all three factors - variety, cold and sulphur dioxide - resulted in significant changes in the carbohydrate content of isolated chloroplast suspensions both singly and in combination.

5.8.1 Carbohydrate Content per Unit Leaf Weight

The mean values for carbohydrate content per gram leaf weight are shown in Figures 5.31 and 5.32 for chloroplasts isolated from Dylan and Aquadulce Claudia plants; the standard errors of the mean are also shown. It can be seen from the figures that in the absence of either cold or pollutant stress, plants of the variety Dylan had a significantly higher carbohydrate content than plants of the variety Aquadulce, the mean value for Dylan being 12.5 mg g⁻¹ and 3.7 mg g⁻¹ for Aquadulce Claudia plants. A two sample t test gave a t value of 2.569 which was significant at the 95% level.

The effects of a four hour fumigation with 100 ppb sulphur dioxide in the absence of added cold stress, were to reduce carbohydrate contents in both varieties of <u>Vicia faba</u> (t = 2.263, p < 0.025 for Dylan plants; t = 4.555, p < 0.001 for Aquadulce plants). Dylan plants exhibited a greater reduction than plants of Aquadulce, the mean values after SO_2

TABLE 5.11

Summary of Multivariate Analysis of Variance of Data for Carbohydrate Content of Two Varieties of <u>Vicia faba</u> Subject to both Low Temperature (10°C: 0, 24 h, 72 h or 1 week) and/or SO_2 (0, 100 ppb: 4 h) where Carbohydrate Content of Isolated Chloroplast Suspensions is Expressed per Unit Leaf Fresh Weight (mg g⁻¹).

Source of Variation	DF	SS	MS	VR	Sig.
Sulphur dioxide	1	63 - 48	63 • 48	6.024	0.025
Variety	1	79.32	79.32	7.528	0.01
Cold	3	416·19	138 • 73	13.166	0.001
SO ₂ * variety	1	65 • 74	65 74	6 • 239	0.025
SO ₂ * cold	3	156.03	52.01	4 • 936	0.01
Variety * cold	3	334-26	111.42	10.574	0.001
SO ₂ * Variety * cold	3	1 13 · 22	37.74	3.582	0.025
RESIDUAL	112	1180-18	10.74		
TOTAL	127	2408 • 42	18.96		
Grand Mean	= 5	• 79			
Total No. Observation	s = 12	28			

TABLE 5.12

As for Table 5.11 but Data is Summary of Multivariate Analysis of Carbohydrate Content per Unit Leaf Area (mg cm⁻²).

Source of Variation	DF	S S	MS	VR	Sig.	
Sulphur dioxide	1	0.036	0.035	6•588	0.05	
Variety	1	0.009	0.009	1.834	ns	
Cold	3	0.365	0.122	22.352	0.001	
SO ₂ * variety	1	0.026	0.026	4 · 781	0.02	
SO ₂ * cold	3	0.064	0.021	3.913	0.025	
Variety * cold	3	0·142	0.047	8 • 719	0.001	
SO ₂ * Variety * cold	3	0.062	0.021	3.962	0.025	
RESIDUAL	112	0.610	0.005			
TOTAL	127	1.319	0.010			
Grand Mean	= 0	• 14				
Total No. Observations = 128						
Missing Values	= 4					





Figures 5.31 and 5.32.

Carbohydrate content of isolated chloroplast extracts of two varieties of Vicia faba, Dylan (Fig. 5.31) and Aquadulce Claudia (Fig. 5.32) exposed to SO_2 (0, 100 ppb: 4 h) and/or a range of low temperature (10°C) pretreatments. Results are expressed per unit leaf weight (mg g⁻¹) leach point is a mean of eight values: standard errors of the means are also shownl. fumigation being 4.7 mg g⁻¹ for Dylan and 2.6 mg g⁻¹ for Aquadulce. These figures represented a 60% reduction in Dylan plants and a 30% reduction in Aquadulce Claudia plants. However, carbohydrate content was still significantly higher in Dylan than in Aquadulce Claudia plants (t = 4.434, p < 0.002).

The imposition of cold stress in the absence of SO_2 fumigations resulted in significant changes in the carbohydrate contents of both Dylan and Aquadulce plants. However, both varieties were seen to respond differently to the cold temperature stress. In Dylan plants (Fig. 5.31) cold periods of 24 h and 72 h led to significant reductions in carbohydrate content (t = 2.873 for 24 h and t = 2.415 for 72 h, p < 0.05), although periods of 1 week at 10°C did not result in any significant changes in carbohydrate content in Dylan plants. In plants of Aquadulce Claudia (Fig. 5.32) the imposition of 24 h cold stress did not result in significant changes in carbohydrate content, however, both 72 h and 1 week at 10°C resulted in significant increases in carbohydrate content (t = 2.660, p < 0.05 for 72 h and t = 8.559, p < 0.001 for 1 week).

These changes in carbohydrate content in response to cold stress resulted in plants of Aquadulce Claudia having a significantly higher carbohydrate content than plants of the variety Dylan following periods of cold stress of 24 h and 72 h (t = $2 \cdot 141$, p < $0 \cdot 05$ for 24 h; t = $1 \cdot 983$, p < $0 \cdot 10$ for 72 h). Following the 1 week cold pre-treatments the relationship between the varieties was that observed without cold temperature stress ie. Aquadulce plants were seen to have significantly less carbohydrate per gram leaf tissue than plants of the variety Dylan (t = $2 \cdot 773$, p < $0 \cdot 05$).

The cold temperature pre-treatments were found to influence the effects of sulphur dioxide on carbohydrate content in both varieties of <u>Vicia</u>. In Dylan plants the decrease in carbohydrate content due to SO_2 observed in the absence of cold stress was not seen to occur following periods of 24 h or 72 h at 10°C prior to SO_2 fumigations. A small but significant increase in carbohydrate content was observed in response to SO_2 for the plants subject to 24 h cold stress (t = 1.522, p < 0.10). Sulphur dioxide was still seen to reduce carbohydrate content in plants subject to 1 week cold stress (t = 2.01, p < 0.05) although this reduction of 35% was significantly less than that observed in the absence of cold stress (60%). In plants of the variety Aquadulce Claudia the decreases in carbohydrate content in response to SO_2 without cold pre-treatments were not seen to

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occur in plants subject to 24 h cold stress prior to SO_2 fumigations. Sulphur dioxide exposure increased the carbohydrate content of the 24 h cold-stressed plants from a mean value of 4.1 mg g⁻¹ to 6.1 mg g⁻¹ (t = 2.488, p < 0.05). In plants subject to 72 h cold stress, sulphur dioxide was still seen to significantly reduce carbohydrate contents in Aquadulce (t = 1.951, p < 0.05); however, because carbohydrate content was increased in response to the 72 h cold stress alone, values for plants subject to both 72 h cold and 100 ppb SO₂ were not significantly different to those for unstressed plants. As for the 24 h cold pre-treatment, exposure to SO_2 after a 1 week cold pre-treatment lead to increased carbohydrate contents in Aquadulce (t = 2.018, p < 0.05).

In the absence of either cold or pollutant stress Dylan plants were shown to have significantly higher carbohydrate contents per gram leaf fresh weight than Aquadulce plants. However, the influence of both cold temperature and pollutant stress resulted in the variety Aquadulce Claudia having significantly higher carbohydrate content than Dylan plants after the 24 h cold and 100 ppb SO₂ treatment (t = 3.792, p < 0.01). There were no significant differences in the carbohydrate contents of either variety following both 72 h and 1 week cold treatments with 100 ppb SO₂.

5.8.2 Carbohydrate Content per Unit Leaf Area

When carbohydrate content was calculated per cm² leaf area, multivariate analysis produced similar results (Table 5.12) to those obtained for carbohydrate content per gram leaf weight (Table 5.11); however, varietal differences were not found to be significant when results were calculated per cm² leaf area. When the mean data for each treatment were examined it was seen that all the varietal differences described above still occurred when data were expressed per unit leaf area. Figures 5.33 and 5.34 show this measure of carbohydrate content for all cold treatments, with or without SO₂, for both varieties of <u>Vicia</u>. It can be seen from the figures that in the absence of either stress, isolated chloroplasts from Dylan plants still had significantly more carbohydrate per cm² leaf area than Aquadulce plants (t = 2.322, p < 0.05). The responses of both varieties to either cold or pollutant stress alone were not significantly different to the responses described above for carbohydrate content per unit leaf weight. The only significant difference in the data for carbohydrate

Carbohydrate content – Dylan 0.45 CONTROL 0.40 cm -2 POLLUTED 0.35 0.30 mg carbohydrate 0.25 0.20 0.15 0.10 0.05 0.00 no cold 24h cold 72h cold 1 week cold Length of cold pre-treatment Carbohydrate content - Aquadulce 0.45 0.40cm⁻2 0.35 0.30 mg carbohydrate 0.25 0.20 0.15 0.10 0.05 0.00 24h cold 72h cold 1 week cold no cold Length of cold pre-treatment

Figures 5.33 and 5.34.

Carbohydrate content of isolated chloroplast extracts of two varieties of <u>Vicia faba</u>, Dylan (Fig. 5.33) and Aquadulce Claudia (Fig. 5.34) exposed to SO_2 (0, 100 ppb: 4 h) and/or a range of low temperature (10°C) pretreatments. Results are expressed per unit leaf area (mg cm⁻²) leach point is a mean of eight values: standard errors of the means are also shown]. Results

content per unit leaf weight and per unit leaf area is that for Aquadulce Claudia plants subject to 1 week cold stress. When data were expressed per unit leaf weight a significant increase in carbohydrate content due to SO_2 was observed; however, when results were expressed per unit leaf area there were no significant differences in carbohydrate content of polluted or control plants subject to 1 week cold stress.

It can be seen from the results described above that both cold and pollutant stress affect carbohydrate contents of chloroplasts isolated from both varieties of <u>Vicia</u>. Sulphur dioxide reduced carbohydrate content in both Dylan and Aquadulce but varietal differences in response to cold stress were observed. The combination of both cold and pollutant stress also resulted in varietal differences in carbohydrate content.

5.9 CONCLUSIONS & DISCUSSION

As stated earlier, the aims of this section of the experimental work were to gain information as to the site and mechanisms of interaction of both low temperature and pollutant stress influencing photosynthetic rates in the two varieties of <u>Vicia faba</u> studied. Firstly, chlorophyli content was measured to give an indication of the plants ability to trap light energy. Measurement of Hill reaction activity gave an indication of the ability to transfer light energy to the chemical energy necessary to drive photosynthetic carbon reduction. An indication of the plant's capacity to incorporate CO_2 in the Calvin cycle was possible through measurement of total soluble leaf protein, the large part of which is RuBP carboxylase, the enzyme which effects the first step in CO_2 fixation in C_3 photosynthesis. Finally, chloroplast carbohydrate concentration provided a measure of photosynthetic efficiency, being a measure of the end products of photosynthesis. The effects of both cold and pollutant stress alone and in combination on each of the above parameters are discussed in turn below.

All of the above reactions take place within the chloroplasts of plant leaves. It is considered that photosystem I is located towards the outer surface of the chloroplast thylakoids and photosystem II towards the inner surface; whilst RuBP carboxylase is thought to occur on the outer surface of the thylakoid membrane (Whittingham, 1977). Thus, it is apparent that apart from purely biochemical effects of cold and pollutant stresses on the photosynthetic mechanism, any stress induced structural or morphological changes could influence photosynthetic rates. Indeed, morphological changes in response to cold temperature stress were observed in both varieties of Vicia faba used in this study and are outlined below. Several authors have observed structural changes in response to cold stress (eg. Graham & Patterson, 1982). However, more importantly, changes in chloroplast ultrastructure have been observed (eg. Taylor & Craig, 1971); these changes include chloroplast swelling and unravelling of the thylakoid system whilst the thylakoid intraspaces dilate markedly. Similarly, sulphur dioxide has been shown to alter chloroplast ultrastructure and to cause swelling of the chloroplasts and thylakoid membranes (eg. Wellburn, Majernik & Wellburn, 1972; Black & Black, 1979a,b). Such physical changes must be borne in mind when considering the effects of pollutant and cold stress.

5.9.1 Morphological Changes in Response to Cold Stress

The data presented in 95.4 showed how the imposition of a one week period of low temperature (10°C) stress resulted in significant morphological changes in the leaves of both varieties of <u>Vicia faba</u>. Measurement of leaf area and leaf weight of both cold-stressed and non cold-stressed plants ascertained that cold treated leaves had increased leaf weight per unit leaf area implying that leaves were thicker in cold treated plants. This was confirmed when measurements of leaf thickness were made. However, comparison of both sets of data produced some interesting differences. In the absence of cold stress, leaves from plants of the variety Aquadulce Claudia were found to be significantly thicker than leaves from plants of the variety Dylan; analysis of the data showing the thickness of Aquadulce leaves to be, on average, 8.8% greater than those of Dylan leaves. However, analysis of the data for ratio of leaf weight to leaf area showed Aquadulce leaves to have 20% more leaf weight to leaf area than Dylan plants.

These data suggest differences in leaf densities between varieties. The reliability of these data is obviously open to question because leaf fresh weight was measured and not dry weight, therefore these anomalies could merely be due to differences in leaf water content. But differences in leaf densities could well be due to differences in leaf structure between the varieties ic. a greater density of cells in the leaves of Aquadulce plants or a greater amount of photosynthetic machinery per unit leaf area. Either of these structural differences would have significant consequences on internal resistances to CO_2 and SO_2 transfer as if leaves are more dense ie. cells are more tightly packed, it would be expected that there would be a greater resistance to SO_2 uptake. Indeed, this would explain in part, the data presented in chapter 3 which showed SO₂ flux to Aquadulce leaves to be significantly less than that to Dylan leaves. However, it may be supposed that any increase in the amount of photosynthetic machinery would result not only in significant increases in the ratio of leaf fresh weight but also increases in the amount of soluble leaf protein (mainly enzymes of carbon metabolism) or chlorophyll content (Badger et al., 1982); but, as discussed below, this was not seen to occur in Aquadulce plants in comparison to Dylan plants.

The imposition of cold temperature stress did not alter this

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difference between the varieties. Following the 1 week cold treatment leaves of Aquadulce plants were found to be 7.8% thicker than Dylan leaves but there was 16% more leaf weight per unit leaf area in Aquadulce plants. However, the effects of cold stress were to increase mean leaf thickness by 22.3% and 21.1% in Dylan and Aquadulce plants respectively but the ratio of leaf weight to leaf area was increased by mean values of only 14.1% and 11-0% respectively suggesting a reduction in leaf density due to the imposition of cold stress. It may be that these differences are due to changes in plant water relations as the development of secondary water stress is a characteristic of low temperature stress (Levitt, 1980) but no visible signs of wilting or loss of turgor was apparent in either Dylan or Aquadulce plants subject to 1 week cold stress. Similarly, Musser et al. (1983) studied the short and long term effects of a period of 1 week at 10°C on soybean plants. When plants were harvested 65 days after the end of the cold treatment there was a significant increase in the ratio of leaf dry weight to leaf area in comparison to non cold-stressed plants. These authors also found cold stress to have no significant effect on leaf emergence but leaf elongation rate was much reduced. Cell enlargement depends on biochemical factors controlling cell wall extensibility and deposition of wall components, and physical factors controlling actual expansion. Musser et al. also found that sufficient turgor for enlargement was maintained in chilled plants and thus they concluded that low temperature inhibited the biochemical factors governing leaf enlargement.

The increases in leaf thickness in response to cold stress observed in this study are typical of structural changes occurring during cold acclimation in chilling resistant plants (Crookston <u>et al.</u>, 1974; Levitt, 1980; Graham & Patterson, 1982). Graham & Patterson also described increases in mesophyll cell size in response to cold stress and increases in specific leaf dry weight have been attributed to an accumulation of starch grains within the leaves of cold stress plants as a result of decreased translocation (Crookston <u>et al.</u>, 1974).

Although, in this study, cold stress did not result in obvious wilting or loss of turgor at the end of the 1 week cold period, the appearance of visible injury symptoms within 24 h of plants being returned to warmer temperatures was noted (Plate 5.1). These necrotic lesions at the leaf margin are consistent with symptoms of water stress which have often been found to appear in response to cold stress and it was apparent that the plants had been water stressed at some time during the cold treatment. These data are consistent with the results of Musser <u>et al.</u> (1983) as described above, these authors observing no wilting or loss of turgor in soybean plants at the end of a 1 week cold temperature period although wilting was observed during the first afternoon following the initiation of chilling, this proving to be temporary. Crookston <u>et al.</u> (1974) also observed signs of wilting in <u>Phaseolus vulgaris</u> plants exposed to 5°C overnight but as for <u>Vicia</u>, these decreases in water potential and obvious signs of wilting were not apparent at the end of the cold temperature treatment but occurred on rewarming.

Secondary water stress as a result of chilling temperatures is thought to be due to changes in root permeability thus plant roots can not absorb water sufficiently rapidly at chilling temperatures to keep up with transpirational loss even though this is also decreased (Levitt, 1980). The development of injury symptoms in <u>Vicia faba</u> observed in this study, may therefore be due to increased transpiration as stomatal resistance was decreased on rewarming. It is reasonable to suppose that on transfer to the exposure chamber, leaf temperature was increased almost immediately but root temperature would increase much more slowly. This temperature differential between the leaves and roots would exacerbate the development of water stress symptoms.

The development of water stress and changes in leaf water potential in response to chilling temperatures further complicate the elucidation of pollutant/temperature interactions since water stress has been shown to alter plant pollutant responses. The resulting reduction in leaf turgor may have resulted in changes in stomatal behaviour and thus have affected pollutant uptake. Water stress may have also altered leaf cellular components leading to further changes in plant pollutant responses (see TERG, 1988). However, as for other environmental stresses, there is still insufficient information available to provide a full understanding of how water stress may modify plant pollutant responses or, indeed, vice versa. It is clear that water stress development must not be overlooked when the mechanisms behind pollutant/low temperature stress interaction are being considered since all parameters investigated in this study may be influenced by changes in plant water relations.

5.9.2 Chlorophyll Content

Significant varietal differences in the chlorophyll content of whole leaves were observed in the absence of either cold or pollutant stress. The data in §5.5.1 showed that plants of the variety Dylan had significantly greater chlorophyll content per unit leaf weight than plants of the variety Aquadulce Claudia. However, when results were expressed per unit leaf area no significant varietal differences in chlorophyll content were observed. As described above, these data do not support the theory that the greater density of each Aquadulce leaf was a result of increased photosynthetic capacity per unit leaf area.

This difference in the data for chlorophyll content per unit leaf weight and per unit leaf area may well result from the location of the chlorophyll containing chloroplasts within the leaf. It is well documented that in the dorsi-ventral leaves characteristic of dicotyledons such as beans, the majority of chloroplasts are to be found in the palisade cells directly below the upper epidermis of the leaf, the inner spongy mesophyll cells containing fewer chloroplasts. Figure 5a shows a schematic of a transverse section of such a leaf and the implications of the greater leaf thickness and density found to occur in Aquadulce plants in comparison to Dylan plants. It can be seen that one unit of leaf weight from an Aquadulce leaf incorporates significantly less laminar area than one unit of leaf weight from Dylan plants; thus less chlorophyll containing tissue is contained in 1 g of Aquadulce leaf tissue in comparison to 1 g of Dylan tissue. However, the same amount of chlorophyll containing tissue would be present per unit leaf area.

Similarly, the data presented in chapter 3 showed that Aquadulce plants had significantly greater natural photosynthetic rates than plants of the variety Dylan prior to the imposition of sulphur dioxide stress. The data for chlorophyll content showed that this higher photosynthetic rate was not due to increased chlorophyll content ie. energy trapping facility. However, it is well known that a large part of the chlorophyll in a photochemical system is not reactive, much of it existing as absorbing or receptor molecules all of which are interconnected in a unit through which energy is readily transferred from one molecule to another (Whittingham, 1977). Ultimately, this energy finds its way to a specific molecule which is a reactive form and constitutes a reaction centre. It is thought that less



DYLAN

Because Aquadulce plants have thicker leaves, it can be

seen that 1 g of leaf weight from Dylan plants includes

more of the palisade layer ie. more chlorophyll containing tissue than that of Aquadulce leaves; whilst leaf area

assumes the same amount of chlorophyll containing tissue

in leaves of each variety.

Figur: ί Π

unit density Schematic lea Mg. when measuring weight. T.S. оf Leaf: 5 to show the significance leaf chlorophyll content per Р**f** leaf unit thickness leaf area and and per

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than 1% of the total pigment is concerned with this reaction centre and that several hundred molecules of chlorophyll are organised together into a single group, maximum photosynthetic yield being of the order of one molecule of oxygen for every two thousand molecules of chlorophyll. Thus a lower total leaf chlorophyll content may not necessarily be reflected by a lower photosynthetic rate under optimum environmental conditions.

The effects of a range of sulphur dioxide concentrations on the chlorophyll content of whole leaves of both varieties of Vicia faba under optimum environmental conditions were described in \$5.5. It may have been expected that fumigations of such short duration (4 h) would have little influence on leaf chlorophyll content but, as the data presented in Figures 5.3 to 5.6 showed, not only were significant changes in chlorophyll content observed but significant varietal differences in response to SO₂ were also detected. The imposition of 100 ppb SO₂ for 4 h did not induce changes in the chlorophyll content of leaves of Dylan plants but was seen to induce significant increases in the chlorophyll content of Aquadulce leaves. Although there did not appear to be a direct link between chlorophyll content and photosynthetic rate in all cases, these changes correlate to the data presented in chapter 3 which showed net photosynthetic rates in Dylan plants to decrease by up to 10% in response to 100 ppb SO₂ but the same concentration of SO₂ resulted in enhanced net photosynthetic rates in Aquadulce plants.

Both varieties also showed significant differences in their response to 4 h fumigations with 300 and 500 ppb SO_2 . There was no apparent effect of 300 ppb SO_2 on the chlorophyll content of Aquadulce leaves but a significant increase in the chlorophyll content of Dylan leaves subject to the same treatment. Similarly, exposure to 500 ppb SO_2 resulted in an increase in the chlorophyll content of Aquadulce leaves but induced a significant decrease in the chlorophyll content of Dylan leaves. These changes do not correlate with changes in net photosynthetic rates as described in chapter 3 where increasingly severe photosynthetic impairment was seen to occur in both varieties in response to increased sulphur dioxide concentrations. It may be concluded that the effective mode of SO_2 induced impairment of net photosynthetic rates was not solely that of chlorophyll destruction resulting in reduced energy trapping facility.

A review of the available data for SO_2 effects on leaf chlorophyll content showed that many authors have observed reductions in chlorophyll

content in response to SO_2 . However, the majority of data appeared to reflect changes in response to long-term fumigations and not short SO, episodes. A number of authors have concluded that chlorophyll a is much more sensitive to SO, than chlorophyll b. Rao & LeBlanc (1965) found SO, to induce the degradation of chlorophyll a to phaeophytin; this was thought to be the result of reduced pH thus chlorophyll degradation was explained by acidification. Malhotra (1977) also observed chlorophyll a to be more sensitive to aqueous SO₂ than chlorophyll b. The concentration of phaeophytin a was relatively unaffected by low SO₂ concentrations but was seen to increase with increasing SO_2 concentration. Whilst phaeophytin b was absent, chlorophyllide b content increased slightly. However, Malhotra found that the SO₂ effects on pigment breakdown were not a function of acidification but were a specific effect since the use of HCl to reduce pH to that of 500 ppm aqueous SO_2 resulted in only 5% degradation but 500 ppm aqueous SO₂ resulted in 50% chlorophyll degradation. Malhotra suggested that low SO₂ concentrations effected degradation by means of increased chlorophyllase activity since phaeophytin a formation was low at low SO_2 concentrations. The preferential sensitivity of chlorophyll a to SO_2 in comparison to chlorophyll b was also observed by Rabe & Kreeb (1979) and Shimazaki et al. (1980a,b). In contrast, other researchers have concluded that whilst chlorophyll content is decreased in response to SO_2 , it is the result of a more general degradation rather than a differential sensitivity of chlorophyll a and chlorophyll b to acidification of cell contents due to SO₂ fumigation (Horseman & Wellburn, 1975; Bell, Rutter & Relton, 1979; Suwannapinunt & Kozlowski, 1980; Pratt, Kromroy & Krupa, 1983; Saxe, 1983b; Norby, Richter & Luxmoore, 1985). No significant differences in SO_2 -induced changes in chlorophyll a and chlorophyll b were observed in the two varieties of Vicia faba used in this study. Hällgren & Gezelius (1982) did not observe differences in Chl a/b ratios in Pinus sylvestris in response to SO_2 , nor was chlorophyll concentration different at the end than at the start of the SO₂ fumigation: but chlorophyll concentration was reduced in comparison to control plants and these authors suggested that SO₂ effects were on de novo synthesis rather than on degradation of chlorophyll.

As observed in this study, many of the authors cited above have correlated reduced chlorophyll contents with reduced photosynthetic activity or plant yield but it is generally agreed that the SO_2 effect on chlorophyll destruction is not the primary cause of photosynthetic inhibition. Bell <u>et al</u>. (1979) suggested that chlorophyll degradation or inhibition of synthesis by SO_2 is probably a secondary manifestation of injury, which is not directly associated with reductions in plant growth.

Very few reports of increased chlorophyll concentration in response to SO_2 , as observed in this study, were found in the literature. However, Beckerson & Hofstra (1979) exposed <u>Phaseolus vulgaris</u> to 0.15 ppm SO_2 for 5 d and observed a marked increase in both chlorophyll *a* and chlorophyll *b* content within the first 6 h of fumigation; there being no difference in Chl *a/b* ratio. Zeleňáková & Polek (1982) examined the longterm effects of ambient SO_2 concentrations on the chlorophyll content of apricot leaves and found significant increases in both chlorophyll *a* and chlorophyll *b* in comparison to control plants. Of particular interest is that these authors found SO_2 -induced pigment increases to be most marked during the autumn months in comparison to summer months thus implying an environmental interaction with pollutant effects.

It was also shown in §5.5 that the imposition of a period of low temperature stress in the absence of SO_2 , induced changes in leaf chlorophyll content in both varieties of Vicia faba; the nature of change depending both on variety and on the length of the cold period. For Dylan plants, low temperature stress resulted in marked reductions in leaf chlorophyll content, decreases being greatest in the 72 h and 1 week cold treatments. Aquadulce plants also exhibited marked reductions in chlorophyll content in response to cold stress, decreases being similar for the 24 and 72 h cold treatments but more severe as a result of the 1 week cold treatments. As outlined in the introduction to this chapter reduction in chlorophyll content is a typical response to chilling stress effected both by degradation and reduced synthesis (Berry & Björkman, 1980; Levitt, 1980). Levitt noted that chilling injury has been explained by the accumulation of cell toxins due to disturbances in the normal balance of biochemical processes. More specifically, chilling temperatures have been shown to result in inhibited aerobic respiration thus allowing anaerobic respiration to proceed resulting in the formation of toxic intermediates such as acetaldehyde and ethanol which contribute to chilling injury. Such inhibition of aerobic respiration would lead to an increase in a higher than normal oxygen content in the tissues and Levitt suggested that this O_2 would be available to oxidases other than the cytochrome system normally metabolizing O_2 in an aerobically respiring unstressed plant. Peroxides

would be amongst the products formed by some of the oxidases which may contribute to chlorophyll degradation. Photo-oxidation may also inhibit chlorophyll synthesis at chilling temperatures.

The results obtained in this study showed that low temperature stress prior to pollutant fumigation resulted in significant alterations in the responses of both varieties of Vicia faba to SO2. The significant increase in chlorophyll content in Dylan leaves in response to 300 ppb SO₂ was not observed when plants had been previously subjected to low temperature stress. Nor did 500 ppb SO2 result in reduced chlorophyll content in cold-stressed Dylan plants, in fact, chlorophyll content was increased in response to 500 ppb SO₂ in plants previously subjected to 24 or 72 h at 10°C. It would appear that prior exposure to low temperatures reduced the sensitivity of Dylan plants to SO₂ such that the effects of 500 ppb following cold temperature stress were similar to the effects of 300 ppb SO₂ under optimum environmental conditions. Although increased chlorophyll content in response to SO_2 has been reported in the literature (see above), no explanation of possible mechanisms was offered. The results presented here showing significant increases in chlorophyll content suggest that SO_2 has a marked effect on chlorophyll synthesis, particularly low concentrations. In contrast, higher SO_2 concentrations in the absence of cold temperature stress lead to chlorophyll degradation. However, relatively little SO₂-induced chlorophyll degradation was observed in plants of either variety that had been previously subjected to cold temperature periods of 24 h or 72 h and no degradation was observed in plants of either variety that had been previously subjected to 1 week at 10° C prior to SO_2 fumigation. One possible mechanism could be that longer periods at low temperatures inhibited the activity of enzymes, such as chlorophyllase, responsible for the breakdown of chlorophyll to chlorophyllides or phaeophytins. It is also possible that the effects of chilling stress in reducing chlorophyll synthesis were moderated by SO₂ exposure such that the application of SO₂ stimulated synthesis in cold-stressed plants.

Some differences in the data obtained from determinations of the chlorophyll content of isolated chloroplast suspensions were observed in comparison to that for whole leaves although certain trends identified above were still apparent. Reductions in chlorophyll content in response to cold stress were observed and no SO_2 -induced chlorophyll degradation was observed in plants previously subjected to 1 week cold periods. Due to the

nature of the chloroplast extraction procedure in which substantial amounts of chlorophyll were lost, chlorophyll determinations from whole leaves were thought to be a more reliable indicator of pollutant and/or cold stress effects

5.9.3 Hill Reaction Activity

It was described in \$5.3.5 how numerous attempts failed to provide isolated chloroplasts that would evolve oxygen upon illumination in oxygen electrode experiments even when suitable electron acceptors (eg. ferricyanide) were present. Therefore, it was surprising that healthy Hill reaction rates were obtained in isolated chloroplast suspensions of both varieties in the absence of either cold or pollutant stress since it is the photolysis of water in the Hill reaction that produces oxygen. It would appear that the photoreduction of DCPIP via the electron flow from water was able to occur in chloroplast suspensions but no reasonable explanation for the absence of concomitant oxygen evolution could be established. It may have been that respiratory activity was enhanced as a consequence of the extraction procedures since decreases in oxygen content were observed in the oxygen electrode experiments in the dark; this would be of even more significance if there had been a high presence of mitochondria in the plant extracts. This consumption of oxygen was seen to continue when chloroplasts were illuminated and this may suggest enhanced photorespiration was occurring in chloroplasts in preference to photosynthesis. Photorespiration is known to be favoured by low CO₂ concentrations, high light intensity and temperatures whereas photosynthesis is favoured by high CO₂ concentrations and moderate light and temperature (Walker, 1979). However, temperature in the oxygen electrode was carefully controlled by means of continually circulating water in the jacket surrounding the cell, supplied from a water bath set at 22°C. Also, the addition of bicarbonate solution to the electrode cell should have ensured sufficient CO₂ for photosynthesis to take place.

As for chlorophyll content, significant varietal differences in Hill reaction activity were observed in the absence of either cold or pollutant stress. Chloroplasts isolated from Aquadulce Claudia plants had significantly greater Hill reaction rates per unit chlorophyll than those isolated from Dylan plants. This was interesting because Aquadulce plants had been found to have a lower leaf chlorophyll content than Dylan plants. Thus it would appear that reduced energy trapping facility in Aquadulce plants was compensated for by an increased ability per unit chlorophyll to transfer light energy to chemical energy. This increased efficiency ie. greater activity in Aquadulce plants may have contributed to the greater photosynthetic rates observed in chapter 3 in Aquadulce plants in comparison to Dylan plants.

Varietal differences were also observed in changes in Hill reaction activity in response to either 500 or 100 ppb SO_2 . Reaction rates in Dylan chloroplasts were unaffected by the imposition of 500 ppb SO₂ but were slightly decreased in response to 100 ppb SO₂. In contrast, reaction rates in Aquadulce chloroplasts were severely reduced in response to 500 ppb SO₂ and were also substantially reduced in response to 100 ppb SO₂. The marked reduction in activity observed in isolated Aquadulce chloroplasts in response to 500 ppb SO₂ correlate well with the data presented in Chapter 3 showing net photosynthetic activity to be markedly inhibited by such high sulphur dioxide concentrations. Conversely, the reduction in activity in Aquadulce plants in response to 100 ppb SO_2 do not correlate with the enhancement in net photosynthetic rates observed earlier. Similarly, the absence of any effect of 500 ppb SO_2 on Hill reaction activity in isolated Dylan chloroplasts could not be associated with the substantial photosynthetic inhibition observed in Dylan plants in response to high SO₂ levels. Thus it would appear that sulphur dioxide effects on electron transport in Vicia faba are not the principle mechanism effecting net photosynthetic inhibition.

The results for Vicia did not show any stimulation in Hill reaction activity at low SO₂ concentrations as has been shown by several authors (eg. Libera <u>et al.</u>, 1973; Malhotra, 1976). However, these authors used sulphite solutions on isolated chloroplasts and not gaseous SO₂. Thus differences in observed response may arise because in the use of sulphite solutions immediate responses are measured whereas in the use of gaseous' SO₂ (as used for this study) it is post-fumigation responses that are being monitored. The use of sulphite solutions has also provided results which show no overall effect on Hill reaction activity (Asada <u>et al.</u>, 1965) and inhibition of activity especially at high sulphite concentrations (Silvius <u>et al.</u>, 1975; Malhotra, 1976). Both non-cyclic photophosphorylation and cyclic photophosphorylation were found to be inhibited by the application of sulphite solutions (Asada et al., 1965; Libera et al., 1973; Silvius et al., 1975). Studies of Hill reaction activity of chloroplasts isolated from SO₂fumigated plants have shown electron transport from H_2O to DCPIP to be inhibited by SO₂ (Shimazaki & Sugahara, 1980a,b; Miszalski, 1983). Shimazaki & Sugahara concluded that SO_2 fumigation under illumination inhibited the activity of Photosystem II and non-cyclic photophosphorylation but not Photosystem I and cyclic photophosphorylation. Malhotra & Khan (1984) found the differences between the in vivo effects of SO₂ on both photoelectron transport and phosphorylations and the in vitro effects of sulphite solutions difficult to reconcile. These authors suggested that the effects observed in vitro were the effects of free radicals and sulphate ions formed from the photooxidation of sulphite since sulphate ions have been shown to irreversibly inhibit both cyclic and noncyclic photophosphorylations. As stated above, such discrepancies may arise from the time delay in extracting isolated chloroplasts from fumigated plants where post-fumigation responses are then monitored as opposed to the immediate effects of SO_2 observed through the use of sulphite solutions. However, such discrepancies could also arise from several factors including SO₂ concentrations used, length of the exposure period and, of course, prevailing environmental conditions. Of importance also is the plant species studied and, as has been shown in this study, the cultivar.

The reported effects of SO_2 (or sulphite) on photosynthetic electron transport as described above have been proposed as a major site of SO_2 -induced photosynthetic inhibition since the electron transport system, which supplies ATP and NADPH₂ for the CO₂-fixation system, has been shown to limit the whole process of photosynthesis under light limited conditions (Shimazaki & Sugahara, 1980a); but these authors did not preclude the possible contribution of other SO_2 effects such as inactivation of Calvin cycle enzymes in causing photosynthetic inhibition. However, Barakhtenova & Nikolaevskii (1983) studied the effects of SO₂ on the photochemical activity and photophosphorylation of C_3 and C_4 plants and concluded that the most important cause of SO_2 -mediated inhibition of photosynthesis in plants is the inhibition of photophosphorylation and primarily non-cyclic photophosphorylation. These authors found low SO₂ concentrations to increase Hill reaction activity in all species and attributed enhanced photosynthetic rates to this activation of Photosystem II. Similarly, photosynthetic inhibition at high SO₂ concentrations was attributed to

direct inhibition of photochemical activity. As stated above, in this study photosynthetic inhibition in <u>Vicia</u> could not be attributed solely to SO_2 effects on electron transport but were more likely to be the result of a combination of a wide variety of factors.

The imposition of low temperature stress alone was seen to alter Hill reaction rates in both varieties of <u>Vicia faba</u> and, again, the nature of the change was dependent on both variety and the length of the cold period. Short periods at 10° C (ie. 24 h) did not induce changes in reaction rates of Aquadulce plants. However, the activity of Aquadulce chloroplasts was seen to be decreased by exposure to low temperatures for 72 h or 1 week. Conversely, the Hill reaction rate per unit chlorophyll of Dylan plants was seen to be enhanced by cold temperature periods of 72 h or 1 week whereas exposure to 10° C for 24 h reduced Hill reaction activity in Dylan plants.

In his review of the reported effects of low temperature on the mechanisms of photosynthesis Oquist (1983) suggested inhibition of photosynthetic electron transport as a primary target for chillinactivation of photosynthesis. More specifically, low temperature effects on Photosystem II activity in preference to Photosystem I activity have been identified by a number of authors as contributing to photosynthetic inhibition (eg. Margulies, 1972; Smillie & Nott, 1979; Martin et al., 1981) although the extent of the inhibition was dependent- on species, age of leaves and the nature and duration of the chilling temperature regime (Oquist, 1983). Low temperature inhibition of PS II has been associated with the oxygen evolving side or at the reaction centre of PS II in chilling sensitive plants and inhibition at this site has been used to classify a large number of plants according to their chilling sensitivity (Smillie, 1979). Whilst no data appeared to show enhanced activity in response to chilling temperatures, Garber (1977) did not find chilling stress to influence electron transport in isolated cucumber chloroplasts. It may be that the differences in low temperature-induced changes in Hill reaction activity in the two cultivars of <u>Vicia</u> faba studied here arise from their differences in their relative sensitivities to chilling stress. The preferential sensitivity of Photosystem II to chilling stress has marked implications for pollutant/environmental stress interaction since it was shown above that Photosystem II is also far more sensitive to SO_2 than Photosystem I.

Low temperature stress prior to SO_2 fumigation was seen to result in significant changes in response to SO_2 in both varieties. The severe reduction in Hill reaction rate per unit chlorophyll in Aquadulce plants in response to 500 ppb SO_2 was not seen to occur when plants had been previously exposed to low temperatures, indeed, the reverse was seen to occur and sulphur dioxide stimulated activity in all cold-stressed plants. Similarly, the reduced activity observed in Aquadulce plants in response to 100 ppb SO₂ did not occur when plants had been cold-stressed and this concentration of SO_2 did not then induce any changes in reaction rates in Aquadulce chloroplasts. The imposition of low temperature appeared to sensitize the Hill reaction activity of Dylan plants to SO2. In non coldstressed plants or in plants subjected to 24 h at 10°C, 500 ppb SO₂ was not seen to influence reaction rates. However, following 72 h cold treatments SO_2 -induced increases in activity were observed. Conversely, periods of 1 week at 10°C resulted in SO₂-induced decreases in activity but since cold alone had resulted in increased activity, the combined SO₂/cold stress resulted in reaction rates that were not significantly different from control plants. This is of significance since if determinations of activity had been made only from plants subjected to both stresses and not also from plants subjected to each stress individually it may have been concluded that there was no cold or pollutant effect on Hill reaction activity. Cold temperature stress was also seen to moderate the effects of 100 ppb SO₂ on the reaction rates of Dylan chloroplasts such that the small decrease observed in the absence of cold stress did not occur in plants subjected to 1 week cold stress but rather activity was stimulated.

Although these data for Hill reaction activity could not be correlated exactly with the observed low temperature-induced changes in net photosynthetic inhibition in response to SO_2 it is interesting to note that the data do concur with the conclusions presented in the previous chapter. In chapter 4 it was concluded that reduced pollutant sensitivity following cold stress periods was not the result of reduced pollutant sensitivity in plants of the variety Dylan, but rather reduced flux. The data for Hill reaction suggest enhanced sensitivity of Dylan plants to SO_2 following cold temperature stress. It was also concluded in chapter 4 that reduced photosynthetic inhibition in Aquadulce plants was the result of both reduced pollutant sensitivity and reduced flux. The data for Hill reaction activity also reflect reduced pollutant sensitivity of Aquadulce plants following cold temperature stress. Thus it would appear that one of the mechanisms behind reduced pollutant sensitivity following cold temperature stress was the prevention of SO_2 -induced inhibition of photosynthetic electron transport. However, this mechanism was observed only in plants of the variety Aquadulce Claudia and not Dylan.

5.9.4 Protein Content

As for chlorophyll content and Hill reaction activity, the data for total soluble leaf protein showed significant varietal differences in the absence of either cold or pollutant stress as the protein content of Dylan plants was found to be significantly greater than that of Aquadulce plants. This was a surprising result in view of the data presented in chapter 3 which showed Aquadulce plants to have significantly higher natural photosynthetic rates than Dylan plants. Since a large proportion of soluble protein in leaves is RuBP carboxylase, the enzyme responsible for carbon dioxide fixation, it may have been expected that a higher protein content would have been found in Aquadulce leaves rather than Dylan leaves. Thus it is unlikely that higher photosynthetic rates can be attributed to greater RuBPcase content in Aquadulce leaves; however these data do not preclude the possibility that differences in RuBPcase activity contribute to enhanced photosynthetic rates.

Exposure to 500 ppb SO₂ for 4 h induced significant increases in total soluble leaf protein content in both varieties of <u>Vicia faba</u>. However, comparison of these data with that from other studies is complicated by the fact that reported data are conflicting. In many investigations SO₂induced decreases in total protein content have been reported (eg. Godzik & Linskens, 1974; Jäger & Klein, 1977; Hällgren & Gezelius, 1982; Murray, 1984; Malhotra & Khan, 1984); such changes have been attributed to both decreases in the rate of protein synthesis and/or increases in the rate of protein degradation. Increases in free amino acid content have been proposed to result from such protein degradation (Godzik & Linskens, 1974; Cowling & Bristow, 1979). However Rowland <u>et al.</u> (1989) noted that such increases in amino acid concentration can reflect a number of processes including increased nitrate assimilation or a decrease in transport out of the leaf and may not necessarily reflect protein degradation. Other researchers have not found SO₂ to alter total soluble protein content (eg.
Horsman & Wellburn, 1975; Beckerson & Hofstra, 1979; Rabe & Kreeb, 1979; Tschanz, Landolt, Bleuler & Brunold, 1986). There are also a number of reports which show pollutant induced increases in total soluble leaf protein content (eg. Horsman & Wellburn, 1977; Beckerson & Hofstra, 1979 [ozone]; Sárdi, 1981; Saxe, 1983b). Sárdi (1981) suggested that SO₂-induced increases in protein content result from stimulation of the synthesis of amino acids containing sulphur. The amino acids cysteine and methionine have been reported to contain approximately 90% of the total sulphur in most plants and almost all of these amino acids are found in protein (Rowland, Borland & Lea, 1989). Several authors have found increased content of these amino acids in plants exposed to SO₂ (eg. Ziegler, 1975; Malhotra & Sarkar, 1979). In contrast, Pierre & Queiroz (1981) did not find SO₂ to alter concentration of sulphur-containing amino acids in Phaseolus vulgaris but noted a rapid increase in enzyme capacity as a primary response to subnecrotic SO₂ fumigation. These authors suggested that such an increase in the metabolic potentiality in response to the onset of low levels of SO₂ afforded a temporarily increased capacity of resisting pollution by a faster metabolisation of SO_2 . In the introduction to this chapter it was reported that Levitt (1980) suggested increased protein synthesis in response to chilling stress may aid chilling stress tolerance by compensating for reduced enzyme activity. Since SO₂ has generally been reported to inhibit RuBPcase activity in plants (ref: Miszalski & Ziegler, 1980; Hällgren & Gezelius, 1982) and RuBPcase is the major component of total soluble leaf protein, the observed increase in protein content in Vicia faba in response to SO₂ may result from increased synthesis to counteract reduced enzyme activity. Support for this idea may come from reports that several enzymes involved in amino acid metabolism have been shown to be influenced by SO, fumigations (ref. Malhotra & Khan, 1984). It is clear that the marked SO_2 induced net photosynthetic inhibition observed in both Dylan and Aquadulce plants in response to 500 ppb SO_2 was unlikely to be a result of reduced RuBPcase content given the observed increases in protein. However, it is possible that reduced enzyme activity contributed to photosynthetic inhibition and because of this, protein synthesis was stimulated as a mechanism of reducing SO₂ phytotoxicity.

The imposition of cold stress alone resulted in changes in protein content that were seen to be dependent on both variety and length of cold temperature period. Soluble leaf protein content was increased in all

plants of the variety Aquadulce Claudia subjected to cold stress, the increase being independent of the length of the cold period. However, in Dylan plants, 24 h at 10°C decreased leaf protein content, 72 h at 10°C resulted in increased protein content and 1 week cold temperature periods did not appear to influence the protein content of Dylan plants. The increased protein content of Aquadulce leaves agrees with reported cold temperature effects showing a general increase in soluble proteins at low temperatures (Levitt, 1980; Graham & Patterson, 1982). As stated above such increases may be a compensatory mechanism for reduced enzyme activity to minimise cold temperature-induced photosynthetic inhibition. However the low temperature induced decrease in protein content in Dylan plants subjected to 24 h cold stress do not concur with the reported effects cited above. Graham & Patterson (1982) suggested that increased total soluble leaf protein could result from low temperature effects on both protein degradation and synthesis but with rates of synthesis being less affected than rates of degradation resulting in a greater pool of soluble protein in the tissue. It may be that, in Dylan plants, the first response to the onset of low temperature stress is enhanced protein degradation and that enhanced protein synthesis occurs in response to this enhanced degradation when low temperature stress continues. This would explain the loss of protein observed in Dylan plants subjected to only 24 h cold stress and the increase after 72 h cold temperature stress. If low temperature stress persists then rate of degradation equals rate of synthesis and no apparent change in protein content in relation to unstressed plants is observed as was seen to occur in response to the 1 week cold temperature treatments. Levitt (1980) noted that protein breakdown at low temperatures without an equally rapid resynthesis has been suggested as a cause of injury although experimental evidence was insufficient to prove a chilling-induced protein breakdown. However, he also noted that on the basis of respiratory upset (as described earlier in reference to chlorophyll content) it would be expected that any decrease in the aerobic phase of respiration must result in decreased oxidative phosphorylation. This would decrease the supply of ATP and, therefore, the rate of protein synthesis resulting in a net protein breakdown. It is of interest to note the reduced Hill reaction activity in chloroplasts of Dylan plants in response to 24 h chilling stress described above. This reduced electron transport concomitant with reduced soluble protein content lends credence to the above hypothesis and emphasises the

wide disparity in response between the two varieties of <u>Vicia</u> studied.

Once again, cold temperature stress was found to modify plant responses to SO_2 such that no significant increase in protein content in response to SO₂ was observed in previously cold-stressed Aquadulce plants. Nor was an SO₂-induced soluble protein content increase observed in Dylan plants previously exposed to 24 h or 1 week cold treatments although this increase was still present in plants subjected to 72 h cold temperature periods. The interaction between cold temperature and pollutant stress in determining changes in total leaf soluble protein are not readily explicable. The absence of SO₂-induced protein increases in cold-stressed plants may be the result of the reduced pollutant flux to both varieties observed in chapter 4. If protein increases are due to the metabolism of SO_2 into sulphur containing amino acids as a detoxification mechanism then reduced flux following low temperature stress could minimise the need for detoxification: hence the absence of an increase in protein content. However, this does not explain reduced photosynthetic inhibition in response to SO₂ in cold-stressed plants. It is clear that stimulation of protein synthesis by exposure to SO_2 in the absence of cold stress did not prevent marked photosynthetic inhibition. Similarly, although SO₂ did not alter the protein content in plants previously subjected to 1 week cold stress, photosynthetic inhibition was seen to occur, albeit less severe than in the absence of cold stress. These differences in photosynthetic inhibition may be related to the energy supply and requirements of the plant. It may be that ATP production is insufficient to provide both the energy requirements for enhanced protein synthesis and photosynthetic carbon fixation. This would mean that in non cold-stressed plants the energy requirements of protein synthesis are met at the expense of the energy requirements of photosynthesis thus contributing to net photosynthetic inhibition. The absence of enhanced protein synthesis in cold-stressed plants may therefore contribute to observed lessening of SO₂induced net photosynthetic inhibition since there is no added energy requirement for protein synthesis and more energy is available to drive photosynthetic carbon dioxide fixation.

5.9.5 Carbohydrate Content

Analysis of leaf carbohydrate content gave an indication of photosynthetic efficiency since carbohydrates are the products of photosynthetic activity. However, since changes in carbohydrate content not only reflect changes in production but may reflect changes in assimilate transport into and out of the leaf in response to cold and/or pollutant stress (Minchin & Gould, 1986), care must be taken in interpretation of data. The extent of the differences between the two cultivars of Vicia faba studied here were once again reflected in marked varietal differences in carbohydrate content. Unstressed plants of the variety Dylan were found to have a significantly higher leaf carbohydrate content than plants of the variety Aquadulce Claudia. These differences do not reflect relative photosynthetic rates since Aquadulce plants were shown earlier to have significantly higher natural net photosynthetic rates than Dylan plants. Therefore, the lower carbohydrate content of Aquadulce leaves is not an indicator of lower photosynthetic rates. The difference in carbohydrate content suggests differences in the rate of translocation of photosynthate out of the chloroplasts between the varieties. Indeed, a slower rate of translocation in Dylan leaves may account for the slightly lower net photosynthetic rate in comparison to Aquadulce plants since accumulation of carbohydrate in leaves has been shown to inhibit photosynthesis by feedback inhibition (Levitt, 1980).

The results presented in this chapter showed that the relatively low concentration of sulphur dioxide used (100 ppb) had significant effects on the carbohydrate content of both Dylan and Aquadulce plants. The imposition of SO₂ was seen to reduce the carbohydrate content of both varieties by 30% in Aquadulce leaves and 60% in Dylan leaves. The large decrease in Dylan leaves can be correlated to the observed enhancement of dark respiration rates ie. an increase in consumption of carbohydrate as respiratory substrate. However, since no changes in dark respiration rate of Aquadulce plants were found it might be assumed that reduced carbohydrate content reflected decreased production and that this was also a factor contributing to the large carbohydrate reduction in Dylan plants. These data for Dylan plants agree, in part, with the findings of Koziol & Jordan (1978) in their study of <u>Phaseolus vulgaris</u> when decreased carbohydrate content was correlated with both net photosynthetic depression and increased dark respiration in response to SO_2 . However, these decreases occurred at high SO₂ concentration (above 1.53 ppm) and at lower concentrations these authors found enhanced carbohydrate content in response to SO₂. These authors interpreted these findings in terms of functional changes in the energy budget of the plant whereby the energy requirements for repair or replacement of damages tissue in response to SO_2 are met by keeping the products of photosynthesis within the leaves or by translocating sugars from storage in the stems or roots to the leaves. The use of this energy in repair or replacement processes would be reflected in increased respiration. Although enhanced respiration was found in Dylan plants, which may have been due to repair processes, no increase in carbohydrate content was observed in plants of either variety in response to low SO_2 concentration. As stated above, the absence of respiratory stimulation in Aquadulce plants together with reduced carbohydrate content suggested reduced carbohydrate production. However, since photosynthetic enhancement was observed in Aquadulce plants in response to 100 ppb SO₂ the observed reductions in carbohydrate content may arise from increased translocation out of the chloroplast in response to SO_2 or reduced photosynthetic efficiency whereby carbon dioxide is being fixed but not necessarily resulting in carbohydrate formation. The findings of Lorencplucińska (1983b) in her study of the effects of SO_2 on the dynamics of '*C incorporation into photosynthates in Scots pine, lend support to reduced photosynthetic efficiency. SO_2 was found to inhibit 14C incorporation into the products of the C_3 pathway of carbon reduction but there was an increase in activity of β -carboxylation pathway products resulting from SO₂ effects on secondary metabolic pathways. Lorenc-plucińska concluded that SO_2 funigations resulted in the carbon flow during photosynthesis being directed to β -carboxylation with a restriction of Calvin pathway of carbon reduction.

There are relatively few other reports concerning changes in carbohydrate content in response to SO_2 but it has generally been accepted that low SO_2 concentrations result in increased leaf carbohydrate content (Khan & Malhotra, 1977; Koziol & Cowling, 1980; Saxe, 1983b; Marie & Ormrod, 1988). Many studies have observed pollutant-induced alterations in the pattern of photosynthate allocation in plants whereby photosynthates are retained in leaves and shoots rather than being translocated to roots or fruits (eg. Shimizu, Furakawa & Totsuka, 1980; Jones & Mansfield, 1982; McLaughlin & McConathy, 1983; Freer-Smith, 1985; Murray, 1985; Lorencplucińska, 1986; Mansfield <u>et al.</u>, 1986; Wright <u>et al.</u>, 1986; Marie & Ormrod, 1988). Such changes in resource allocation have been associated with a necessity to provide the energy for repair processes to be carried out.

Of particular interest is that the carbohydrate content of leaves has been implicated in determining plant pollutant sensitivity. As early as 1962, Dugger, Taylor, Cardiff & Thompson found a relationship between leaf carbohydrate content and the sensitivity of pinto beans to ozone: these authors found ozone damage to occur only when carbohydrate content was between 1 and 4 mg g⁻¹ fresh weight; young plants not susceptible to ozone contained high sugar levels. Dugger <u>et al</u>. (1966 see Koziol & Jordan, 1978) also observed that the ozone-sensitive variety of tobacco, Bel-W3, had much higher levels of soluble sugars in leaves than did the 0_3 -resistant, Bel-B. The issue was also raised by Koziol & Jordan (1978) but little further work seems to have been undertaken to clarify earlier observations. The results obtained in this study of Vicia seem to concur with the findings of Dugger et al. since Dylan leaves had significantly higher carbohydrate content than Aquadulce plants and Dylan plants were found to be more sensitive to low SO₂ concentrations. Dylan plants exhibited photosynthetic inhibition in response to 100 ppb SO_2 whereas photosynthetic stimulation was observed in Aquadulce plants.

Low temperature stress alone was found to influence leaf carbohydrate content although the nature of the change depended on both the length of the cold period and plant cultivar. Exposure to 10°C for 24 h or 72 h resulted in marked decreases in carbohydrate content in Dylan leaves whereas the longer cold period of 1 week was not seen to influence leaf carbohydrate content. In contrast, Aquadulce leaves were unaffected by the 24 h cold period but carbohydrate content was seen to increase in response to both the 72 h and the 1 week cold treatments. The increase in carbohydrate content in Aquadulce leaves in response to cold stress is typical of reported chilling stress responses and is likely to be due to decreased translocation of photosynthates out of the leaf (Crookston et al., 1974; Levitt, 1980). The decreases in carbohydrate content in Dylan plants in response to 24 h and 72 h cold temperature stress do not concur with data available in current literature. These decreases may result from the enhanced rates of dark respiration observed in cold-stressed plants when translocation of photosynthates out of the leaf is unaffected by cold

Discussion

Chapter 5

temperature; however, dark respiration rates were also found to increase in response to the 1 week cold treatments when carbohydrate content was unaffected. It may be that Dylan plants are able to acclimate to low temperature stress during longer exposure periods but it is more likely that the levels of carbohydrate in Dylan leaves exposed to 1 week cold stress result from both reduced translocation and increased dark respiration such that the net result is not significantly different from that of non cold-stressed plants. It is of interest to note that in Aquadulce plants exposed to 72 h cold, dark respiration rates were enhanced in comparison to non cold-stressed plants but respiration rates were unaffected by the 1 week cold periods suggesting Aquadulce plants were able to acclimate to reduced temperature. These differences in rates of Rd in Aquadulce were not paralleled by differences in leaf carbohydrate content between the two cold treatments where increased carbohydrate content was observed. Reduced translocation and the associated accumulation of carbohydrate within the leaf have been proposed to cause the decreased photosynthetic rates observed in response to cold stress. However, most reports of this nature concern C₄ species and Crookston et al. (1974) observed that in C₃ plants carbohydrate accumulation has little or no influence on photosynthesis or subsequent growth. As decreased photosynthetic rates in <u>Vicia</u> faba in response to cold stress were found in this study to occur concomitant with both decreased (Dylan) and increased (Aquadulce) leaf carbohydrate content, it may be concluded that net photosynthetic inhibition was not a result of feed-back inhibition in response to low temperature stress.

The effects of 100 ppb SO_2 on leaf carbohydrate content were found to be modified when plants had been previously exposed to low temperature stress. The large decreases observed in Dylan plants were not seen to occur when plants had been pre-stressed by 24 h or 72 h at 10°C and although SO_2 -induced decreases were observed in the 1 week coldstressed plants, these decreases were not as marked as those observed in the absence of cold stress. Similarly, SO_2 did not reduce the leaf carbohydrate content in Aquadulce plants previously subjected to low temperature stress although decreases in non cold-stressed plants had been observed. It would appear that low temperature conditions had reduced the sensitivity of plants to SO_2 and, for Dylan plants, these data would concur with observations of net photosynthetic inhibition in response to 100 ppb

SO₂ which was also found to be lessened in cold-stressed plants. However, the imposition of cold stress was also seen to alter dark respiration responses to SO_2 such that no enhancement was observed. Therefore reduced photosynthetic inhibition and the absence of further decreases in carbohydrate content in Dylan plants in response to SO₂ result from the absence of increased carbohydrate consumption via respiratory stimulation in cold-stressed plants. In contrast, the absence of SO_2 -induced decreases in carbohydrate content of cold-stressed Aquadulce leaves cannot be related to changes in dark respiration response since SO₂ did not induce increased respiration in either cold-stressed or non cold-stressed plants. Nor did the imposition of cold temperature stress alter net photosynthetic responses to 100 ppb SO₂ in Aquadulce plants so that the implied reduced sensitivity to SO₂ from carbohydrate data was not supported by reduced net photosynthetic sensitivity to low SO_2 concentrations. It may be concluded that the absence of decreased carbohydrate content in response to SO₂ in cold-stressed Aquadulce plants resulted from reduced translocation of photosynthates since the effects of SO₂ on dark respiration and net photosynthetic rates were not found to differ in cold-stressed and non cold-stressed plants.

The apparent lower sensitivity of Aquadulce plants to low SO₂ concentrations in comparison to Dylan plants may arise from differences in carbohydrate content as stated above. However, for all environmental regimes Aquadulce plants were shown to have significantly higher rates of dark respiration than Dylan plants. Since detoxification and repair capacity have been associated with increased respiratory activity (Mansfield & Jones, 1985), it may well be that the naturally higher respiratory rates of Aquadulce plants confer reduced sensitivity to low pollutant concentrations via increased ability to carry out repair and detoxification mechanisms. Although there are apparently no specific reports in current literature regarding environmental/pollutant interaction on carbohydrate concentration, several reports, mostly from the Lancaster research group, have shown that environmental conditions prior to and during pollutant exposure may predispose plants to pollutant stress via modification of the amount of metabolic intermediates necessary for repair processes (Davies, 1980; Jones & Mansfield, 1982; Freer-Smith, 1985; Mansfield & Jones, 1985; Mansfield et al., 1986). These authors have proposed enhanced pollutant sensitivity under conditions of slow growth (ie. low light/temperature or short photoperiod) to result from low photosynthetic rates, a reduced store

of carbohydrates for respiratory substrate and thus a reduction in repair capacity; although alterations in assimilate transport and resource allocation via investment in photosynthetic material at the expense of root growth may compensate for impaired photosynthetic capacity. The result for <u>Vicia faba</u> obtained in this study do not support these observations; however since the results from Lancaster reflect long-term SO₂ fumigations and not the short exposure periods used in this study and since different species were studied, it is not surprising that data are not comparable.

The most outstanding feature of the data presented in this chapter was the marked varietal differences occurring in all parameters studied in the absence of either cold or pollutant stress. Therefore varietal differences in pollutant sensitivity before and after cold temperature stress were not surprising. Differences in stomatal and residual resistances leading to differences in pollutant flux have already been shown to contribute to differential sensitivity and the results presented in this chapter have shown that other physiological and metabolic factors contribute to varying pollutant sensitivity between cultivars. For example, the greater sensitivity of Dylan plants to low SO_2 concentrations in comparison to Aquadulce plants can be linked to decreased chlorophyll content, reduced Hill reaction activity and decreased leaf carbohydrate content together with enhanced rates of dark respiration.

The imposition of cold temperature stress was found to alter pollutant sensitivity such that SO_2 -induced chlorophyll degradation did not occur in previously cold-stressed plants nor were marked SO_2 -induced decreases in carbohydrate content observed. Similarly, enhanced protein synthesis in response to SO_2 did not occur in cold-stressed plants. All these factors contributed to reduced photosynthetic inhibition in response to SO_2 in cold-stressed plants. In contrast, the imposition of low temperature stress was seen to enhance the sensitivity of the Hill reaction of Dylan plants to SO_2 whilst pollutant sensitivity was reduced in Aquadulce plants.

CHAPTER SIX

GENERAL CONCLUSIONS

Much attention has been given to the elucidation of the mechanisms of pollutant action on plants to enable the prediction of the effects of a known amount of pollutant on crop yield. However, of the extensive research undertaken, many of the data are conflicting. Differences in species studied are known to contribute to differences in experimental data. However, reported data for single species have also been conflicting. Certain of the discrepancies between the results of different experiments are now known to arise from the unique characteristics of the wide variety of exposure systems used. Such systems can range from laboratory based cuvettes or plant chambers to open-top chambers and other field exposure systems (Unsworth, 1982). As has been discussed earlier, a common oversight in many early studies was the failure to realise the significance of adequate air flow across plant leaves in determining actual pollutant flux into the plant. Similarly, environmental parameters of exposure systems were often poorly defined. However, what has become apparent from such diverse studies, is the significance of the prevailing environmental conditions in determining plant responses to pollutants. Differences in data from experimental fumigations have been shown to arise from differences between environmental factors within the ranges actually encountered in the field. Such differences are of importance because they may reflect possible responses of plants in the outside environment -(Mansfield & Jones, 1985).

Field exposures and the use of open-top chambers have enabled the effects of long-term pollutant exposures to be studied in environmental conditions which are very close to ambient. In recent years, such experimental designs have shown the effects of air pollutants to be modified by suboptimal environmental conditions such as low light intensities and low temperatures commonly occurring in winter. There is evidence to suggest that environmental conditions which result in slow growth can result in enhanced sensitivity to pollutants (eg. Bell, Rutter & Relton, 1979; Davies, 1980; Mansfield & Jones, 1985). However, the mechanisms contributing to such environmental/pollutant interactions are as yet, poorly understood. Therefore, this study was undertaken to quantify the effects of suboptimal environmental conditions on plant responses to sulphur dioxide. Because the study was laboratory based, it was possible to minimise variability in exposure system parameters such as air-flow rates, temperature, humidity, light intensity and photoperiod and pollutant concentration all of which are known to influence plant responses and to contribute to the variability in published response data. In this way, the impact of the imposition of low light stress and low temperature stress could be defined. Comparison of the data obtained with that for the effects of a range of SO₂ concentrations under optimum environmental conditions provided an insight into possible interactive mechanisms leading towards a greater understanding of plant responses to pollutants in the field.

There are many reports in the literature of intra-specific plant responses to pollutants. Thus two cultivars of <u>Vicia</u> <u>faba</u> were studied to determine the extent of intra-specific variability and also to determine if the relative sensitivities of each cultivar to SO_2 were influenced by the imposition of environmental stress.

The data obtained in this study have served to highlight the complexity of the mechanisms of SO_2 action on plants even when environmental conditions are optimal and therefore, not limiting. Events are further complicated when environmental conditions are suboptimal since a number of different factors are affected which result in changes in the observed effects of SO_2 on net photosynthetic rates. It has been shown that the imposition of low light intensities or low temperatures limit photosynthetic rates. Since SO_2 has also been shown to inhibit photosynthesis, it may have been expected that combinations of both environmental and pollutant stresses would have had even more severe consequences. However, this was not found to occur in the two cultivars of <u>Vicia faba</u> examined. The main conclusions derived from this study are outlined below. The implications of these data in relation to current knowledge of air pollution effects on plants in the field are then discussed.

The first and most obvious conclusion to be drawn from the data obtained in this study was that marked varietal differences occur between the two cultivars of <u>Vicia faba</u> examined. These varietal differences were also expressed in differences in pollutant sensitivity and response to environmental stresses such as low light and low temperature. Not surprisingly, differential sensitivity to combined environmental/pollutant stresses was also prevalent. The varietal differences observed in the absence of either pollutant or environmental stress were as follows:

• Aquadulce plants had significantly higher rates of net photosynthesis (Pnet) and dark respiration (Rd) than Dylan plants.

• These differences in Pnet and Rd were found to be associated with a higher stomatal resistance but a smaller mesophyll resistance to H_2O and CO_2 transfer in Aquadulce plants.

• Leaves of Aquadulce Claudia plants were found to be significantly thicker and of greater density than leaves of Dylan plants. Such differences may have significant consequences for gaseous transfer and may have contributed to the observed differences in internal resistance to CO_2 transfer between varieties.

• Biochemical analysis determined Aquadulce plants to have significantly lower chlorophyll, protein and carbohydrate content per unit leaf fresh weight in comparison to Dylan plants, all of which would suggest a reduced photosynthetic capacity. However determinations of Hill reaction activity showed Aquadulce plants to have significantly higher rates per unit chlorophyll than Dylan plants. Thus reduced energy trapping facility in Aquadulce plants was compensated for by increased ability to transfer light energy to chemical energy and this greater efficiency in electron transport contributed to the greater photosynthetic rates observed in Aquadulce plants.

6.1 Varietal Differences in Response to SO₂ Alone

Measurement of the effects of a range of sulphur dioxide concentrations (0 - 600 ppb: 4 h) on both cultivars of <u>Vicia faba</u> determined that net photosynthetic rates in both varieties were inhibited by SO₂ and inhibition increased with increasing SO₂ concentration. Differential pollutant sensitivity was found to depend on pollutant concentration such that at low SO₂ concentrations (\cong 100 ppb) Aquadulce plants exhibited enhancement in photosynthetic rates that were not observed in Dylan plants and Aquadulce plants were considered to be less sensitive to SO₂. However at high SO₂ concentrations (\geqslant 300 ppb) Aquadulce plants were considered to be more sensitive to SO₂ than Dylan plants since greater SO₂-induced photosynthetic inhibition was observed. Full recovery to Conclusions

pre-fumigation rates was observed in Dylan plants within 15 h of the end of the SO_2 fumigation period but much slower recovery rates were found in Aquadulce Claudia plants. Examination of a number of leaf parameters to determine the mechanisms behind this differential sensitivity led to the following conclusions being drawn:

• Dylan plants were found to exhibit SO_2 -induced increases in dark respiration rates that were large and independent of the SO_2 concentration supplied whilst no SO_2 effects on Rd in Aquadulce plants were observed. Such increases in Rd contributed to the net photosynthetic inhibition in Dylan plants.

• Stomatal resistances (r_s) to H_20 transfer of both varieties were found to be altered in response to SO_2 fumigations and the magnitude of stomatal response was found to much greater in Dylan plants than Aquadulce plants. High SO_2 concentrations induced enhanced stomatal closure in both varieties. However, variable responses to concentrations below 400 ppb were observed although, in general enhanced stomatal opening was seen to occur. SO_2 -induced changes in stomatal resistance to CO_2 transfer alone could not account for the observed SO_2 effects on net photosynthetic rates.

• The residual (mesophyll) resistance (r_r) to CO_2 transfer was found to be altered in response to SO_2 with high SO_2 concentrations inducing a large increase in r_r in both varieties of <u>Vicia faba</u>. SO_2 induced changes in net photosynthetic rates in Aquadulce plants were attributed, in part to changes in stomatal resistance but changes in r_r were considered to be the predominant factor influencing CO_2 exchange. In Dylan plants, net photosynthetic inhibition was attributed equally to changes in r_s and r_r although at low SO_2 concentrations changes in r_s . The effects on CO_2 exchange were moderated by SO_2 -induced effects on dark respiration and at low SO_2 concentrations, net photosynthetic inhibition in Dylan plants resulted from enhanced respiratory activity.

• Differences in pollutant uptake have often been proposed as a mechanism contributing to differences in relative pollutant sensitivities in plants. Examination of pollutant flux to both varieties in relation to ambient SO_2 concentrations showed these differences to occur in <u>Vicia faba</u>. There was significantly less SO_2 flux into Aquadulce leaves in comparison to Dylan leaves for any given ambient SO_2 concentration and Aquadulce plants were found to have significantly higher stomatal resistances to SO_2

transfer. However, since varietal differences in the extent of Pnet inhibition were observed when fluxes were equal it may be concluded that apart from differences in pollutant flux, differential sensitivity must arise from different sites/modes of action of SO_2 on the metabolic processes within each variety. Threshold values for SO₂ flux, above which photosynthetic inhibition was seen to occur differed between varieties such that threshold flux values for Aquadulce plants were more than double those obtained for Dylan plants. These values for threshold flux explain, in part, the lower sensitivity of Aquadulce plants in comparison to Dylan plants at SO_2 concentrations below 300 ppb SO_2 . However, once the threshold flux had been exceeded, these relative sensitivities were reversed and Aquadulce plants showed significantly greater net photosynthetic inhibition than Dylan plants for any given flux value. This reversal in relative sensitivities explains, in part the greater inhibition of net photosynthetic rates in Aquadulce plants at SO₂ concentrations above 300 ppb in comparison to Dylan plants.

• Analysis of data for stomatal resistance to SO₂ uptake showed flux to be only partly correlated to stomatal conductance. These data lead to the conclusion that stomatal resistance was not the only factor determining pollutant flux to both varieties. Less flux than would be expected from r_s data was observed at low SO₂ concentrations (§ 300 ppb) when enhanced stomatal opening occurred whilst significantly greater flux was observed at higher SO_2 concentrations when enhanced stomatal closure was observed. Comparison of resistance data from mass balance calculations and from analogy to water vapour transfer showed discrepancies which were attributed to a significant residual resistance to SO_2 uptake which became increasingly negative with increasing pollutant concentration. Explanations for this residual resistance include incorrect assumptions of leaf surface deposition and significant cuticular transport of SO_2 ; a shorter diffusive pathway for SO_2 in comparison to that for effluxing H_2O molecules has also been proposed. However, these factors would not explain the lower flux into leaves than would be expected from r_s measurements when ambient SO₂ was § 300 ppb. Pollutant flux decreased after the first hour of the 4 h fumigation period and then stabilised, the initial higher flux measurements were attributed to surface deposition hence flux measurements after 3% h of SO₂ fumigation were used for resistance analyses when a steady state situation in the exposure chamber was achieved. Thus, in this study, leaf

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surface deposition was discounted as contributing to higher flux values than would be expected from stomatal resistance data. Possible contributing mechanisms behind this residual resistance are outlined later in this section.

• It was concluded that pollutant flux to both varieties of <u>Vicia</u> <u>faba</u> was governed by both residual and stomatal resistances to SO_2 transfer but residual resistances had the greatest influence. Since residual resistances were similar in magnitude in both varieties, lower flux to Aquadulce plants in comparison to Dylan plants was attributed to significantly greater stomatal resistances to SO_2 uptake. Thus differential sensitivity between Dylan and Aquadulce plants could be attributed, in part, to differences in pollutant uptake and avoidance mechanisms. However, differential sensitivity must also arise from SO_2 effects on metabolic processes and differing pollutant tolerance since greater photosynthetic inhibition was observed in Aquadulce plants when flux measurements were equal.

Biochemical analyses determined there to be significant varietal differences in the effects of SO_2 on leaf pigments and metabolites and electron transport capacity.

• Low SO₂ concentrations (\leq 100 ppb) did not alter the chlorophyll content of Dylan leaves but was seen to increase total chlorophyll content in Aquadulce leaves suggesting enhanced chlorophyll synthesis which correlated with the photosynthetic enhancement observed in Aquadulce plants at low SO₂ levels. However, exposure to 300 ppb SO₂ did not alter chlorophyll content of Aquadulce leaves but induced significant increases in Dylan leaves. Exposure to 500 ppb SO₂ resulted in a significant decrease in chlorophyll content in Dylan plants but chlorophyll synthesis was stimulated in Aquadulce plants. Because these changes did not correlate with the increasing photosynthetic impairment observed in both varieties in response to increasing SO₂ concentration, it was concluded that the effective mode of SO₂ action was not solely that of chlorophyll destruction resulting in reduced energy trapping facility. No evidence was found to suggest that chlorophyll a was more sensitive to SO₂ than chlorophyll b and chlorophylleb ratios were not influenced by SO₂ fumigations.

• Varietal differences in the effects of SO₂ on Hill reaction activity of isolated chloroplasts were also found to occur. Rates in Aquadulce plants were found to be significantly more inhibited by exposure to either 100 or 500 ppb SO_2 in comparison to Dylan plants which showed little inhibition at low SO_2 and no inhibition at high SO_2 levels. It may be concluded that differential sensitivities of Aquadulce and Dylan plants in the degree of SO_2 -induced photosynthetic inhibition were due in part to differential sensitivities of the photosynthetic electron transport systems which supply ATP and NADPH₂ for the CO_2 -fixation system.

• Measurement of total soluble leaf protein provided an indication as to CO_2 -fixation capability since a large proportion of soluble protein is RuBP carboxylase; although total protein content measurements did not provide information regarding enzyme activity. Exposure to 500 ppb SO_2 for 4 h induced large increases in protein content. Since marked net photosynthetic inhibition occurred in both varieties at this high SO_2 concentration and RuBPcase has been shown by many workers to be inhibited by SO_2 , it was concluded that enhanced protein synthesis may have occurred as a compensatory mechanism for reduced enzyme activity. Also, SO_2 may have induced enhanced synthesis of sulphur containing amino-acids, almost all of which are found in proteins.

• Determinations of total leaf carbohydrate content gave indications of photosynthetic efficiency since carbohydrates are the products of photosynthetic activity. Sulphur dioxide was seen to reduce the carbohydrate content of both varieties but the reduction was greater in Dylan plants. The larger decrease in Dylan plants was though to result from the increases in dark respiration rates in response to SO_2 since carbohydrates provide respiratory substrates. As net photosynthetic inhibition occurred in Dylan plants in response to 100 ppb SO_2 , decreased carbohydrate content was thought to result from decreased production. However, since exposure to 100 ppb SO₂ was seen to enhance photosynthetic activity in Aquadulce plants but carbohydrate content was reduced, it was concluded that decreased carbohydrate content arose, not from decreased production but from SO_2 effects on translocation of carbohydrates out of the chloroplasts. Alternatively, reduced photosynthetic efficiency may have contributed to the observed decrease in carbohydrate content of Aquadulce leaves whereby CO₂ is fixed but is not incorporated into carbohydrate production but rather is incorporated into secondary metabolic pathways.

It is possible that an explanation of a residual resistance to SO_2 uptake, which inhibits pollutant flux at low concentrations and facilitates pollutant uptake at higher concentrations, lies with internal detoxification mechanisms and the incorporation of the products of SO_2 solubility into plant metabolites or arises from purely physical characteristics of SO₂ diffusivity. Sulphur dioxide is known to dissolve in the surface water of the substomatal cavity to form sulphite and bisulphite ions. Since the major active site of SO_2 action is within the chloroplast, then these ions must be transported across several membranes to reach sites of action within the chloroplasts. Transport of these ions away from the deposition site maintains a concentration gradient allowing further dissolution of SO_2 . The residual resistance may, therefore arise from purely physical factors relating to the solubility of SO_2 in water and the surface area for deposition available within the substomatal cavity and the rate of diffusion away from the deposition site. Membrane permeability to SO_2 products must therefore provide an extra resistance to diffusion and transport which may serve to explain the positive residual resistance noted at low SO₂ concentrations. In contrast, damage to internal membranes has often been observed in plants exposed to SO_2 and at high SO_2 concentrations loss of membrane integrity may allow greater diffusion of soluble SO₂ products into the chloroplasts thus facilitating pollutant uptake. Such disruptions in cell membrane structure would contribute to the severe photosynthetic inhibition observed at high SO₂ concentrations. Alternatively, apart from purely physical aspects, a residual resistance to SO_2 uptake may be related to biochemical factors. If, as has been reported, sulphite ions directly compete with bicarbonate ions for binding sites on the enzyme RuBP carboxylase, it is possible that high concentrations of SO₂ render more sulphite ions that bind to the enzyme. Little is known regarding the fate of sulphite ions bound to this enzyme and it has yet to be proved whether sulphite is further metabolised in this way. However, if this 'binding' is permanent, this will maintain a concentration gradient thus facilitating SO_2 flux and at the same time, rendering the enzyme less able to incorporate CO₂ thus contributing to photosynthetic inhibition. This enzyme deactivation may then stimulate protein synthesis as a compensatory mechanism. The data obtained in this study, showing enhanced protein synthesis in response to SO₂ would support this theory. The apparent positive residual resistance at low SO₂ concentrations may result

from too few sulphite ions being present to effectively compete with bicarbonate ions for binding sites on the enzyme.

6.2 Responses to Environmental Stress Alone

When <u>Vicia</u> <u>faba</u> plants were grown under low light intensities it was found that:

• Net photosynthetic rates were much reduced in both varieties to rates less than half those observed under optimum environmental conditions.

• Dark respiration rates were also much lower in plants of both varieties grown under low light intensities.

• Reduced photosynthetic activity could be correlated with an effective doubling in stomatal resistance to H_2O and CO_2 transfer in low light stressed plants but changes in stomatal resistance accounted for only 10% of photosynthetic inhibition in Dylan plants.

• Changes in residual resistance to CO_2 transfer were found to account for 90% of the reduction in photosynthetic rates in Dylan plants. For Aquadulce plants photosynthetic depression under low light intensities was correlated equally with changes in stomatal and residual resistances to CO_2 transfer.

• Plants of both varieties showed visible signs of low light stress and were much smaller and 'weaker' than plants of comparable age grown under high light conditions. Leaves were very pale in colour indicating a marked reduction in total leaf chlorophyll content which would have contributed to depressed photosynthetic rates.

The effects of low temperature stress on both Dylan and Aquadulce plants were dependent on the length of the cold period such that:

• Periods of 24 h at 10°C did not influence net photosynthetic rates in either variety; however, 72 h or 1 week cold temperature periods resulted in a 20% depression in net photosynthetic rates in comparison to non cold-stressed plants. Recovery to pre-stress rates occurred more quickly in the 72 h cold treated plants than in plants exposed for 1 week.

• Varietal differences in dark respiration responses to cold stress were observed. Dark respiration rates were enhanced in Dylan plants in response to all cold treatments. This respiratory stimulation declined upon rewarming but the rate of recovery was found to be dependent on and inversely proportional to the length of the cold treatment. In contrast, respiratory stimulation was not observed in Aquadulce plants exposed to 24 h or 1 week of cold temperature stress. Enhanced respiratory activity occurred in response to the 72 h cold treatments and was seen to persist up to 3 d after rewarming. Such respiratory stimulation contributed to the depression of net photosynthetic rates observed in both varieties but was not the sole cause of Pnet inhibition.

• Cold temperature stress resulted in much increased variability in data for stomatal resistance to CO_2 transfer but, in general, stomatal resistance was substantially increased in cold-stressed plants.

• Residual resistances to CO_2 transfer were also found to be variable following cold temperature stress but were not found to be significantly higher than values obtained for non cold-stressed plants and in some cases were much lower. It was concluded that low temperatureinduced net photosynthetic depression largely resulted from increased stomatal resistance to CO_2 transfer and, in Dylan plants, respiratory stimulation.

• Changes in leaf structure were observed in both varieties in response to the 1 week cold treatments which resulted in increased leaf thickness but a reduction in leaf density in cold-stressed plants. This apparent reduction in leaf density, together with the development of injury symptoms in some plants which were characteristic of water stress symptoms, suggested that low temperature stress had influenced plant water relations. The development of secondary water stress is typical of reported responses to low temperature and must be considered to have contributed greatly to the noted increases in stomatal resistance.

Cold temperature-induced changes in plant pigments and metabolites were also found which may have contributed to changes in photosynthetic rates.

• Chlorophyll content was significantly reduced in both Dylan and Aquadulce plants in response to low temperatures, the lowest chlorophyll content being found in the longer cold treatments. Reduced chlorophyll content was attributed both to degradation and reduced synthesis but it was not thought likely that changes in chlorophyll content alone had effected net photosynthetic depression.

• Marked varietal differences in cold temperature-induced changes in Hill reaction activity were noted which were dependent on the length of the cold treatment period. Rates of Hill reaction per unit chlorophyll of chloroplasts isolated from Aquadulce plants were not influenced by exposure to 10°C for 24 h periods but were significantly reduced in response to both the 72 h and 1 week cold treatments. In contrast, 72 h of cold temperature stress induced significant increases in the rate of Hill reaction activity in isolated Dylan chloroplasts but periods of 24 h or 1 week inhibited activity. Although reduced Hill reaction activity could have contributed to depressed photosynthetic rates in both varieties in the long-term, as indicated by data from 1 week cold periods, the effects of cold temperature on electron transport do not serve to explain the effects of shorter cold stress periods.

• Total soluble leaf protein content was also altered in response to low temperature stress but, again, changes were dependent on both variety and the length of the cold treatment period. Protein content was increased in all cold temperature-stressed Aquadulce plants, these increases being seemingly independent of the length of the cold period. However, in Dylan plants, 24 h cold resulted in decreased protein content, 72 h cold resulted in increased protein content and 1 week cold did not influence total soluble leaf protein content in Dylan plants. It was apparent that low temperature stress influenced rates of protein synthesis in both varieties of Vicia faba. Enhanced protein synthesis is consistent with reports in current literature which suggest increases in protein content to be a compensatory mechanism to counteract reduced enzyme activity occurring in response to low temperature stress and the data showing net photosynthetic inhibition in response to both the 72 h and 1 week cold periods would correlate with this hypothesis. However, although this mechanism may have occurred in plants subjected to longer periods of low temperature stress, it did not serve to explain the reduced protein content of Dylan plants exposed to only 24 h of low temperature stress. Since low temperature stress has been reported to influence both protein synthesis and degradation it was thought that the initial response of Dylan plants to the imposition of low temperature stress was enhanced protein degradation and that enhanced protein synthesis occurred some time after the onset of degradation when cold stress continued. As low temperatures persisted it was thought that

rates of synthesis and degradation stabilised and became equal such that no apparent change in leaf protein content was observed. It may be assumed that any enhanced protein degradation occurring in Aquadulce plants was outweighed by enhanced rates of synthesis occurring in response to cold stress and that the initial lag in the onset of enhanced synthesis occurring in Dylan plants did not occur in Aquadulce plants. Since the degradation of protein in Dylan plants in response to 24 h of cold temperature stress was not accompanied by any observable decline in net photosynthetic rates it may be possible that net photosynthetic inhibition occurring in response to low temperature stress results from increased energy consumption for protein synthesis occurring at the expense of the energy requirements of photosynthesis.

• Low temperature stress alone was also found to influence total leaf carbohydrate content but responses were again seen to be cultivar specific. Reduced carbohydrate content was observed in Dylan plants following the 24 h or 72 h cold treatments but the 1 week cold treatment had no apparent effect. Conversely, the carbohydrate content of Aquadulce chloroplasts was unchanged upon exposure to 24 h of low temperatures but was significantly increased in plants subjected to the 72 h and 1 week treatments. A general increase in carbohydrate content, as observed in Aquadulce plants, was consistent with reports in the literature of decreased translocation of photosynthates out of the leaf in response to cold stress; hence, the reduced carbohydrate content in Dylan plants was surprising. Such decreases may have resulted from the observed low temperature-induced respiratory stimulation. It was thought that the absence of any apparent effect of the 1 week cold treatments on carbohydrate content of Dylan plants was a product of both reduced translocation and respiratory stimulation. It was concluded that feed-back inhibition was not operating since low temperature-induced net photosynthetic depression was associated with both decreased and increased leaf carbohydrate content in Vicia faba.

6.3 Responses to Combined Environmental/Pollutant Stress

The imposition of either low light or low temperature stress prior to pollutant fumigation was found to moderate the responses of both varieties of <u>Vicia</u> <u>faba</u> to sulphur dioxide. When plants were grown under low light intensities the degree of SO_2 -induced net photosynthetic inhibition as compared to plants grown under high light intensities, was reduced. This lessening in inhibition was most marked in plants of the variety Dylan such that significantly less inhibition was observed in comparison to Aquadulce plants at all SO_2 concentrations below 400 ppb. Examination of several leaf parameters to determine the mechanisms behind this reduced photosynthetic sensitivity showed that:

• Dark respiration rates in Dylan plants grown under low light intensities were lessened in response to SO_2 ; this occurred independently of SO_2 concentration. This response was the reverse of that found in Dylan plants grown under high light intensities and contributed significantly to the lessening in the observed degree of SO_2 -induced net photosynthetic inhibition. The greater photosynthetic sensitivity of low light-stressed Aquadulce plants in comparison to Dylan plants was attributed, in part, to SO_2 having no effect on dark respiration rates of Aquadulce plants.

• Stomatal responses to SO_2 were significantly altered as a consequence of plants having been grown under low light intensities. This modification was most marked in Dylan plants where stomatal responses were the reverse of those seen in high light grown plants and increasing SO_2 concentration resulted in decreasing stomatal resistances to H_2O and CO_2 transfer. This reversal in response contributed to the lessening of SO_2 induced net photosynthetic inhibition in low light-grown Dylan plants in comparison to high light-grown plants. This reversal in stomatal response also contributed to the lesser sensitivity of Dylan plants in comparison to Aquadulce plants since, as for high light-grown plants, stomatal resistances were still seen to increase in response to SO_2 in low lightgrown Aquadulce plants.

• Under low light intensities, residual resistances (r_r) to CO_2 transfer were still found to increase markedly in both varieties at higher SO_2 concentrations and to contribute largely to SO_2 -induced net photosynthetic inhibition. However, increases in r_r in both varieties were found to be less than occurred under high light conditions and this contributed, in part, to the lessening in SO_2 -induced inhibition of net photosynthetic rates particularly at higher SO_2 concentrations. However, it was concluded that the reduced sensitivity to SO_2 of low light-grown Dylan plants in comparison to high light-grown plants was largely attributable to decreases in dark respiration rates and, particularly at low SO_2 concentrations, changes in stomatal resistance.

• The imposition of low light stress led to differences in the relationship between pollutant flux and ambient SO_2 concentrations. Although flux to Dylan plants was not determined to be significantly different to that for high light-grown plants, increased variability in data obtained was observed, this being attributed to the effects of low light stress in increasing stomatal resistance. Thus reduced net photosynthetic inhibition in Dylan plants could not be attributed to reduced flux. In contrast, the effect of added low light stress on Aquadulce plants was to significantly reduce pollutant flux in comparison to that to high light-grown plants. This reduction in flux was thought to account for the lessening of SO_2 -induced inhibition of net photosynthetic rates in low light-stressed Aquadulce plants in comparison to high light-grown plants.

• Although fluxes to Dylan plants were not found to be significantly different when plants were grown under high or low light intensities, the relationship between ambient SO₂ concentration and residual and stomatal resistances to SO₂ flux was found to be reversed. Under low light intensities r_rSO_2 became less negative as SO₂ concentration increased whilst stomatal resistance decreased. It was concluded that pollutant flux to Dylan plants was governed equally by stomatal and residual resistances whereas, under optimum environmental conditions fluxes were seen to be largely governed by residual resistances to pollutant transfer. Varietal differences were significant since low light stress was not seen to alter residual resistances to SO₂ transfer in Aquadulce plants. However, stomatal resistances were higher than those observed in high light conditions which resulted in the reduced flux to Aquadulce plants under low light conditions.

• The imposition of low light stress was also seen to alter the relative sensitivities of each variety of <u>Vicia faba</u> to sulphur dioxide. For Dylan plants, reductions in SO_2 -induced net photosynthetic inhibition under low light intensities did not arise from reductions in flux and less inhibition was seen for the same range of flux measurements in comparison to that for high light intensities. It was concluded that low light stress had reduced the sensitivity of Dylan plants to sulphur dioxide and increased plant tolerance. Conversely, reductions in SO_2 -induced net photosynthetic inhibition in low light-stressed Aquadulce plants were correlated with reduced pollutant flux and greater net photosynthetic

inhibition was observed for any given measure of flux in comparison to that for high light-grown plants. Similarly, the threshold flux value, above which net photosynthetic inhibition was seen to occur was substantially lowered under low light conditions. It was concluded that low light stress had increased the sensitivity of Aquadulce plants to SO_2 but injury was reduced via avoidance mechanisms since flux into the plants was reduced under low light conditions.

The imposition of periods of low temperature stress prior to SO₂ fumigation was also seen to alter plant pollutant responses. The degree of SO_2 -induced net photosynthetic inhibition was found to be dependent on both variety and the length of the cold pre-treatment (10°C: 24, 72 h or 1 week). Exposure to 10°C for only 24 h was seen to reduce the degree of SO₂induced net photosynthetic inhibition occurring in Dylan plants when data were compared with those for non cold-stressed plants and enhanced rates were observed at low SO_2 concentrations which had not been observed in non cold-stressed plants. Conversely, although a 24 h cold period did not significantly alter the responses of Aquadulce plants to SO₂, the data were increasingly variable and no photosynthetic enhancement was observed although this was seen to occur at low SO₂ concentrations in non coldstressed plants. SO_2 -induced net photosynthetic inhibition was also much reduced in Dylan plants previously subjected to either 72 h or 1 week of low temperature prior to SO_2 fumigation. This reduction occurred at all SO_2 concentrations used. In contrast, exposure to 72 h or 1 week at 10°C reduced SO₂-induced net photosynthetic inhibition of Aquadulce plants only when ambient SO_2 concentrations exceeded 250 ppb. At lower concentrations there did not appear to be any significant differences in the photosynthetic responses of cold-stressed or non cold-stressed Aquadulce plants. These low temperature modifications in net photosynthetic responses to SO₂ were associated with:

• Low temperature stress resulted in changes in the dark respiratory responses of Dylan plants to SO_2 such that no SO_2 -induced respiratory stimulation was observed in cold-stressed Dylan plants although this had occurred in non cold-stressed plants. However, low temperature stress alone had already induced respiratory stimulation in Dylan plants. The absence of enhanced dark respiration rates could explain, in part, the lessening of SO_2 -induced net photosynthetic inhibition in low temperaturestressed Dylan plants. However, modification of net photosynthetic responses to SO_2 in low temperature-stressed Aquadulce plants could not be attributed to changes in SO_2 effects on dark respiration rates.

• Low temperature stress significantly altered the stomatal responses of both varieties to SO, such that, unlike for non cold-stressed plants, there was little apparent correlation between ambient SO₂ concentration and % change in stomatal resistance and no obvious threshold concentration, indicating a switch in stomatal response from enhanced opening to enhanced closure. Varietal differences were highlighted since low temperature stress was seen to markedly reduce the magnitude of stomatal response to SO₂ in Dylan plants whereas the magnitude of stomatal response was markedly increased in Aquadulce plants. Of significance also is that following exposure to 1 week at 10°C enhanced stomatal closure in response to SO₂ was observed in all plants of both varieties at all SO₂ concentrations. Such changes in stomatal response to SO, may have been expected to profoundly alter net photosynthetic responses to SO₂ in coldstressed plants. Remarkably, however, little correlation between SO₂-induced net photosynthetic inhibition and changes in stomatal resistance was found and it was concluded that, in plants subjected to longer periods of cold stress, changes in Pnet in response to SO₂ occur seemingly independently of changes in rs suggesting low temperature stress has a profound effect on that part of the stomatal mechanism sensitive to SO₂. However, some degree of stomatal control in determining net photosynthetic rates was still observed in plants subjected to shorter cold stress periods.

• Since stomatal resistance was not the major controlling factor determining environmental modification of photosynthetic responses to SO_2 , other factors must have been exerting a controlling influence. Examination of residual resistances (r_r) to carbon dioxide transfer showed that the marked SO_2 -induced increases in r_r seen to occur in non cold-stressed plants were not as apparent in cold-stressed plants of either variety. SO_2 induced changes in r_r were found to be dependent on the length of the cold pre-treatment, and variety. In Dylan plants subjected to 24 h at 10°C much smaller SO_2 -induced increases in r_r occurred in relation to those observed in non cold-stressed plants which contributed significantly to the lessening in SO_2 -induced net photosynthetic inhibition. Interestingly, although there were no apparent significant differences between the degree of SO_2 -induced net photosynthetic inhibition in Aquadulce plants subjected to 24 h at 10°C and that of non cold-stressed plants, analysis of resistance data showed significant changes to have occurred. In contrast to non cold-stressed plants there were no large increases in r_rCO_2 in response to SO₂ in cold-stressed Aquadulce plants and at SO₂ concentrations below 285 ppb r_rCO_2 was found to decrease; these decreases in r_r were concomitant with increases in stomatal resistance and it was concluded that stomatal resistances played a larger role in determining the degree of SO₂-induced net photosynthetic inhibition in Aquadulce plants subjected to 24 h of low temperature stress. In plants of both varieties subjected to longer periods of cold temperature stress reductions in SO₂-induced net photosynthetic inhibition were associated with much lower residual resistances to CO₂ transfer in comparison to those of non cold-stressed plants.

• Low temperature stress was also seen to alter the relationship between pollutant flux and ambient sulphur dioxide concentrations as the responses observed became increasingly variable with increasing length of the cold pre-treatments. This increased variability was attributed to low temperature effects on stomatal resistance outlined above. Surprisingly, unlike the relationship observed in non cold-stressed plants, no significant differences between measured and calculated pollutant fluxes were found in cold-stressed plants which may have suggested the absence of a residual resistance to SO_2 transfer. The correlation between flux and ambient SO_2 concentration was not significantly altered by the imposition of 24 h of low temperature stress in either variety, nor were the any significant differences in flux to plants of the variety Dylan following the 72 h cold treatments. However flux was significantly reduced in Aquadulce plants subjected to the 72 h cold treatments and following the 1 week cold treatments SO₂ flux into leaves of both varieties was significantly reduced in comparison to that to non cold-stressed plants. These reductions in flux could account for the lessening of SO₂-induced net photosynthetic inhibition in cold-stressed plants of the variety Aquadulce Claudia and Dylan plants exposed to the 1 week cold pre-treatments. However, reduced photosynthetic inhibition in Dylan plants exposed to 24 h or 72 h of low temperature stress prior to SO₂ fumigation could not be attributed to reductions in pollutant flux.

• Despite the absence of significant differences between pollutant flux as derived from mass balance calculations and flux as derived from analogy to water vapour transfer, analysis of the resistance data for pollutant uptake in cold-stressed plants showed a residual resistance to SO₂ uptake to still be evident. However, all three cold treatments resulted in much increased variability in both residual and stomatal resistances to pollutant uptake. Despite flux being apparently unaltered following the 24 h cold treatments, it was found that the increased stomatal resistance occurring in response to the cold treatments was matched by residual resistances to SO₂ uptake being increasingly negative. Following the 1 week cold treatments when flux to both varieties was significantly reduced significant varietal differences in residual and stomatal resistance to SO_2 uptake were observed. In Dylan plants residual resistances were seen to be largely negative at SO_2 concentrations below 300 ppb (the converse of that occurring in non cold-stressed plants) when stomatal resistance was seen to increase. In Aquadulce plants both stomatal and residual resistances increased with increasing SO₂ concentration thus resulting in much reduced flux.

• Low temperature stress influenced the relative sensitivities of both Dylan and Aquadulce plants to SO_2 . Lower SO_2 -induced inhibition of net photosynthesis in Dylan plants exposed to either 24 h or 72 h of low temperature stress was attributed to increased pollutant tolerance since fluxes were unchanged. Conversely, following the 1 week cold treatments, the lessening in response to SO_2 fumigations in Dylan plants was a product of reduced flux and increased sensitivity to SO_2 was found to have occurred. The lessening in responses to SO_2 of Aquadulce plants exposed to either 72 h or 1 week of low temperature stress was the result of both reduced flux and reduced pollutant sensitivity ie. a combination of avoidance and tolerance mechanisms.

The observations of increased pollutant tolerance indicated that low temperature stress had uncoupled the relationship between pollutant flux and plant response and suggested that the mechanisms of stress interaction arose not only from changes in resistance to gas exchange but from other biochemical or physiological factors. Low temperature stress was seen to result in modification of SO_2 effects on leaf pigments, electron transport and metabolites as follows:

• The imposition of low temperature stress was found to reduce the sensitivity of Dylan plants to SO_2 when changes in chlorophyll content were considered. SO_2 -induced reductions in chlorophyll content were not observed in cold-stressed plants although they had been present in non cold-stressed plants indicating that prior exposure to low temperature stress lessened SO_2 effects on chlorophyll degradation. SO_2 was seen to result in a stimulation of chlorophyll synthesis in non cold-stressed Aquadulce plants but this was not seen to occur in cold-stressed plants. Such changes in chlorophyll content did not serve to explain low temperature-induced changes in photosynthetic response to SO_2 .

• Varietal differences were again apparent when rates of Hill reaction activity were considered. The marked sensitivity of Hill reaction rates of Aquadulce plants to SO_2 in the absence of cold-stress was not observed in cold-stressed plants and rather than inhibition, exposure to 500 ppb SO_2 was found to stimulate activity in cold-stressed Aquadulce plants. Conversely, exposure to low temperature stress appeared to sensitize the Hill reaction activity of Dylan plants to SO_2 , These data correlate with the low temperature-induced changes relative sensitivities to SO_2 described above. It was concluded that one of the mechanisms contributing to reduced pollutant sensitivity following cold temperature stress in Aquadulce plants was the prevention of SO_2 -induced inhibition of photosynthetic electron transport.

• Analysis of total soluble leaf protein content showed that low temperature stress prevented the marked SO₂-induced increases in protein content observed in non cold-stressed plants. Considered in terms of the energy budget of the plant, this absence of stimulation in protein synthesis was thought to contribute to reduced pollutant sensitivity by removing the added energy requirements for enhanced protein synthesis resulting in more energy being available to drive photosynthetic carbon fixation; this was thought to occur in Aquadulce plants particularly. Cold stress alone was seen to inhibit Hill reaction activity and it may be assumed that as a consequence, ATP formation was also inhibited by low temperature stress. If ATP production is limited then its utilisation in protein synthesis would probably result in not enough energy being available to drive photosynthetic carbon fixation. The preferential utilisation of ATP in driving CO₂-fixation rather than protein synthesis would be of benefit to the plant in the short-term. However, if as is suggested, enhanced protein synthesis is a compensatory mechanism for SO₂induced inhibition of enzyme activity, then the absence of enhanced protein synthesis may be detrimental to the plant in the long-term.

• Analysis of leaf carbohydrate content showed that low temperature stress prevented the SO₂-induced reductions in carbohydrate content observed in non cold-stressed plants. For Dylan plants, this was thought to be the result of the absence of increased respiratory consumption via respiratory stimulation since SO_2 did not increase respiration rates in cold-stressed Dylan plants and this had been observed in non cold-stressed plants. However, since SO_2 did not provoke a respiratory response in Aquadulce plants then the absence of respiratory stimulation could not explain the absence of SO_2 -induced decreases in carbohydrate content. Under optimum environmental conditions it was thought that SO₂-induced reduced carbohydrate content of Aquadulce leaves was the result of enhanced translocation of photosynthates out of the leaf. Thus the imposition of low temperature stress was thought to prevent this enhanced translocation and since carbohydrate content was seen to increase it was thought that SO₂ inhibited carbohydrate translocation in coldstressed Aquadulce plants. The SO₂-induced decrease in carbohydrate content of Aquadulce plants subjected to 72 h cold-stress was thought to be the result of the observed respiratory stimulation in these plants.

6.4 OVERVIEW

Given the range of responses to either environmental or pollutant stress alone, as summarised above, it was not surprising to find that prior exposure to environmental stress modified the responses of both varieties of <u>Vicia faba</u> to sulphur dioxide and that significant varietal differences were observed. What was surprising was that contrary to most reports, environmental stress appeared to reduce plant pollutant responses. However, this did not necessarily mean reduced sensitivity since lessening in photosynthetic inhibition was in some cases the result of reduced pollutant flux. Plant responses to combined environmental/pollutant stress were, therefore, the result of a combination of both avoidance and tolerance mechanisms. Differential sensitivity of the two cultivars of <u>Vicia faba</u>, Dylan and Aquadulce Claudia was found to result from both differential SO₂ uptake and different tolerances to actual pollutant uptake. The mechanisms employed by each variety to counteract the presence of SO₂ were found to be dependent on prevailing environmental conditions. Since this study dealt with laboratory based relatively short environmental stress and pollutant funigation periods these data are not readily comparable with that obtained, long-term field funigation experiments. Also, low light and low temperature stress were investigated separately whereas these conditions usually occur together in winter conditions in the field. However, these data do serve to emphasise the influence of prevailing environmental conditions in determining plant pollutant responses and it is by investigations of each stress individually that insight into interactive mechanisms can be gained. Possible interactive mechanisms are discussed below which also provided some explanation for the discrepancies between data for long-term and shortterm exposure experiments.

In the search for the identification of a mechanism giving an indication of plant pollutant sensitivity, the data obtained from this study would preclude the use of analysis of pollutant effects on plant pigments and metabolites as reliable indicators of sensitivity. The effects of sulphur dioxide with or without low temperature stress were found to be the result of a combination of a number of subtle effects rather than a specific effect on any single parameter and net photosynthetic inhibition could not be correlated exactly with changes in chlorophyll, protein or carbohydrate content or changes in Hill reaction activity. Therefore, changes in net photosynthetic rates are considered to be a significant indicator of plant pollutant sensitivity. However, such responses must be related to actual pollutant fluxes rather than ambient pollutant concentrations since differential sensitivity as related to ambient concentrations has been shown to be correlated, in part, with differences in pollutant uptake.

However, careful consideration must be given to the manner in which pollutant fluxes are calculated. Gas phase resistance, principally at the stomata is commonly thought to be the predominant factor limiting the diffusion of SO_2 and it is often assumed that stomatal resistances to pollutant uptake are proportional to stomatal resistances for water vapour efflux, allowing for differing molecular diffusivities. However, the data from this study have confirmed the existence of a residual resistance to SO_2 uptake which, when environmental conditions are not limiting, appeared to be the predominant factor governing SO_2 uptake by both Aquadulce and Dylan leaves since flux was only partly correlated with stomatal resistance.

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Thus the resistance pathways for water vapour transfer and pollutant uptake are not completely synonymous. Of significance is that the imposition of either low light or low temperature stress prior to pollutant exposure alters the relative contributions of both the stomatal and residual resistances to SO_2 uptake.

Under low light conditions, for Dylan plants, stomatal and residual resistances were equally important in governing pollutant flux; although, in comparison to high light-grown plants, changes in both resistances with increasing SO_2 concentration were reversed such that flux was apparently unchanged. Conversely, the residual resistance to SO₂ uptake was unchanged in low-light grown Aquadulce plants whilst stomatal resistance was increased thus flux was reduced. Short periods of low temperature stress alone increased stomatal resistances to SO₂ uptake but these were concomitant with residual resistances being increasingly negative, thus flux was unchanged. However, longer periods of cold temperature stress induced changes in both stomatal and residual resistances to SO_2 uptake such that flux to both varieties was significantly reduced. Again, residual resistances to SO₂ transfer in Dylan plants, in relation to increasing SO_2 concentration, were the reverse of those observed in non cold-stressed plants ie. they were largely negative at low SO₂ concentrations.

Such changes in the relative contributions of both the residual and stomatal resistances to pollutant flux when environmental conditions are limiting may have possible long-term consequences in plant pollutant responses. If, as for the stomatal resistance, the residual resistance is a purely physical resistance related to SO_2 diffusivity, then changes in the relative proportions of each which do not result in changes in flux, will not have significant effects on the overall plant response to the pollutant. However, if as suggested earlier, the residual resistance arises from internal detoxification mechanisms or the incorporation of the products of SO_2 solubility into plant metabolites, then a large decrease in this resistance (ie. r_r becoming largely negative) must indicate a significant change in plant metabolism. This can more easily be explained if we consider an hypothetical example of resistances in differing environmental regimes: Conclusions

Firstly,

(i) Optimum environmental condit	ions: stomatal resistance = 5 s cm	1 ⁻¹
	residual resistance = -3 s cm	1-1
Therefore,	net resistance to $SO_2 = 2 s cm$	، _1
Secondly,	-	
(ii) Low Light or 1 week cold:	stomatal resistance = 10 s cm	<u>'_1</u>
	residual resistance = -8 s cm	1 _1
Again, net	resistance to SO_2 uptake = 2 s cm	ت ،

In both instances, net resistance to SO_2 uptake is the same and flux is unchanged. However, the residual resistance to SO_2 uptake may be related to the detoxification of sulphite ions, possibly their oxidation to sulphate. In this case, in suboptimal environmental conditions when the residual resistance is seen to largely decrease, these data would lead to the conclusion that rates of detoxification had been enhanced. The observation of reduced net photosynthetic inhibition in response to high SO_2 concentrations when environmental conditions were limiting would lend credence to this conclusion.

Alternatively, if as outlined earlier, the site of the residual resistance is the incorporation of sulphite ions onto the binding sites on the enzyme RuBP carboxylase, then these data would suggest that under low light conditions or following low temperature stress, the rate of sulphite incorporation was increased. This hypothesis can be correlated to the observed low temperature-induced increases in total soluble leaf protein content since a large proportion of soluble protein is the enzyme RuBP carboxylase. Thus, following low temperature stress, there is an increase in the amount of this enzyme resulting in an increase in the number of binding sites available for reaction with sulphite ions which would correlate with large decreases in observed residual resistances to SO_2 uptake. Such action would inevitably lead to enhanced pollutant sensitivity since fewer bicarbonate ions would be able to bind with the enzyme resulting in enhanced net photosynthetic inhibition. Interestingly, the data for both Dylan and Aquadulce plants subjected to 1 week cold temperature stress showed enhanced pollutant sensitivity to low SO_2 concentrations when residual resistances were largely negative. The observed reduction in the degree of SO₂-induced net photosynthetic inhibition, as related to ambient SO_2 concentration, in such plants was

attributed to reduced pollutant flux related to low temperature-induced increased stomatal resistance. It would be of interest to determine what effect low light stress has on the total soluble protein content of <u>Vicia</u> <u>faba</u> CV. Dylan since reductions in SO₂-induced net photosynthetic inhibition were found to be the result of reduced sensitivity to the pollutant and not reduced flux. There is evidence in the literature to suggest that the synthesis of RuBP carboxylase within the chloroplast is strongly light dependent (Fitter & Hay, 1981). Assuming there is such a reduced synthesis of the enzyme under low light intensities in <u>Vicia faba</u>, this would provide a plausible explanation for reduced sensitivity to high SO₂ concentrations in Dylan plants in comparison to high light-grown plants.

The idea of of pollutant sensitivity being related to carbohydrate content was introduced by Dugger et al. (1962) in their study of ozone damage to pinto beans. The results obtained in this study also indicate that carbohydrate content of leaves prior to pollutant exposure is implicated in the differential sensitivity to SO₂ in cultivars of <u>Vicia faba</u>. In the absence of either cold or low light stress, Dylan plants were found to be more sensitive to low SO_2 concentrations than plants of the variety Aquadulce Claudia. Analysis of leaf carbohydrate content showed Dylan leaves to contain almost three times the carbohydrate content of Aquadulce leaves. Of significance is that exposure to either 24 h or 72 h of low temperature stress was seen to reduce the carbohydrate content of Dylan leaves. In such cold-stressed Dylan plants, SO₂-induced net photosynthetic inhibition was found to be lessened in relation to non cold-stressed plants. This reduced response was not found to result from reduced flux but from reduced sensitivity to the pollutant. Similarly, 1 week cold stress did not reduce the carbohydrate content of Dylan leaves although a reduced photosynthetic response to the pollutant was observed, this was found to result from reduced pollutant flux and not reduced pollutant sensitivity.

Cold temperature stress was not seen to reduce the carbohydrate content of Aquadulce leaves and no reduced sensitivity to low SO_2 concentrations was observed in Aquadulce plants. Indeed, exposure to either 72 h or 1 week cold temperature stress was seen to increase the carbohydrate content in Aquadulce plants when increased photosynthetic sensitivity to low SO_2 concentrations was noted. However, since reduced sensitivity to SO_2 concentrations above 300 ppb was observed in Aquadulce

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plants previously subjected to the 1 week cold treatments, it must be concluded that at high SO_2 concentrations some other mechanism is acting to confer this reduced sensitivity. Nevertheless, it would appear that the modification of plant responses to SO_2 by low temperature stress could be related to low temperature-induced changes in carbohydrate content. Thus it is apparent that further investigations into the link between carbohydrate content and sensitivity to SO_2 are merited.

Modification of plant responses to SO_2 by the imposition of low light or low temperature stress was also found to be related to significant effects on stomatal control of gas exchange. In the absence of environmental stress there were found to be relatively good correlations between SO₂-induced changes in net photosynthetic rates and SO₂-induced changes in stomatal resistance. Similarly, there were good correlations between changes in stomatal resistance and actual pollutant flux. These data indicate that the stomata play a significant part in governing both CO₂ exchange and SO₂ uptake although the major controlling factors for the exchange of both gases was found to be the residual resistances. However, when plants were grown under low light conditions or were subjected to low temperature stress prior to pollutant fumigation, no correlation between SO₂-induced changes in net photosynthetic rates and changes in stomatal resistances were found. Similarly, there was very little correlation between SO₂-induced changes in stomatal resistance and pollutant flux. The implication of these data is that the imposition of environmental stress had removed a degree of stomatal control over pollutant entry and CO₂ exchange. Since changes in stomatal resistance seemed to occur independently of pollutant flux or concentration it may be concluded that environmental stress had had a profound effect on that part of the stomatal control mechanism sensitive to SO₂. The observed reductions in flux in some plants were due to increased stomatal resistance resulting from the environmental stress and not the pollutant exposure.

In chapter 4 (S4.10.5) the significance of direct stomatal responses to SO₂ rather than effects mediated solely through changes in internal carbon dioxide concentration were discussed. Several authors dispute the existence of a direct effect of SO₂ on stomata; however, it was concluded that, under optimum environmental conditions, there was a direct stomatal response to SO₂ in <u>Vicia faba</u> and that the stomata exert some control over pollutant uptake. The observation that the residual resistance to CO_2 is a major controlling factor for gas exchange indicated that the stomata were also responding to changes in the intracellular CO_2 concentration. However, it would appear that following environmental stress, stomatal control is diminished. Although SO_2 elicited a direct stomatal response in low light-grown Dylan plants, the effects were small in comparison to that for high light-grown plants. The imposition of low temperature stress apparently resulted in loss of a direct stomatal response to SO_2 given the responses outlined above. It is thought that increased stomatal resistance resulting from exposure to SO_2 in plants subjected to longer periods of cold temperature stress was a secondary response to pollutant fumigation, possibly arising from changes in the intracellular CO_2 concentration as net photosynthetic rates were inhibited.

It was also shown that the magnitude of stomatal responses to SO₂ was much reduced in Dylan plants following the longer cold temperature pre-treatments but that the magnitude of stomatal responses to SO₂ was much increased in cold-stressed Aquadulce plants. These data would suggest that the reduction in independent stomatal control in cold temperaturestressed Aquadulce plants was accompanied by an increase in the sensitivity of the stomata to CO_2 .

The loss of a direct stomatal response to SO₂ following environmental stress could have severe consequences for the yield and growth of plants in the field since the stomata are important in pollutant avoidance. Mansfield & Freer-Smith (1984) pointed out that a true pollutant avoidance mechanism would involve stomatal closure in avoidance of stress in the mesophyll rather than as an event secondary to that stress. Thus stomatal closure which occurs as a result of inhibitory action of a pollutant on photosynthesis in the mesophyll cannot be looked upon as a desirable way of avoiding stress due to the pollutant. In Vicia faba it can be seen that, when environmental conditions are not limiting, the stomata respond to SO₂ directly thus acting as a true avoidance mechanism. However, following environmental stress, it would appear that this true avoidance mechanism is lost and stomata are reacting to SO_2 effects on the mesophyll. The effects of environmental stress on the residual resistance to SO_2 uptake, as outlined above, indicating a greater mesophyll effect, would support this theory. An increase in the effects of SO_2 in the mesophyll could go someway towards explaining the discrepancies in the observed

modification of pollutant responses by environmental stresses between short-term laboratory based experiments and long-term field fumigations.

This theory would suggest that suboptimal environmental conditions result in enhanced pollutant sensitivity ie. a greater inhibitory response when flux is unchanged which would concur with the observations of the Lancaster University group in their long-term experiments. However, since the results obtained in this study show <u>Vicia faba</u> to be less responsive to SO_2 following environmental stress, it is obvious that other mechanisms are acting to confer apparent reduced sensitivity. The data from this study show that apart from reduced pollutant flux when environmental conditions are suboptimal, reduced sensitivity may be linked to leaf carbohydrate content as discussed above.

The range of sulphur dioxide concentrations used in this study were in excess of ambient atmospheric concentrations based on annual, monthly or daily means. However, when hourly means are considered, it is still not uncommon to find ambient SO_2 concentrations to exceed 200 ppb for short periods of time especially close to point sources (see Chapter 1). The experiments performed in this study were designed to reflect such short, episodic fumigations and the higher concentrations were used to identify marked varietal differences and possible mechanisms of SO₂ action. The use of very high SO₂ concentrations in laboratory based experiments has often been justified as necessary to determine the physiological mechanisms of SO_2 on plants; the assumption is that the mode of SO_2 action is the same regardless of concentration. However, the data obtained in this study have shown this original premise not to hold true since plant responses to 100 ppb and 500 ppb were often the reverse of each other suggesting different modes of action depending on pollutant concentration. Thus high SO₂ concentrations can not be reflecting the mechanisms of SO_2 action at low ambient concentrations as found in the field.

Although, mean ambient SO_2 concentrations are low, as is described earlier, it is well documented that the SO_2 concentrations in ambient air are constantly fluctuating. Thus it is difficult to extrapolate from responses to constant levels of SO_2 observed in controlled experiments to predict responses to ambient SO_2 levels in the field even though the mean concentrations are very similar. It is possible that the observed pollutant effects in field exposures are arising from acute effects of
occasional short-term peaks. There is relatively little information regarding the effects of fluctuating SO_2 concentrations on plants. However, recent data from the Imperial College Research group at Silwood Park suggests that for long-term SO_2 injury, the over-riding parameter is the overall mean and not episodic high SO_2 peaks (Bell, 1985). A recent report from Ashmore, Bell & Minmack (1988) showed that plant responses are not significantly influenced by episodic SO_2 exposure and confirmed the earlier experimental results of Garsed & Rutter (1984, in Ashmore <u>et al.</u>, 1988). These data would suggest that results from fumigation experiments can be extrapolated to field conditions although it would still be particularly difficult to extrapolate from short experimental exposures to season-long responses.

However, it is recognised that if fumigation results are to be usable for predictive modelling of crop loss in the field then pollution exposure must be described in terms of foliar flux density and not ambient concentration. Total dose (mean concentration × duration) is often used to describe plant exposure over a growing season but this method assumes a proportional relationship between ambient pollutant concentration and flux to the plant. Similarly, this approach assumes there to be a strong general relationship between flux into the leaf and the amount of injury or yield loss. The data from this study have shown that such correlations do occur when environmental conditions are not limiting but there are significant interactive effects of environmental factors. These interactive effects include low temperature effects on stomatal and residual resistances which determine flux and changes in the relationship between flux and plant. response. Total dose also ignores the importance of peak concentrations which, as has been shown in this study, are likely to have a significant impact on plant productivity. It has been shown that the effects of 4 h SO₂ fumigations are often severe and, particularly for Aquadulce plants at high concentrations, are not readily reversible.

This type of study is designed to investigate interactive mechanisms of environmental and pollutant effects on plants, where environmental and pollutant variables can be carefully controlled, and not to develop predictive models for crop loss assessment. It would be very difficult to extrapolate the data obtained in this study, involving the monitoring of the effects of short SO_2 fumigations together with the interactive effects of short-term low temperature exposures in depressing net photosynthetic rates, to predict long-term consequences on plant yield under field conditions. It has yet to proved whether there is a proportional correlation between depressed photosynthetic rates as a result of pollutant fumigation and ultimate reductions in yield since the relationship between carbon dioxide uptake and eventual yield is not direct. The situation is further complicated by the certain knowledge that both SO_2 and temperature influence photosynthate allocation and assimilate distribution, a point highlighted by significant changes in leaf carbohydrate content in these short experiments.

In the field, effects on yield over a growing season would depend on how often these episodic high pollutant peaks occur, remembering that such responses to pollutant peaks would be set against a background of low ambient SO₂ concentrations since SO₂ is present continually. Reduced yield has been shown to occur in plants exposed to 43 μ g m⁻³ SO₂ for 173 days in winter (Bell, Rutter & Relton, 1979), thus it can be assumed that the continuous presence of low ambient SO₂ concentrations is stressful to plants under certain environmental conditions. It is highly probable that continual exposure to low ambient SO₂ concentrations. Also, SO₂ seldom, if ever, occurs alone and ambient air is usually a cocktail of low pollutant concentrations, particularly ozone and nitrogeA oxides which have both been shown to influence plant growth and yield.

Another difficulty in extrapolating these data to field conditions is that environmental variables are constantly fluctuating and, in winter, temperatures will be below 10°C for periods of weeks not days. Low light intensities, low humidity and high CO_2 levels also typify winter conditions, all of which influence leaf diffusive resistances thus influencing pollutant flux and CO_2 exchange.

It is clear that if dose-response relationships are to be clearly defined, leading to the accurate prediction of yield losses then several research approaches must be undertaken together. Chamberless field-based studies can provide information as to crop loss over a single, or several growing seasons. However, such studies can provide little information regarding mechanisms of pollutant action or environmental modification since there is little control over levels of pollutants; also such experiments Conclusions

are not readily replicated since environmental conditions and ambient pollutant levels are constantly fluctuating. Secondly, field-based open-top chambers can be used where a wide range of treatments can be imposed and the effects of air filtration can be well defined. However, the presence of the chamber may result in changes in the climate surrounding the plants in comparison to field conditions. Such modifications include reduced air-flow over leaves which may alter pollutant flux characteristics. Air temperatures, especially in winter, may be higher than in the open field and even a small difference of 1 or 2°C may affect crop responses to air pollutants (Roberts, 1984). Both these approaches can provide data regarding dosc-response relationships and effects on yield but results are confounded with the effects of environmental factors and as such, are not readily replicable. It is also very difficult in such field-based studies to define the pollutant dose over the growing season which, as described earlier, is often expressed in terms of total dose and relies heavily on ambient pollution concentrations. Thirdly, laboratory based chambered systems can be used where plants can be exposed to a wide range of pollutant treatments and environmental treatments and other variables can be well controlled. Because environmental and pollutant exposure characteristics can be well controlled, such experiments can easily be replicated and can provide information regarding mechanisms of pollutant action and how plant pollutant responses are modified by prevailing environmental conditions. Another advantage of such controlled experiments is that actual pollutant uptake by the plant can be accurately determined. and plant responses quantified in terms of pollutant flux.

A knowledge of interactive mechanisms can resolve some of the difficulties in interpreting data from field-fumigation studies and explain discrepancies in data between studies. The data from all research approaches together will bring us closer to being able to predict possible crop loss in a given area with correlation to environmental factors.

As described above this study has highlighted several factors which contribute to environmental modification of plant pollutant responses and has gone someway to explaining the wide range of responses to pollutants observed in the field.

It is clear that the direction of future research must be towards elucidation of the effects of both low light/short photoperiod and low temperature conditions together in moderating plant pollutant responses. The converse also applies in that exposure to pollutants may alter plant responses to environmental stresses and may predispose the plant to injury, thus this area also requires careful investigation. The use of unrealistic high SO₂ concentrations should be avoided in an attempt to approximate field conditions if precise mechanisms of pollutant action are to be elucidated. Of importance also is that SO₂ seldom occurs alone and the interactive effects of other gaseous pollutants must be considered. Given the marked cultivar specific responses to combined environmental/pollutant stresses observed in this study, it is evident that generalisations as to the mechanisms of pollutant effects on plants can not be made. Separate investigations of economically important species must be undertaken to provide a database of information which is of use to agriculturalists in identifying sensitive or tolerant strains and achieving maximum productivity.

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Computer Calculation of Gas Exchange Parameters

The computer programme presented below was originally devised by V.J. Black & D. Ormrod (*pers. comm.*) and was adapted for use on a BBC Master microcomputer by Mr P. Geissler. The programme enabled the computation of all the gas exchange parameters defined in §2.3 both directly and quickly. The units required for input of data into the programme are:-

- (i) C_i, water vapour content of air entering chamber as dew point temperature in °C.
- (ii) Co, water vapour content of air leaving chamber as dew point temperature in °C.
- (111) CiCO, CO₂ uptake in ppm (difference between CO_2 concentration in incoming air and CO_2 concentration in outgoing air).
- (iv) Tamb, chamber temperature in *C.
- (v) T_{1} , leaf temperature in ^{*}C.
- (vi) LA, leaf area, cm².
- (vii) FR, flow rate, f min⁻¹.
- (viii) r_a, aerodynamic resistance, s cm⁻¹.
- (ix) Sin, SO₂ concentration of incoming air, ppb.
- (x) Sout, SO_2 concentration of air leaving chamber, ppb.
- (xi) Ssorb, SO₂ concentration adsorbed onto chamber walls, ppb.

PROGRAMME

5 REM ***COPYRIGHT G. A. HUNT & P. GEISSLER 1989***

- 10 *****TVO, 1
- 20 MODEO
- 30 VDU5
- 40 PRINT' TAB(20)"GAS EXCHANGE PARAMETERS"

- 70 INPUT'"DATE ? "D\$;:VDU11:INPUTTAB(25)"Title ? "T\$
- 80 PRINT''"Ci(H";:VDU25,0,0;-12;:PRINT"2";:VDU25,0,0;12;:INPUT"O) = "Ci
- 90 PRINT''Co(H";:VDU25,0,0;-12;:PRINT"2";:VDU25,0,0;12:INPUT"0) = "Co

- 100 PRINT' "CiCo(CO";: VDU25, 0, 0; -12;: PRINT"2";: VDU25, 0, 0; 12;: INPUT") = "CiCo
- 110 PRINT' "Ambient Temperature (T";: VDU25,0,0;-12;: PRINT"Amb";: VDU25,0,0; 12;: INPUT") = "Tamb
- 120 PRINT'"Leaf Temperature (T";:VDU25,0,0;-12;:PRINT"L";:VDU25,0,0;12; :INPUT") = "TL
- 130 INPUT' "Flow Rate (FR) = "FR
- 140 INPUT'"Leaf Area (LA) = "LA
- 150 PRINT' "Aerodynamic Resistance (r";:VDU25,0,0;-12;:PRINT"a";:VDU25,0,0;12;:INPUT") = "ra
- 160 PRINT' TAB(15)"Do you want SO";: VDU25,0,;-12;: PRINT"2";: VDU25,0,0; 12; : PRINT"fluxes, luv? (Y/N) ";
- 170 Ans\$=GET\$: IF Ans\$="Y" or Ans\$="y" F%=1: PRINT"Yes" ELSE F%=0: PRINT"No" :GOTO210
- 180 PRINT' "SO"; : VDU25, 0, 0; -12; : PRINT"2"; : VDU25, 0, 0; 12; : INPUT" in = "Sin
- 190 PRINT' "SO";: VDU25, 0, 0; -12;: PRINT"2";: VDU25, 0, ; 12;: INPUT" out = "Sout
- 200 PRINT' "SO"; : VDU25, 0, 0; -12; : PRINT"2"; : VDU25, 0, ; 12; : INPUT" chamber sorption = "Ssorb
- 220 Ci=FNmb(Ci):Co=FNmb(Co)
- 230 Tamb=Tamb+273.2: TLk=TL+273.2
- 240 Cav = (Ci+Co)/2
- 250 E=((Co-Ci)/Tamb)*((FR*1000)/(LA*60))
- 260 EH20=E*7812
- 270 eSTL=FNmb(TL)
- 280 Xo=eSTL/TLk
- 290 Xi=Cav/Tamb
- 300 rtH2O=(Xo-Xi)/E
- 310 rs=rtH2O-ra
- 320 Gs=1/rs
- 330 vpd=eSTL-Cav
- 340 IF F%=0 GOTO 380
- 350 flux=((Sin-Sout-Ssorp)*FR*2.86)/(LA*6)

```
360 rtS02=((Sout*2.86/flux)-(ra*157))/100
```

370 GtS02=1/rtS02

380 Pnet=(CiCo*FR*60*1.83)/(LA*100)

400 @%=&20310

- 410 PRINT' TAB(15)"Do you want to print the results ";
- 420 IF F%=1 PRINT"too ? (Y/N)"ELSE PRINT"then ? (Y/N)"

430 Ans\$=GET\$: IF Ans\$="y" OR Ans\$="Y" P%=1 ELSE P%=0

- 440 CLS
- 450 IF P%=1 VDU2
- 460 VDU1, 27, 1, 87, 1, 1, 1, 27, 1, 71: PRINT' D\$TAB(20); T\$: VDU1, 27, 1, 87, 1, 0, 27, 1, 72
- 470 PRINT''"Transpiration Rate (EH";: VDU25,0,0;-12; 1, 27, 1, 83, 1, 1: PRINT"2";: VDU25,0,0; 12; 1, 27, 1, 84: PRINT"O) "; TAB(40); EH20; "gm";: VDU25,0,0; 12; 1, 27, 1, 83, 1, 0: PRINT"-2";: VDU25,0,0;-12; 1, 27, 1, 84: PRINT"hr";: VDU25,0,0; 12; 1, 27, 1, 83, 1,0
- 480 PRINT"-1";: VDU25, 0, 0; -12; 1, 27, 1, 84
- 490 PRINT''"Stomatal resistance (rs) "; TAB(40); rs; " s cm"; : VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT"-1; : VDU25, 0, 0; -12; 1, 27, 1, 84
- 500 PRINT''"Stomatal conductance (1/rs) "; TAB(40); GS; " CM S"; : VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT "-1"; : VDU25, 0, 0; -12; 1, 27, 1, 84
- 510 PRINT''"Vapour Pressure Deficit (vpd) "; TAB(40); VPD; " mb "
- 520 IF F%=0 GOTO 590
- 530 PRINT' "SO";: VDU25, 0, 0; -12; 1, 27, 1, 83, 1, 1: PRINT"2";: VDU25, 0, 0; 12; 1, 27, 1, 84: PRINT" flux "; TAB(40); flux; " ug m";: VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT"-2";: VDU25, 0, 0; -12; 1, 27, 1, 84: PRINT"S";: VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT"-1";
- 540 VDU25, 0, 0; -12; 1, 27, 1, 84
- 550 PRINT''"SO";: VDU25, 0, 0; -12; 1, 27, 1, 83, 1, 1: PRINT"2";: VDU25, 0, 0; 12; 1, 27, 1, 84: PRINT" resistance "; TAB(40); rtSO2; " s cm";: VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT"-1";: VDU25, 0, 0; -12; 1, 27, 1, 84
- 560 PRINT''"SO";: VDU25, 0, 0; -12; 1, 27, 1, 83, 1, 1: PRINT"2";: VDU25, 0, 0; 12; 1, 27, 1, 84: PRINT" / H";: VDU25, 0, 0; -12; 1, 27, 1, 83, 1, 1: PRINT"2";: VDU25, 0, ; 12; 1, 27, 1, 84: PRINT"O resistance"; TAB(40); rs*1.98; " s cm";: VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT"-1;
- 570 VDU25,0,0;-12; 1,27,1,84
- 580 PRINT''"SO";: VDU25, 0, 0; -12; 1, 27, 1, 83, 1, 1: PRINT"2";: VDU25, 0, 0; 12; 1, 27, 1, 84: PRINT" conductance"; TAB(40); GtS02; " cm s";: VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT"-1";: VDU25, 0, 0; -12; 1, 27, 1, 84
- 590 PRINT''"Net Photosynthesis (Pnet) "TAB(40); Pnet;" g m";: VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0: PRINT"-2";: VDU25, 0, 0; -12; 1, 27, 1, 84: PRINT" hr";: VDU25, 0, 0; 12; 1, 27, 1, 83, 1, 0
- 600 PRINT"-1";: VDU25, 0, 0; -12; 1, 27, 1, 84, 1, 13
- 610 IF P%=1 VDU3
- 620 PRINT '''TAB(10)"PRESS BREAK TO CONTINUE"
- 625 *KEY10 OLDIIMCLSIIMRUNIIM
- 630 END
- 640 DEF FNmb(X) :REM ***CONVERTS DEW POINT TO MILLIBARS***
- 650 =6.1078*EXP(17.2694*(X/(X+238.3)))