STUDIES ON THE DEVELOPMENT OF SULPHUR DIOXIDE TOLERANCE IN GRASSES

by

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ABSTRACT

The level of chronic and acute SO_2 tolerance in populations and clones of the economically important grass species *Agrostis tenuis, Dactylis glomerata, Festuca rubra, Lolium multiflorum, L. perenne, Phleum pratense* and *Poa pratensis,* was determined by fumigation with 300-350 μgm^{-3} SO_2 for several weeks (chronic) and 4000-5500 μgm^{-3} for 6 hrs (acute). In several cases chronic and acute fumigation caused differential growth reductions and % leaf length injury, respectively, between populations from polluted and unpolluted sources, indicating the development of tolerance. Difficulty was encountered in differentiating between clones for chronic tolerance. In an attempt to develop a rapid screening technique, the relationships of chronic tolerance with fluorescence and reflectance of leaves were established.

The development of SO_2 tolerance was investigated by 4 approaches:

a) The minimum time for tolerance to occur, by annual comparison between plant samples from plots sown at a polluted site and from the original seed sown.

b) The long term development of tolerance, by comparison of 5 lawns of different ages (1-200 yrs) from a polluted area.

c) Analysis of tolerance along a transect from a point source.

d) Investigation of the genetic control of acute SO_2 tolerance by diallel analysis.

The effects of SO_2, the orders of tolerance between species, the relationship between chronic and acute injury and between plant growth and chronic injury are discussed. The occurrence and rate of development of SO_2 tolerance, are also discussed, and considered in relation to their possible practical significance.

LIST OF CONTENTS

		PAGE
ABSTRACT		*i*
CHAPTER 1:	INTRODUCTION	1
CHAPTER 2:	GENERAL MATERIALS AND METHODS	14
	2.1 Potting Mix	14
	2.2 Choice of Grass Species	14
	2.3 Field Sites	15
	2.3.1 Helmshore	15
	2.3.2 Askern	19
	2.3.3 Philips Park	25
	2.3.4 The Temple	30
	2.4 SO_2 Sampling System	36
	2.5 Fumigation Systems	36
	2.5.1 Outdoor Perspex Chambers	36
	2.5.2 Outdoor Glass Chambers	41
	2.5.3 Controlled Environment Chamber	44
	2.6 Assessment of SO_2 Effects	47
	2.6.1 Chronic Fumigation	47
	2.6.2 Acute Fumigation	48
CHAPTER 3:	AN INVESTIGATION OF THE RATE OF DEVELOPMENT OF SO_2 TOLERANCE	50
	3.1 Screenings for Acute SO_2 Tolerance in Philips Park Populations	50
	3.1.1 Method	50
	3.1.2 Results and Discussion	51

LIST OF CONTENTS (Continued)

PAGE

3.2 Repeat Screenings for Acute SO_2 Tolerance 53

3.2.1 Results and Discussion 53

3.3 Screening for Chronic SO_2 Tolerance in the Philips Park Populations 54

3.3.1 Method 54

3.3.2 Results and Discussion 56

3.4 Investigation of the Genotypic Structure of the 1979 *Lolium perenne* Population from Philips Park 67

3.4.1 Morphology 68

3.4.2 Electrophoretic Technique 68

3.4.3 Results of Morphological Observations.. 70

3.4.4 Results of Isozyme Analysis 71

3.4.5 Discussion of Genotypic Structure 75

3.5 General Discussion 77

CHAPTER 4: THE DEVELOPMENT OF ACUTE SO_2 TOLERANCE IN LAWNS OF DIFFERENT AGES 80

4.1 Introduction 80

4.2 Method 81

4.3 Results and Discussion 81

CHAPTER 5: DEVELOPMENT OF SO_2 TOLERANCE IN POPULATIONS AT DIFFERENT DISTANCES FROM A POINT SOURCE 89

5.1 Introduction 89

5.2 Screenings for Acute SO_2 Tolerance in Populations Along the Askern Transect 89

LIST OF CONTENTS (Continued)

PAGE

5.2.1 Method 90

5.2.2. Results and Discussion 90

5.3 Screening for Chronic SO_2 Tolerance in *Festuca rubra* Populations Along the Askern Transect 97

5.3.1 Method 97

5.3.2 Results and Discussion 97

5.4 Comparison of Acute and Chronic SO_2 Tolerance with Vegetative Characters in *F. rubra* Along the Askern Transect. 101

5.4.1 Clinal Patterns 102

5.4.2 Character Correlations 104

5.4.3 Population Variability 106

5.5 General Discussion 106

CHAPTER 6: AN INVESTIGATION OF SOME OF THE GENETIC ASPECTS OF ACUTE SO_2 TOLERANCE 109

6.1 Methods 109

6.1.1 Preparation of Selected Clones 109

6.1.2 Crossing 110

6.1.3 Screening of Parents and Progeny for Acute SO_2 Tolerance 111

6.2 Biometrical Analysis of Diallel 113

6.2.1 Analysis of Variance 114

6.2.2 The Relationship of Variance and Covariance of Arrays 117

6.2.3 Estimation of the Main Components of Variation 122

6.3 General Discussion 124

LIST OF CONTENTS (Continued)

PAGE

CHAPTER 7: SELECTION OF TOLERANT AND NON TOLERANT GENOTYPES 126

7.1 Introduction 126

7.2 Screening for Chronic SO_2 Tolerance of 19 Clones of *Lolium perenne* from Helmshore. 126

7.2.1 Method 126

7.2.2 Results and Discussion 127

7.3 Screening for Acute SO_2 Tolerance of 19 Clones of *Lolium perenne* from Helmshore. 131

7.3.1 Method 131

7.3.2 Results and Discussion 131

7.4 Screening for Chronic SO_2 Tolerance of *Lolium perenne* Clones from Several Populations 133

7.4.1 Method 133

7.4.2 Results and Discussion 134

7.5 General Discussion 140

CHAPTER 8: AN INVESTIGATION OF REMOTE SENSING TECHNIQUES FOR ESTIMATING CHRONIC SO_2 TOLERANCE IN *LOLIUM PERENNE*. 141

8.1 Introduction 141

8.1.1 Fluorescence 141

8.1.2 Reflectance 145

8.2 An Investigation of the Relationship Between Chlorophyll Fluorescence and Chronic SO_2 Tolerance 147

8.2.1 Method 147

8.2.2 Results and Discussion 149

LIST OF CONTENTS (Continued)

PAGE

8.3 An Investigation of the Relationship Between Reflectance and Chronic SO_2 Tolerance 154

8.3.1 Method 154

8.3.2 Results and Discussion 156

8.4 Conclusions 159

CHAPTER 9: DISCUSSION OF SO_2 EFFECTS 164

9.1 Chronic SO_2 Injury 164

9.2 Acute SO_2 Injury 183

9.3 Comparison of Chronic and Acute SO_2 Injury. 186

9.4 Conclusions 189

CHAPTER 10: DISCUSSION OF SO_2 TOLERANCE 192

SUMMARY 205

ACKNOWLEDGEMENTS 209

REFERENCES 210

APPENDICES

1. Regression of SO_2 Concentration Against Averaging time for SO_2 Pollution Characteristics at Philips Park. 228

2. Assessment of Acute Injury 229

3. Mean Fluorescence and Reflectance Values of Leaves of *L. perenne* After Chronic Fumigation 235

4. Growth of Plants in First and Second Screening Experiments 238

LIST OF CONTENTS (Continued)

PAGE

5. Enzyme Staining Solution Recipes for Starch Gel Electrophoresis 241

6. SO_2 Concentrations during Chronic Fumigations 242

LIST OF TABLES

PAGE

2.1 Plant Material Used in Fumigation Experiments and its Sources. 16

2.2 SO_2 Levels at Philips Park 29

3.1 % Leaf Length Injury of Philips Park and Original Seed Samples After Acute Fumigation 52

3.2 Repeated Acute Fumigations 55

3.3 Growth of Original Seed and Philips Park Populations after Fumigation with 317 μgm^{-3} SO_2 for 123 days c f. Growth in Clean Air. 57

3.4 Comparison of % Change in Growth of Original Seed and Philips Park Grass Populations Subjected to a Chronic SO_2 Fumigation of Mean Concentration of 317 μgm^{-3} for 123 days. 60

3.5 Acute Exposure from Chronic Fumigation. 64

3.6 Growth of Philips Park Populations in the Clean Air Chamber for 123 days. 66

3.7 Frequency of Different Morphological Groups in the P.P. Population of *Lolium perenne* 71

3.8 Frequency of Different Isozyme Patterns in the P.P. Population of *L. perenne* 75

3.9 Combination of Morphological and Electrophoretic Techniques for Clonal Recognition to Give the Least Number of Distinct Genotypes in the Sample of *L. perenne* from P.P. 76

3.10 % Ground Cover by Grass Species on Plots at Philips Park 79

LIST OF TABLES (Continued)

		PAGE
4.1	SO_2 Concentrations and % Mean Acute Leaf Injury for Each Fumigation of Temple Populations	82
4.2	Analysis of Variance of Acute Injury of The Temple Populations	85
5.1	SO_2 Concentrations and Mean % Injury for Acute Fumigations of the Askern Populations.	90
5.2	Analysis of Variance of Acute Injury of the Populations Along the Askern Transect	92
5.3	Growth of Askern Populations After Fumigation with 299 μgm^{-3} SO_2 or Clean Air for 139 days	98
5.4	Within Site Correlations and Between Site Correlations for a Range of Growth Parameters and SO_2 Injury	105
6.1a	Numbers of Progeny Produced from Diallel Cross	112
6.1b	Numbers of Progeny Produced from Self Pollination.	112
6.2	Hayman Analysis Adapted for the Half Diallel of Acute SO_2 Injury	116
6.3a	Analysis of Variance of Wr + Vr.	118
6.3b	Analysis of Variance of Wr - Vr.	118
6.4	Half-Diallel Table Pooled over Plots and Fumigations	119
6.5	Analysis of Variance of Wr/Vr Regression	119
6.6	Estimates of Components of Variation	123
7.1	Classification of Clones of *L. perenne* Chosen for Screening for Chronic SO_2 Tolerance	128
7.2	Analysis of Growth of Selected Clones of *L. perenne* in a Mean of 354 μgm^{-3} SO_2 for 140 days Compared with Filtered Air.	128

LIST OF TABLES (Continued)

		PAGE
7.3	Analysis of Acute Fumigation of 19 Clones	131
7.4	Analysis of Growth of 19 Clones of *L. perenne* in 336 μgm^{-3} SO_2 for 130 days Compared With Clean Air.	135
7.5	Single Degree of Freedom Comparisons Between Populations of *L. perenne* for Chronic SO_2 Injury..	138
8.1	Leaf Absorption Spectra	146
8.2	Analysis of Fluorescence Measurements of Single Leaves of *L. perenne* after Chronic Fumigation	151
8.3	Coefficients of Correlation Between Parameters of Chronic SO_2 Injury and Fluorescence	153
8.4	Regression of Fluorescence Against Growth in SO_2 Treatment cf. Growth in Control	155
8.5	Analysis of Hemispherical Reflectance of Single Leaves of *L. perenne* after Chronic SO_2 Fumigation.	157
8.6	Coefficients of Correlation Between Parameters of Chronic SO_2 Injury and Reflectance	158
8.7	Regressions of Reflectance Against Growth Response to Chronic SO_2 Concentrations	159
9.1	Summary of Growth Analyses from Fumigations Involving Several Grass Species	166
9.2	Summary of the Growth of Grass Species of Chronic SO_2 Fumigations Compared to Growth in Clean Air	171
9.3	Regression of Clonal Mean Growth in SO_2 Against Growth in Clean Air for Screening Experiment 1.	176

LIST OF TABLES (Continued)

PAGE

9.4 Regressions of Growth in SO_2 Against Growth in Clean Air for Screening Experiment 2. 177

9.5 Regressions of Growth in SO_2 Against Growth in Clean Air for Chronic Fumigation of the Askern Populations. 178

9.6 Regressions of Growth in SO_2 Against Growth in Clean Air for the Philips Park Populations 179

9.7 Correlation Coefficients Between the Levels of Sensitivity to Acute and Chronic Injury for Several Grass Species 187

LIST OF FIGURES

		PAGE
1.1	Mean SO_2 Levels at Selected Sites in the U.K. During the Period 1968-79.	2
2.1	Map of the Location of the Great House E.H.F., Helmshore and the Surrounding Area.	17
2.2	Mean Winter (October - March inclusive) SO_2 Concentrations at Helmshore.	20
2.3	Location of the Collection Site at Helmshore	21
2.4	Map Showing the Collection Sites (A-H) Along the Transect at Askern, in Yorkshire.	22
2.5	Location of Philips Park in Manchester Showing the Surrounding Industrial Premises	26
2.6	Location of the Temple in London and the Lawns Sampled.	32
2.7	Annual Average of SO_2 at Ground-Level for 7 Inner-London National Survey Sites	33
2.8	SO_2 Sampling System	37
2.9	Outdoor Perspex Fumigation Chambers	39
2.10	Outdoor Glass Fumigation Chambers	42
2.11	Controlled Environment Fumigation System	45
3.1	Chronic Injury/Acute Injury	65
3.2	Interpretation of Enzyme Banding Patterns for PGI and GOT from Replicate Starch Gels After Electrophoresis of Leaf Samples of 21 Clones of *L. perenne*	73
4.1	Acute SO_2 Injury in The Temple Lawns	84

LIST OF FIGURES (Continued)

		PAGE
4.2	Distribution of Acute SO_2 Injury in the Lawn Populations from The Temple	86
5.1	Acute SO_2 Injury of Populations Along the Askern Transect.	93
5.2	Distribution of Acute SO_2 Injury in the Populations Along the Askern Transect.	95
5.3	Growth of Askern Transect Populations of *Festuca rubra* Subjected to 299 μgm^{-3} SO_2 for 130 days Compared to Growth in Clean Air.	100
5.4	Growth of Askern Transect Populations in Filtered Air Chamber.	103
5.5	Population Variation Over the Askern Transect Expressed as a % of the Site with the Maximum Value..	107
6.1a	Wr/Vr Graph for Acute SO_2 Injury	121
6.1b	Relationship of Wr + Vr and Mean Acute SO_2 Injury.	121
7.1	Acute Injury of 19 Selected Clones of *Lolium perenne*.	132
7.2	Growth of 19 Clones of *Lolium perenne* Subjected to a Mean of 336 μgm^{-3} SO_2 for 130 days Compared to Growth in Clean Air.	137
8.1	The 'Z Scheme' of Electron Flow in Photosynthesis, Showing the Involvement of the Two Photoreactions	142
8.2	Action Spectra for Photosystem 1(PS1) and Photosystem 2 (PS2).	142
8.3	The Kautsky Effect as Observed for *Lolium perenne*.	144
8.4	Spectral Reflectance Curve of *Mimulus cardinalis*..	146

LIST OF FIGURES (Continued)

PAGE

8.5 Apparatus for Dual Beam Excitation of Chlorophyll Fluorescence. 148

8.6 Typical Fluorescence Curve from Dual Beam Method. 150

9.1 Dose Response Curve for Chronic SO_2 Fumigations of Shoot Dry Wt. of *Lolium perenne* 170

9.2 Dose Response of Populations to Acute SO_2 Fumigation for Each Species 184

LIST OF PLATES

		PAGE
2.1	View of the Rossendale Valley Above Helmshore Showing the Collection Site of *L. perenne* sample.	18
2.2	View of Askern with Coalite Smokeless Fuel Plant in the Centre Background and the Clinic, to the Right of the Middle, Where the Meloy Continuous SO_2 Monitor is in Operation.	24
2.3	The Plots at Philips Park, Sown by the S.T.R.I. (1975)	27
2.4	Lawns of The Inner Temple in London.	34
2.5	Lawns of The Middle Temple in London	37
2.6	Outdoor Perspex Fumigation Chambers	40
2.7	Outdoor Glass Fumigation Chambers	43
2.8	Environmentally Controlled Fumigation Chamber.	46
3.1	Starch Gel Showing Electrophoretic Variants of PGI for 21 Clones of *L. perenne* from the 1980, Philips Park Population.	72
3.2	Starch Gel Showing Electrophoretic Variants of GOT for 21 Clones of *L. perenne* from the 1980, Philips Park Population	74

CHAPTER 1

INTRODUCTION

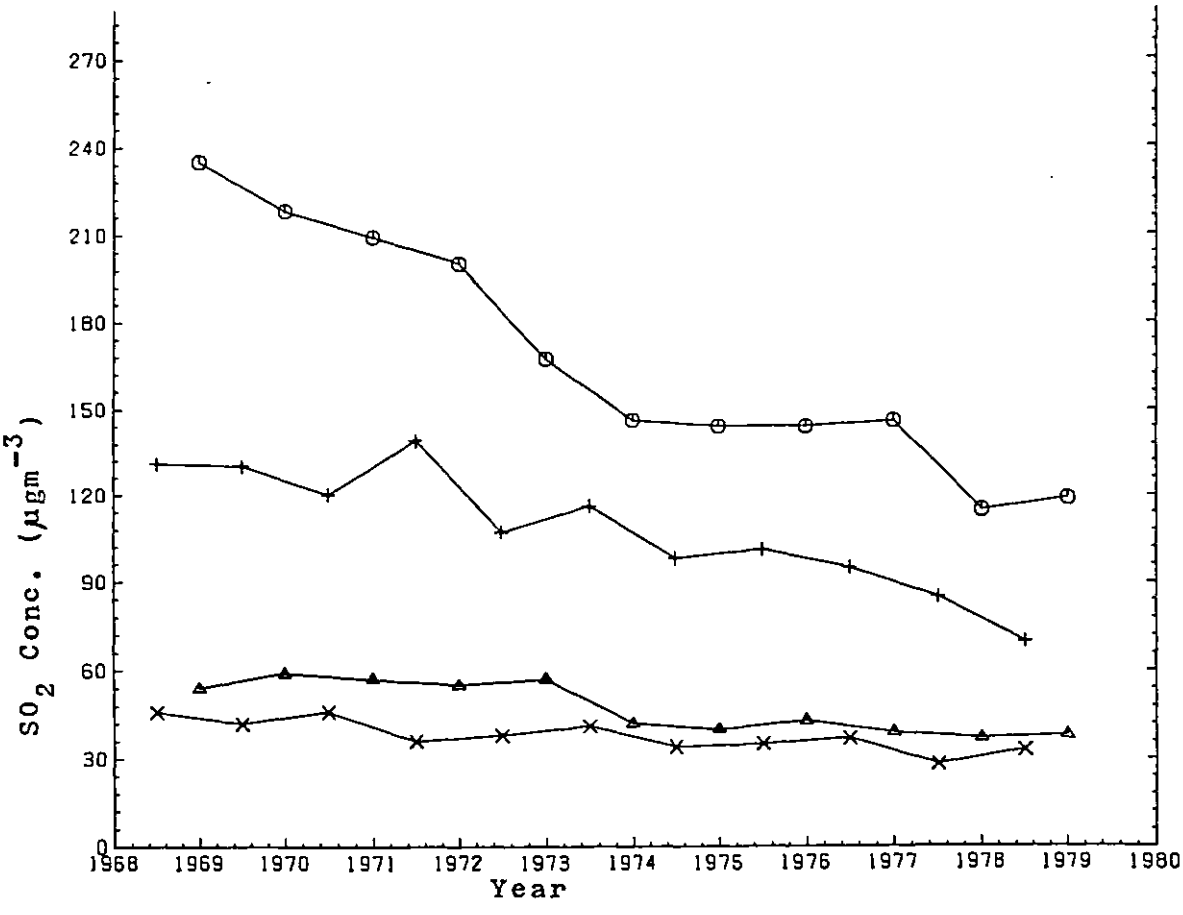
The most important gaseous air pollutants currently found in the United Kingdom are carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NO_x), ozone (O_3)and sulphur dioxide (SO_2), of which the last three can occur at phytotoxic concentrations on occasions. SO_2 is formed principally as a result of the combustion of coal and oil and currently emitted at an annual rate of 5×10^6 tonnes in this country.

Though the national output of SO_2 has remained fairly constant, since "The Clean Air Act" (1956), there has been an overall decrease in urban and rural ground level concentrations (Roberts and Lane, 1981), probably as a result of increased efficiency of dispersion (Fig. 1,1). However, this involves the emission of gas higher into the atmosphere, so that it travels further, and is thought to be the cause of increased acidity of precipitation in Scandinavia.

The removal of SO_2 and its derivatives from the atmosphere by wet deposition accounts for about 17% of SO_2 deposited on land surfaces in the U.K. The remaining 83% is removed from ambient air by dry deposition on soil, water and vegetation. It has been estimated that much of rural Britain will receive an atmospheric input of about 110 kg ha^{-1} $year^{-1}$ of sulphur, assuming a mean SO_2 level of $40\mu gm^{-3}$(Garland, Clough and Fowler, 1973).

The route of SO_2 uptake into plants, which is primarily through the stomata, can be considered using a resistance model. Firstly there

Fig. 1.1 Mean SO_2 Levels at Selected Sites in the U.K. During the Period 1968-79.



○- Urban, Winter means

+- " ", Summer means

△- Rural, Winter means

×- " ", Summer means

From data collated by Roberts and Lane (1981)

is the aerodynamic resistance (r_a), created by the boundary layer close to the leaf surface, where the transition from turbulent to molecular diffusion takes place. The stomatal pore offers resistance (r_s) to the movement of gas through it, which depends on the degree of stomatal opening. This in turn is influenced by environmental factors such as light, water status, CO_2 concentration and the presence of pollutant gases themselves. When exposed to SO_2 stomata are known to open (Majernik and Mansfield, 1970; Biscoe, Unsworth and Pinckney, 1973) although this response may be modified by CO_2, SO_2 concentrations and humidity. A further factor, internal resistance to SO_2, is probably normally negligible, because it is rapidly oxidised to sulphate on the walls of the substomatal cavity. However, when subjected to high concentrations or prolonged exposure to relatively high levels of SO_2, the internal resistance may increase substantially (Unsworth, Biscoe and Black, 1976).

Visible damage caused by SO_2 on plants is usually in the form of necroses on the tips and along the lengths of the leaves on monocotyledons and intercostally on dicotyledons (Jacobson and Hill, 1970; Van Haut and Stratmann, 1970; Guderian, 1977). These necroses are typical of acute injury, believed to be the result of the accumulation of sulphite, to toxic concentrations in the leaf. This probably occurs because the plant absorbs SO_2 at a faster rate than it is metabolized to less toxic forms (Thomas, Hendricks and Hill, 1950; Thomas, 1951). Acute SO_2 injury occurs after exposures to SO_2 concentrations of about 600μgm^{-3} or greater for relatively short periods. In the field it is restricted to sites where very high concentrations of SO_2 occur, such as within major cities or near industrial point sources.

A second form of SO_2 injury, chronic injury is the result of long term exposure to relatively low levels of the gas. It takes the form of a yellowing of the leaves (chlorosis) and premature senescence or else is only detectable as decreased growth, without any visible symptoms. The existance of invisible injury was disputed until the early 1970's (Thomas and Hill, 1937; Setterstrom, Zimmerman and Crocker, 1938; Thomas et al, 1950; Thomas, 1951). One of the first demonstrations of invisible injury was by Tingey, Reinert, Dunning and Heck (1971) who exposed *Raphanus sativus* cv. Cherry Belle to 143μgm^{-3} SO_2 for 40 hrs per week for 5 weeks, which reduced hypocotyl yield significantly more than clean air controls, but without any visible symptoms appearing. Further experimental fumigations have been carried out in Britain on various grass species and shown growth reductions (Bell and Clough, 1973; Lockyer, Cowling and Jones, 1976; Ashenden and Mansfield, 1978; Bell, Rutter and Relton, 1979; Ashenden, 1979; Ashenden and Williams, 1980), using a range of SO_2 concentrations between 40 and 450μgm^{-3} applied continuously for periods ranging from 43 to 180 days. These SO_2 concentrations were similar to contemporary ambient SO_2 levels found in urban and urban fringe areas of Britain suggesting that effects of chronic SO_2 may be widespread.

Evidence of the effects of ambient air on grasses had been previously demonstrated by Bleasdale (1973) in an experiment carried out in the early 1950's. *L. perenne* cv S23 was reduced in growth, by the ambient air of a Manchester suburb containing a mean SO_2 concentration of between 137 and 175μgm^{-3}, by between 16 and 57% compared to plants of the same cultivar grown in purified air at the same site. Recently Crittenden and Read (1978, 1979) have shown reduced growth and yield of

L. perenne, *L. multiflorum* and *Dactylis glomerata* caused by the ambient air of a Sheffield suburb in which the mean SO_2 concentrations were no greater than 70$\mu gm^{-3} SO_2$. Both experiments showed greater growth reductions than have generally been found with fumigations using pure SO_2. It was considered that SO_2 was the predominant agent in the depression of yield, but that fluctuations in ambient SO_2 levels and the presence of other pollutant gases enhanced this effect (Bell, 1982).

The majority of grasslands in urban areas are parks and sports-fields, and the above evidence suggests that ambient SO_2 may contribute to their degradation. This is especially likely under winter conditions when regeneration of turf is slow, increasing the effects of wear from trampling. SO_2 levels are higher in the winter due to an increased combustion of coal and oil, and furthermore there is good evidence that grass species are more susceptible to chronic SO_2 injury under winter conditions (Bell, Rutter and Relton, 1979; Davies, 1980).

The sensitivity of plants to SO_2 is affected by a wide range of environmental factors. Toxic effects of acute SO_2 have been shown to be minimised at low temperatures, increasing with rising temperature, although relatively stable between 10°C and 40°C (Wells, 1917; Stoklasa, 1923; Davies, 1968). Very little SO_2 is absorbed at night when stomata are closed (Garsed and Read, 1977) and plants are more resistant. *Phleum pratense* has been shown to be more sensitive to chronic SO_2 injury under a winter light regime (125$\mu E\ m^{-2} s^{-1}$ PAR for 12 hd^{-1}) than a summer regime (480$\mu Em^{-2} s^{-1}$ PAR for 16 hd^{-1}) (Davies, 1980; Jones and Mansfield, 1981). It was suggested that this may have been caused by the plant producing insufficient reducing power, under the low light regime, to metabolize the SO_2 to less toxic compounds.

Ashenden et al (1977) have demonstrated growth of *L. perenne* to be reduced by fumigation with 285 to 340μgm^{-3} SO_2, in a wind of 25 m min^{-1}, but not at one of 10 m min^{-1} probably as a result of decreased r_a.

Water status may affect the response of plants to SO_2 : under drought conditions stomata close, increasing tolerance (Markowski, Grzesiak and Schramel, 1974; Schramel, 1975) while in a relative humidity of 30% or less, plants may be 3 or more times resistant than at 100% RH, also as a result of stomatal closure (Setterstrom and Zimmerman, 1939; Zahn, 1963).

Investigations of nutrient status have generally shown reduced SO_2 sensitivity with increased fertility of the soil (Zahn, 1963; Enderlein and Kastner, 1967; Berge, 1970; Leone and Brennan, 1971).

Another factor which may influence the degree of response of plants to SO_2 is the presence of other gases, which have been shown to cause synergistic, additive or antagonistic effects. Other major pollutants which may be of concern in this respect, are O_3 and NO_x.

O_3 levels are low in the U.K. and significant peaks only occur in the summer, while SO_2 concentrations are generally at a maximum in the winter. Therefore, the occurrence of SO_2 and O_3 together in phytotoxic concentrations is rare in Britain.

NO_x and SO_2 commonly occur together, often at similar concentrations between 0.04ppm (107.0μgm^{-3} SO_2) and 0.08ppm (213.0μgm^{-3} SO_2), but in the countryside NO_x concentrations may be 50% higher than SO_2 (v/v) (Mansfield and Freer-Smith, 1981).

The action of NO_x gases on plants is complicated because NO and

NO_2 always occur together, but in different ratios at different types of sites, and are absorbed by vegetation at different rates. However, it has been shown that concentrations of only 0.25ppm NO_2 can cause appreciable yield reductions in tomato crops (Taylor and Eaton, 1966; Spierings, 1971).

The majority of investigations on acute injury caused by mixtures of NO_x and SO_2 demonstrate a synergistic or at least an additive effect (Tingey et al, 1971; Fujiwara, 1975; Bennett, Hill, Soleimani and Edwards, 1975). The most extensive study on the effects of moderate levels of SO_2 and NO_2 mixtures on plants has been carried out at the University of Lancaster (Ashenden and Mansfield, 1978; Ashenden, 1979; Ashenden and Williams, 1980). Four grass species were subjected to 0.11ppm ($234\mu gm^{-3}$) SO_2 and/or 0.11ppm NO_2, for 103.5 hrs per week, from October 1976 to March 1977. NO_2 alone gave little or no effect while SO_2 caused some reduction in the growth of all species. However, SO_2 + NO_2 caused highly significant reductions ranging from 52% to 86% in *L. multiflorum* and *Phleum pratense* respectively. The concentrations of SO_2 and NO_2 were similar to those found in polluted urban areas and suggests that NO_2 may play an important role in the apparent effects of SO_2 in the field.

Considerable difficulty has been encountered in attempting to develop clear dose-response relationships for SO_2 and chronic injury on plants. In many cases interactions with climatic conditions, both as a result of seasonal influences and chamber design, obscure the influence of SO_2 on plant growth in consecutive experiments. This is further complicated because response to SO_2 varies both between and within species.

Extensive lists of species and their sensitivities to acute SO_2 have been made by Linzon et al (1972) and Wood (1968). Ayazloo, Bell and Laycock (1980) compared the relative susceptibility to acute SO_2 of turf grass cultivars of 6 species, giving the following order of increasing sensitivity: *F. rubra* ssp. *rubra* cv. Engina, *Poa pratensis* cv. Baron, *Poa annua*, *L. perenne* cv. S23, *L. multiflorum* cv. R.V.P., *Phleum pratense* cv. S48. Two fumigations, with chronic concentrations of SO_2 administered to several grass species concurrently have been carried out: Ayazloo and Bell (1981) subjected *D. glomerata* , *F. rubra*, *H. lanatus* and *L. perenne* to 367μgm^{-3} SO_2 for 131 days: while Ashenden (1979) and Ashenden and Williams (1980) exposed *D. glomerata*, *L. multiflorum*, *Phleum pratense* and *Poa pratensis* to 194μgm^{-3} SO_2 for 140 days. Comparing the results of both fumigations reveals *F. rubra* and *L. multiflorum* to be relatively tolerant to chronic SO_2 and *D. glomerata*, *H. lanatus* and *Poa pratensis* to be highly sensitive, but *L. perenne* had an intermediate tolerance.

Interspecific variation in response to SO_2 pollution may lead to changes in the structure of the ecosystem at polluted sites, as the more sensitive species are liable to disappear with a possible influx of new more resistant species (Smith, 1974; McClenahen, 1978).

Differential susceptibility to SO_2 pollution within species has only recently been demonstrated (Brennan and Halisky, 1970), but has been recorded for other pollutants for some time. In particular a large volume of knowledge has accrued concerning differential tolerance to heavy metals in soil.

Bradshaw (1952) discovered differential lead and zinc tolerance between populations of *Agrostis tenuis* growing on contaminated mine

soil and uncontaminated pasture soil only 100 m apart. This tolerance was found to be specific in most cases to one metal (Jowett, 1958; 1963). Heavy metal tolerance is heritable (Gregory and Bradshaw, 1965) and shows, in *F. rubra*, a mainly additive genetic component with partial dominance, of tolerance, and some small maternal effects (Urquehart, 1971; Gartside and McNeilly, 1974).

There is ample evidence showing that selection maintains the tolerant populations on the mines (Jowett, 1958, 1963; Gregory and Bradshaw, 1965; Antonovics, Bradshaw and Turner, 1975) and that tolerant phenotypes are at a competitive disadvantage to non-tolerant phenotypes on uncontaminated soil (Antonovics, 1966; McNeilly, 1968).

The rate of evolution has been a major concern of heavy metal tolerance research. Thus Wu, Bradshaw and Thurman (1975) investigated a series of grasslands of different ages contaminated with copper, compared with unpolluted grasslands. The polluted sites had a range of plant cover from large patches of bare ground in the youngest (5 yrs old) to complete cover in the oldest (70 yrs old). *Agrostis stolonifera* populations were non-tolerant at uncontaminated sites, but showed at least some degree of tolerance, with a positive relationship of this with age of site, at the polluted sites. It has been shown that tolerant seedlings exist in a non-tolerant population at the rate of 1 to 2%, although this may depend on the toxic element and the species (Walley, Khan and Bradshaw, 1974; Gartside and McNeilly, 1974).

Heavy metal tolerance demonstrates the action of selection caused by a pollutant, its ability to maintain differences between interbreeding populations and the rapidity with which it can produce changes in a

population, so ameliorating the effect of the pollutant.

Intraspecific differential sensitivity to acute SO_2 injury has been demonstrated between cultivars of several grass species (Brennan and Halisky, 1970; Murray, Howell and Wilton, 1975). Taylor and Murdy, (1975) examined populations of *Geranium carolinianum* collected at various distances from a 31 year old coal-burning power station and determined their response to acute SO_2 fumigation. Less acute injury was found for those populations close to the power station than those from clean air sites, and it was concluded that acute SO_2 tolerance had developed at the sites proximal to the power plant, due to high ambient concentrations of SO_2.

The existence of intra-specific chronic SO_2 injury was established by Bell and Clough (1973) and Bell and Mudd (1976) after observations that native *L. perenne* plants suffered little damage after a pollution incident which caused chlorosis on the leaves of cv. S23 in an adjacent field at Helmshore (ref section 2.3.1). Four selected native pasture clones and samples of cv. S23 grown from seed were subjected to summer and winter fumigations of $343\mu gm^{-3}$ SO_2 for 9 weeks and $191\mu gm^{-3}$ SO_2 for 26 weeks, respectively. In both experiments the S23 plants showed significant decreases in growth of over 40%, while the Helmshore clones showed no change. This is strong evidence of the existence and evolution of chronic SO_2 tolerance in the field, but it did not indicate the level of tolerance in the population as a whole. Horsman, Roberts and Bradshaw (1978) fumigated 36 randomly samples clones of *L. perenne* from clean air and polluted sites in Lancashire and Merseyside ($650-700\mu gm^{-3}$ for 8 weeks). Comparison of clean and polluted sites gave similar results to Bell and Clough (1973) and also showed a positive relationship between

mean tolerance and ambient SO_2 concentrations at the different sites. This evidence confirmed that natural populations of *L. perenne* have the capability to respond to selection in response to chronic SO_2 pollution.

The most extensive study of SO_2 tolerance was carried out by Ayazloo in 1979 (Ayazloo and Bell, 1981; Ayazloo, Garsed and Bell, 1982). This involved the screening, for acute and chronic SO_2 tolerance, of populations of *D. glomerata, F. rubra, H. lanatus, L. perenne* and *Phleum bertolonii* from one or more of three sites in the North of England with differing pollution characteristics. Their response was compared to that of either bred cultivars or populations of the same species collected from clean air sites. The chronic fumigations involved concentrations between 250μgm^{-3} and 480$\mu gm^{-3} SO_2$ over periods of 61 to 180 days, while during acute fumigations plants were subjected to approximately 5320$\mu gm^{-3} SO_2$ for 6 hrs. The results revealed acute and chronic SO_2 tolerance to have developed at all sites and in all species, although some species were not tolerant at every site. This and the previous evidence indicates that selection for SO_2 tolerance appears to be widespread in polluted areas of the U.K. It was observed that all populations had a wide range of variability to both acute and chronic injury. Similar heterogeneity has been observed by Taylor and Murdy (1975) and was considered important for the rapid evolution of tolerant populations. Horsman et al (1979) suggested a relationship between reduced variability and intensity of selection, as a result of the elimination of sensitive genotypes, but no consistent relationship in this respect was shown by Ayazloo and Bell (1981).

Ayazloo et al (1982) investigated some of the possible mechanisms

of SO_2 tolerance. They found that the rankings of the rate of ^{14}C and ^{35}S uptake and of acute SO_2 sensitivity (*H. lanatus* < *D. glomerata* < *F. rubra* < *L. perenne*) were strikingly similar and that this was related to decreasing rs at the inter and intra-specific level. Also it was shown that stomata of more tolerant species tend to close when exposed to acute SO_2 levels. It was concluded that avoidance mechanisms may play an important role in tolerance to acute SO_2 injury. Similar mechanisms are unlikely for chronic tolerance, since the order of chronic tolerance between species is almost the opposite to acute tolerance.

Investigations of the hereditary nature of SO_2 tolerance, have so far only examined acute tolerance. Repeatability estimates have been calculated for *Pinus strobus* (0.8) and *Populus tremuloides* (0.6) to assess the possibilities of breeding tolerant cultivars (Houston and Stairs, 1973; Karnosky, 1977). Acute tolerance in *Geranium carolinianum* was shown to be polygenically controlled and genetic variation to be mainly additive (Taylor, 1978). However, in *Oryza sativa* strains, acute tolerance is controlled by only one pair of genes and the genetic component of variation is mainly additive, although in one strain it has been shown that acute tolerance is controlled by a single dominant gene (Omura, Satoh, Fujinuma and Aiga, 1980). It is evident that the genetical control of SO_2 tolerance varies between and within species.

The work of Ayazloo, (1979) raised a number of questions which merited further investigation and these form the basis of the present work. It is of particular interest to understand the rate at which SO_2 tolerance can evolve under field conditions. So far the minimum age demonstrated for this to occur in grass species is 17 - 25 years

(Ayazloo and Bell, 1981) although shorter periods might be expected.

The main objectives of the present work are:

1) To determine the minimum time for the development of SO_2 tolerance in grass populations.
2) To study the long term establishment of acute SO_2 tolerance in grass populations.
3) To investigate the development of SO_2 tolerance over a range of SO_2 concentrations.
4) To elucidate the genetic characteristics of SO_2 tolerance in grasses.

The procedure to determine chronic SO_2 tolerance involves prolonged fumigation with a large number of replicates, thus limiting the number of populations and genotypes that could be examined. Thus it was decided to investigate the feasibility of developing a simple rapid method of screening for chronic SO_2 tolerances, and several possibilities in this respect were investigated.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 *Potting mix.*

All plants were grown in the University of California D1 mix (Matkin and Chandler, 1957), except where stated. This mix has the following composition for a volume of 100*l*.

Peat	75*l*
Sand	25*l*
Superphosphate	120g
Potassium Nitrate	15g
Potassium Sulphate	15g
Magnesium Carbonate	295g
Calcium Carbonate	235g
Fritted Trace Elements	37g

2.2 *Choice of Grass Species*

Seven species of grass were used in this investigation. The Gramineae is the most important plant family with respect to variability, value of products and percentage of land surface covered. Its members are important for food, a variety of fibre products and binding top soil to the land. Large areas of rural Britain are devoted to grasslands, primarily as herbage for sheep and cattle and in towns for sports and recreation. Agricultural and amenity grasslands contain a relatively small range of grass species, mainly due to the small number able to withstand wear from trampling, and frequent cutting or grazing. It was previously suggested, in the Introduction, that SO_2 pollution may contribute to the deleterious effects of these factors on grasslands, in or near urban areas.

The species of grass used in this project were primarily chosen for their importance to British agriculture and amenity grasslands; they are listed with their sources in Table 2.1.

2.3 *Field Sites*

2.3.1 *Helmshore (Grid ref. SD 773 205)*

The Great House Experimental Husbandry Farm (Ministry of Agriculture, Fisheries and Food) is situated 275m above sea level, on an east facing side of the Rossendale valley in the Southern Pennines, about 1.4 km southwest of Helmshore village (Grid ref. SD 785 214) (Fig. and Plate 2.1). The soils consist of loams to clay loams over Carboniferous boulder clay. The site has a combination of high rainfall (1.63 m.p.a.) and low sunshine hours (1180 hrs. p.a.), which, aggravated by smoke pollution, has in the past, caused a farming problem unique to the north west of Britain (Anon, 1970).

The area has a long history of air pollution, probably since the early 19th century, from both domestic and industrial sources. The small mill towns of Ramsbottom, Haslingden and Rawtenstall are situated 4.5 km to the south east, 2.25 km to the north and 5.5 km to the north east of the Great House E.H.F. respectively. Within a 15 km radius are the towns of Bolton, Bury, Rochdale, Burnley, Blackburn and Accrington. Manchester is only 24 km to the south and Liverpool 48 km to the south west. Thus the site is surrounded by major pollution sources.

Helmshore is on the southern edge of the Rossendale sub-region of the National Survey of Smoke and SO_2. The only rural SO_2 and smoke pollution monitoring site for this sub region is operated at the Great House E.H.F..

The winter mean SO_2 levels are generally high for a rural site

TABLE 2.1 Plant Material Used in Fumigation Experiments and its Sources

Species	Source	Stock Derived from
Agrostis tenuis Sibth.	The Temple, London.	Tillers
Dactylis glomerata L.	Askern, Yorkshire The Temple, London	Tillers Tillers
Festuca rubra L.	Askern, Yorkshire Philips Park, Manchester The Temple, London c.v. Engina *	Tillers Tillers Tillers Seed
Lolium multiflorum Lam.	Philips Park, Manchester c.v. R.V.P. *	Tillers Seed
Lolium perenne L.	Askern, Yorkshire Helmshore, Lancashire Philips Park, Manchester c.v. S23 * c.v. S23 res	Tillers Tillers Tillers Seed Tillers
Phleum pratense L.	Philips Park, Manchester c.v. S48 *	Tillers Seed
Poa pratensis L.	Philips Park, Manchester c.v. Baron *	Tillers Seed

* Seed was provided by the Sports Turf Research Institute, Bingley Yorkshire.

Fig. 2.1 Map of the Location of the Great House E.H.F., Helmshore and the Surrounding Area

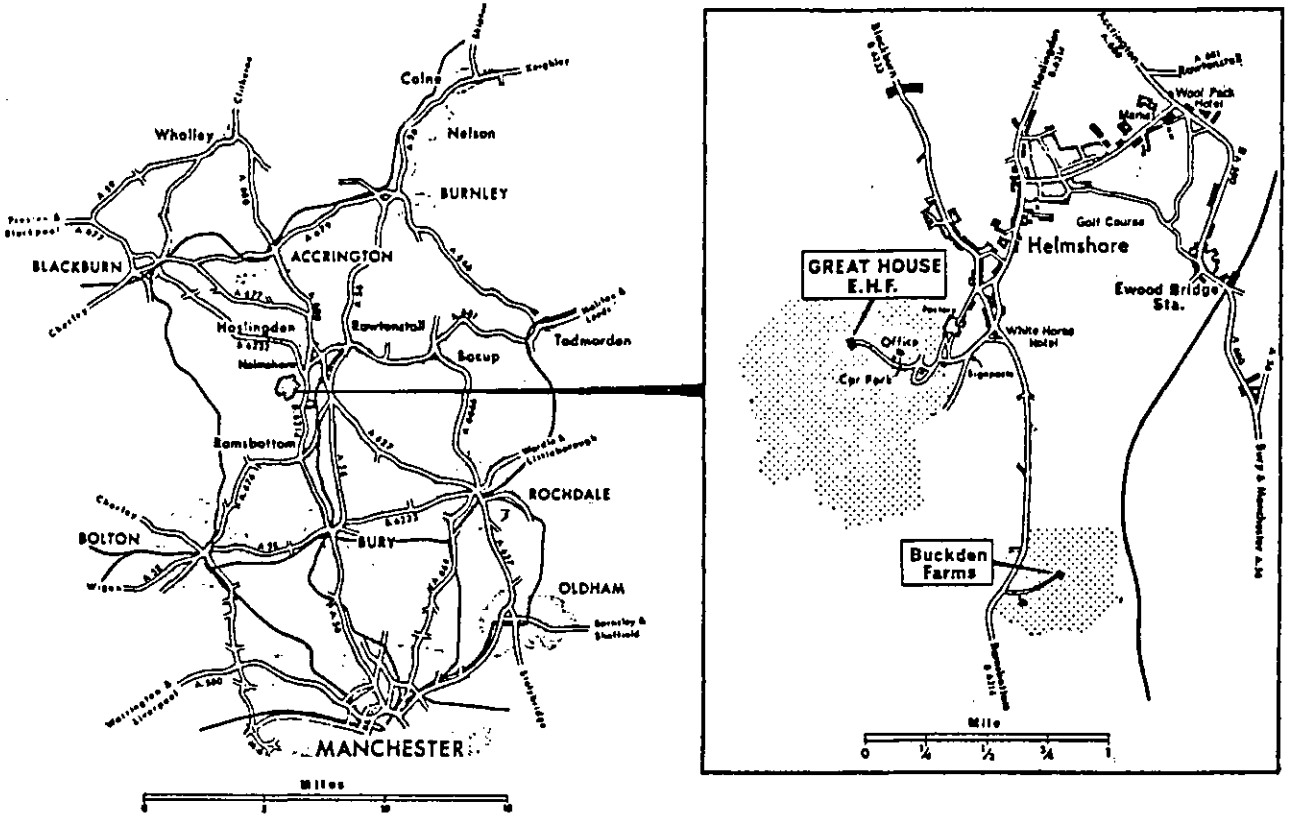


Plate 2.1 View of the Rossendale Valley Above Helmshore Showing the Collection Site of *L. perenne* (Great House Meadow on the Right of the Woods).



(Fig. 2.2), although lower than the regional average. The mean winter concentration between 1959 and 1977 was 110 μgm^{-3}, with the highest winter mean of 165 in 1963 and the lowest in 1975 of 75 μgm^{-3}. The highest daily concentrations rarely exceed 500 μgm^{-3}. Smoke levels have been decreasing since the initiation of monitoring at the site, but SO_2 levels have only shown a decline since 1973.

Observations of the effects of air pollution at this site, have been made after attempts to reseed some of the pastures at Helmshore, with bred cultivars after World War Two : characteristic yellowing of leaves and necrotic lesions have appeared after air pollution incidents in resown pastures, but not in the adjacent native pastures of the same species (Bell and Mudd, 1976). This suggested that plants in the latter had some degree of SO_2 tolerance.

On the basis of the results of work by Bell and Mudd (1976) on the tolerance of a few selected native *L. perenne* clones from Helmshore, Ayazloo (1979) collected samples in 1977 of several grass-species from the Great House Meadow (Fig. 2.3). The *L. perenne* population sample from this collection was used in some of the present experiments.

2.3.2 *Askern (Grid ref. SE 564 132)*

The coal mining village of Askern (Fig. 2.4) is situated 12 km north of Doncaster in South Yorkshire. It is in a flat rural area mostly devoted to cereals and grassland, about 8 m above sea level, although in the centre of the village is a solitary hill over 30 m high. The area is fairly dry (0.578 m p.a. rainfall) and has a low sunshine record, compared to the national average (1266 hrs. p.a.). The wind blows almost 50% of the time from between southwest and northwest, mainly at speeds of 6-8 ms^{-1} (Met. Office Finningly).

Fig. 2.2 Mean Winter (October - March inclusive) SO_2 Concentrations at Helmshore (Based on data from the Warren Spring Laboratory and the Great House E.H.F.).

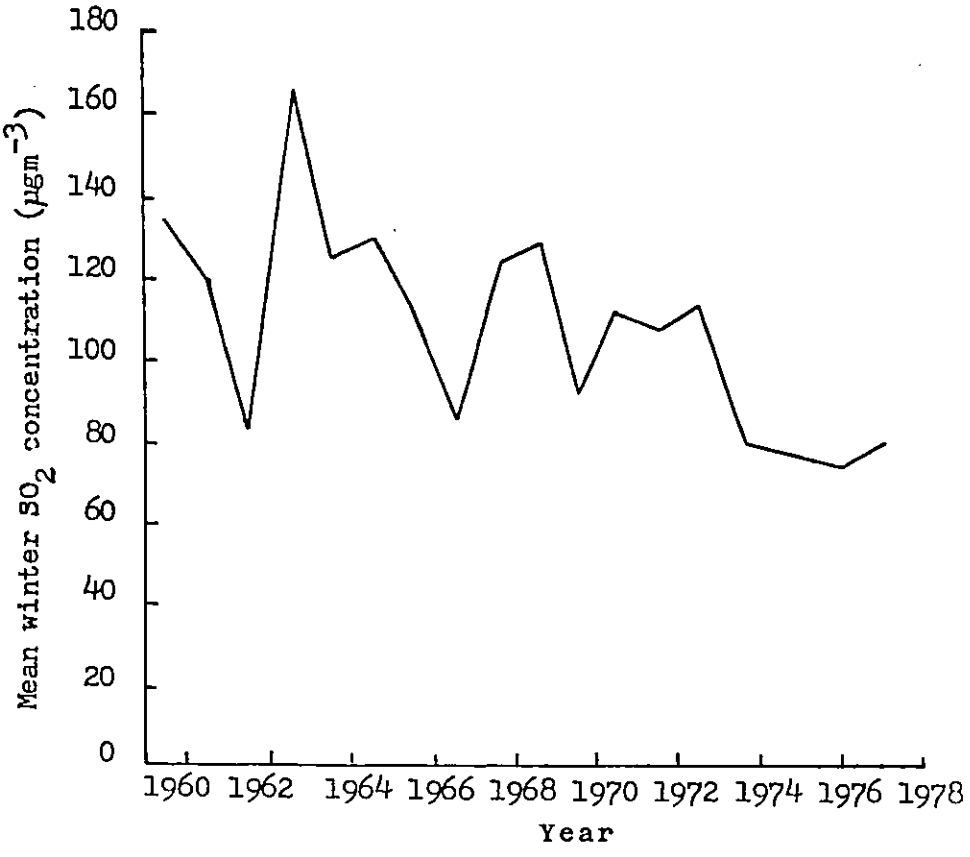
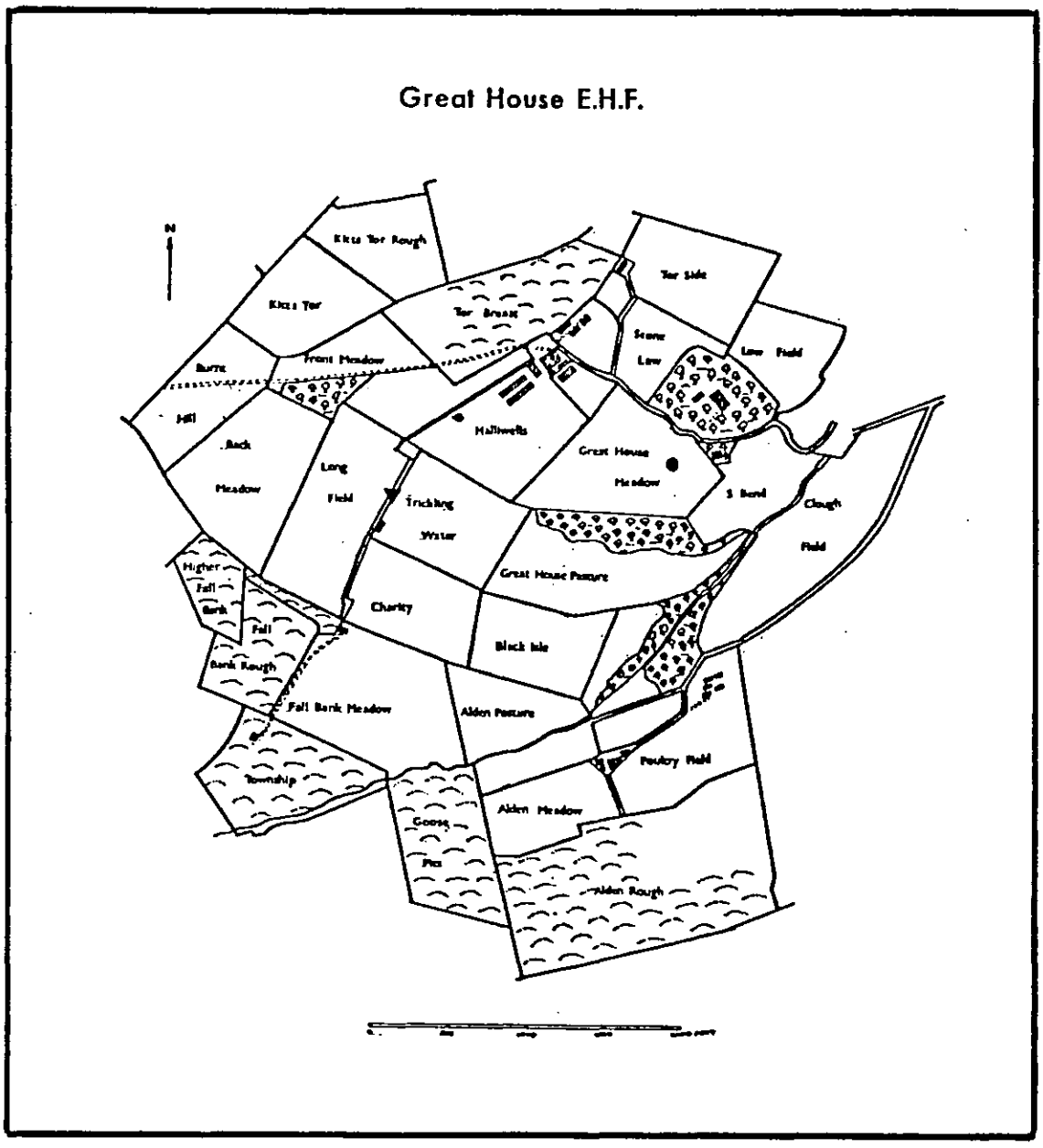
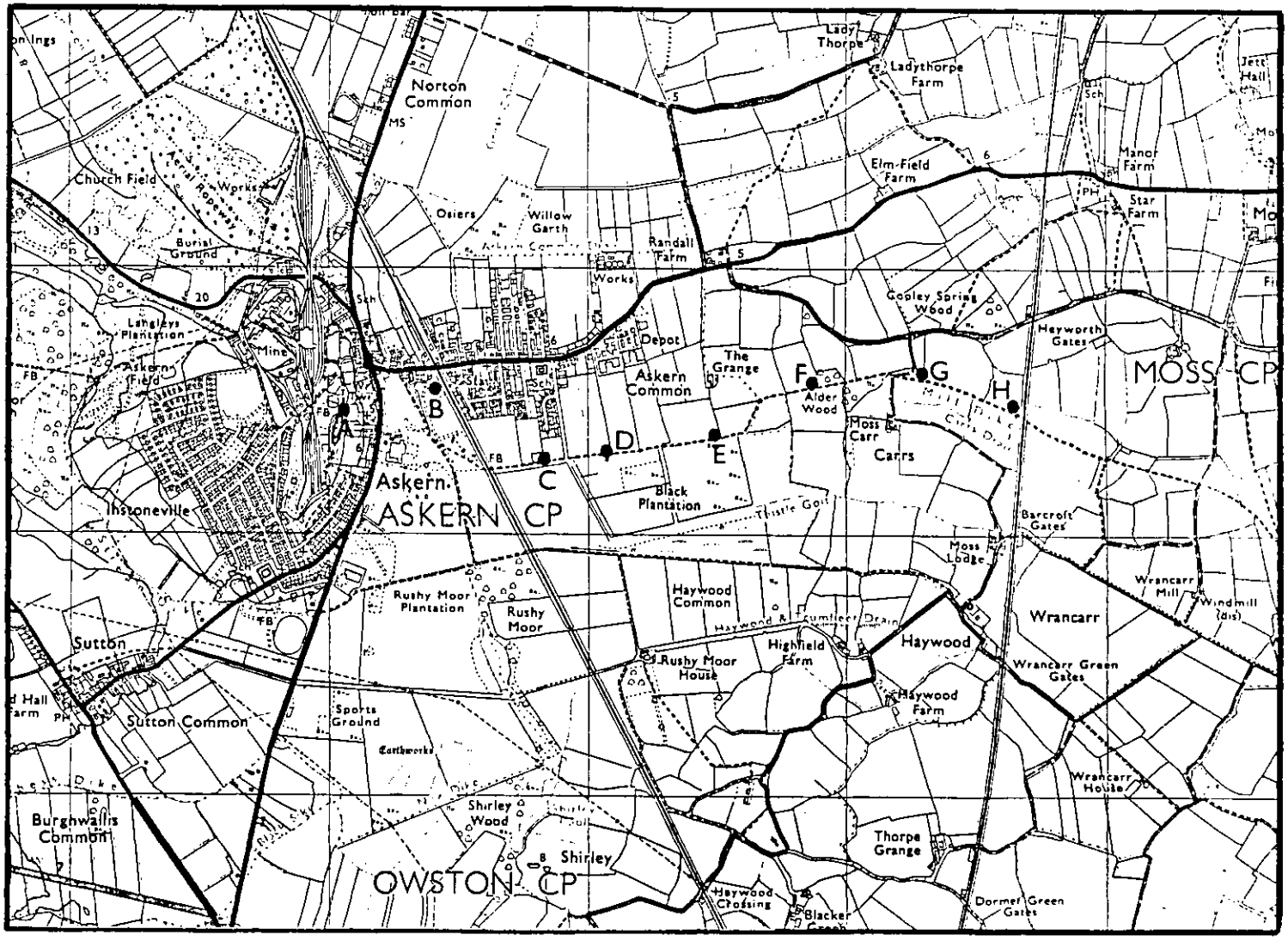


Fig. 2.3 Location of the Collection Site at Helmshore



●-Site of collection of Lolium perenne (Ayazloo,1979)

Fig. 2.4 Map Showing the Collection Sites (A - H) Along the Transect at Askern in Yorkshire. Scale 1:25,000



It was not until 1926 when a Coalite Smokeless fuel plant was constructed on Askern hill, that pollution problems began (plate 2.2). The Coalite factory currently releases 3,000 tonnes of SO_2 into the atmosphere per annum (Mr. P. Read pers. comm.). 400 m. to the east of the factory the Doncaster Metropolitan Borough Council operate a Meloy continuous sulphur monitor, and conventional National Survey SO_2/Smoke monitoring apparatus, installed in a clinic. Data for the years between 1970 and 1979 show summer means ranging from 108 to 131 μgm^{-3} SO_2 and winter means from 128 to 146 μgm^{-3}. Daily means occasionally occur up to 1,000 μgm^{-3} SO_2 and the highest hourly mean of over 2,000 μgm^{-3} was only recorded in the winter of 1979-80 (Mr. P. Read pers. comm.). The background SO_2 level for the area, from domestic sources, is about 60 μgm^{-3}, which is recorded at Askern when the Coalite plant is shut down. In recent years alterations have been made to the stacks in an attempt to improve the dispersion of the air pollutants. Originally, the factory had 3 stacks of about 37 m. In 1978 one of the stacks was closed and another extended to 51 m. Following these alterations there was a fall of SO_2 levels in the winter of 1978/9, but over the 1979/80 winter, levels rose again. One possible reason for this recent increase is the building of a washery, by the National Coal Board, next to the Coalite plant. It is believed that the washery channels the wind over the east of the village from a greater range of wind directions than before and that the wind speed is diminished, causing more rapid deposition of SO_2 (Mr. P. Read pers. comm.). The overall effect of this seems to have nullified the improvements made at the Coalite factory. At present, efforts are being made to reduce ground level emissions, which cause problems in the immediate area around the plant, by enclosing conveyors

Plate 2.2 View of Askern with Coalite Smokeless Fuel Plant in the Centre Background and the Clinic, right of the middle, where the Meloy continuous SO_2 monitor is in operation.



carrying cooling coke (Mr. P. Read pers. comm.).

The concentrations of SO_2 found at Askern are well in excess of those known to cause acute and chronic injury over the appropriate period of time, in fumigation experiments. Ayazloo (1979) randomly sampled 20 specimens of several grass species, at 2 sites in 1976: from wasteland adjacent to the clinic (Plate 2.2) and a 'clean site' (grid ref SE 609 141) 5 km east of the clinic. Plants from the Askern site *Lolium perenne* population sample, were used in some of the following experiments.

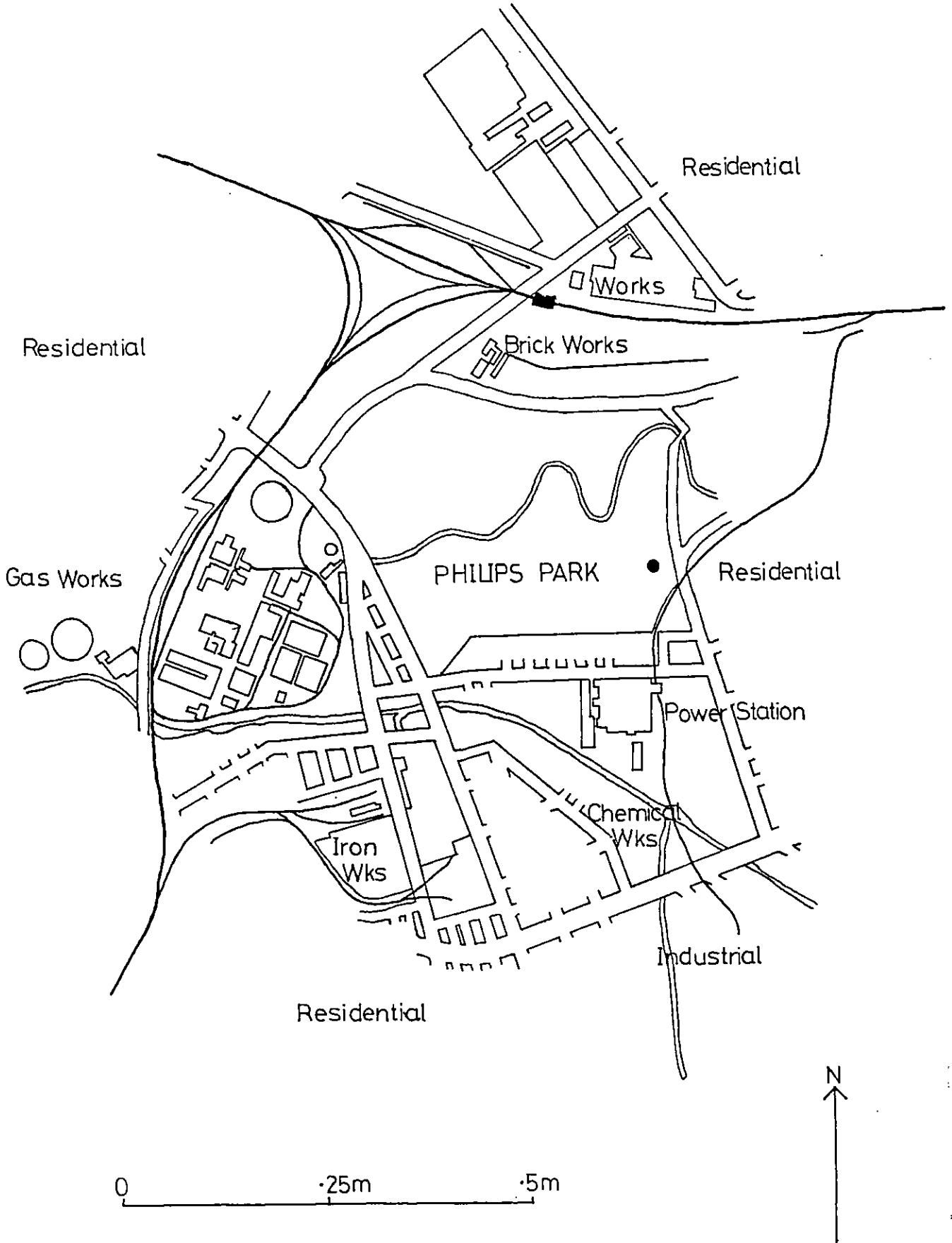
The Coalite factory is the only major source of SO_2 in the vicinity of Askern, making it suitable for a transect study, from the factory. Thirty five tillers of *Festuca rubra* and *Dactylis glomerata* were randomly collected in the summer of 1979, from each of 8 sites between 60 m (site A) and 2700 m (site H) east of the factory (Fig. 2.4). Site A was adjacent to the factory and the soil contained a high proportion of coal dust. Sites B and C were in wasteland areas in or close to the village but the remaining sites were along the edges and sides of drainage ditches.

2.3.3 *Philips Park (Grid ref SJ 872 994)*

Philips Park is situated in an industrial area 20 m above sea level, 4 Km east of Manchester city centre (Plate 2.3; Fig. 2.5). It receives air pollution from the bulk of the conurbation due to the prevailing westerly winds which average between 3.6 and 8.2 ms^{-1} (Met. Office Station, Manchester Ringway). It receives about 0.8 m of rainfall a year with about 1150 hrs of sunshine.

The site has been described by Pettigrew (1928) who was the

Fig. 2.5 Location of Philips Park in Manchester Showing the Surrounding Industrial Premises.



●- The site of the field trial set up by the S.T.R.I.

Plate 2.3 The Plots at Philips Park, Sown by the STRI (1975).



contemporary Superintendent of Manchester Parks, as follows: "Vegetation is perhaps more adversely affected by air pollution in Philips Park than any other open space in Manchester City. This is largely accounted for by the fact that on its western boundary and not a stone's throw from the main gates stands a very extensive gas works, while on its southern side, less than 100 yards away, is a large electricity works, either of which alone must bring about a heavy mortality among plants in the neighbourhood." However, the gas works and power station have closed in recent years.

The nearest National Air Pollution Survey station "Manchester 15, Clayton" is 450 m to the south of the park. SO_2 records (Table 2.2) from here show that annual levels fluctuated between 120 and 152 μgm^{-3} from the early 1970's until 1978, with a subsequent fairly marked decrease below 100 μgm^{-3}. These winter and annual means are within the range known to reduce growth of grass species in fumigation experiments. As a record of higher levels of SO_2 the National Survey provides the maximum daily mean for each month, but this does not necessarily reveal the occurrence of possible acute levels of SO_2, which may be of a few hours duration. However, the probable maximum hourly mean can be estimated from Larsen's (1969) formula:

$$Cmax(t) = Cmax(h)\ t^{b} \qquad \text{(appendix 1)}$$

Cmax(t) = Maximum concentration of pollutant to be expected during a year for average time t

Cmax(h) = Annual maximum hourly concentration

t = Averaging time

b = Exponent which characterises variability of pollutant. The slope of the log graph of maximum concentration over t.

The calculated maximum hourly means (Table 2.2) for 1974-7 are well

TABLE 2.2 SO_2 Levels at Philips Park (μgm^{-3})

	1974 - 75	1975 - 76	1976 - 77	1977 - 78	1978 - 79	1979 - 80
Annual mean	144	120	152	124	97	71
Winter mean	175	159	186	135	112	72
Max daily mean	448	494	515	344	339	278
Est. max. hrly. mean	887	978	1020	681	671	550

over 600 μgm^{-3} SO_2, which is the generally accepted threshold level for acute SO_2 injury. Since the winter of 1976-77 these estimates have dropped to around 600 μgm^{-3} but it is possible that SO_2 concentrations below this may be sufficient to cause acute injury under environmental conditions which predispose plants to sensitivity. A range of turfgrass cultivars were sown, in 4 m^2 plots in Philips Park on the 13th August 1975, by the Sports Turf Research Institute (Laycock 1980), as part of a programme designed to assess their relative performance in a polluted area. These plots were weeded selectively and mown in an attempt to maintain them as monocultures, with minimal flowering.

It was decided to study the minimum time for the development of SO_2 tolerance in the plots at Philips Park. Random samples were taken of 30 tillers of 5 species (*Lolium perenne* c v. S23, *L. multiflorum* c v. RvP, *Poa pratensis* c v. Baron, *Phleum pratense* c v. S48, *Festuca rubra* c v. Engina) each from a single plot every year, since 1975, except 1977. The first two years screenings for tolerance were carried out by Ayazloo (1979).

2.3.4 *The Temple (TQ 314 810)*

The Temple is situated on the northern embankment of the River Thames, in London. The grounds include several lawns which date from 1770 to the present day (pers. comm. Rear Admiral T.B. Homan* and Capt. J.B. Morison, R.N. +): the youngest lawn (E) sown by the present Head Gardener in 1977; the main Middle Temple lawn (D) was relaid after the

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*Sub Treasurer "The Honourable Society of the Inner Temple"

+Under Treasurer "The Honourable Society of the Middle Temple".

completion of a library in 1958; the centre of the main Inner Temple lawn was the site of a barrage balloon mooring in World War 2, which was recovered in 1946 (C); the proximal lawn to the embankment was conveyed to The Inner Temple under "The Thames Embankment Act 1862", and the lawn (B) is therefore of approximately 1875 vintage; the oldest lawn (A) was established between 1767 and 1780, when The Inner Temple was allowed by the City to extend the garden, after a new embankment wall was built in 1767, (Plates 2.4 and 2.5; Fig. 2.6).

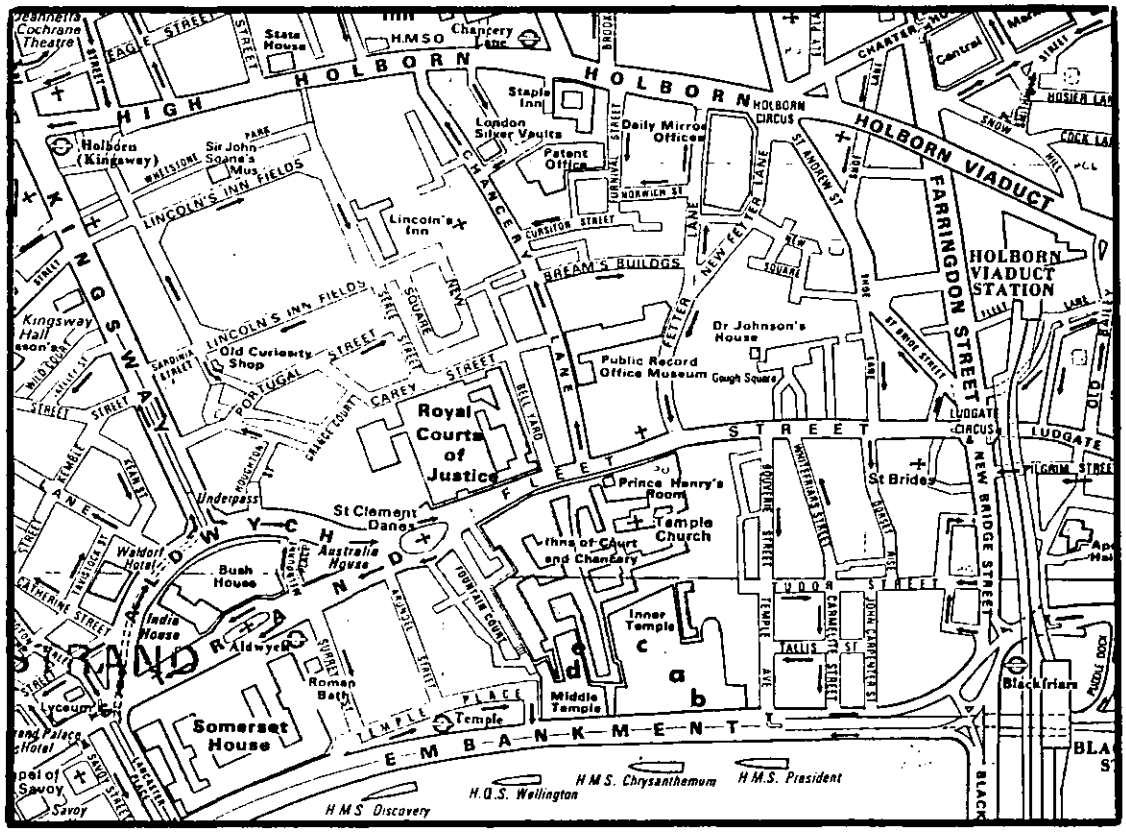
The main present day sources of pollution in central London arise from office space heating and road traffic, but in the 18th century domestic and industrial burning of coal was predominant. Brimblecombe (1977) predicted SO_2 concentrations in the City, from the quantities of imported coal. It was concluded that from 1690 the annual average levels of SO_2 were relatively constant at about 200 μgm^{-3}. It was not until 1965 when the controls of the "Clean Air Act" (1956) began to take effect that the ambient concentrations for the inner capital started to decrease (Fig. 2.7). The mean annual concentrations dropped below 100 μgm^{-3} SO_2 in the mid 1970's and are presently at 60 - 70 μgm^{-3} per annum.

It was decided to investigate the acute SO_2 tolerance of *Agrostis tenuis*, *Festuca rubra* and *Dactylis glomerata* populations of 5 lawns dated 1770(A) 1875(B) 1946(C) 1958(D) and 1977(E).

Apart from lawn E, which was sown with a mixture of *A. tenuis*, *F. rubra* and *Poa pratensis*, the method of preparation of each lawn was unknown. However, it is known, that the laying of turf was practiced before 1665 (Anon, 1982), and sowing with seed was first recorded in the early 18th Century using seed from wild and pasture populations. Reliable grass cultivars were not developed until 1930 and most lawns

Fig. 2.6 Location of The Temple in London and the Lawns Sampled.

Scale 7":1mile



N

Lawns	Age
a	1780
b	1875
c	1946
d	1958
e	1977

Fig 2.7 Annual Averages of SO_2 at Ground-Level for 7 Inner-London National Survey Sites.



Plate 2.4 Lawns of the Inner Temple in London

Lawn	Date
A.	1770
B.	1875
C.	1946



Plate 2.5 Lawns of The Middle Temple in London

Lawn	Date
D.	1958
E.	1977



were prepared using turf previous to this time. Thus lawns A and B were probably turfed while C and D were more likely sown.

Over 30 random samples of each species, were collected in April of 1979, from each lawn except lawn E, on which *D. glomerata* was absent. These samples were potted up and grown in the greenhouse for at least 3 months.

2.4 *SO_2 Sampling System*

SO_2 concentrations for all experiments were measured using a modification of the technique employed by the National Survey of Smoke and SO_2 (Anon, 1966).

The sampling system (Fig. 2.8) consisted of a diaphragm pump which drew air through a drechsel bottle containing a 1 Vol. solution of hydrogen peroxide (H_2O_2), adjusted to a pH of 4.5, followed by a second bottle of silica gel (drying agent). The air was finally sucked through a gas meter, giving an accurate measure of the volume of air sampled. The drechsel bottle containing H_2O_2 was placed near the centre of the chamber during fumigation, with the inlet at plant height.

After fumigation the H_2O_2 solution was titrated against N/250 sodium tetraborate, using B.D.H. "4.5" indicator. The average SO_2 concentration was calculated as follows:

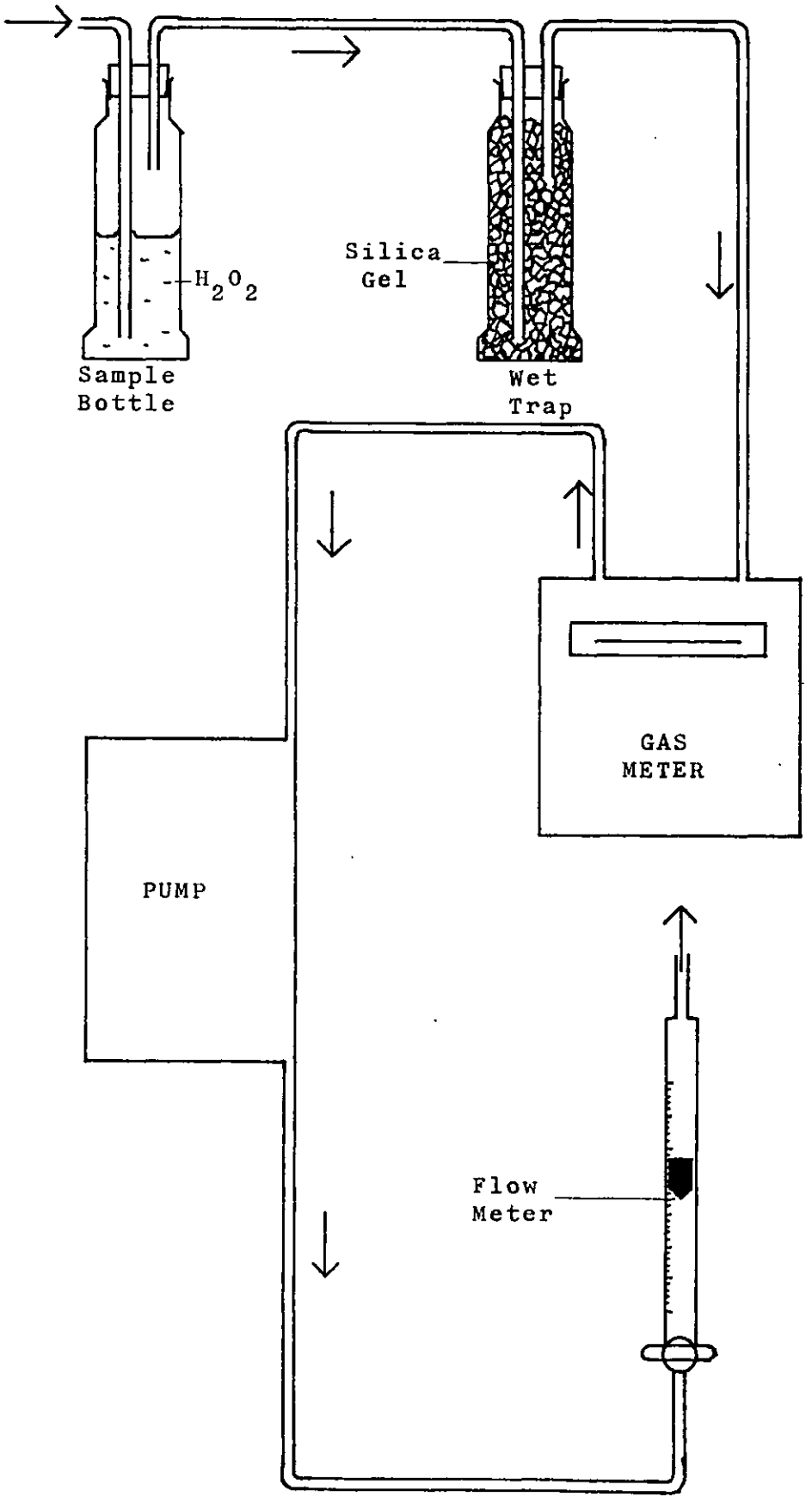
$$SO_2\ (\mu gm^{-3}) = \frac{452 \times \text{Vol. of } H_2O_2 (cm^3) \times \text{Vol. of borate } (cm^3)}{\text{Vol. of air sampled } (ft^3)}$$

2.5 *Fumigation Systems*

2.5.1 *Outdoor Perspex chambers*

The fumigation system consisted of a pair of identical Perspex

Fig. 2.8 SO_2 Sampling System.



chambers (Fig. 2.9; Plate 2.6) each of a volume of 2.25 m^3. These were supplied with purified air, which had been passed through an activated carbon pack filter. The inlet pipes had a cross sectional area of 0.0095 m^2 pointing towards the roof of the chamber, causing an even distribution of turbulent air, which was checked using a hot wire anemometer. The incoming flow of air was measured using an electronic vane anemometer at the end of the inlet pipe. The flow rate was adjusted in both chambers to provide an inlet flow of 7.8 ms^{-1} in each, equivalent to 2 air changes per minute. This rate was sufficient to prevent fluctuations of CO_2 concentrations in the chamber and create enough turbulence to reduce the aerodynamic resistance to SO_2 uptake by a mature sward of *Lolium perenne* to less than 38 sm^{-1} (Bell, Rutter and Relton, 1979). At this level the aerodynamic resistance is not limiting to SO_2 uptake (Ashenden and Mansfield, 1977). The air exited via three small holes in the sides of each chamber.

Comparisons of temperature and humidity were made between the two chambers and between the chambers and outside, using a thermohygrograph and gun hygrometer (an aspirated wet and dry thermistor instrument). Similar values for relative humidity were obtained in both chambers, but there was a reduction of 3-18% in comparison with ambient air. Although temperature was never more than 1^oC different between chambers it was generally greater by 2.0 - 8.0^oC than outside. Both results of temperature and humidity agreed with similar measurements made by Ayazloo (1975), who also demonstrated that temperature was uniform within a single chamber. Farrar, et al (1977) showed outside temperatures to be consistently higher, even at night when the difference was between 2^oC and 3^oC. They also showed that incoming solar radiation was reduced by 15% of the ambient values.

Fig. 2.9 Outdoor Perspex Fumigation Chambers.

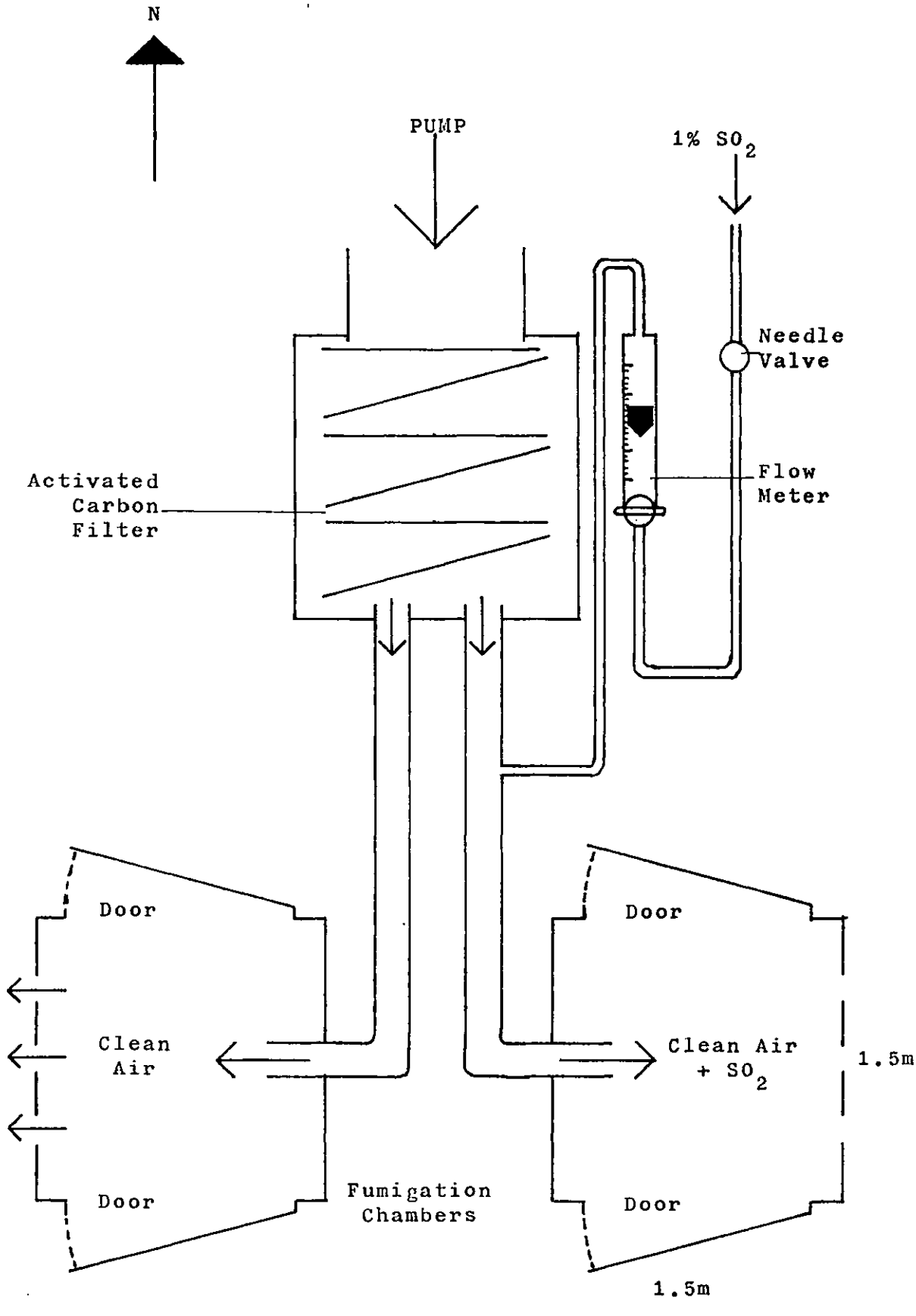


Plate 2.6 Outdoor Perspex Fumigation Chambers



SO_2 was supplied by a cylinder containing 1% SO_2 (balance nitrogen) to one of the inlet pipes and controlled by a needle value. Thus one chamber was supplied with a determined level of SO_2, while the other remained as a clean air control. After changing cylinders, the SO_2 in the chamber was monitored for a short period with a Meloy SA 285 continuous sulphur monitor, thus allowing rapid readjustment to the correct concentrations. During fumigation SO_2 levels were recorded over consecutive periods of at least one week, using the H_2O_2 bubbler system (Section 2.4).

The chambers each had two gas tight doors, with glove holes to allow manipulation of plant material and equipment, with minimal disturbance of internal environmental conditions. The plants were watered by subirrigation, via a bed of sand in each chamber.

2.5.2 *Outdoor glass chambers*

The system comprised two identical glass houses (Fig. 2.10, Plate 2.7),each with an internal volume of 6.5 m^3. The chambers were originally designed for fumigation of tree seedlings and had a height of 2 m. For economic reasons (i.e. rate of SO_2 consumption), a false roof of polythene sheeting was installed in each chamber, reducing the height of each to 1 m and the volume to 3.1 m^3.

Each chamber was supplied with air through an activated carbon filter. This entered the chambers through inlets of 0.0095 m^2 cross sectional area at a flow rate of 8.2 ms^{-1}, giving 1.5 air changes per minute. This low rate of air flow was used to reduce the consumption of SO_2; it was believed that this would not cause CO_2 fluctuations since the system was used mainly under winter conditions when photosynthesis is low. The inlet pipe pointed towards the roof of the chamber,

Fig. 2.10 Outdoor Glass Fumigation Chambers.

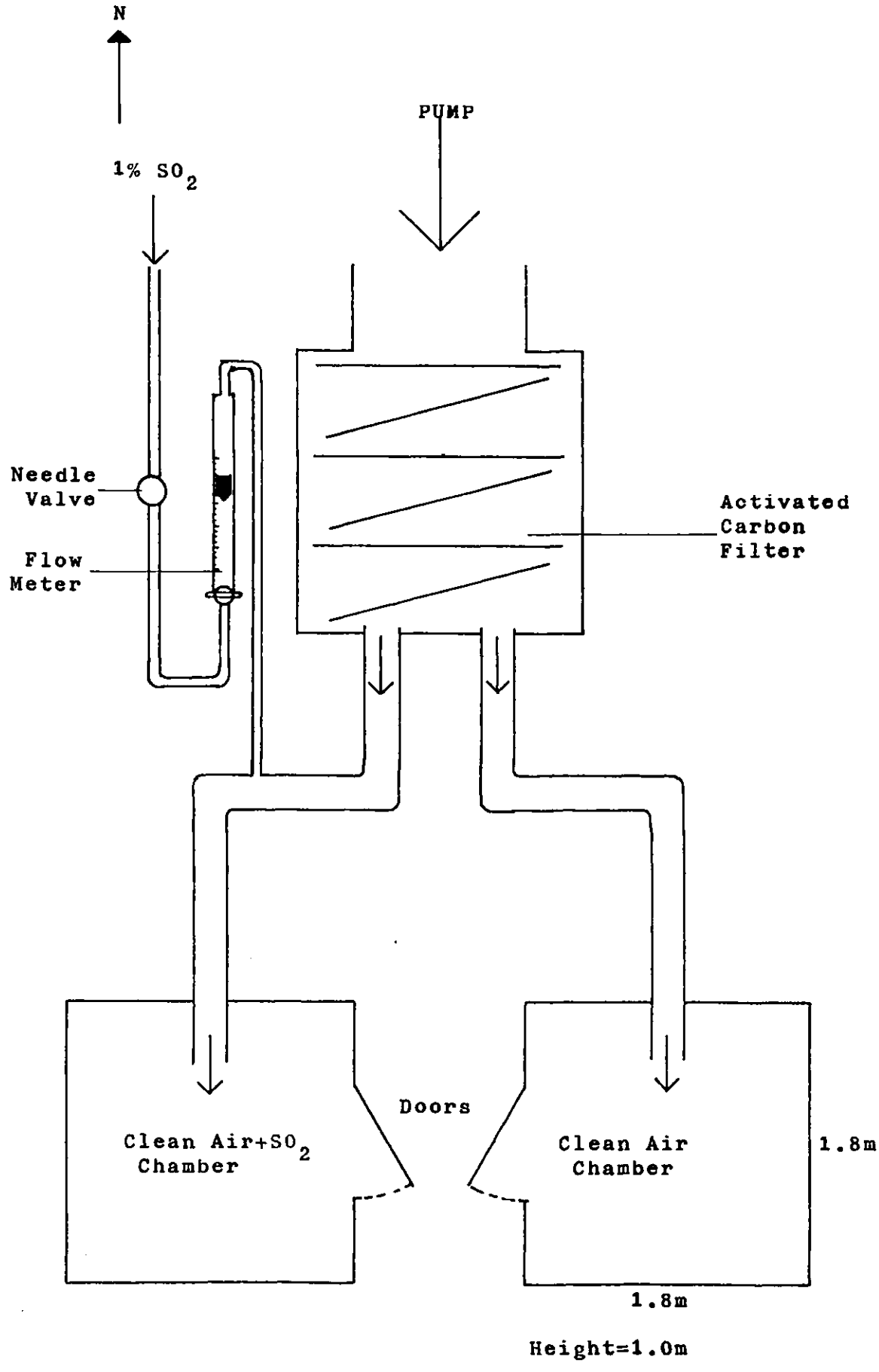


Plate 2.7 Outdoor Glass Fumigation Chambers



giving an even distribution of air and enough turbulence to cause leaf flutter.

Environmental comparisons of light, temperature and humidity were made between the two chambers, and between the chambers and outside. Readings from thermohygrographs and a gun hygrometer showed the chambers' relative humidities to be the same and their temperatures to vary by no more than 1.5°C. However, in comparison with outside, the chambers maintained a consistently higher temperature by 2°C to 10°C and lower R.H. by between 5% and 20%.

SO_2 was supplied by cylinders containing 1% SO_2 (balance nitrogen) to one of the inlet pipes, controlled by a needle valve and flowstat. Thus, one chamber was supplied, with a mixture of SO_2 and purified air, while the other was supplied with purified air only. Distribution of SO_2 in the chambers was originally tested using a Meloy SA. 285 continuous sulphur monitor. Measurements of SO_2 during fumigations were taken consecutively over at least weekly periods, using the H_2O_2 bubbler system (Section 2.4).

Access to the chambers was through doors, facing each other. Watering was by subirrigation via a bed of sand in each chamber.

2.5.3 *Controlled Environment Chamber*

All acute SO_2 fumigations were carried out in a 0.25 m^3 perspex chamber inside a Fison's controlled environment cabinet. This was set to give standard conditions of 21°C, 80% R.H. and a uniformly distributed P.A.R. of 420 $\mu Em^{-2}s^{-1}$ at plant height, provided by two banks of fluorescent tubes (Fig.2.11; Plate 2.8).

Air was sucked through the chamber at 1.8 air changes per minute,

Fig. 2.11 Controlled Enviroment Fumigation System.

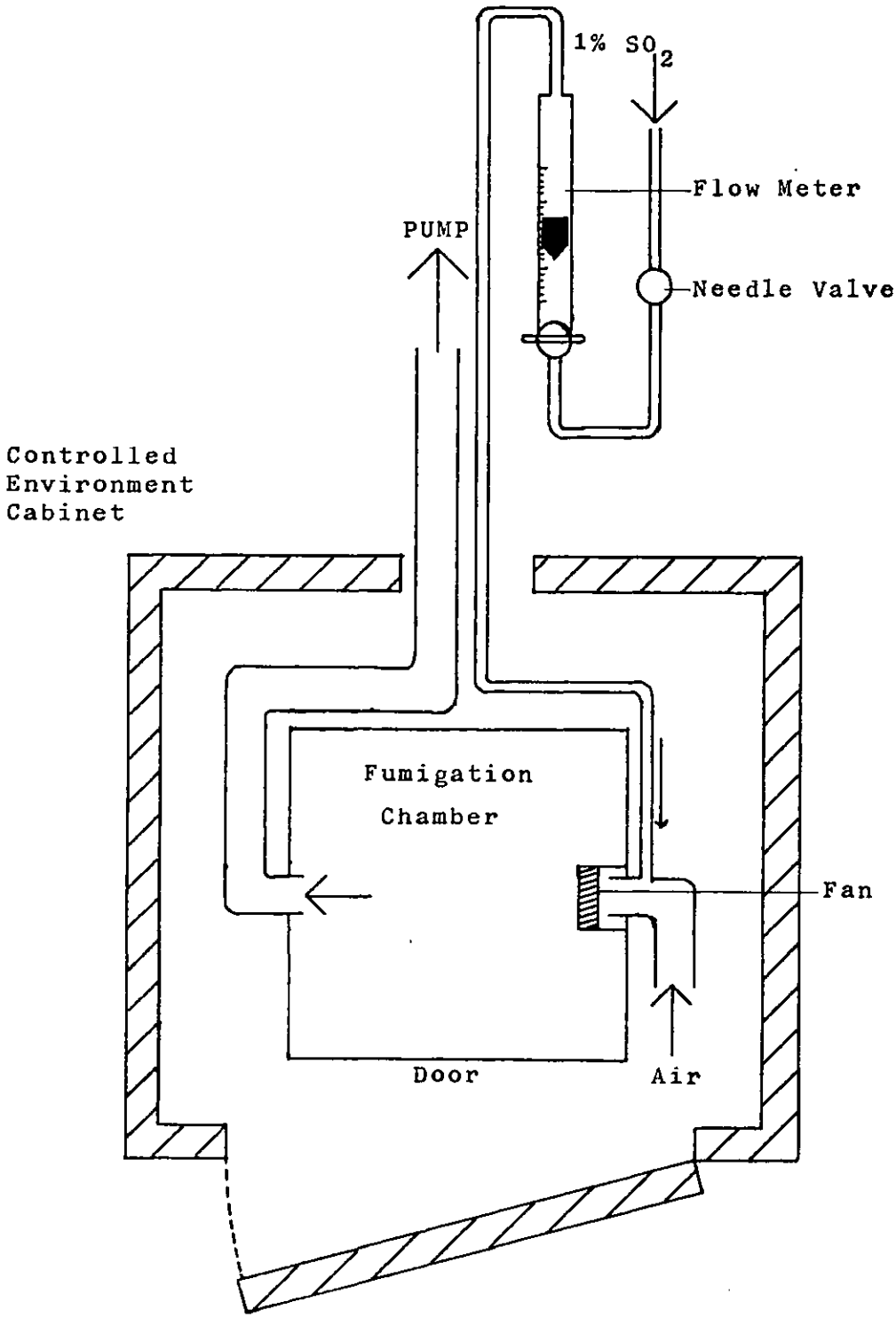
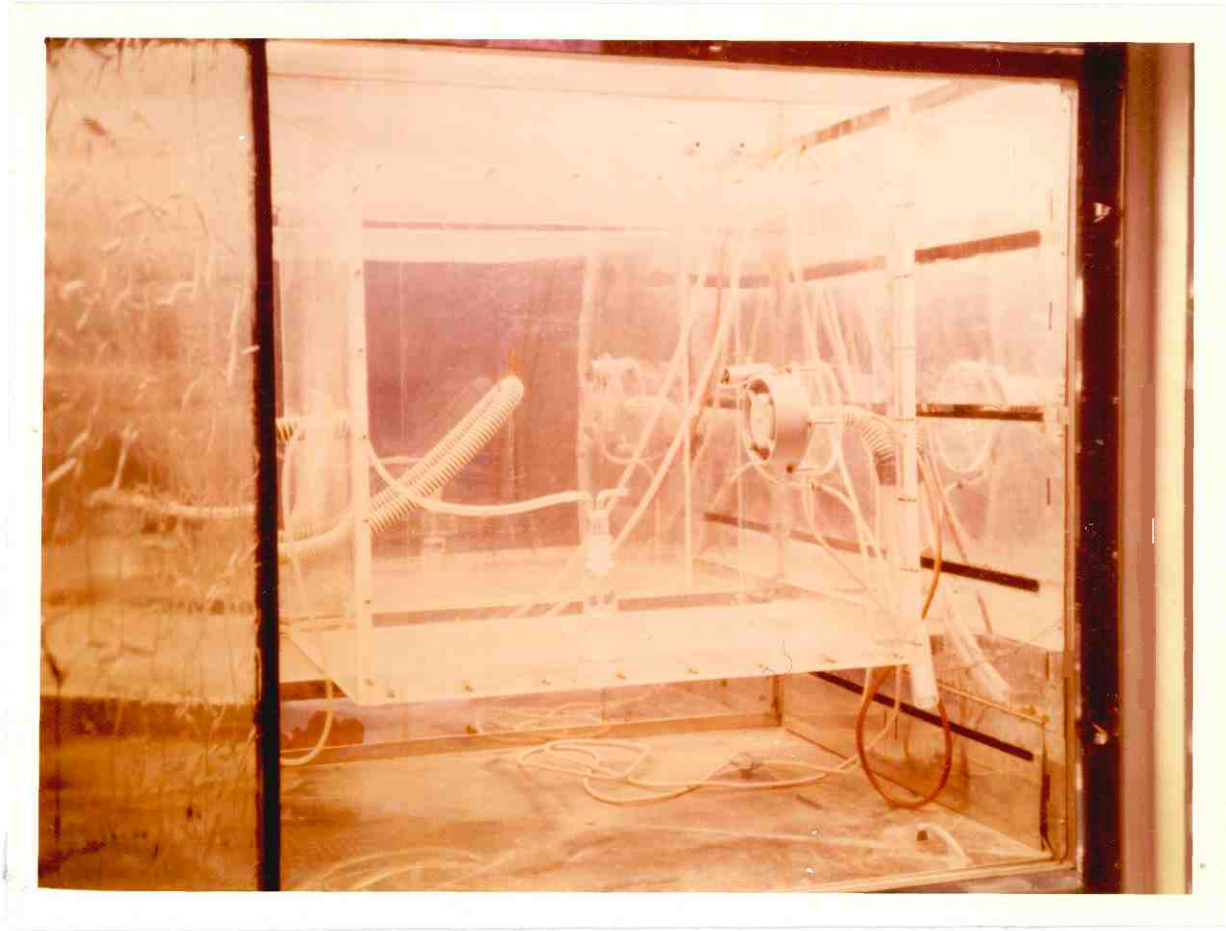


Plate 2.8 Environmentally Controlled Fumigation Chamber



by a pump outside the Fison's cabinet. A fan was mounted at the inlet pipe in order to mix the incoming gases and provide sufficient turbulence to cause leaf flutter.

SO_2 was bled into the inlet pipe from a cylinder of 1% SO_2 (balance nitrogen), with the flow rate regulated by a needle valve and measured by an accurate flowmeter. The concentration of SO_2 in the chamber was assessed using the H_2O_2 bubbler system during fumigation.

Manipulation of plants and equipment within the chamber during a fumigation, was facilitated via two glove holes in the gas tight-door of the perspex chamber. This allowed access with minimum disturbance of internal conditions.

2.6 *Assessment of SO_2 Effects*

2.6.1 *Chronic fumigation*

Assessment of chronic injury was carried out by measurement of plant growth in SO_2 compared with clean air, using either the glass or Perspex outdoor chambers (Sections 2.5.1 and 2.5.2).

Two uniform tillers were taken from each plant and potted in "Long Tom" pots, of volume $10^6 mm^3$ and soil surface area 7900 mm^2, the shoots and roots having been clipped to 30 mm each. One tiller of each plant was placed in each chamber and grown for at least 3 months until differences in yield were apparent.

The plants were harvested at the end of the experiment, by separating them into live shoot, dead shoot and root, the latter being thoroughly washed in tap water to remove any adhering soil particles. The plant material was then dried in an oven at 80°C for 3 days, after which the

dry wts were estimated. In addition to dry wts. the number of tillers and leaves were recorded for each plant in all fumigations, excluding that in Section 7.1. .

2.6.2 *Acute fumigation*

A single tiller of a standard size was taken from each plant, its roots trimmed to 30 mm, senescent leaves removed, and then potted in a plastic beaker, of diameter 65 mm and volume 20 x $10^3 mm^3$. Following this the plants were grown in the greenhouse for 3 weeks prior to fumigation.

SO_2 exposures were carried out in the controlled environment chamber, with a concentration of SO_2 ranging between 4000 and 5000 μgm^{-3}, a level which has been shown to cause a measurable degree of injury on grass species (Ayazloo, 1979). The environmental cabinet was operated for a few weeks before the experiments in order to gain as stable as possible environmental conditions and also to calibrate the control of SO_2 concentrations.

Before placing the plants in the chamber, each plant was reduced to the youngest three leaves of the largest tiller and then the soil surface of each pot was covered in Alkathene granules to minimize SO_2 absorption by the wet surfaces. Plants were well watered by sub-irrigation 24 hrs. prior to fumigation.

The plants were placed in the fumigation chamber the evening before exposure at 1800 hrs, to allow the plants time to aclimatize to the environmental conditions of the chamber. The beakers were arranged randomly, ensuring no leaves were trapped between pots or touching the sides of the chamber.

Fumigations were conducted continuously between 1100 hrs and 1700 hrs, following which the plants were transferred to the greenhouse and scored for injury 3 days later.

Leaf injury caused by acute SO_2 takes the typical form of white necroses on the tips and points of bending on the leaves, sometimes extending down the blade. Since the species used had narrow leaf blades, the length of injury rather than the area was measured, along with the total leaf length.

The degree of injury for each leaf and plant was expressed as a percentage of the total leaf length.

Prior to this investigation, acute injury was assessed on all leaves and tillers produced in the 3 weeks before fumigation (Ayazloo, 1979). Preliminary experiments described in Appendix 2 showed that single tillers and the age of the leaf did not affect its response to SO_2 treatment.

CHAPTER 3

AN INVESTIGATION OF THE RATE OF DEVELOPMENT OF SO_2 TOLERANCE

It has been known for some time, that tolerance to heavy metals can develop within a period as short as 5 years in grass populations of *Agrostis stolonifera* (Wu, Bradshaw and Thurman, 1975), and that the genes for tolerance may exist in a non-tolerant population at a frequency of about 1 to 2% (Walley, Khan and Bradshaw, 1974). Few data are available however, concerning the rate of evolution of SO_2 tolerance. The shortest period in which SO_2 tolerance has been shown to develop in a polluted area is 20 years, in the case of both acute and chronic injury for *Phleum bertolonii* and *Lolium perenne* from Helmshore and Philips Park, respectively (Ayazloo, 1979).

In this section, a study is described in which the possibility of the development of tolerance over a much shorter time scale was investigated. Plots at Philips Park were sampled annually from 1975, the year of sowing, until 1981 excluding 1977 (ref Section 2.3.3). It was hoped that this would enable observation of the minimum time for plants to evolve tolerance, and provide information of how SO_2 tolerant phenotypes spread through a population, these being of special interest in evolutionary studies. It was also considered that the knowledge of the rate at which a grass population is able to adapt to the deleterious effects of SO_2, would be of importance for agricultural and amenity grassland management in polluted areas.

3.1 *Screenings for Acute SO_2 Tolerance in the Philips Park Populations*

3.1.1 *Method*

Samples of the 5 species, from the stock of original seed sown in

the plots at Philips Park (Section 2.3.3), were sown in separate seed trays and grown in the greenhouse for 2 months. At the time that the plants were collected from Philips Park (late February each year), 30 random individuals of the plants grown from seed were selected from each species and grown subsequently alongside the samples from Philips Park. The plants were grown in the greenhouse for at least 3 months, to minimise carryover effects.

Each fumigation used 30 tillers from the Philips Park (PP) population and 30 from the original seed (OS) of a single species. Acute fumigations were carried out as described in Section 2.6.2 and the OS and PP populations were compared for the degree of acute injury, in order to determine whether tolerance had developed.

3.1.2 *Results and discussion*

Over the first two sampling periods no differences were observed between the two populations, OS and PP, of any species (Ayazloo, 1979), but in 1979 the sample of *Lolium perenne* from Philips Park revealed a significantly smaller degree of acute injury than the OS population (Table 3.1). Fumigations for 1980 showed this difference was maintained in *L. perenne* and in addition, interpopulation differences were detected in *Phleum pratense* and *Poa pratensis*. In the screening of the 1981 collection, only *Phleum pratense* showed differential acute injury between populations.

The results presented here suggest that *L. perenne* was able to develop tolerance to acute SO_2 injury within a period of $3\frac{1}{2}$ years and that this tolerance was maintained for at least one year, during which *Poa pratensis* and *Phleum pratense* also developed tolerance, $4\frac{1}{2}$ years after sowing. However, in the following year, 1981, only *Phleum pratense*

TABLE 3.1 % Leaf Length Injury of Philips Park and Original Seed Samples After Acute Fumigation

SPECIES	POP	1976	1978	1979		1980		1981	
		% INJURY	% INJURY	SO_2 μgm^{-3}	% INJURY	SO_2 μgm^{-3}	% INJURY	SO_2 μgm^{-3}	% INJURY
Lolium	O.S.	20.5	23.5		9.7		42.8		6.0
				4184	**	4568	*	4907	
perenne	P.P.	18.5	20.1		4.3		33.0		6.7
Lolium	O.S.	50.7	48.0		20.0		56.0		7.3
				4087		4503		4762	
multiflorum	P.P.	52.5	44.7		14.9		64.1		12.1
Poa	O.S.	59.8	67.0		3.4		44.5		12.7
				4032		5317	*	4149	
pratensis	P.P.	61.4	62.5		4.4		35.0		10.7
Festuca	O.S.	4.5	4.4		4.1		24.5		9.9
				5139		5816		3949	
rubra	P.P.	4.4	3.9		8.1		20.7		9.7
Phleum	O.S.	6.1	9.4		5.8		67.2		4.6
				3924		5052	*	4641	*
pratense	P.P.	5.8	8.8		9.0		54.3		0.8

Data for years 1976 and 1978 from Ayazloo (1979) after fumigation with 5320 ± 140 μgm^{-3} SO_2

* = $P < 0.05$ ** = $P < 0.01$

showed acute SO_2 tolerance, suggesting *L. perenne* and *Poa pratensis* had lost their tolerance.

The large variability, between fumigations in successive years, of injury response within species was the cause of some concern (e.g. *Phleum pratense* OS:1981 4.6%, 1980 67.2%). This makes it difficult to compare consecutive years' fumigations, but individual year's results are valid because each fumigation was a separate entity. The cause of the variation is not certain. It does not appear to be the result of differences in SO_2 concentrations during fumigations, because these are not related to the overall degree of acute injury. Since this experiment was conducted in an environmentally controlled cabinet it seems unlikely that the differences observed could have resulted from different conditions during the experiment. One possibility is that differences in environmental conditions in the glasshouse prior to fumigation, may have modified the sensitivity of the plants to SO_2 injury.

3.2 *Repeat Screenings for Acute SO_2 Tolerance*

Fumigations for *L. perenne* 1979 and 1981 were repeated in order to confirm previous findings and also to investigate the persistence of acute tolerance in the population samples maintained for a prolonged period in a clean air environment.

3.2.1 *Results and discussion*

All fumigations of the *Lolium perenne* 1979 populations showed a differential response, between OS and PP, to acute SO_2 (Table 3.2). These differences were consistent between the populations at 5, 16 and 20 months after the PP population had been moved from its polluted environment in Manchester and grown in the clean air of the greenhouse

at Ascot. The repeat fumigation of the 1981 populations of *L. perenne* confirmed the lack of tolerance in the PP population (Table 3.2).

These repeat fumigations provide further evidence of the development and subsequent loss of tolerance in the PP population of *L. perenne*. Furthermore the persistance of this character in the population samples grown in clean air for over 1½ years, suggests that it has a genetic basis. However, the relevant breeding experiments were not carried out.

It should be noted, that there were no repeat fumigations of the *Poa pratensis* 1980 population, and thus the same confidence cannot be placed on the observations of tolerance development as for the other two species.

3.3 *Screening for Chronic SO_2 Tolerance in the Philips Park Populations*

It is surprising that tolerance to acute SO_2 injury has been selected for so rapidly in the Philips Park populations, considering that SO_2 incidents with the potential to cause this type of injury are probably rare at Philips Park (Section 2.3.3). It would seem far more likely, in view of the prevailing SO_2 levels at the site, that chronic injury tolerance may have developed.

The number of plants and time length involved in a chronic fumigation prohibited the screening of each year's collection. However, it was possible to fumigate all species from the 1980 collection, together with the 1979 collection of *Lolium perenne*.

3.3.1 *Method*

The 5 species, each with 2 populations (original seed and 1980

TABLE 3.2 Repeated Acute Fumigations of Philips Park and Original Seed Populations

Species	Collection -Screening (mths)	Pop	% Leaf Injury	SO_2 (ugm^{-3})
Lolium perenne 1979	5	O.S.	9.7 **	4184
		P.P.	4.3	
	16	O.S.	37.4 ***	5629
		P.P.	21.1	
	20	O.S.	58.5 **	5083
		P.P.	43.3	
Lolium perenne 1981	3	O.S.	4.8	5038
		P.P.	4.9	
	7	O.S.	6.0	4907
		P.P.	6.7	

** = $P < 0.01$ *** = $P < 0.001$

collection), except *Lolium perenne* which had the extra 1979 collection, all with 30 replicates, were potted up. These pots were placed in a totally randomised order in one of the glass chambers with a similar number of plants in the other, identically randomised. The plants were fumigated (ref. Section 2.6.1) with a mean of 317 μgm^{-3} SO_2 for 123 days between January and June of 1981, exchanging the plants and treatments between chambers each month, in order to eliminate any possible differences in climatic conditions between the chambers.

The fumigation was terminated a week earlier than planned due to the air pump shutting down, following a strike by lightning. As a result of this, the plants were accidentally subjected overnight to a concentration of SO_2, estimated to be approximately 12000 μgm^{-3}. It was decided that this would not affect the measurements of growth if the plants were harvested immediately.

In addition to growth measurements, visual estimates were made of the amount of acute injury caused by the accidental fumigation.

3.3.2 *Results and discussion*

There were three sources of variation of interest from this experiment: gas treatment, gas treatment x species and gas treatment x species x populations (Table 3.3).

The variation due to gas treatment was significant in all characters except root/live shoot ratio, dead shoot dry wt. and the number of live leaves per tiller. SO_2 caused an overall reduction in total dry wt. of all plants of 28%. This was reflected in both the live shoot dry wt. (29%) and root dry wt. (32%). The decrease in live shoot dry wt. was reflected in a reduction in the number of tillers (17%) and the

TABLE 3.3 Growth of Original Seed and Philips Park Populations after Fumigation with 317 μgm^{-3} SO_2 for 123 days c.f. Growth in Clean Air.

Growth Parameter	Treat-ment	Mean Growth per plant	% Change of Growth in SO_2 c.f. Clean Air		Gas Treatment x Species	Gas Treatment x Species x Populations
No Live Leaves	C SO_2	47.28 38.25	-19.10	**	*	N.S.
No Live Tillers	C SO_2	14.75 12.17	-17.49	**	**	N.S.
Live Shoot dry wt. (g)	C SO_2	0.961 0.680	-29.24	**	**	**
Dead Shoot dry wt. (g)	C SO_2	0.173 0.175	+ 1.16	N.S.	**	**
Root dry wt. (g)	C SO_2	1.101 0.745	-32.33	**	**	*
Live leaves per tiller	C SO_2	0.317 0.323	+ 1.89	N.S.	N.S.	N.S.
Mean Live Leaf dry wt (g)	C SO_2	0.022 0.019	-13.64	**	**	**
Mean Tiller dry wt.(g)	C SO_2	0.071 0.061	-14.08	**	*	N.S.
Dead/Live Shoot	C SO_2	0.184 0.263	+42.93	**	**	N.S.
Root/Live Shoot	C SO_2	1.394 1.317	-5.52	N.S.	**	N.S.
Total Shoot dry wt. (g)	C SO_2	1.134 0.855	-24.60	**	**	**
Total Plant dry wt. (g)	C SO_2	2.234 1.600	-28.38	**	**	**

* = $P < 0.05$ ** = $P < 0.01$

mean live leaf dry wt. (14%). There was also an increase in senescence (43%).

The second source of variation, the interaction of gas treatments and species, is significant for all characters, except the number of live leaves per tiller (Table 3.3). This indicates a differential response, between species, to the chronic SO_2 fumigation. Means of % change due to treatment, for each species, include OS. and PP. values. The effects of the SO_2 treatment on the O.S. population are discussed here (Table 3.4).

The O.S. population of *Festuca rubra* suffered the largest reduction (-57%) of all the species studied, exhibiting significant changes in all growth parameters, except dead shoot dry wt., number of live leaves per tiller, senescence and root/live shoot ratio. *Phleum pratense* showed the least amount of chronic injury (-21%) in the total plant dry wt., with significant changes in senescence (+94%) and root dry wt. (-30%). There was also a decrease in the number of leaves (-29%), although there was no significant change in live shoot dry wt. (-19%), the number of leaves per tiller (+19%) or the number of tillers (-18%). Thus it seems that *Phleum pratense* was primarily affected by SO_2, by a reduction of root growth and an increase in senescence. *Poa pratensis* was significantly affected in the root dry wt. only (-42%) with a corresponding reduction in the root/live shoot ratio (-25%), this largely contributing to the 34% decrease in total plant dry wt., which was the second highest SO_2 induced reduction, amongst the species examined. Apart from a reduction in the number of leaves per plant (-22%) for *Lolium multiflorum*, both *Lolium* species showed decreases in the same growth characters. The effects of the fumigation were distributed

between increases in senescence (+44%; +35%), and decreases in live shoot dry wt. (-20%;-31%) and root dry wts. (-32%; -28%) (Table 3.4), for *L. multiflorum* and *L. perenne* respectively.

The comparison of the five species demonstrates the variability of interspecific response to chronic concentrations of SO_2. In the present investigation, one species was affected primarily in the roots (*Poa pratensis*), whereas the other four species showed a variety of combinations of responses of live shoots, roots and senescence.

A further observation from the effects of chronic SO_2 on the O.S. populations of each species, was the order of chronic tolerance: *F. rubra* is the most sensitive, with *Poa pratensis*, *L. perenne*, *L. multiflorum* and *Phleum pratense* ranked in order of increasing tolerance. These differences were, however, only significant between *F. rubra*, *Poa pratensis* and *L. multiflorum*.

Variation attributed to gas treatment x species x populations was significant for 6 growth characters : live shoot, dead shoot, root, mean live leaf, total shoot and total plant dry wts. (Table 3.3). This shows differential susceptibility of the O.S. and P.P. populations to chronic SO_2 fumigation.

Comparison of population means for growth in SO_2 with growth in clean air revealed 3 species with differential susceptibility (Table 3.4).

L. perenne was the only population to show strong evidence of tolerance to chronic concentrations of SO_2, when compared to the O.S. population of the same species. The O.S. population was reduced by 27% in total dry wt. compared with 9% for the P.P. population (Table 3.4), which can be mainly attributed to differential live shoot injury between

TABLE 3.4 Comparison of % Change in Growth of Original Seed and Philips Park Grass Populations Subjected to a Chronic SO_2 Fumigation of Mean Concentration of 317 μgm^{-3} for 123 Days

Species	Pop.	No. of Live Leaves	No. of Tillers	Live Shoot Dry Wt.	Dead Shoot Dry Wt.	Root Dry Wt.	Live Leaves Per Tiller	Mean Live Leaf Dry Wt.	Mean Tiller Dry Wt.	Dead/ Live Shoot	Root/ Live Shoot	Total Shoot Dry Wt.	Total Plant Dry Wt.
Poa	O.S.	-15.25	-11.02	-22.15_a	+ 4.33	-41.55_a *	+ 7.43	+ 5.42	- 3.36	+28.73	-25.56 *	-19.50	-33.91 *
pratensis	P.P.	-30.87 *	-23.80 *	-39.01_b *	+18.71	-57.43_b *	+ 9.31 *	-12.49	-21.29	+73.52 *	-32.64 *	-31.59 *	-48.11 *
Phleum	O.S.	-29.20 *	-17.58	-19.46	+95.09 *	-30.60 *	+18.58 *	+ 9.68	- 7.95	+94.46 *	+ 6.98	- 6.70	-20.80 *
pratense	P.P.	-16.82	- 5.32	- 3.51	+54.73 *	-18.14 *	+12.97	+16.67	+ 5.78	+79.38 *	-11.55	+ 3.52	- 9.47
Festuca	O.S.	-34.52 *	-34.39 *	-61.71 *	-49.22	-52.98 *	- 7.23	-40.83 *	-45.51 *	+26.81	-30.54	-61.05 *	-57.27 *
rubra	P.P.	-33.92 *	-33.24 *	-50.25 *	-43.14	-53.98 *	- 0.91	-25.54	-25.36	+39.83	-13.32	-49.88 *	-51.99 *
Lolium	O.S.	-22.30 *	-12.84	-20.11 *	+ 7.75_a	-32.00 *	+ 7.57	- 8.10_a	- 4.29	+44.00 *	- 8.50	-15.04 *	-21.96 *
multiflorum	P.P.	-19.74 *	-23.61 *	-45.42 *	-22.33_b *	-36.40 *	- 5.61	-31.76_b *	-26.51 *	+33.24 *	+13.66	-41.09 *	-39.56 *
Lolium	O.S.	-13.45	- 8.72	-30.96_a *	- 7.45_a	-28.00 *	+ 3.31	-18.20	-20.20	+35.37 *	+19.25	-27.05_a *	-27.43_a *
perenne	79	- 4.14	- 9.98	-23.56_{ab} *	- 2.32_a	-22.14	- 7.54	-27.02 *	-17.65	+37.69 *	+13.25	-19.52_{ab}	-19.17_{ab} *
	80	- 1.02	+ 6.58	-13.10_b	-30.63_b *	-12.31	+ 7.99	- 8.00	-14.75	+53.08 *	- 2.03	- 5.58_b	- 8.73_b

* = $P < 0.05$ For % change in growth between treated and clean air plants.

Differences of alphabet symbols indicate interpopulation differences within species.

the populations. Dead shoot dry wt. was significantly higher in the P.P. population than the O.S., but not significant for senescence.

The tolerance mechanism which, to some extent, has ameliorated the chronic effects of SO_2 on the live shoot dry wt. of the P.P. population of *L. perenne* did not reduce these effects on senescence. This suggests that the effects of moderate concentrations of SO_2 on senescence and shoot growth, may involve two separate mechanisms.

Finally, the 1979 population of L. perenne shows no significant tolerance compared to O.S., although it occupies a position midway between O.S. and the 1980 population. It can be concluded therefore, that tolerance to SO_2 injury appears to have developed in the P.P. population of *L. perenne* over a period of 4½ years.

The results of *Poa pratensis* (Table 3.4) reveal, that several growth parameters of the P.P. population have been reduced significantly in the SO_2 treatment chamber, and that these were reduced to a significantly greater extent than the O.S. population for the live shoot dry wt. and root dry wt. This suggests that the P.P. population has become less tolerant to chronic SO_2. However during the fumigation, *Poa pratensis* only, was infected by a powdery mildew, this occurring only in the treatment chamber. This powdery mildew had previously been observed on *Poa pratensis* in the greenhouse, where it had been noticed that infection seemed greater in populations collected from the field, than those grown from commercial seed. Some months after the chronic fumigation, this mildew was observed on the original seed and P.P. stock cultures of *Poa pratensis*, in the greenhouse. Visible estimates of the percentage leaf area of mildew infection, were recorded

for each plant, without knowing which population each came from. A non-parametric analysis revealed, mildew infection in the P.P. population (median 30%) to be highly significantly greater ($P < 0.001$) than the O.S. population (median 5%). Assuming that initial inoculation of the populations with mildew spores, was evenly distributed amongst the two populations, it may be considered that the O.S. population is more tolerant to powdery mildew than the P.P. population. Therefore, it seems possible that the greater reductions in live shoot and root dry wts. of the P.P. population, compared to the O.S. population of *Poa pratensis*, may have been a result of differential mildew infection. It may also be concluded that the P.P. population of *Poa pratensis* has lost its tolerance to powdery mildew over a period of 4½ years. This may indicate that mildew tolerance was of no selective advantage under the environmental conditions at Philips Park.

Lolium multiflorum was differentially affected, in two growth characters, between populations by the fumigation (Table 3.4). However, there is an element of conflict in the results, in that a decrease in senescence (O.S. + 8%; P.P. -22% dead shoot) is indicative of less injury, whereas a decrease in mean live leaf dry wt. (O.S. + 8%; P.P.-32%) suggests an increase in chronic injury. Although overall, there seems to be a greater degree of injury in the P.P. population the results are generally inconclusive.

Of the two remaining species, *Phleum pratense* and *F. rubra*, neither show any significant differential chronic tolerance between populations.

The results of the visual assessment of acute injury from the accidental fumigation, generally agreed with the findings of previous

acute fumigations (Table 3.5). *L. perenne* (1979 & 80) and *Phleum pratense* populations from P.P. showed acute tolerance while *F. rubra* and *L. multiflorum* did not. The P.P. population of *Poa pratensis* was an exception, in that it did not show tolerance to this accidental fumigation, but had previously shown tolerance in the acute screening. However, this may again be attributed to differential mildew sensitivity. From the above observations, it seems that pretreatment with a prolonged concentration of SO_2 did not affect the differential manifestation of the tolerance to acute injury.

Previous investigators (Ayazloo and Bell, 1981; Horsman et al, 1979) have compared the response of the same genotypes to acute and chronic SO_2 fumigations and found no relationship. In the present experiment, it was possible to compare chronic and acute response for the same plant. No significant correlations were found for any populations. Fig. 3.1 is a typical example of the lack of relationship found for all species. This strengthens the argument that the mechanisms for selection of acute and chronic SO_2 injury tolerance are independant, as suggested by Horsman et al (1979) and Ayazloo and Bell (1981).

An examination of the growth of both populations, of each species, in the clean air chamber was made in order to evaluate the effect of selection on growth characters in the P.P. population (Table 3.6). *Phleum pratense* was the only species not to show any significant difference in growth between the two populations. This suggests that environmental conditions at Philips Park have favoured smaller phenotypes in *L. perenne* and *F. rubra*, but larger phenotypes in *L. multiflorum* and *F. rubra*. Thus, significant changes have taken place in the growth characteristics of these populations, within a period of 4½ years.

TABLE 3.5 Acute Exposure from Chronic Fumigation

Species	Pop	Scored % Injury	
Lolium perenne 1979	O.S.	40	***
	P.P.	10	
Lolium perenne 1980	O.S.	40	**
	P.P.	20	
Lolium multiflorum 1980	O.S.	55	
	P.P.	50	
Poa pratensis 1980	O.S.	30	
	P.P.	30	
Festuca rubra 1980	O.S.	30	
	P.P.	30	
Phleum pratense 1980	O.S.	50	**
	P.P.	40	

** $P < 0.01$

*** $P < 0.001$

Fig. 3.1 Chronic Injury/Acute Injury (after accidental acute SO_2 incident during chronic SO_2 Fumigation).

Species	Correlation Coefficients O.S.	P.P.	
F. rubra	-0.027	0.169	
L. perenne	-0.059	-0.019	
L. multiflorum	0.189	0.056	All n.s.
Phleum pratense	0.033	-0.067	
Poa pratensis	-0.219	-0.174	

Example: L. perenne P.P. (1980).

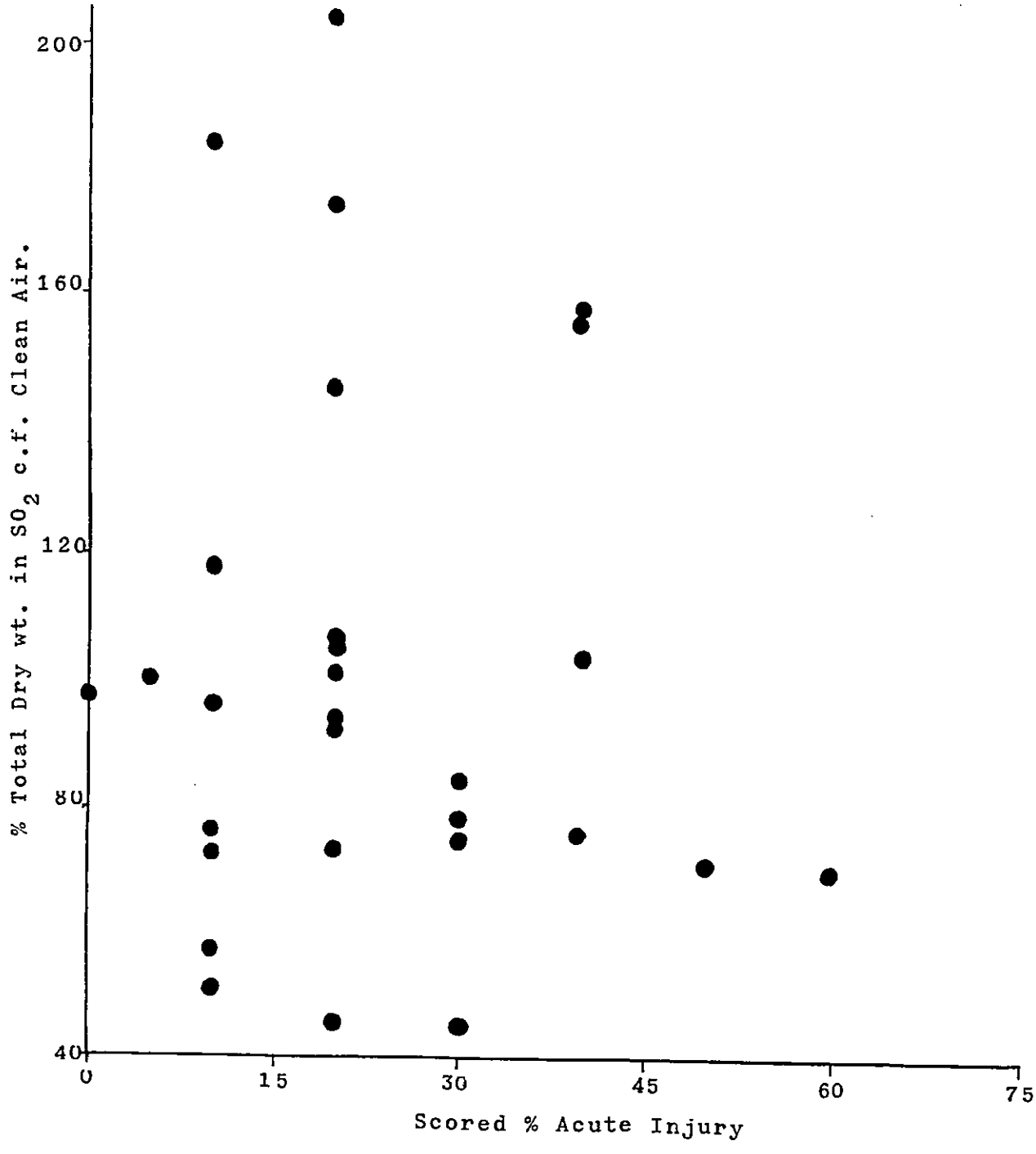


TABLE 3.6 Growth of Philips Park Populations in the Clean Air Chamber for 123 days.

Species	Pop.	No. of Leaves	No. of Tillers	Live Shoot Dry Wt. (g)	Dead Shoot Dry Wt. (g)	Root Dry Wt. (g)	Leaves / Tillers	Mean Leaf Dry Wt. (g)	Mean Tiller Dry Wt. (g)	Dead / Live Shoot	Root / Shoot	Total Shoot Dry Wt. (g)	Total Plant Dry Wt. (g)
Poa	O.S.	28.50 a	9.80 a	0.485	0.074	1.119 a	0.343	0.018	0.054	0.159	2.316	0.559	1.678 a
pratensis	P.P.	43.83 b	14.37 b	0.664	0.099	1.353 b	0.333	0.015	0.047	0.165	2.148	0.763	2.116 b
Phleum	O.S.	44.73	9.33	0.771	0.096	1.245	0.209	0.018	0.091	0.175	1.748	0.866	2.111
pratense	P.P.	45.17	9.40	0.798	0.111	1.373	0.211	0.019	0.092	0.144	1.827	0.909	2.282
Festuca	O.S.	42.01	12.62 a	0.922	0.052	0.857	0.297	0.024 a	0.082 a	0.057	0.979	0.973	1.830 a
rubra	P.P.	59.44	17.81 b	0.800	0.046	0.891	0.303	0.014 b	0.048 b	0.058	1.254	0.846	0.782 b
Lolium	O.S.	56.85	19.40	1.422 a	0.320 a	1.091	0.361	0.029 a	0.079 a	0.255	0.859	1.742 a	2.833 a
multiflorum	P.P.	58.40	20.63	1.915 b	0.441 b	1.170	0.369	0.037 b	0.101 b	0.258	0.659	2.356 b	3.526 b
Lolium	O.S.	52.65	16.51	1.476 a	0.291 a	1.193	0.324 a	0.030 a	0.092 a	0.211	0.826	1.767 a	2.960 a
perenne	79	49.73	17.33	1.023 b	0.237 b	1.061	0.358 b	0.023 b	0.073 b	0.253	1.094	1.260 b	2.321 b
	80	55.64	17.33	1.023 b	0.213 b	1.113	0.313 a	0.019 b	0.061 b	0.223	1.180	1.236 b	2.348 b

Significant differences ($P < 0.05$) between populations of the same species are indicated by different letters.

In all cases where there were significant differences of growth in SO_2 compared with growth in clean air, between populations, there was a corresponding difference of growth in clean air (Tables 3.4; 3.6) It is interesting, that this difference was such that the population which suffered the greatest reduction in the treatment chamber of each of the 3 species, *L. perenne*, *L. multiflorum* and *Poa pratensis*, produced the largest plants in the control chamber. This relationship holds for non-significant susceptibility of *F. rubra*, but not for *Phleum pratense*. Although there seems to be some relationship between plant growth with chronic SO_2 injury, it is not possible to draw conclusions for all species because of the complications from mildew infection of *Poa pratensis*. To examine this relationship further it is necessary to conduct a regression analysis within each species (ref Section 9.1).

3.4 *Investigation of the Genotypic Structure of the 1979 Lolium perenne Population from Philips Park.*

Charles (1961), when investigating the differential survival of cultivars of *Lolium*, *Dactylis* and *Phleum*, discovered that considerable depletion of seedlings, of the order of 80%, occurred in the first two months after sowing, and that this increased to 90% within one year. The plots at P.P. had been present for 3½ years before acute SO_2 tolerance was observed, and it is reasonable to assume that the number of genotypes present on the plots, was probably less than 10% of the number of viable seeds sown. The number of genotypes present on the plot has a certain bearing on the effects of selection. The method of sampling (ref Section 2.3.4) from the plots, makes the mean SO_2 injury effectively a reflection of the degree of vegetative cover attributable to tolerant genotypes. An increase in "tolerance cover"

must either involve the loss of susceptible genotypes or their reduction in growth.

It was decided to investigate the variability, between individual plants of the P.P. 1979 sample of *L. perenne*. The plants were assessed on the basis of morphological and electrophoretic variants.

3.4.1 *Morphology*

Differences of plant morphology between 21 individuals of *Lolium perenne* were assessed using a similar method to Harberd and Owen (1969). Three tillers of the same size were removed from stock cultures and potted separately, on the 27th June 1981. Each plant was randomly numbered, so that the classification was blind. Two months after potting, each plant was compared on the basis of visual appearance with all others, and assigned to distinct morphological groups. Any disagreements between replicates, were checked before the final groupings. The whole process was repeated several weeks later.

3.4.2 *Electrophoretic Technique*

The electrophoretic isomers of two enzyme systems, glutamate oxaloacetate-transaminase (GOT) and phosphoglucoisomerase (PGI) were distinguished for 21 individuals of *Lolium perenne* from the P.P. 1979 population, using a horizontal starch gel method (Hayward and McAdam, 1977; pers comm, Dr. M.D. Hayward).

Three stock buffer solutions were prepared and stored away from direct sunlight in a cool place:

1. the "crushing buffer", 0.605g of TRIS mixed with 50 µl of 2 mercaptoethanol in a final volume of 50 µl adjusted to pH 7.2, giving a concentration of 0.1M TRIS,

2. the "bridge buffer" for the electrode chambers was prepared from 12.36g of boric acid, dissolved in 1M lithium hydroxide and adjusted to a pH of 8.3 in a final volume of 1 litre, giving a concentration of 0.2M borate,
3. TRIS citric acid buffer, comprised of 6.05g of TRIS dissolved in 1M citric acid to a volume of 1 litre, with the pH adjusted to 8.3, giving a concentration of 0.05M TRIS.

The starch gel was prepared (Smith, 1976) by mixing 19.2g of hydrolyzed starch in a small amount of "electrode buffer" (9 parts TRIS buffer to 1 part "bridge buffer") to a smooth consistency, then a further buffer added to a volume of 150 ml. The starch suspension was poured into a round-bottomed flask and attached to a rotary evaporator with the flask resting in a water bath at 95^{o}C. The gel was mixed continually for 4 minutes, during which time it became viscous and opaque, shortly afterwards returning to a mobile semi transparent state, at which time a negative pressure was applied inside the flask, causing the mixture to boil. The vigorous boiling was allowed to continue for several seconds, ensuring the removal of air bubbles in the liquid, but also taking care to prevent excess dehydration of the gel. The liquid was poured into a 220 mm x 130 mm x 2.5 mm glass plate and a glass sheet gradually lowered, from one end first, over the gel, ensuring no air was trapped underneath. This was held down flat with a heavy object, and given time to set at room temperature. The gel was stored overnight, sealed in "clingfilm", in a refrigerator.

Crude extracts of the plants were obtained by macerating with a glass rod in a solid watch glass, approximately 7 cm of the youngest leaf of a randomly selected tiller in two drops of "crushing buffer".

The extract was absorbed onto a 5 mm strip of filter paper, which was inserted in one of 21 5mm slits spaced evenly along the length of the gel, 3 cm from the glass edge.

Once the slits in the gel all contained extracts, each from a different plant, the plate was transferred to the electrode chamber. The gels were connected to the "bridge buffer" via two wide strips of filter paper, which stretched the length of the gel, overlapping on one side by 1 cm and with the other side dipped in the buffer, thus completing the electric circuit. The gels were run at 325v and 4°C for 20 minutes, after which the extract wicks were removed. Electrophoresis was continued for 4 to 5 hrs until the borate front had migrated 8 cm; all systems migrated anodally.

After electrophoresis the gels were subjected to staining, in order to visualize the banding patterns. G.O.T. stain was mixed (Appendix 4), then poured over the gel in a polythene container and left in the dark at room temperature for 3 hrs to develop. P.G.I. (Appendix 4) was treated similarly, but assayed at 37°C for 2 hrs.

After staining, the gels were drawn and photographed as soon as possible. The whole procedure was repeated to confirm results. During the process plastic gloves were worn and glassware cleaned to analytical standards to avoid contamination with alien amino acids.

3.4.3 *Results of Morphological Observations*

Very little flowering occurred in the plants during the period of the investigation and thus comparisons were restricted to the vegetative parts. However, even using only vegetative appearance 8 obvious morphological groups were distinguished (Table 3.7) labelled A to E

respectively.

The assessments were made on the general appearance of individual plants, but it was evident that some characters exerted a major influence in classifying groups. Many of the differences were based on height and width of clones, which in some cases, was related to habit (prostrate or erect): a particular plant '24' was extremely prostrate in habit. Other characters of importance included the density and width of tillers and width and greenness of leaves.

TABLE 3.7 Frequency of Different Morphological Groups in the P.P. Population of *L. perenne*

Arbitrary symbol for morphological group	A	B	C	D	E	F	G	H
Number of plants	1	3	6	3	2	1	1	4

It was assumed that the repeatability of these groups (Table 3.7) between replicates indicated possible genetic differences, but these were not tested with the appropriate breeding experiments.

3.4.4 *Results of Isoenzyme Analysis*

Differences between clones were recognised by distinction of electrophoretic variants of two enzyme systems : PG1 (Fig. 3.2; plate 3.1) and GOT (Fig. 3.2; plate 3.2). It was assumed that the different enzyme bands, were the products of different genes, but the nature of the genetic control of these bands could only be proven using breeding experiments, which were not performed.

On the electrophoretic plates of both PG1 and GOT, there were several vague bands which were excluded, since they were difficult to

Plate 3.1 Starch Gel Showing Electrophoretic Variants of PG1 from 21 Clones of the 1980 Population from Philips Park.

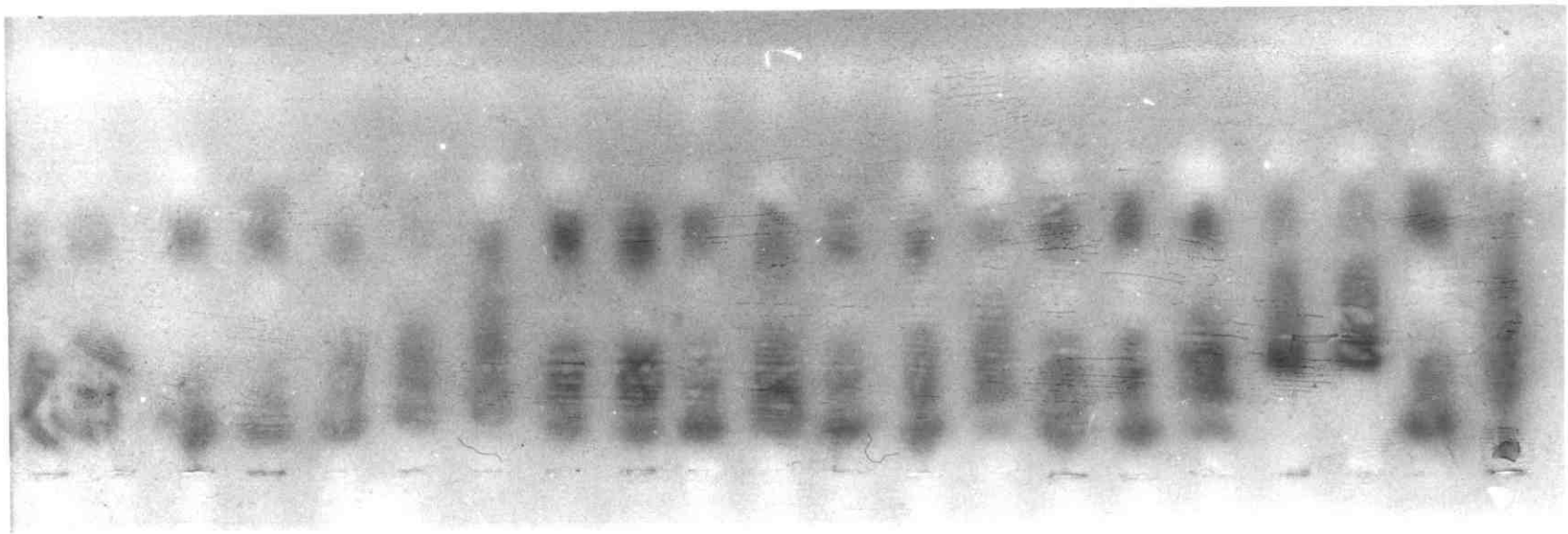
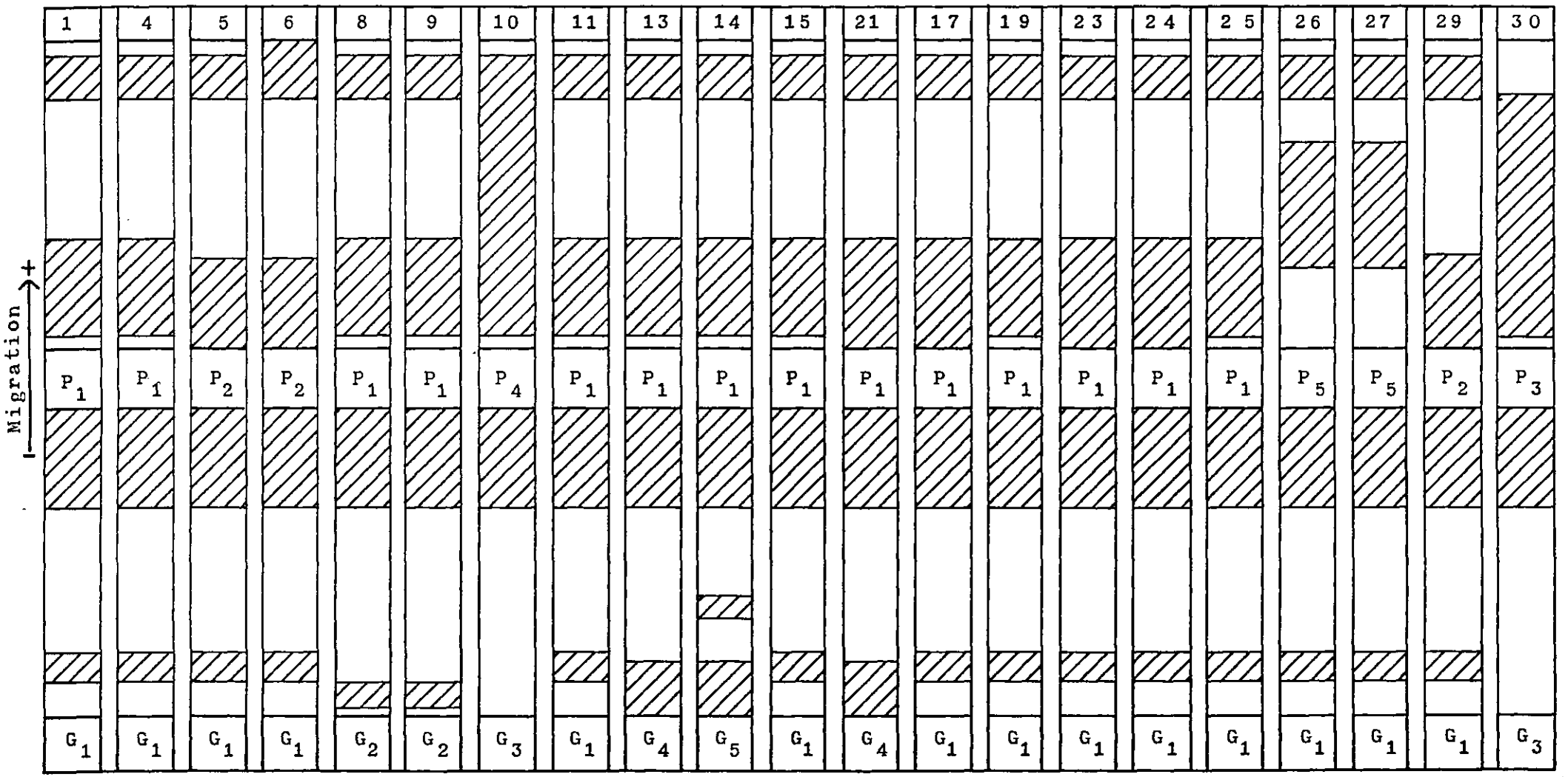
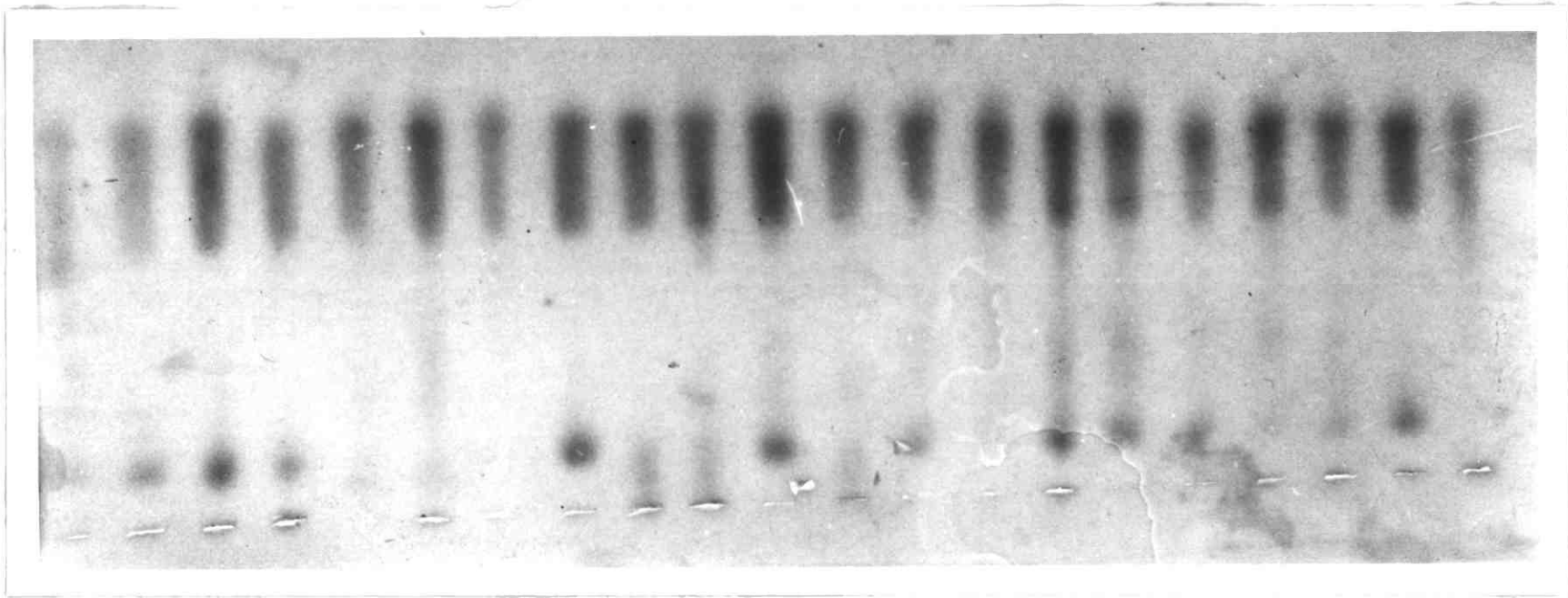


Fig. 3.2 Interpretation of Enzyme Banding Patterns for PGI (top) and GOT (bottom) from Replicate Starch Gels After Electrophoresis of Leaf Samples of 21 Clones of L. perenne.



1-30 Clones (top line). P_{1-5} and G_{1-5} are Electrophoretic Variants for PGI and GOT, Respectively.

Plate 3.2 Starch Gel Showing Electrophoretic Variants of GOT from 21 Clones of the 1980 Population from Philips Park.



distinguish (Plate 3.1; 3.2). . Repeat experiments gave similar results.

For each of the two enzyme systems, 5 variants were recognised and labelled P_1 to P_5 for PGl and G_1 to G_5 for GOT (Table 3.8), which resulted in 8 different combinations of variants. For both enzyme systems one pattern accounted for 2/3 of the plants, but in combination P_1 : G_1 accounted for just under half (Table 3.9). These results indicated the restricted variation of PGl and GOT electrophoretic variants within the P.P. population of *L. perenne*.

TABLE 3.8 Frequency of Different Isozyme Patterns in the P.P. Population of *L. perenne*

Arbitrary symbols for isozyme patterns		1	2	3	4	5
Number of plants	PGl(P)	14	3	1	1	2
	GOT(G)	14	2	2	2	1

3.4.5 *Discussion of Electrophoretic and Morphological Variants*

The results of the morphological investigation did not complement those of electrophoresis, in that there were no strongly dominant morphological groups. This is interesting because it suggests that selection at Philips Park has maintained a heterogeneous morphology.

The morphological and electrophoretic groups show no easily discernible relationships; there are a number of subdivisions of morphological groups by isoenzyme groups and vice versa. None of these subdivisions have more than 3 plants.

Assuming that each subdivision in Table 3.9 indicates the least

number of genotypes present in the P.P. population sample of *L. perenne*, it seems there were a large number of genotypes in the population at the time of sampling. This would tend to suggest that individual plants are fairly small, and that changes in population tolerance as a result of selection, would involve the relatively frequent loss of genotypes.

TABLE 3.9 Combination of Morphological and Electrophoretic Techniques for Clonal Recognition to Give the Least Number of Distinct genotypes in the sample of *L. perenne* from P.P.

Electrophoretic Variants		Morphological Variants							
		A	B	C	D	E	F	G	H
PG1	GOT								
P_1	G_1	24	17	25,4,1		11,15	23		19
P_1	G_2				9,8				
P_1	G_4		13		21				
P_1	G_5								14
P_2	G_1			6					5,29
P_3	G_3			30					
P_4	G_3					10			
P_5	G_1		26	27					

3.5 *General Discussion*

The evidence presented in this chapter indicates that bred cultivars of *Lolium perenne*, *Phleum pratense* and possibly *Poa pratensis* developed tolerance to acute SO_2 injury, over a period of $3\frac{1}{2}$, $4\frac{1}{2}$ and $4\frac{1}{2}$ years respectively. Furthermore there is evidence suggesting that chronic SO_2 tolerance developed in *L. perenne* in $4\frac{1}{2}$ years.

Tolerance to acute injury was not found in the 1981 *L. perenne* population from Philips Park. This may have been due to a lowering of the selection pressure which maintained tolerance, possibly as a result of decreases in the ambient SO_2 concentrations at the site (ref. Section 2.3.3). If this is true, it seems likely that acute tolerance in *Phleum pratense* will also diminish if current trends of ambient SO_2 levels continue.

Several other, presumably adaptive, changes were observed amongst the species grown at Philips Park. *Poa pratensis* showed decreased tolerance to powdery mildew, while all species except *Phleum pratense* exhibited changes in growth characteristics. None of these characters seemed to have any relationship with SO_2 tolerance, but it was noted that, between O.S. and P.P., the population that suffered the significantly greater chronic injury tended to have larger plants. This evidence suggests the presence of other selective factors operating in the plots at Philips Park.

L. perenne developed tolerance to both acute and chronic concentrations of SO_2 more rapidly than any other species. The *F. rubra* O.S. population was more susceptible to chronic injury than *L. perenne* O.S. and may have been expected to show change more rapidly considering the greater scope for tolerance. However, it is possible that the presence

of differential tolerance may have been obscured, by the high degree of chronic injury sustained by this species after experimental fumigation, which might have been detectable at a lower SO_2 concentration. The speed of adaption by *L. perenne* is probably partially due to genetic variability and availability of tolerance genes. *L. perenne* S23 is noted for its high variability, originating from about 100 parental families compared to 4 or 5 in most other bred cultivars. It is also important to note that tolerance to SO_2 has previously been reported for *L. perenne* at Philips Park (Ayazloo and Bell, 1981) in a lawn only 20 m from the experimental plots. This lawn may have been a source of tolerance genes to the plot population, but under the mowing regime at Philips Park, it is unlikely that much gene flow would occur.

L. multiflorum, although often considered biennial, was still persistant in the plots nearby 6 years after sowing. It is fairly common for a biennial or an annual species to take on a perennial habit, when flowering is prevented as was probably the situation under the mowing regime at Philips Park. However, forced to adopt a perennial habit for several years, and for which it is probably not adapted, *L. multiflorum* is eventually likely to be at a disadvantage to plants specialized in the perennial habit, or to younger members of the same species. In both chronic and acute fumigations, *L. multiflorum* showed a tendency towards greater susceptibility in the P.P. population. In these experiments comparison of young O.S. plants with 6 year old P.P. plants for SO_2 tolerance, may be biased by their relative ages.

The investigation of the variability of clones indicated, that there were a large number of genotypes still present in the *L. perenne* population in 1979. It was argued that with a large number of genotypes,

most of the plants must have been small, with only a few tillers, and that significant changes detected in SO_2 tolerance or any other character of the population, would probably involve the loss of less competitive genotypes. Laycock (1980) measured ground cover of sown species on the plots, twice a year, up to April 1979. In the last year of recording, *F. rubra*, *L. multiflorum* and *L. perenne* were reduced from 78%, 45%, and 72% to 48%, 13% and 37%, respectively (Table 3.10). This was the same year as the appearance of acute SO_2 tolerance in *L. perenne*, and suggests that the decrease in ground cover was selective, with a greater proportion of tolerant than non-tolerant phenotypes surviving.

The decreases in ground cover by the species originally sown in each plot would probably enable the invasion by seed of other species. However, it was observed during sampling at the plots that most of the area not inhabited by the relevant species was invaded, through vegetative reproduction, by species from adjacent plots.

TABLE 3.10 % Ground Cover by Grass Species on Plots at Philips Park

(from Laycock, 1980)

Species	% Ground Cover					
	16/8/76	8/4/77	12/8/77	17/4/78	12/9/78	17/4/79
Festuca rubra	94	86	68	78	36	48
Lolium multiflorum	42	60	50	45	41	13
Lolium perenne	79	71	62	72	41	37
Phleum pratense	90	79	82	82	79	66
Poa pratensis	96	88	88	88	79	85

CHAPTER 4

THE DEVELOPMENT OF ACUTE SO_2 TOLERANCE IN LAWNS OF DIFFERENT AGES

4.1 *Introduction*

In 1975, Wu, Thurman and Bradshaw reported the results of an investigation into the copper tolerance of *Agrostis tenuis* populations, from a series of lawns of different ages. These had experienced deposition of atmospheric copper pollution, from a nearby cable factory, for different lengths of time. The *A. tenuis* populations showed a range of tolerance, positively correlated with the age of lawn, representing an evolutionary series in which tolerant plants were being selected and spreading, both by seed and vegetatively, thus founding new tolerant populations.

It was decided to study the development of acute SO_2 tolerance, with time, in a series of lawns of different ages at The Temple in Central London, an area with a long history of air pollution (ref Section 2.3.4). All the lawns were well tended and provided a good ground cover, unlike in the study of Wu et al (1975), where vegetation cover increased with age; thus these lawns gave no visible indication of differential tolerance to SO_2, or any other prevailing environmental factor.

Investigations were made to determine the level of acute SO_2 tolerance in The Temple lawns, for several grass species, and the relationship of this with the age of each lawn. The lawns were selected with the aim of studying the development of tolerance over a period of almost 200 years.

4.2 *Method*

Samples of *Agrostis tenuis*, *Festuca rubra* and *Dactylis glomerata* were collected from 5 lawns at The Temple, dated 1770, 1875, 1946, 1958 and 1977 and labelled a to e respectively, (ref Section 2.3.4). The plants were grown in the greenhouse for 4 months prior to screening for acute SO_2 tolerance (ref Section 2.6.2), using the following experimental design. *A. tenuis* and *F. rubra* were screened separately over 3 fumigations, 10 plants from each of the 5 population samples per fumigation being randomised in the chamber. There were only 4 populations of *D. glomerata*, this species being absent in the youngest lawn. These were screened over two days using, in each fumigation, 15 plants from each population, randomised together. Mean SO_2 concentrations for each acute fumigation are given in Table 4.1.

4.3 *Results and Discussion*

Differences of % leaf length injury were detected between the populations of *Agrostis tenuis* and *Festuca rubra*, but not for *Dactylis glomerata* (Table 4.2). This suggests that differential tolerance to acute concentrations of SO_2 was present in the lawns of The Temple for *A. tenuis* and *F. rubra*, but there is no evidence of a similar development for *D. glomerata*.

There were highly significant differences between fumigations for *A. tenuis* (Table 4.2) which appear to be related to the mean SO_2 concentration (Table 4.1). Percentage leaf length injury on *D. glomerata* was very low (< 3%) which was unlikely to be due entirely to lower SO_2 concentrations during the fumigations and may indicate an inherent tolerance in this species.

TABLE 4.1 SO_2 Concentrations and % Mean Acute Leaf Injury for each Fumigation of Temple Populations

Agrostis tenuis

	SO_2 μgm^{-3}	% Injury
1	5906	34.8
2	5309	22.5
3	4964	16.4

Festuca rubra

	SO_2 μgm^{-3}	% Injury
1	5979	11.3
2	5698	16.3
3	5425	12.8

Dactylis glomerata

	SO_2 μgm^{-3}	% Injury
1	4662	2.6
2	3951	2.3

It was hypothesised that the older lawns having been subjected to selection pressure from ambient SO_2 for a longer period, would show a temporal cline of increasing acute SO_2 tolerance with age. The intensity of selection is related to the gradient of a cline (Clarke, 1966),: therefore it is possible to surmise that if a cline occurred it would be steepest between the youngest, least tolerant of the populations, because these would be subjected to the most intense selection causing the greatest rate of change. As the population becomes more tolerant a decrease in the rate of adaptation would be expected, with lower selection pressures, resulting in a cline with a logarithmic curve. This model is an ideal situation which in the field is complicated by changes in ambient SO_2 concentrations which would affect the shape of the curve, but not the order of tolerance of the populations.

The populations of *F. rubra* show a clinal pattern similar to that hypothesised, possibly accounted for by the length of exposure to ambient SO_2, giving evidence of an evolutionary series of acute tolerance (Fig. 4.1). *A. tenuis* does not entirely fit this pattern: the oldest lawn (1780) shows an intermediate tolerance, although the younger populations are the most susceptible. There were no differences in acute tolerance between the *D. glomerata* populations, but the degree of injury was very small suggesting that all the plants were highly tolerant. *D. glomerata* is not commonly used as a turf grass, so it is probable that the individuals present at The Temple invaded from elsewhere in London. If so, such invaders are liable to be already tolerant to the ambient SO_2 of London.

The distribution of acute SO_2 injury within populations of each

Fig. 4.1 Acute SO_2 Injury in The Temple Lawns (after angular transformation).

Confidence limits at 95%

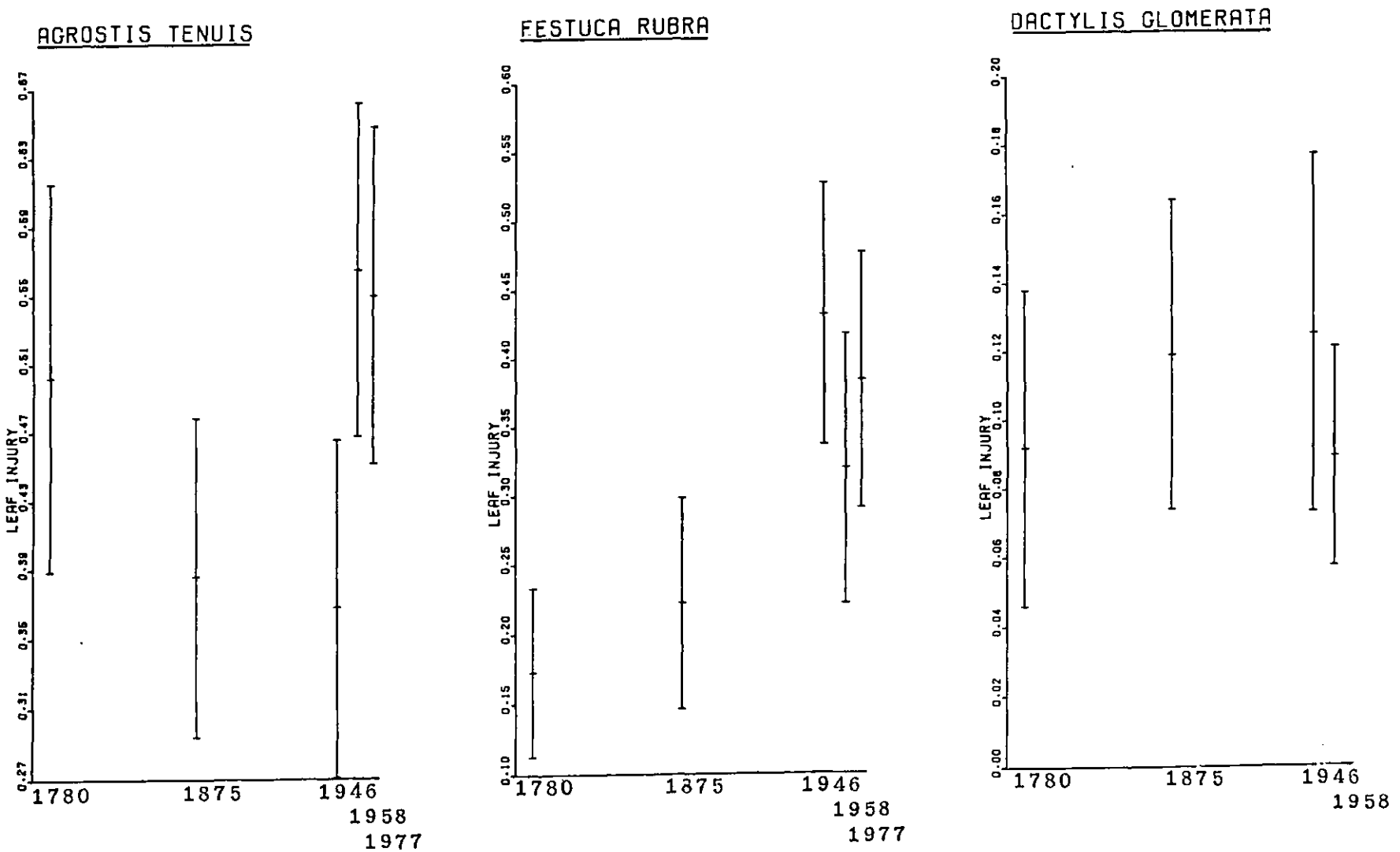


TABLE 4.2 Analysis of Variance of Acute Injury of The Temple Populations

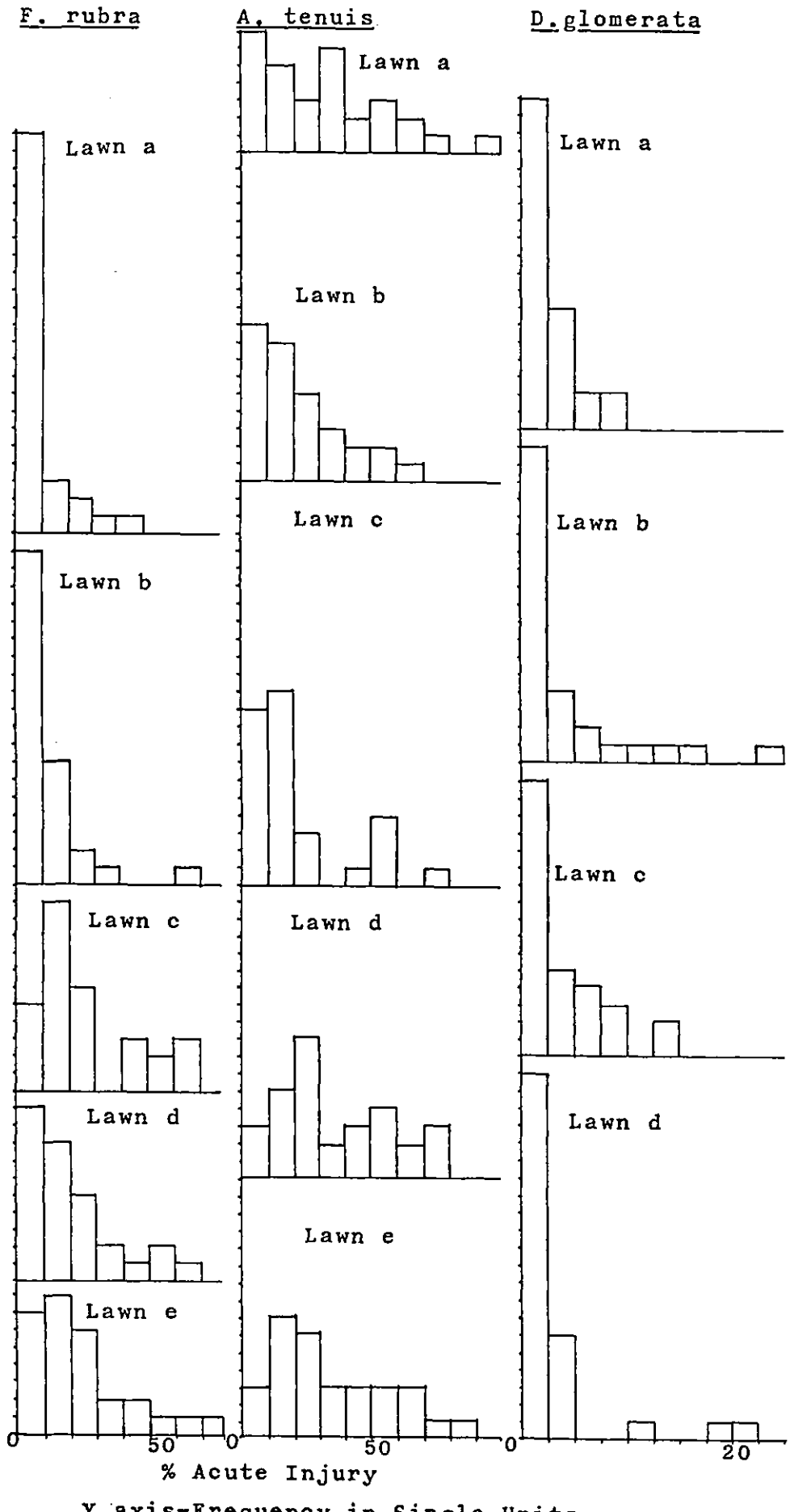
Source	*A. tenuis* F	*A. tenuis* P	*F. rubra* F	*F. rubra* P	*D. glomerata* F	*D. glomerata* P
Lawn Age	4.03	**	6.50	**	0.86	N.S.
Fumigation Date	14.20	**	1.59	N.S.	0.02	N.S.
Lawn Age x Fumigation Date	0.87	N.S.	0.80	N.S.	0.02	N.S.

** = $p < 0.01$

lawn are given in Fig. 4.2. The most tolerant populations have a uniformly low % acute injury, with only a few clones over 20% as in *F. rubra* 1780 and 1875, and *A. tenuis* 1946. The more susceptible lawns exhibited a wider range of injury with medians of greater than 10% acute injury, as in *F. rubra* 1977 and *A. tenuis* 1977 and 1958. In *D. glomerata* the mean % injuries for each population are less than 5%, but there are still several plants showing injury of greater than 20%. Such variation is likely to be important for adaptation to fluctuating ambient SO_2 levels and indicates the ability of non-tolerant genotypes to survive in a polluted atmosphere.

The present investigation demonstrates that acute SO_2 tolerance has evolved within a period of 30 years in *A. tenuis* and over 30 years in *F. rubra*. It is possible that acute SO_2 tolerance has not developed in the younger populations due to the current rarity of acute SO_2 incidents.

Fig. 4.2 Distribution of Acute SO_2 Injury in the Lawn Populations from The Temple.



F. rubra showed a cline of the development of acute SO_2 tolerance, but the hypothesised pattern was not present in *D. glomerata* or *A. tenuis*, although grown under the same environment and subjected to the same SO_2 concentrations. Differences in the gradients of the clines could be due to differential specific response to selection, while differences in the order of tolerance of the populations is possibly due to genetic variability. These lawns were well tended and mown regularly, allowing little gene flow to or from them. In such a situation the population's genetic variability would tend to be highly limited, having a maximum when the lawn was laid and continually decreasing as less fit genotypes are excluded by competition. Differences in genetic variability between the populations are liable to cause differential capacity for adaptation to environmental flux. The existence of such differences is quite likely since the lawns were established over a period of 200 years. Considering the advances made in grass breeding during this period, a lawn laid 200 years ago might be expected to have a very different genetic character to that of a present day lawn. Seed and turf were at that time collected and cut respectively in the wild, while present day seed and turves are derived from highly bred cultivars based on a few original genotypes. There is also likely to be a difference of genetic variability between lawns established from seed or turf, but in the present experiment only the 1977 lawn's origin is known in this respect, it having originated from seed.

Genetic variability may also restrict readaptation of an old tolerant lawn to a cleaner air environment. If, as suggested, the younger populations at The Temple have not developed acute tolerance

because of decreasing ambient SO_2 levels, it would be expected that the old lawns might lose their tolerance. In this event, these populations may be transient, or unable to lose tolerance, having discarded the genetic ability to do so.

CHAPTER 5

DEVELOPMENT OF SO_2 TOLERANCE IN POPULATIONS AT DIFFERENT DISTANCES FROM A POINT SOURCE

5.1 *Introduction*

The existence of a point source of air pollutants at Askern provides a range of SO_2 concentrations decreasing with distance from the stacks (ref. Section 2.3.2). This enabled the investigation of the effect of a range of selective pressures of different intensity, imposed by varying SO_2 levels on grass species growing in the area. The expected result of such selection pressure would be a cline of SO_2 tolerance, decreasing with distance from the stacks.

A cline is defined as "A gradual change in a phenotypic characteristic of a population" (King, 1974). Haldane (1948) classifies clines into two types: a) that caused by migration away from the centre of origin of a gene and b) the situation where one phenotype is favoured, by selection, in one part of a habitat more than another. The type of cline involved in the present study is the latter, known as a morph-ratio cline, which has been studied with respect to heavy metal tolerance (Jain and Bradshaw, 1970; Antonovics and Bradshaw, 1970). However, clinal changes in heavy metal tolerance may occur over only a few metres, due to abrupt changes in soil toxicity. In the present study a cline of 2 km length was investigated, over which the change in pollution levels was continuous.

5.2 *Screenings for Acute SO_2 Tolerance in Populations along the Askern Transect*

5.2.1 *Method*

Samples of *D. glomerata* and *F. rubra* were collected along a transect, at 8 sites of varying distances up to 2 km, east of the Askern Coalite factory (ref Section 2.3.2). After 4 months growing in the greenhouse, the plants were screened for acute SO_2 tolerance (ref Section 2.6.2) using the following design. Each species was screened separately over 4 fumigations, using 8 plants from each population per fumigation, totally randomised in the chamber. The whole procedure was repeated two months later. SO_2 concentrations for each fumigation are presented in Table 5.1.

TABLE 5.1 SO_2 Concentrations and mean % Injury for Acute Fumigations of the Askern Populations.

SCREENINGS	FUMIGATIONS	*DACTYLIS GLOMERATA* $\mu gm^{-3} SO_2$	Mean % Injury	*FESTUCA RUBRA* $\mu gm^{-3} SO_2$	Mean % Injury
1	DAY 1	4895	8.1	5041	9.6
	DAY 2	5773	4.4	4871	11.5
	DAY 3	5857	11.0	5097	7.6
	DAY 4	*	6.1	5392	14.1
2	DAY 1	4737	1.8	5690	9.7
	DAY 2	5424	19.2	5666	11.8
	DAY 3	6088	2.8	7542	16.2
	DAY 4	5702	0.6	5876	5.4

* No reading due to a breakdown in the sampling system.

5.2.2 *Results and discussion*

Analysis of the data indicated large differences between the

populations of *Festuca rubra* and possible differences between the populations of *Dactylis glomerata*, since only the first screening gave a significant result (Table 5.2). This suggested differential susceptibility of the populations, to acute SO_2 injury, along the transect.

In all four analyses the variance ratio for the factor "fumigations" was highly significant, indicating large variation in acute injury between separate fumigations. These variations did not seem to be related to the relevant SO_2 concentrations (Table 5.1). It seems that some other factor(s) may have affected the degree of injury on different days.

Even though there were large differences between fumigations, there were no significant interactions in the analyses, suggesting that the individual fumigations caused a relatively similar response in each population.

Two graphs plotted for *F. rubra* (Fig. 5.1) had similar patterns, with distinct clines of SO_2 tolerance decreasing with distance from the Coalite factory. There were several features of interest in the cline. It is evident that population A, closest to the emission source, was very much more tolerant than its adjacent population B, such that a line between A and B provided the steepest part of the cline. This was probably the area where maximum selection occurred. At the top of Askern hill (Site A) the plants do not experience the emissions from the stacks, except on days with no wind, but are often exposed to the maximum concentrations of the factory ground level emissions (ref. Section 2.3.2). Also at site A the plants are exposed to coal dust which may cause the wedging open of stomata,

enhancing the uptake of SO_2 and increasing possible damage (Williams, Lloyd and Ricks, 1971; Ricks and Williams, 1974).

TABLE 5.2 Analysis of Variance of Acute Injury of the Populations Along the Askern Transect.

Festuca rubra

	Acute 1		Acute 2	
Source	F	P	F	P
Populations	15.54	**	5.55	**
Fumigations	7.34	**	13.59	**
Pops x Fums	0.66	N.S.	1.53	N.S.

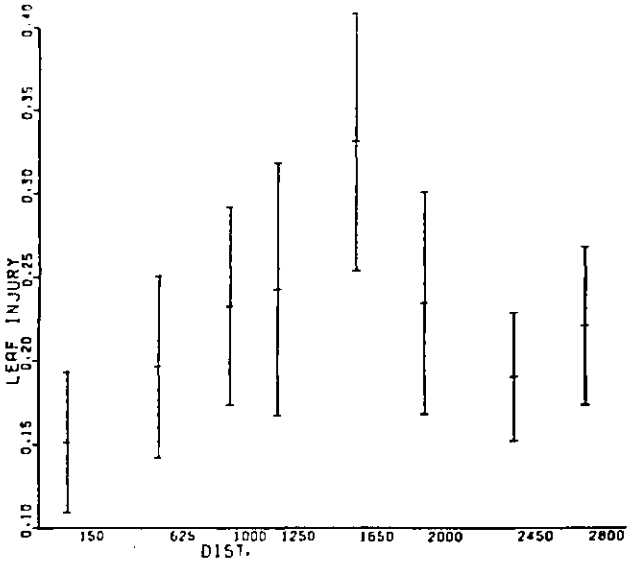
Dactylis glomerata

	Acute 1		Acute 2	
Source	F	P	F	P
Populations	3.29	**	1.14	N.S.
Fumigations	7.05	**	53.82	**
Pops x Fums	1.04	N.S.	1.32	N.S.

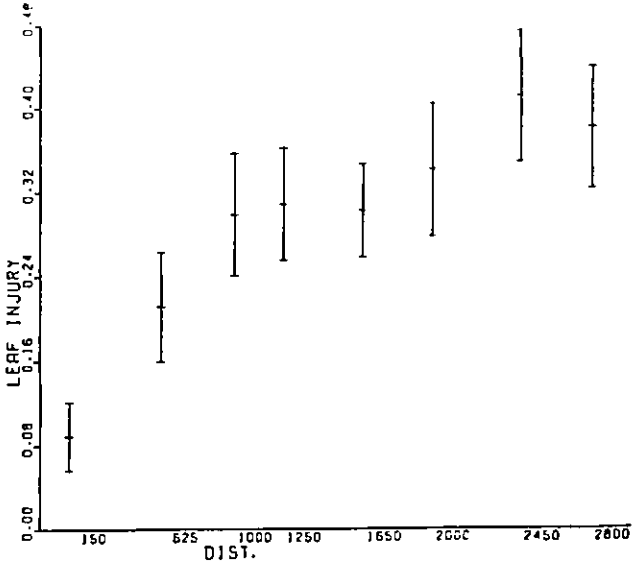
A second interesting feature of the Askern *F. rubra* cline is the plateau from site C to E, a distance of over 600 m. This may possibly relate to the point at which the plumes from the stacks first make contact with the ground. The effective height of the stack is 59 m including the height of the hill, but assuming no plume rise from the metal chimneys, which is probable in view of the emissions being cooled by the conductive properties of the chimney (pers. comm. Prof. R.S. Scorer). A rough estimate of the

Fig. 5.1 %Acute SO_2 Injury of Populations Along the Askern Transect (after angular transformation).

DACTYLIS GLOMERATA 1

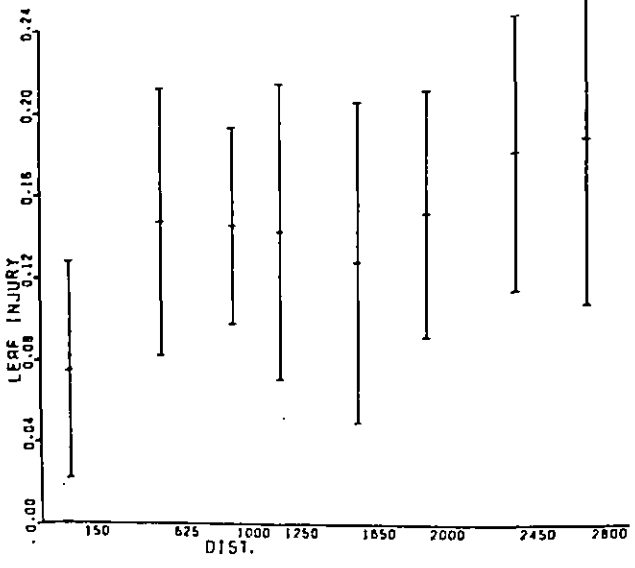


FESTUCA RUBRA 1

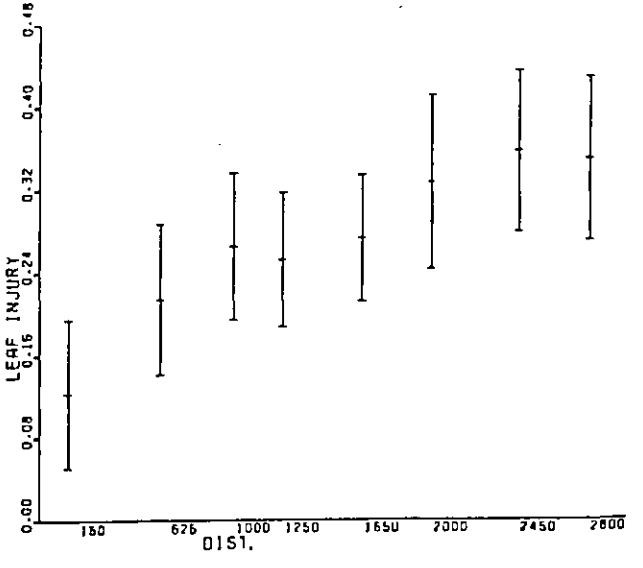


Sites	Distance
A	150m
B	625m
C	1000m
D	1250m
E	1650m
F	2000m
G	2450m
H	2800m

DACTYLIS GLOMERATA 2



FESTUCA RUBRA 2



distance at which the plume first reaches the ground is 12 or 15 times the effective stack height under reasonably stable conditions (pers. comm. Prof. R.S. Scorer). The point of plume descent was calculated as from 703 m to 879 m between sites B and C. This is at the beginning of the observed plateau. In view of the prevailing westerly wind this is likely to be the minimum distance for plume descent. It is therefore possible that the descent of the plume interrupts the progressive decreases in SO_2 concentrations originating from the ground level emissions, thus causing a decrease in gradient of the acute tolerance cline. The width of the plateau reflects the variability of the point of plume descent from the stacks, probably as a result of the variation of wind speed.

The two graphs plotted for *D. glomerata* (Fig. 5.1) show very little similarity, the general shape of the curve for screening 1 being almost the reverse of that for screening 2. The only population to show significant differences in acute SO_2 sensitivity was found in screening 1, where plants from site E showed a greater amount of injury than those from sites A and B. However, in the repeat screening site E plants were among the least injured specimens. One point of interest is that population A showed the lowest injury value in both screenings , thus reflecting the pattern found in *F. rubra*. The general configuration of populations of *D. glomerata*, does not demonstrate any clear pattern of differential tolerance to the relative pollution levels.

The population percentage frequencies of acute SO_2 injury are represented in Fig. 5.2 for both species and both screenings. Examination of the histograms for *F. rubra* reveals trends of decreasing

Fig. 5.2 Distribution of Acute SO_2 Injury in the Populations Along the Askern Transect.

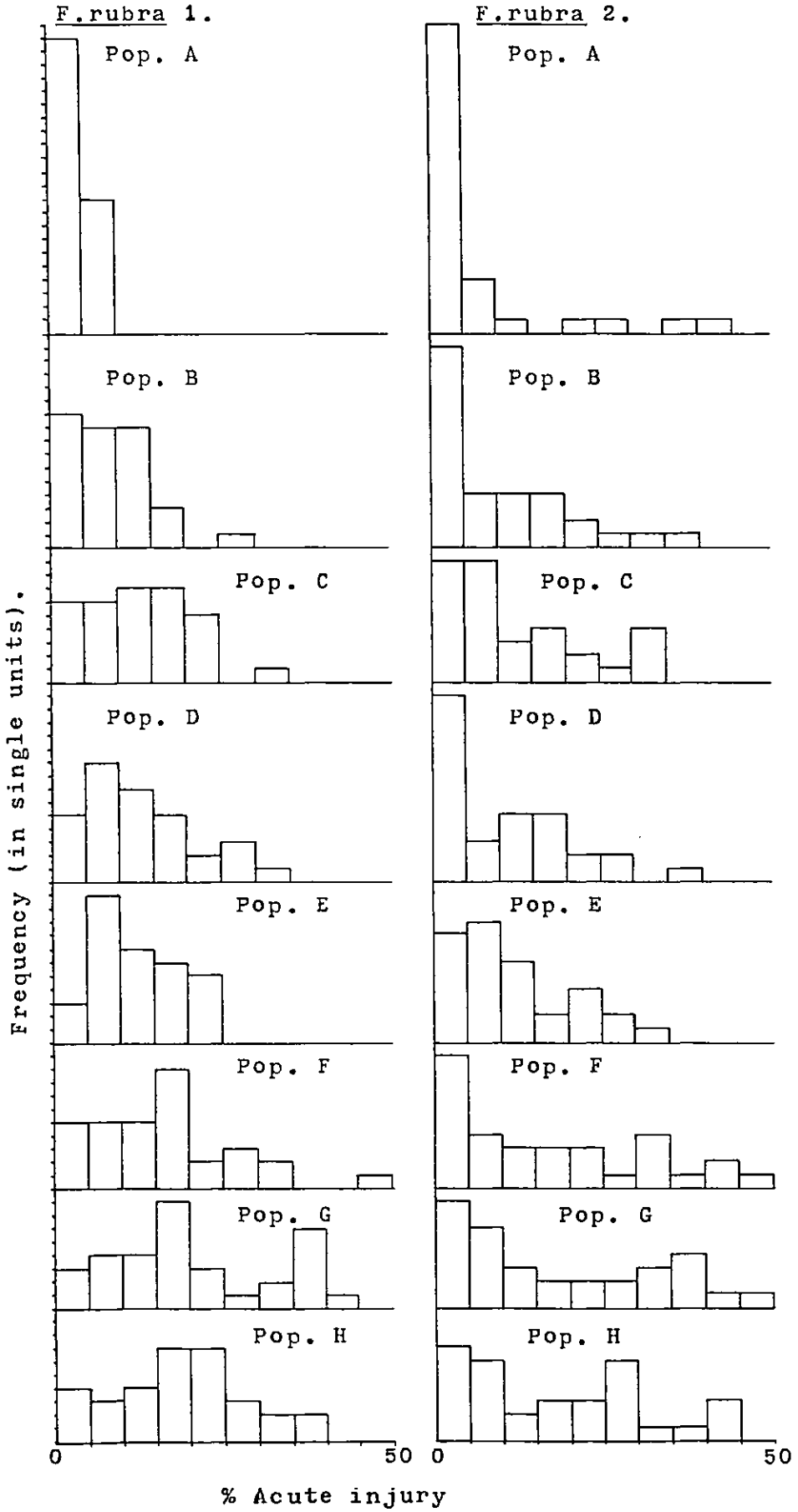
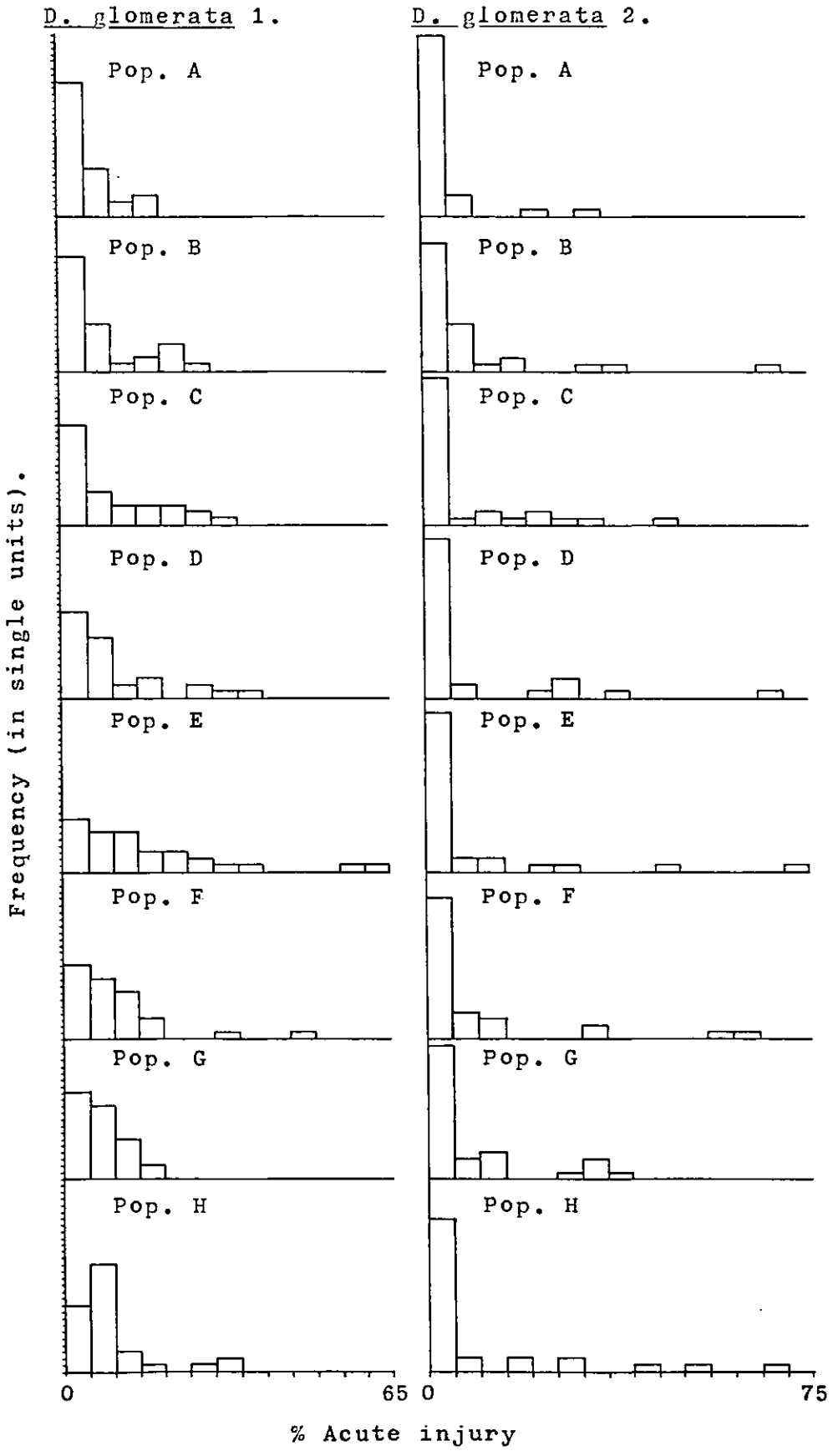


Fig. 5.2 Distribution of Acute SO_2 Injury in the Populations Along the Askern Transect.



frequency of uninjured plants and increasing range of tolerance within populations with increasing distance from Askern. Population histograms for *D. glomerata* also show a relationship of increasing mean % injury with decreasing number of uninjured plants (Fig. 5.2).

5.3 *Screening for Chronic SO_2 Tolerance in Festuca rubra Populations Along the Askern Transect*

The discovery of a cline of tolerance to acute SO_2 injury in *F. rubra* at Askern, prompted an investigation into the possibility of a similar cline of tolerance to chronic SO_2 injury existing for the same species.

5.3.1 *Method*

Two tillers from 30 clones of each of the 8 populations of *F. rubra* collected from Askern (ref. Section 2.3.2), were potted up. One plant of every clone was put in each of the two chambers and randomised in a mirror image pattern (ref. Section 7.4.1). The plants were then subjected to a chronic fumigation (ref. Section 2.6.1) with a mean of 299 $\mu gm^{-3} SO_2$ or clean air, for a period of 139 days (18/3/81 - 4/8/81).

5.3.2 *Results and discussion*

The main analysis (Table 5.3) shows significant decreases in the number of leaves (-8%), live shoot dry wt. (-12%), root dry wt. (-14%), total shoot dry wt, (-8%) and total plant dry wt. (-11%) in the SO_2 treatment chamber compared to the control. This was accompanied by an increase in senescence (+11%), but no change in the dead shoot dry wt. The treatment did not affect the number of

TABLE 5.3 Growth of Askern Populations after Fumigation with 299 μgm^{-3} SO_2 or Clean Air for 139 Days

CHARACTER	TREAT	Mean Growth per plant	% Change in Growth in SO_2 of Control	Significance SO_2 Treat x Pop
Number of leaves	C SO_2	114.3 105.3	-7.87 *	N.S.
No. Tillers	C SO_2	39.82 37.43	-6.00 +	N.S.
Live Shoot dry wt. (g)	C SO_2	1.248 1.111	-11.98 **	N.S.
Dead Shoot dry wt. (g)	C SO_2	1.074 1.019	-5.12 N.S.	N.S.
Root dry wt. (g)	C SO_2	2.539 2.177	-14.26 **	*
No. Leaves/tillers	C SO_2	2.867 2.820	-1.64 N.S.	N.S.
Mean leaf wt. (g)	C SO_2	0.0121 0.0118	-2.48 N.S.	N.S.
Mean tiller wt. (g)	C SO_2	0.0344 0.0328	-4.65 N.S.	N.S.
Scenescence	C SO_2	0.873 0.968	+10.88 **	N.S.
Root/Live Shoot	C SO_2	2.046 2.047	+0.05 N.S.	N.S.
Total Shoot (g)	C SO_2	2.323 2.130	-8.31 **	N.S.
Total Plant (g)	C SO_2	4.862 4.307	-11.42 **	N.S.

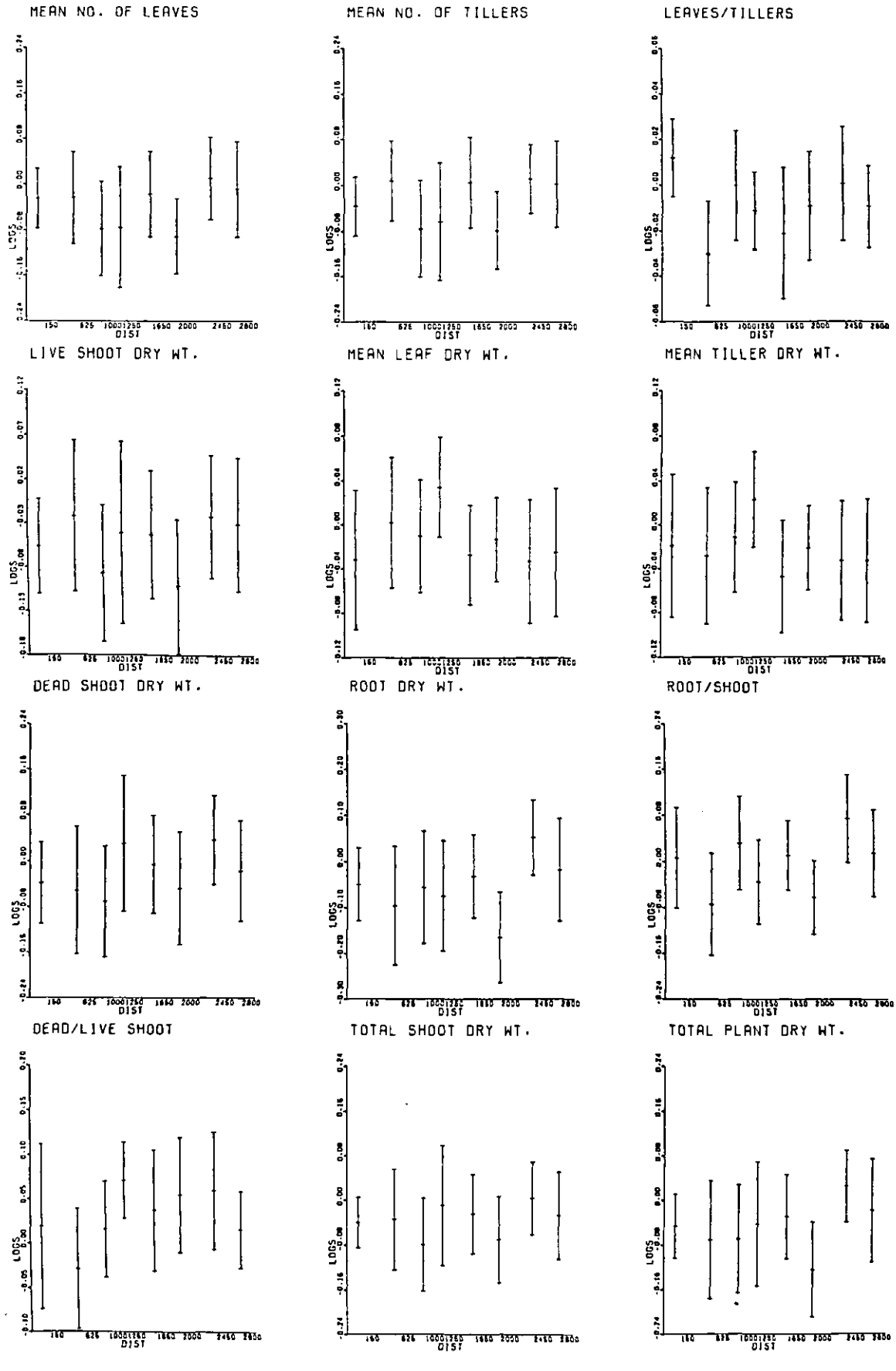
+ = $P < 0.1$ * = $P < 0.05$ ** = $P < 0.01$

leaves per tiller, mean leaf and tiller dry wts. or root/live shoot ratio. The decrease in live shoot dry wt (-11%) must be accounted for by either a decrease in plant size or number of units. There was negligible change in the number of leaves per tiller (-2%), suggesting the decrease in the number of leaves was attributable to the reduction in the number of tillers (-6%), although this was only significant at the 10% level. Since the reductions in live mean leaf dry wt. and number of leaves per tiller are very small the decrease in number of tillers must account for most of the loss of live shoot dry wt. Overall there was a reduction of plant growth related to reductions in tiller number and root dry wts. and increased senescence.

The primary aim of this experiment was to determine the range of tolerance between populations along the Askern transect, expressed in the interaction of SO_2 treatments with populations in the main analysis. No significant interactions were evident for any growth characters except the root dry wt. this also being the character which showed the largest percentage change in response to SO_2 (Table 5.3). Examination of the means (Fig. 5.3) shows the significant interaction to arise from a difference in susceptibility to chronic SO_2 injury between sites F and G only.

Some populations were affected significantly by the SO_2 treatment (Fig. 5.3). In population B there was a decrease in the number of leaves per tiller, but this was not reflected in other characters such as number of leaves or live shoot dry wt. Population C was inhibited in shoot growth with significant effects on live shoot growth, and leaf and tiller number. Population D displayed an increase

Fig. 5.3 Growth of Askern Transect Populations of Festuca rubra Subjected to 299μgm^{-3} SO_2 for 139days Compared to Growth in Clean Air.



Confidence Limits at 95%

in senescence, while F, the only population to show any differential effect compared to the other populations, was also the only one to show a reduction in total plant growth. The remaining populations A, E, G and H showed no significant changes in growth due to chronic SO_2 fumigation. These observations demonstrate an inconsistency in the effects of chronic SO_2 concentrations between populations.

5.4 *Comparison of Acute and Chronic SO_2 Tolerance with Vegetative Parameters in F. rubra along the Askern Transect.*

At least one clear cline (acute SO_2 tolerance in *F. rubra*) was demonstrated in the previous experiments. By using data collected from the plants grown in clean air, it was possible to compare the pattern of population growth characteristics along the transect with SO_2 tolerance. This had the limitations, that only vegetative parameters could be measured at the harvest and that the plants were grown in pots under chamber conditions and therefore their growth characteristics may not necessarily reflect those occurring in the field.

Fifteen parameters were investigated: 12 vegetative characters from the data of the control chamber plus the levels of acute injury from both screenings and the SO_2 induced reduction in root dry wt.

Three approaches were made:

1. Comparison of clinal patterns.
2. Correlation of characters between and within populations.
3. Comparison of population variability.

5.4.1 *Clinal patterns*

Fig. 5.4 shows the clinal patterns of the growth parameters in the control chamber, measured at the end of the experiment. While the equivalent clines for acute SO_2 injury and chronic SO_2 effects on roots, are depicted in Figs. 5.1 and 5.3 respectively.

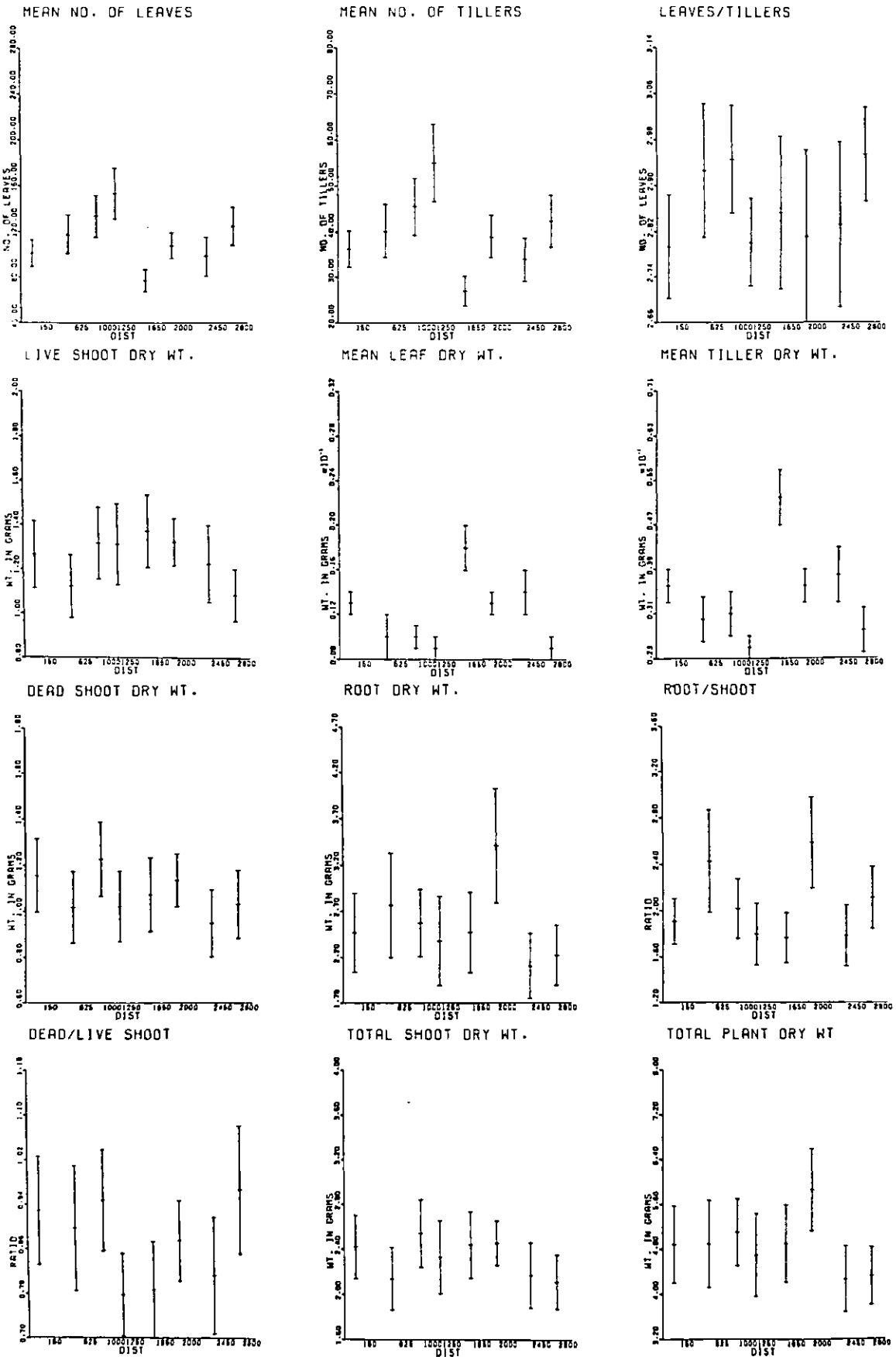
One clinal pattern of possible interest emerges from an examination of these data viz. in tiller number, which increases with distance from the Coalite factory, but with a substantial decrease in this parameter between sites D and E. This decrease is such that the mean tiller no. of site E is less than site A and site H is no greater than site C. This pattern is repeated in the mean live leaf and tiller dry wts, although it is not reflected in the live shoot dry wt.

No other consistent trends were evident along the transect, although there were several cases of significant differences between population means: live shoot dry wt., root dry wt., root/live shoot ratio, senescence and total plant dry wt.

The existence of different clinal patterns along the transect can have several explanations (Antonovics and Bradshaw, 1970):

1. The characters may have a different genetic basis (Haldane 1948). However, this would only account for changes in steepness and position of a cline.
2. Clinal patterns may match the changes in selective environmental factors along the transect.
3. The differing clinal patterns may be a result of differing selection pressures (with equal gene flow) along the transect.

Fig. 5.4 Growth of Askern Transect Populations in Filtered Air Chamber with 95% Confidence Limits.



5.4.2 *Character correlations*

Correlations were made between SO_2 injury parameters and all growth characteristics, between and within populations (Table 5.4). Within site correlations were calculated for each population and averaged over the 8 sites using a Z transformation.

Only 4 significant correlations were revealed, one of which, root dry wt/chronic root injury (N.B. growth in SO_2/growth in clean air ratio), was autocorrelated.

Dead shoot dry wt/chronic root injury exhibited a significant within sites correlation ($r = 0.426$), but not between sites. This suggests that these characters have a physiological or genetical relationship of decreasing dead shoot dry wt. with increasing chronic tolerance, which is broken down by selection between sites. There was no evidence of this relationship between senescence and chronic root injury and therefore it may be of little importance.

Total plant dry wt. was significantly correlated with chronic root injury ($r = -0.893$) between sites, suggesting the larger populations to be more sensitive to SO_2. This was not correlated within sites and thus any physiological/genetic linkage is unlikely. However, it is interesting that most of the correlations between chronic root injury and growth in clean air are negative.

It was surprising that acute 1 and acute 2 were only correlated between populations ($r = 0.975$) and not within ($r = 0.018$). This suggests that either acute SO_2 tolerance is not under genetic control in *F. rubra* or that variability is so great that it obscures any

TABLE 5.4 Within Site Correlations (lower fig.) and Between site Correlations (upper fig) for a range of Growth Parameters and SO_2 Injury

	ACUTE 1	ACUTE 2	CHRONIC (ROOTS)
ACUTE 2	0.975* -0.018		
CHRONIC (ROOTS)	0.381 -0.003	0.225 -0.055	
NO. LEAVES	0.110 0.023	-0.004 -0.002	0.221 -0.176
NO. TILLERS	0.070 0.034	-0.039 0.019	0.134 -0.180
LIVE SHOOT	-0.154 0.057	-0.077 -0.062	-0.492 -0.291
DEAD SHOOT	-0.453 0.148	-0.389 0.040	-0.525 -0.426*
ROOT	-0.189 0.069	-0.058 0.002	-0.870* 0.276
LEAVES/ TILLERS	0.226 -0.016	0.207 -0.083	0.460 0.085
MEAN LEAF WT.	-0.024 0.011	0.037 0.00	-0.203 -0.045
MEAN TILLERS WT.	-0.021 0.007	0.039 -0.036	-0.180 -0.062
$\frac{\text{DEAD}}{\text{LIVE}}$ SHOOT	-0.257 0.125	-0.163 0.093	-0.068 -0.323
$\frac{\text{ROOT}}{\text{LIVE}}$ SHOOT	-0.041 0.087	0.095 0.056	-0.564 -0.103
TOTAL SHOOT	-0.324 0.023	-0.300 -0.045	-0.556 -0.321
TOTAL PLANT	-0.263 0.016	-0.149 -0.033	-0.893* -0.308

* = $P < 0.05$

relationship for this character. It is possible that significant correlations may only be attained, with adequate replication within means.

No significant correlations were observed between acute and chronic injury, suggesting these to involve independent mechanisms; this is in agreement with the findings of Horsman et al (1979) and Ayazloo and Bell (1981).

5.4.3 *Population variability*

Within population variabilities were calculated for all characters, to determine the relationship with clinal pattern. They were plotted (Fig. 5.5) as percentages of the population with the maximum value for each character.

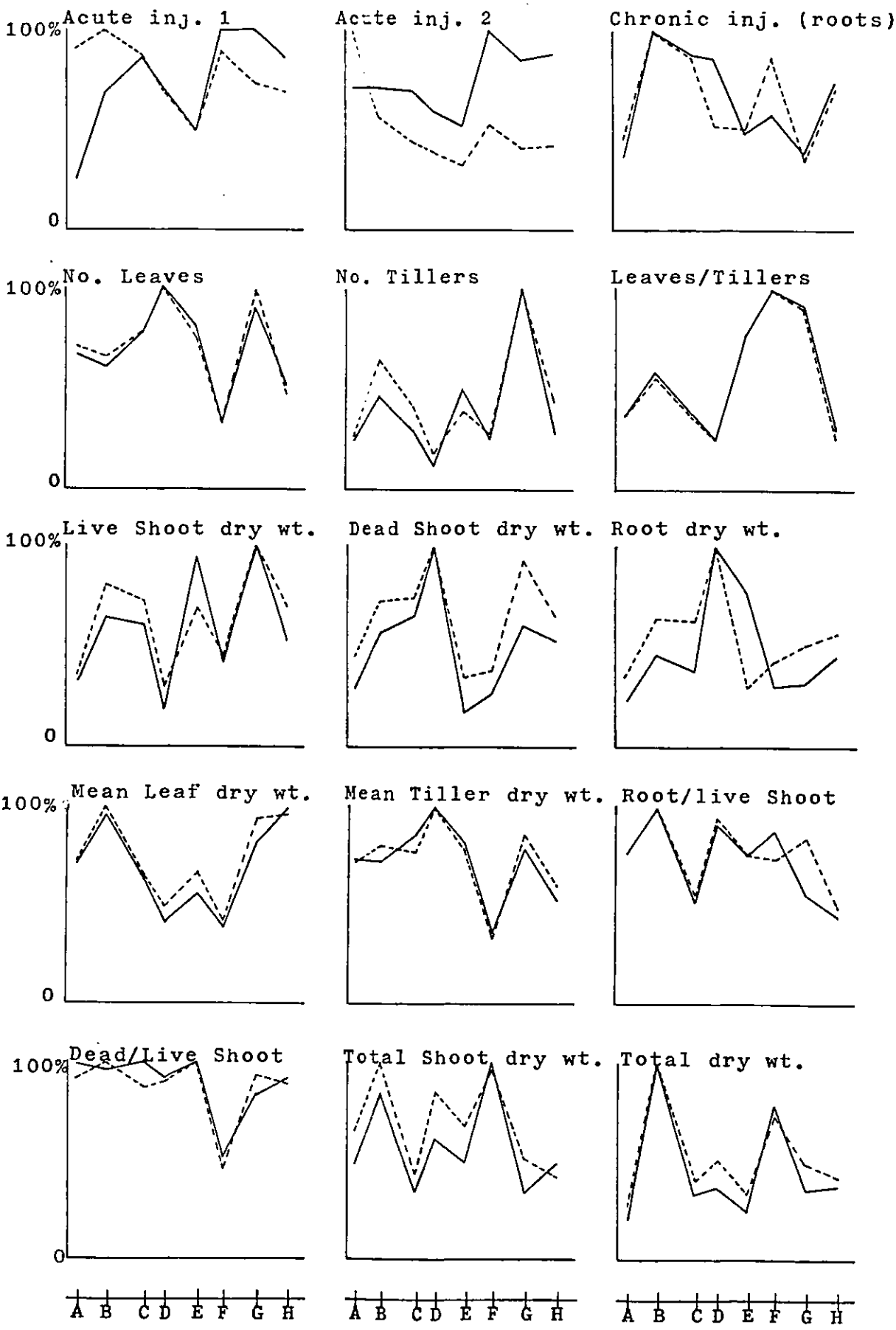
The only clear trend along the transect, was the decreasing coefficient of variance of acute 2 with increasing distance from Askern, which may also be reflected in the coefficients of variance for acute 1. This suggests the greatest variability to occur under the maximum selection pressure by SO_2, a situation that has previously been observed for copper tolerance in *Agrostis tenuis* (Wu et al, 1975).

In general the variability of most parameters showed a high degree of kurtosis, along the transect, with few if any detectable trends.

5.5 *General Discussion*

The Coalite factory at Askern releases considerable quantities of SO_2 into the surrounding atmosphere. This acts as a powerful

Fig. 5.5 Population Variation Over the Askern Transect Expressed as a % of the Site with the Maximum Value.



selective force, which has caused a cline of increasing acute tolerance in *F. rubra* populations, with proximity to the factory chimneys. A similar cline was not observed for *D. glomerata*, nor was there evidence of clines for chronic SO_2 tolerance in *F. rubra* despite differential tolerance between populations F and G.

The lack of differences between populations could be explained in several ways, assuming that the species had sufficient genetic variability, and were not innately tolerant, and SO_2 concentrations during fumigation were conducive to the detection of any differences (ref Chapter 10). Two possible reasons are:

1. The SO_2 concentrations along the transect, did not vary sufficiently to be differentially selective.
2. Clinal patterns may have been disrupted by the presence of other selective agents.

Comparison of SO_2 injury with growth parameters indicated the possible presence of other important selective factors.

Variability in *F. rubra* populations for acute tolerance may increase with proximity to the stacks. This is contrary to the findings of Horsman et al (1979), but Holgate (1964) has shown that population variability may increase, with increasing selection, where gene frequencies are equal.

CHAPTER 6

AN INVESTIGATION OF SOME OF THE GENETIC ASPECTS OF ACUTE SO_2 TOLERANCE

The evolution of a character within a population must ultimately depend on its heritability. In the present chapter some of the genetic qualities of acute SO_2 tolerance are examined in *Lolium perenne* in order to provide further evidence of the nature of selection by SO_2 and to elucidate the heritability of this character. Since time was limited it was decided to adopt a diallel crossing programme in which genetical analysis could be carried out after only one generation.

6.1 *Methods*

6.1.1. *Preparation of selected clones*

Six *L. perenne* clones, from Helmshore (ref Section 2), 3 acute tolerant and 3 acute non-tolerant, were selected on the basis of previous fumigations (Section 7,2). These six clones plus the S23 resistant clone were broken up for transplanting on the 26th of September 1979. Ten replicates of every clone, each of at least 10 tillers were planted in a brown earth soil, collected from Silwood Bottom at Imperial College Field Station, Silwood Park (Nat. Grid. reference SU 945685), in heavy gauge polythene bags, with punched holes for drainage. These were moved into the greenhouse, under fluorescent lighting, and maintained for a month to establish.

For maximum flowering, overwintering conditions of 16 weeks below $4^{\circ}C$ or a 10 hr day were needed. To facilitate this the plants were moved (17/11/79), after being cropped to within 1 cm of the soil, to a walled garden at Silwood Park. This is a wind protected site with no

artificial light sources.

During the summer of 1980 very few flowering heads were produced by the plants, so the crossing was postponed until the following year. From early spring of 1980 onwards the plants were supplied regularly with the fertiliser, "Solufeed".

Since experimental time was limited it was necessary to promote early flowering. The plants were moved from the walled garden to a controlled environment room (15/12/80). Here they were subjected to a regime of 4°C and 6 hr day for 30 days after which the plants were moved to a greenhouse with continuous fluorescent light at approximately 21°C. The first flowering heads appeared 27 days after being placed in the greenhouse.

6.1.2. *Crossing*

The following method, known as Auto-pollination (Jenkins, 1931), was adopted for all crosses. About 12 flowering heads (1 unit) of each of the two clones to be crossed were selected. Each culm of a unit was carefully wrapped, 10 cm from the flowering head, with a 5 cm band of cotton wool and bound together ensuring that none of the culms were crushed. This procedure was repeated for the unit of the second clone and then the shorter plant raised so that the flowering heads of each clone were at similar heights. The two units were then enclosed in a 6" x 22" permeable cellophane bag and tied at the point at which this covered the cotton wool binding. The bag covered the cotton wool by several cm to prevent wetting of the latter during watering, which might attract mildew infection. The units were also dusted with insecticide prior to bagging.

Having bagged the two units a cane was pushed into the soil of one of the clones, to which the bag was attached for support. The plant with the cane was labelled maternal, the other paternal, by convention.

Once the cross had been set up it was inspected each day and the bag shaken once or twice between 10.00 pm and 4.00 am, the hours of anthesis, to aid pollination. When the anthers were dead and flowering had finished, but before the seeds began to ripen, the paternal unit was removed from the bag and bagged separately, enabling the inspection of both maternal and paternal seed. When the culms had dried out they were cut off and the whole apparatus stacked until the seed ripened.

There were originally seven clones to be crossed, but only five produced enough flowering heads to facilitate the necessary diallel crosses. To test that there was no significant degree of selfing occurring, at least two units of each clone were bagged on their own so that the only seed produced would be by selfing.

The seed was separated from the dead flowering heads (23/6/81) and sown in seed trays. These trays were placed in a randomised order in the greenhouse under fluorescent lights to germinate

6.1.3. *Screening of parents and progeny for acute SO_2 tolerance*

When the progeny were two months old they were counted in order to design a screening experiment (Table 6.1a). The numbers of progeny differed widely between parents of the same crosses, indicating strong reciprocal effects; also the number of seedlings was generally low. The number of progeny produced from selfs was also extremely low, indicating

TABLE 6.1a Numbers of Progeny Produced from Diallel Cross

CROSS NO.	PARENTS			NO. OF PROGENY	
	PATERNAL		MATERNAL	PATERNAL	MATERNAL
1	47	x	61	84	76
2	47	x	27	93	36
3	89	x	27	5	58
4	89	x	S23 res	67	0
5	89	x	47	41	9
6	S23 res	x	27	12	34
7	S23 res	x	47	29	23
8	27	x	61	23	19
9	61	x	89	0	44
10	61	x	S23 res	16	30

TABLE 6.1b Numbers of Progeny Produced from Self Pollination

PARENT	NO. PROGENY
S23 res	0
27	1
47	2
61	0
89	1

no significant inbreeding to have occurred during crossing (Table 6.1b).

The lack of large numbers of progeny from both parents made it difficult to carry out a full diallel analysis. However, there were enough seedlings to carry out a half diallel analysis by pooling the maternal and paternal progeny, giving the possibility of 40 replicates. Where one parent had less than 20 seedlings the difference was made up from seedlings of the other parent of the cross.

From each parent and each family 40 tillers of a similar size were removed and potted up for acute fumigation (ref. Section 2.6.2). This gave 600 plants which were fumigated with acute concentrations of SO_2 over 8 fumigations, 5 plants from each family and parent per fumigation. Within each fumigation the 75 plants were divided into 5 plots, with one of each family in each. The plots referred to positions in the fumigation chamber and within each plot the plants were randomised.

After three weeks in the greenhouse the plants were fumigated with acute concentrations of SO_2 over 8 consecutive days and measured for % leaf injury 3 days after each fumigation.

6.2 *Biometrical Analysis of Diallel*

The limited number of progeny from the crosses restricted the analysis to a half diallel, pooling the reciprocal crosses and giving a replication for each family of 40. The half diallel analysis used was based on the additive dominance model which proposes that all variation from the mid parent (m) can be explained in terms of additive (d) and dominance (h) genetic components. If this model is true the

following assumptions must be correct (Kearsey, 1965):

a) Regular diploid behaviour at meiosis

b) No maternal effects

c) No linkage

d) No non-allelic interaction

e) No genotype-environment interactions

f) No multiple alleles

g) Uncorrelated gene distributions.

The present analysis is in three parts: the analysis of variance investigating the significance of differences of the genetical components of variation between families and deviations of progeny from the mid parent, the testing of the model using the relationships of variances and covariances and calculation of the main components. It must be noted that the adoption of the half diallel analysis, by pooling of families, leads to the loss of information of reciprocal effects. Therefore it was assumed that these were negligible as previously found for acute SO_2 tolerance in *Geranium carolinianum* (Taylor, 1978).

6.2.1. *Analysis of variance*

The acute injury data were subjected to a three way analysis with families, fumigations and plots as factors. There were no significant differences between plots or for interactions involving plots, so this factor was removed from the analysis. The subsequent (Table 6.2) anovar table showed highly significant differences between families and between fumigations. The former indicates differences in acute SO_2 tolerance between the families, thus justifying further investigation, involving the genetic components. The latter reveals major differences in acute injury between fumigations. The interaction of Families and Fumigations

is also significant, possibly suggesting genotype-environment interaction. This interaction did not disappear when the data were scaled with square root and antilog transformations.

As previously stated the Families factor contains all the genetic components of variation. This was partitioned, using the method of Hayman (1954), adapted for the half diallel (Morley Jones, 1965), into 'a' the variation between mean effects of each parental line i.e.: additive effects, and 'b' the non-additive genetic component or dominance effect. 'b' was further subdivided into 'b_1', testing the overall deviation of the progeny from the mid parent and therefore unidirectional effects, 'b_2' for the interaction of non-additivity with arrays which relates to gene asymmetry and 'b_3' the remaining non-additive variation testing other causes of dominance.

All the genetic items were significant when tested with the Fumigations x Families interaction (Table 6.2). Thus it was still possible to continue using the additive dominance model in spite of some genotype-environment effects (Table 6.2). The genetic items were then tested using the relevant Fumigations x item mean square. The significance of 'a' and 'b' indicated additive genetical variance among the parents with dominance, at some loci, respectively. 'b_1' and 'b_3' were both significant, the large b_1 indicating strong directional dominance, but there were also other dominance effects (b_3) not attributable to directional dominance (b_1) or asymmetrical distribution of genes (b_2). The latter was not significant and suggested that the parents had similar numbers of dominant alleles. The mean acute injury of parental values (20%) was smaller than the progeny (34%); since b, was highly significant this indicates that an average susceptibility

TABLE 6.2 Hayman Analysis Adapted for the Half Diallel of Acute SO_2 Injury (after Angular Transformation)

Source	df	M.S.	Factor F = Error	Item F = Fum x Families	Item F = Fum x Item
a	4	0.7208		7.9296**	7.4435*
b	10	0.6748		7.4235**	7.6278**
b_1	1	3.9298		43.2321**	48.6018**
b_2	4	0.2556		2.8119*	2.5446N.S.
b_3	5	0.3592		3.9516**	4.4675**
Families	14	0.6879	13.5080**		
Fumigations	7	1.7760	34.8745**		
Fum x a	28	0.0968			
Fum x b	70	0.0885			
Fum x b_1	7	0.0809			
Fum x b_2	28	0.1004			
Fum x b_3	35	0.0804			
Fum x Families	98	0.0909	1.7850**		
Error	480	0.0509			
Total	599	0.0925			

* = $P < 0.05$ ** = $P < 0.01$

to SO_2 injury was dominant and conversely tolerance was recessive. A large value for b_1 can suggest overdominance or heterosis but this should be treated with caution, because it may include a confounded environmental component.

6.2.2. *The relationship of variance and covariance of arrays*

Having established the significance of the genetic components of SO_2 tolerance, it was necessary to test the model further before the calculation of the main components. The relationship of variance (Vr) and covariance (Wr) of members of the same array provide two complementary tests of adequacy: an analysis of variance of Wr + Vr and Wr - Vr and regression of Wr on Vr.

Vr and Wr values for each of the 40 replicate arrays were calculated and then Wr + Vr and Wr - Vr subjected to analyses of variance. It was found that Wr + Vr changed from array to array (Table 6.3a) suggesting the presence of dominance and possibly some forms of non-allelic interaction. However, for Wr - Vr (Table 6.3b) there was no significant difference between arrays, which indicated the absence of non-allelic interaction. Therefore only dominance was present and there was no evidence of the genes being associated in a non-random way between parents, or of any inadequacy of the additive-dominance model.

All the data were pooled to form one half diallel table and then the mean, Vr and Wr values of each array calculated (Table 6.4). A regression (Table 6.5) of Wr and Vr was highly significant and gave a slope of 1.188 which was not significant from 1 ($t = 1.73$) but highly significant from 0 ($t = 10.88$). This suggested that all non-

TABLE 6.3a Analysis of Variance of Wr + Vr

Source	df	S.S.	M.S.	V.R.
Fumigations	7	0.07148	0.01021	2.45 *
Blocks	4	0.01078	0.00270	0.65 N.S.
Arrays	4	0.11950	0.02989	7.18 **
Fum x Blocks	28	0.14630	0.00523	1.25 N.S.
Fum x Arrays	28	0.11750	0.00420	1.01 N.S.
Blocks x Arrays	16	0.05214	0.00326	0.78 N.S.
Fum x Arrays X Blocks	112	0.46650	0.00417	
Total	199	0.98430	0.00495	

TABLE 6.3b Analysis of Variance of Wr - Vr

Source	df	S.S.	M.S.	V.R.
Fumigations	7	0.04880	0.00697	3.78 **
Blocks	4	0.01455	0.00364	1.97 N.S.
Arrays	4	0.01003	0.00251	1.36 N.S.
Fum x Blocks	28	0.11690	0.00417	2.27 **
Fum x Arrays	28	0.08527	0.00305	1.65 *
Blocks x Arrays	16	0.03130	0.00196	1.06 N.S.
Fum x Arrays x Blocks	112	0.20640	0.00184	
Total	199	0.51320	0.00258	

** = $P < 0.01$ * = $P < 0.05$

TABLE 6.4 Half-Diallel Table Pooled over Plots and Fumigations

PARENTS	$S23_{RES}$	27	47	61	89	MEANS	Vr	Wr
$S23_{res}$	0.418	0.544	0.478	0.578	0.558	0.515	0.00436	-0.00553
27		0.413	0.625	0.653	0.539	0.555	0.00875	+0.00076
47			0.636	0.627	0.743	0.622	0.00890	-0.00261
61				0.269	0.471	0.520	0.02450	+0.01630
89					0.313	0.525	0.02417	+0.01944
MEANS						0.547	0.01414	+0.00567

TABLE 6.5 Analysis of Variance of Wr/Vr Regression

Regression Equation = Wr = -0.0111 + 1.188 Vr

Source	df	S.S.	M.S.	VR
Regression	1	0.00050784	0.00050784	118.378 **
Error	3	0.00001288	0.00000429	
Total	4	0.00052072		

** = $P < 0.01$

additive variation could be accounted for by dominance and provides further proof of the adequacy of the model.

The Wr/Vr graph also provided a measure of the average dominance, expressed as the departure of the regression line along the Wr axis. This was - 0.0111 and equals $\frac{1}{4}$ (D - H_1), which since it was negative suggests that $H_1 > D$. However, the value contains an environmental component which must be removed to obtain an estimate of dominance.

The dispersion of points along the Wr/Vr regression line revealed clear differentiation of dominant and recessive groups in the parental lines which may suggest that only a few genes are involved. The lowest values on the regression line have the most dominant genes, whereas the highest points have the least and are therefore the most recessive. In the present case (Fig. 6.1a) clone S23 res had the most dominant genes with clones 47, 27, 61, 89 following in order of decreasing dominance. The clones 61 and 89 were close to the limiting parabola $Wr = \sqrt{Vp.Vr}$ suggesting them to be completely recessive. The relative number of dominant alleles (Wr + Vr) were plotted against the parental means of acute injury (Fig. 6.1b) to determine the relationship of dominance with acute SO_2 injury: i.e. the direction of dominance. A correlation coefficient of r = -0.721, significant only at the 10% level suggested a tentative possibility of a trend of increasing dominance for susceptible plants and increasing recessive genes for acute SO_2 tolerant plants. It is interesting to note that clone S23 res has the greatest dominance but only medium acute injury; this may be a reflection of its different genetic background, being from a different population than the other parents.

Fig. 6.1a Wr/Vr Graph for Acute SO_2 Injury. (after angular transformation)

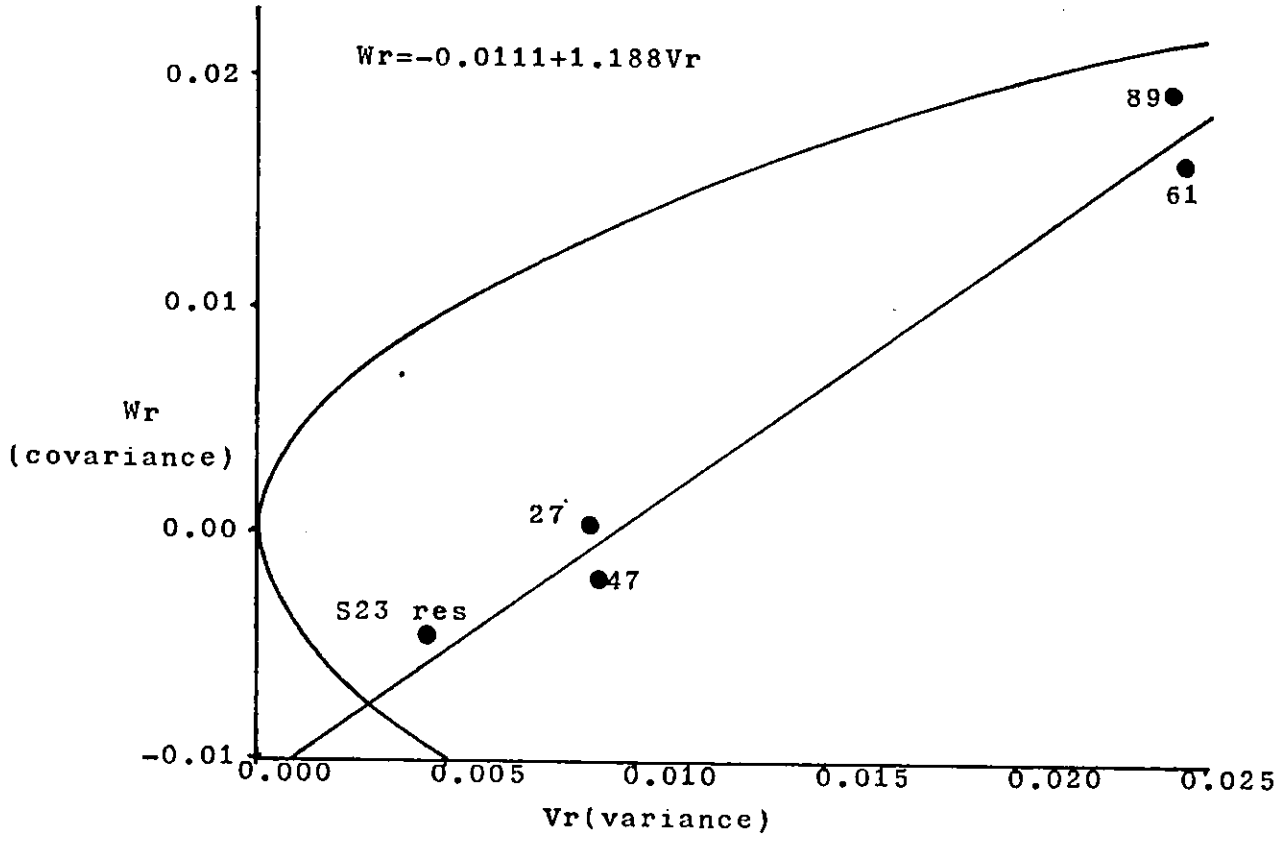
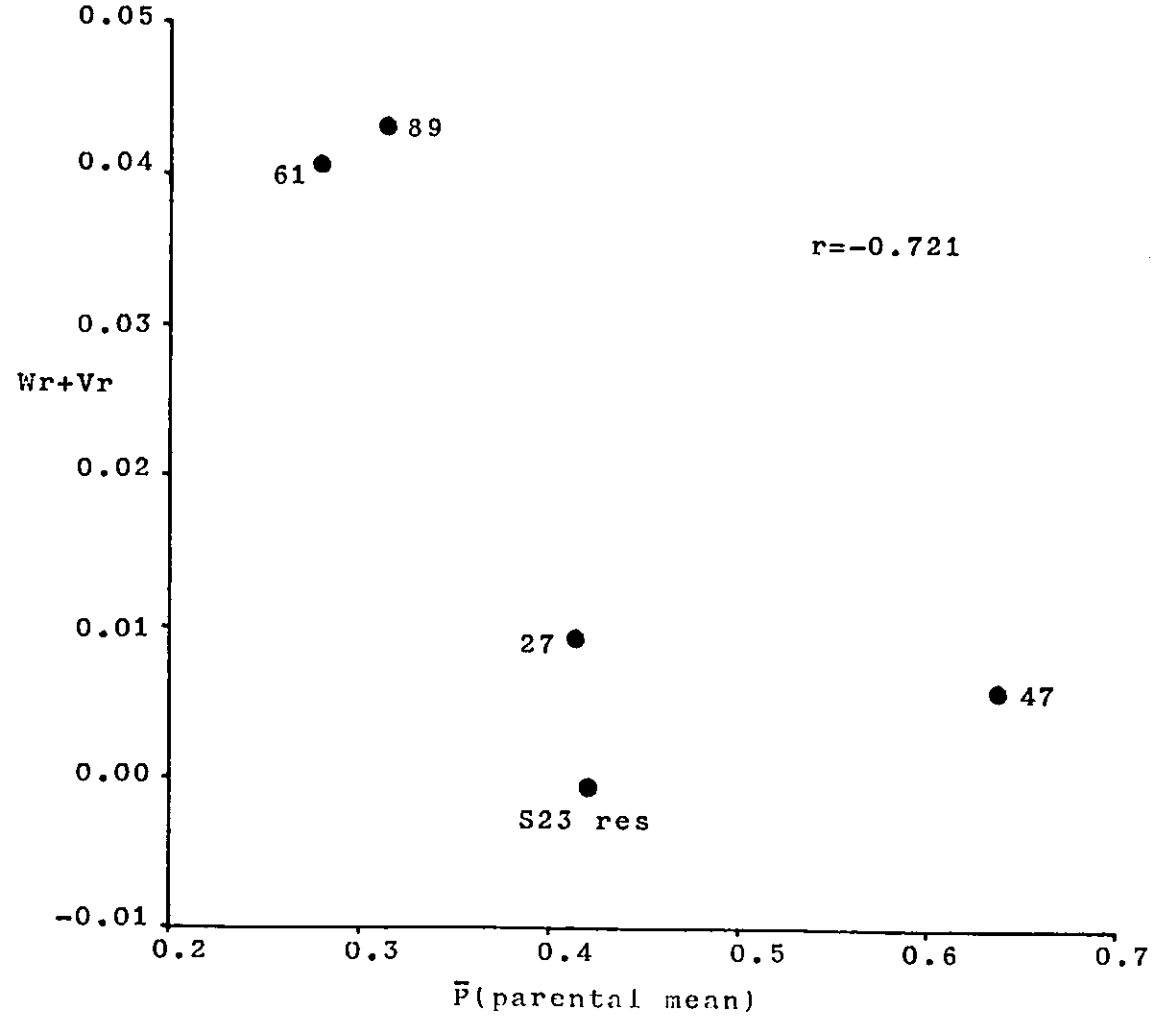


Fig. 6.1b Relationship of Wr+Vr and Mean Acute SO_2 Injury. (after angular transformation)



6.2.3. *Estimation of main components of variation*

The application of various tests indicated the adequacy of the additive-dominance model for describing the acute injury data of the half diallel cross. Accepting this model, it was possible to calculate the main components for each fumigation, from the mean variance of arrays ($\bar{V}r$), the mean covariance of arrays ($\bar{W}r$), the variance of parental means (Vp) and the variance of array means ($V\bar{r}$). All these statistics contained an environmental component (E), taken as the interaction mean square in the analysis of variance. These statistics were used (Table 6.6) to calculate the mean values of four genetic components of variation, additive (D), dominance (H_1 and H_2) and F_1 the sum of additive, dominance products which relate to the consistency of the direction of dominance (Mather and Jinks, 1971).

The relative size of the mean estimates of the genetic components D (0.03 $\pm$ 0.01) and H_1 (0.11 $\pm$ 0.03) (Table 6.6), suggest overdominance controls the response of the plants to SO_2. This is born out by a dominance ratio of greater than 1 ($\sqrt{H_1/D}$ = 2.06 $\pm$ 0.49), and agreed with the Wr/Vr regression, in which the Wr intercept was negative.

The ratio $\frac{1}{4} H_2/H_1$ was almost equal to 0.25 (0.21 $\pm$ 0.02) indicating the allele frequencies for acute SO_2 susceptibility and tolerance are almost equal, as previously predicted by the non-significant b_2 item in the "Hayman Analysis" (Table 6.2).

The positive value of F indicated the presence of more dominant alleles than recessive in the parents, irrespective of whether for tolerance or susceptibility. The ratio $\frac{1}{2}F/\sqrt{D(H_1-H_2)}$ (0.61 $\pm$ 0.47) was highly variable but did suggest that dominance was not completely consistent over all loci.

TABLE 6.6 Estimates of Components of Variation

Components		Estimates with 95% Confidence
D		0.03033 ± 0.01338
H_1		0.10663 ± 0.03106
H_2		0.08888 ± 0.02137
F		0.03157 ± 0.02702
$\sqrt{(H_1/D)}$		2.06447 ± 0.49254
$\frac{1}{4}H_2/H_1$		0.21273 ± 0.02329
$\frac{1}{2}F/\sqrt{D(H_1-H_2)}$		0.61190 ± 0.46917
Heritability	Narrow	22.38% ± 8.99%
	Broad	85.04% ± 4.97%

The narrow sense heritability (h_n^2 = 22.38% ± 8.99%) is several times smaller than the broad sense heritability (85.04% ± 4.97%) reflecting the large dominance component in the genetic control of acute SO_2 susceptibility. Although this may also be partially due to the effect of the equal gene frequencies on these values (Mather and Jinks, 1971).

All mean estimates had large confidence limits reflecting the large environmental component resulting from differences between fumigations.

6.3 *Discussion*

The present chapter describes the analysis of a 5 x 5 diallel cross, to investigate some aspects of the genetic control of acute SO_2 tolerance in *L. perenne*. To obtain accurate estimates of genetic parameters, at least 8 parental lines are necessary (Pederson, 1971). Therefore the present results should be interpreted with a certain degree of caution.

It was found that the response of *L. perenne* to acute SO_2 concentrations is heritable, with a significant additive component, but primarily controlled by dominance. Overdominance was evident, but not at all loci and dominance tended to increase with SO_2 susceptibility. There were possibly only a few genes involved, but it should be noted that low narrow sense heritability and the continuous distribution of this character in wild populations (Ayazloo and Bell, 1981) are more compatible with quantitative inheritance.

Two previous investigations of the genetics of acute SO_2 tolerance

have been carried out by Taylor (1978) and Omura et al (1980). Taylor (1978) showed that SO_2 tolerance in *Geranium carolinianum* plants, from natural populations, was accountable by additive gene effects with neither sensitivity nor resistance exhibiting dominance. It was also argued that tolerance is polygenically controlled. Omura et al (1980) examined selected rice strains and found that SO_2 sensitivity was controlled by a pair of genes, with no evidence of dominance. Thus it appears that the genetic control of $SO_{2_{\wedge}}^{\text{tolerance}}$ varies considerably between species.

The narrow sense heritability was low (22% $\pm$ 8.99) compared to Taylor's (1978) estimates of 50% $\pm$ 14, probably as a result of the large dominance component in the present work. However, this parameter is dependent on environmental conditions of the experiment and may possibly have a higher value in the field.

Directional effects of dominance have not previously been observed for acute SO_2 tolerance (Taylor, 1978; Omura et al 1980), but have for O_3 inheritance (Povilaitis, 1967; Cameron and Taylor, 1973). Both these studies have shown a recessive tendency for O_3 tolerance in tobacco varieties and sweet corn hybrids respectively, as found for SO_2 tolerance in *L. perenne* in the present work. This substantiates the evidence that the character is controlled by only a few genes since the likelihood of recessives surviving against a strong selective force is greater, fewer loci involved.

CHAPTER 7

SELECTION OF TOLERANT AND NON TOLERANT GENOTYPES

7.1 *Introduction*

The duration and size of chronic SO_2 fumigation experiments make it difficult to carry out more than a limited number of these. It is evident that a quicker, more reliable method of screening is necessary. Until recently, it was believed that acute SO_2 injury would reflect the response to chronic SO_2 fumigation, but Ayazloo and Bell (1981) and Horsman, Roberts and Bradshaw (1979) have shown that the two characters are not correlated. This suggests that the mechanisms for acute and chronic SO_2 injury are separate and that one cannot be used to predict the other. Thus it is necessary to seek a physiological, biochemical or even morphological factor, which is easily measured and correlates closely with growth response to chronic SO_2 fumigation.

Before any quick methods of screening can be investigated it is necessary to select some phenotypes clearly demonstrated to be tolerant and non-tolerant, both to acute and chronic SO_2 injury. The present chapter describes experiments in which clones of *Lolium perenne* were fumigated with acute and chronic levels of SO_2.

7.2 *Screening for Chronic SO_2 Tolerence of 19 Clones of Lolium perenne from Helmshore.*

7.2.1 *Method*

Nineteen clones of *L. perenne* were chosen from the Helmshore population sample collected by M. Ayazloo and J.N.B. Bell in 1976 (Ayazloo and Bell, 1981). They were selected on the basis of 4 phenotypes, using

data from previous fumigations by Ayazloo (1979): 1. tolerance to acute injury, but not to chronic, 2. tolerance to chronic injury, but not to acute, 3. tolerance to both acute and chronic injury, 4. high susceptibility to acute and chronic injury. The data used as a guide to choose individuals, was based on a single replicate and therefore there was no absolute proof of the relative sensitivity of the clones. There were five clones chosen for each phenotype, except "acute tolerant" for which there were 4 (Table 7.1).

Ten uniform sized tillers were selected from each clone, 5 replicates for each gas treatment, and potted up. The plants were transferred to the fumigation chambers and arranged in a random order in each. They were fumigated with a mean of 354 $\mu gm^{-3} SO_2$ for 140 days (11/3/79 - 30/7/79).

In the first month of the experiment the plants were accidentally exposed to over 600 μgm^{-3}, causing a small amount of leaf necrosis (< 5%), apparent on the 5th of April. This was taken into account in the discussion of results.

When the plants were harvested, only live shoot, dead shoot and root dry weights were measured.

7.2.2 *Results and discussion*

SO_2 treatment caused an overall increase in senescence of 35%, but without a significant reduction in the live shoot dry wt. The root dry wt. was significantly greater in SO_2, which also resulted in an increased root/live shoot ratio (Table 7.2).

There were no gas treatment/clone interactions, indicating that

TABLE 7.1 Classification of Clones of *L. perenne* Chosen for Screening for Chronic SO_2 Tolerance

Phenotype	Clone No.
Non Tolerant	51, 10, 71, 19, 42
Chronic & Acute Tolerant	35, 75, 37, 27, 17
Chronic Tolerant Only	61, 89, 41, 47, 87
Acute Tolerant Only	66, 86, 58, 50

TABLE 7.2 Analysis of Growth of Selected Clones of *L. perenne* in a Mean of 354 $\mu gm^{-3} SO_2$ for 140 Days Compared with Filtered Air.

Growth Parameter	Treat-ment	Mean Growth per Plant (g)	% Change of Growth in SO_2 cf Clean Air	Interaction Clones x Gas Treatment
Live Shoot dry Wt.	c SO_2	3.17 2.91	-8 N.S.	N.S.
Dead Shoot Dry Wt.	c SO_2	1.20 1.47	+23 ***	N.S.
Root dry Wt.	c SO_2	2.33 2.64	+13 *	N.S.
Root/Live Shoot	c SO_2	0.784 0.964	+23 ***	N.S.
Dead Shoot/Live Shoot	c SO_2	0.415 0.560	+35 ***	N.S.
Total Shoot dry wt.	c SO_2	4.36 4.38	0 N.S.	N.S.
Total Plant Dry wt.	c SO_2	6.69 7.02	-5 N.S.	N.S.

* = $P < 0.05$ *** = $P < 0.001$

the experiment did not distinguish between tolerant and non-tolerant plants.

Increases in senescence due to chronic fumigation with SO_2 have been observed in previous investigations (Bell and Clough, 1973; Ashenden and Mansfield, 1977; Bell et al, 1979; Ayazloo and Bell, 1981), but there have been no reports of a significant increase in the root dry wt., although some workers have shown significant increases in root/live shoot ratio (Bell et al, 1979; Ayazloo and Bell, 1981).

The occurrence of increased senescence and root dry wt. together is surprising, since the former suggests the chronic fumigation to have been deleterious to plant growth, while the latter suggests it was beneficial. One possible explanation of the increased senescence, is the accidental acute incident during the early stages of the experiment, the resulting necroses would have been included in the dead shoot dry wt., although it seems unlikely that the effects of this would still be measurable after 3 months.

The increase in root dry wt. might have occurred, if after several months growth, during which no fertiliser was added, the plants became nutrient deficient. Under such conditions, the low levels of SO_2 in the fumigation chamber may have been metabolized by the plants (Cowling and Jones, 1970), and the plants in the control chamber would have tended to be smaller, as was observed for the roots.

A further explanation is drought, which is well known to cause an increase in both senescence and root dry wts. During the summer, when this fumigation was carried out, soil moisture in the chambers evaporates very rapidly, and may expose the plants to short periods of

drought. It is not unreasonable to suggest, that the effects of this may be enhanced by SO_2, especially when considering that SO_2 tends to exaggerate the effects of other adverse environmental conditions on plants (Baker, Unsworth and Greenwood, 1982; Davison and Bailey, 1982).

The main aim of the experiment was to determine clones tolerant and non tolerant, to chronic SO_2 fumigation. However, it seems the design of the experiment was unable to expose any differences. This may have been due to the small effect of the treatment, probably because the SO_2 concentrations were too low and/or the environmental conditions were not conducive to SO_2 injury: e.g. under a summer light regime of high light intensity and long days, the effects of SO_2 are known to be decreased in comparison to a winter light regime of low light intensity and short days (Davies, 1980).

It is also likely that the inability to distinguish the tolerant clones lies with the selection of the material. It is evident from the variation expressed by the replicates, for the growth response to SO_2, that assignation of a phenotype to an individual plant, based on a single observation of a previous experiment, may be of doubtful value. It also follows that the number of replicates used in the present experiment was not enough to allow statistical differentiation of phenotypes. Thirdly, the range of possible tolerance between clones, was probably restricted due to the selection of all clones from one population. Since there are no available replicated harvest data, from the original experiments, for individual plants, it is not possible to correct the first problem, but any further investigation along these lines should employ greater replication and use several populations.

7.3 *Screening for Acute SO_2 Tolerance of 19 Clones of Lolium perenne from Helmshore.*

7.3.1 *Method*

Replicates from the 19 clones selected in Section 7.2.1 were screened for acute SO_2 tolerance. Six fresh uniform tillers of each plant were potted up three weeks before fumigation. The 114 plants were screened in two fumigations, three replicates of each clone in each, using the method described in Section 2.6.2. The first fumigation was at 4870 μgm^{-3} SO_2, and the second at 5019 $\mu gm^{-3} SO_2$.

7.3.2 *Results and Discussion*

Analysis of the % leaf injury (Table 7.3), showed highly significant differences between clones, but no interaction of clones and fumigations, or differences between fumigations. This indicates differential acute SO_2 tolerance between clones and that both fumigations show the same results.

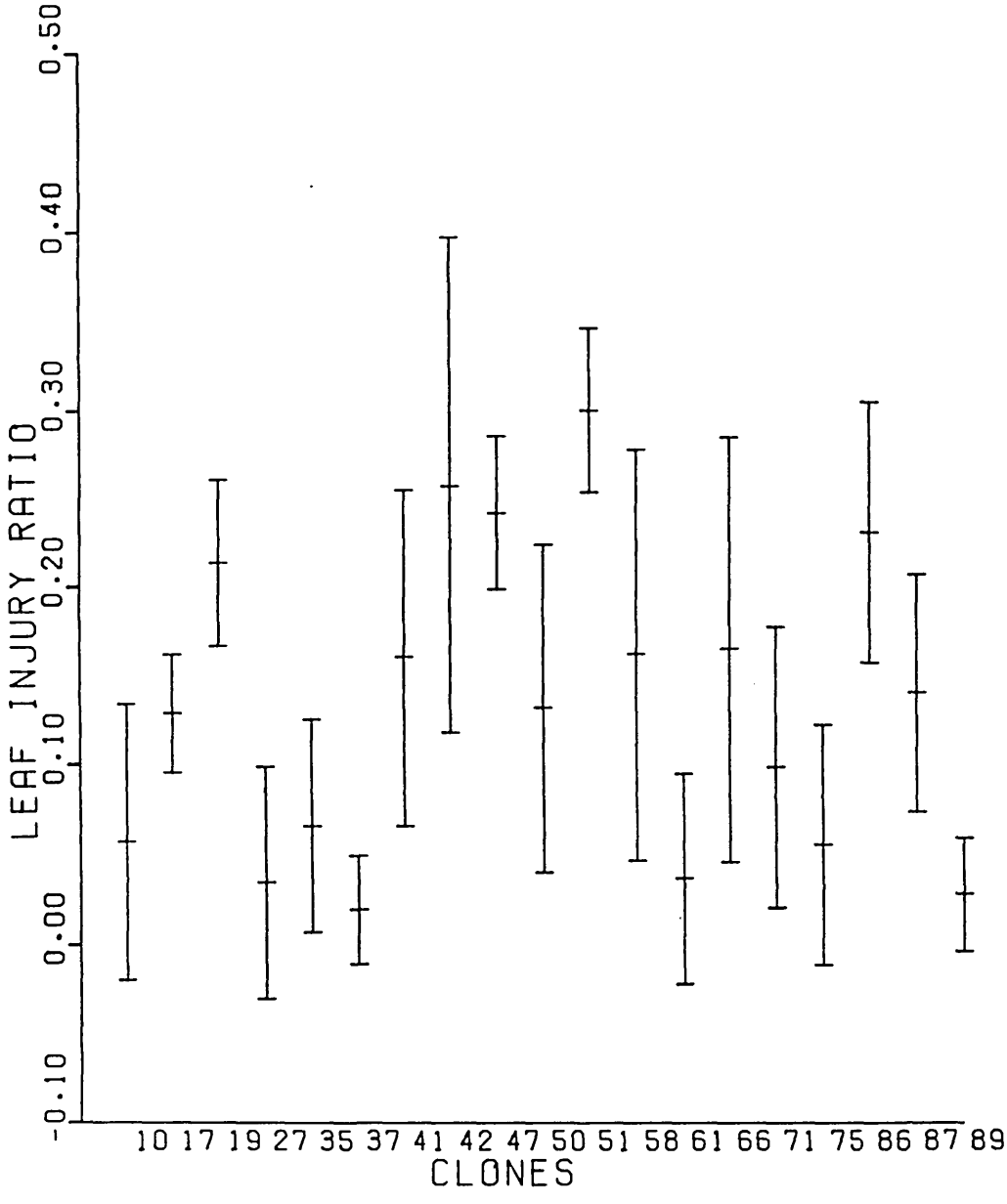
TABLE 7.3 Analysis of Acute fumigation of 19 Clones (after Angular Transformation)

Source	df	VR	P
Clones	18	8.94	***
Fumigations	1	2.89	N.S.
Clones x Fumigations	18	1.76	N.S.

*** = $P < 0.001$

The range of tolerance of the clones is depicted in Fig. 7.1 and on the basis of these results, suitable clones were selected for further investigation. Clones 89, 58, 37 and 27 were considered tolerant whereas clones 42, 47, 51 and 86 were considered non-tolerant.

Fig. 7.1 Acute Injury of 19 Selected Clones of Lolium perenne (after angular transformation).



Confidence limits at 95%

The clones were originally chosen, from data of an acute fumigation by Ayazloo (1979), by taking the most and least injured individuals. Single degree of freedom comparisons were made between the chosen acute tolerant and susceptible phenotypes. Also the ranking of tolerance of the results of Ayazloo (1979), was correlated with the ranking of tolerance based on the replicated fumigation. Neither analysis showed a significant result ($F = 0.294$, $r_s = 0.17$), indicating no relationship between the selection classifications and the results of the fumigations. This emphasises the large variability of the character of acute SO_2 tolerance, and therefore the unreliability of predicting tolerance without adequate replication.

7.4 *Screening for Chronic SO_2 Tolerance of Selected Clones of Lolium perenne from several populations*

In the discussion in Section 7.2.2 it was suggested that the range of chronic tolerance within one population, may not be sufficient to detect differential tolerance between genotypes, without very high replication. In the present investigation, clones were chosen from several populations and the replication doubled in an attempt to overcome this problem.

7.4.1 *Method*

The following 19 clones of *Lolium perenne* were selected, from stock cultures, for a range of chronic SO_2 sensitivities: 5 apparently tolerant clones from Askern (Ayazloo, 1979), selected by examination of results of previous fumigations; 5 apparently tolerant clones from Helmshore (Ayazloo, 1979) selected on the basis of the results from Section 7.2; 8 clones of c.v. S23 selected randomly from seedlings,

and grown up in the greenhouse in pots for several months before fumigation: these were assumed to be non-tolerant; 1 clone of S23 resistant, a tolerant clone selected from a non-tolerant S23 population, after chronic fumigation (pers. comm. Dr. J.N.B. Bell).

Twenty uniform sized tillers were removed from each individual plant, 10 for each treatment. The potted tillers for a single treatment, were arranged in a randomised order in one chamber, while the other 10 replicates were placed in the same random order, but as a mirror image, in the second chamber. This experimental design using a mirror image pattern was employed, because it was considered that the most important environmental factor varying across the chamber was the air flow, and the inlet pipes entered from opposite sides in the two chambers (ref. Section 2.5.1).

The plants were subjected to a chronic fumigation (ref. Section 2.6.1), with a mean concentration of 336 μgm^{-3} SO_2 for 130 days (17/4/80 - 25/7/80).

7.4.2 *Results and discussion*

The results in Table 7.4 show that SO_2 caused an overall reduction in growth of 13% compared to growth in the control chamber. The dry wt. of the live shoots was significantly reduced in the SO_2 treatment by 23%. Such a reduction must be due to a decrease in size of plant units (leaves or tillers and/or a reduction in the number of units). The mean live leaf and tiller dry wts. and number of leaves per tiller were not significantly reduced, which suggests that the dry wt. decrease of the live shoots can be accounted for mainly by the significant reduction in the number of tillers (17%). No deaths of tillers were observed during the experiment, and therefore it can be concluded that

TABLE 7.4 Analysis of Growth of 19 Clones of *Lolium perenne* in 336 μgm^{-3} SO_2 for 130 Days Compared with Clean Air.

GROWTH CHARACTER	CONTROL MEAN	TREATMENT MEAN	% CHANGE OF GROWTH IN SO_2 cf. CLEAN AIR	P	CLONES X GAS TREAT.
No. of Tillers	40.8	34.0	-16.67	***	*
No. of Leaves	121.3	99.4	-18.05	***	N.S.
Live Shoot Dry Wt (g)	1.387	1.068	-23.00	***	N.S.
Dead Shoot Dry Wt (g)	1.487	1.434	- 3.56	N.S	N.S.
Root Dry Wt (g)	2.257	1.878	-16.79	***	N.S.
No. Leaves per Tiller	3.011	2.953	- 1.93	N.S	N.S.
Mean Live Leaf Dry Wt. (g)	0.011	0.010	- 9.09	N.S	N.S.
Mean Tiller Dry Wt. (g)	0.034	0.032	- 5.88	N.S	N.S.
Dead Shoot/ Live Shoot	1.335	1.658	+24.19	**	N.S.
Root/Live Shoot	1.582	1.348	-14.79	***	N.S.
Total Shoot Dry Wt (g)	5.131	4.381	-14.62	***	N.S.
Total Plant Dry Wt (g)	2.874	2.502	-12.94	***	N.S.

*** = $P < 0.001$

** = $P < 0.010$

* = $P < 0.050$

fewer tillers were initiated in SO_2 than clean air.

The dead/live shoot ratio is the only character which showed an increase (+ 24%), as a result of the SO_2 treatment, indicating a higher degree of senescence under the chronic SO_2 regime, compared to clean air.

Accompanying the large reduction in the shoots, there was also a large decrease in root dry wt. (-17%) and a significant reduction of root/live shoot ratio (-15%) suggesting a greater effect on roots than shoots.

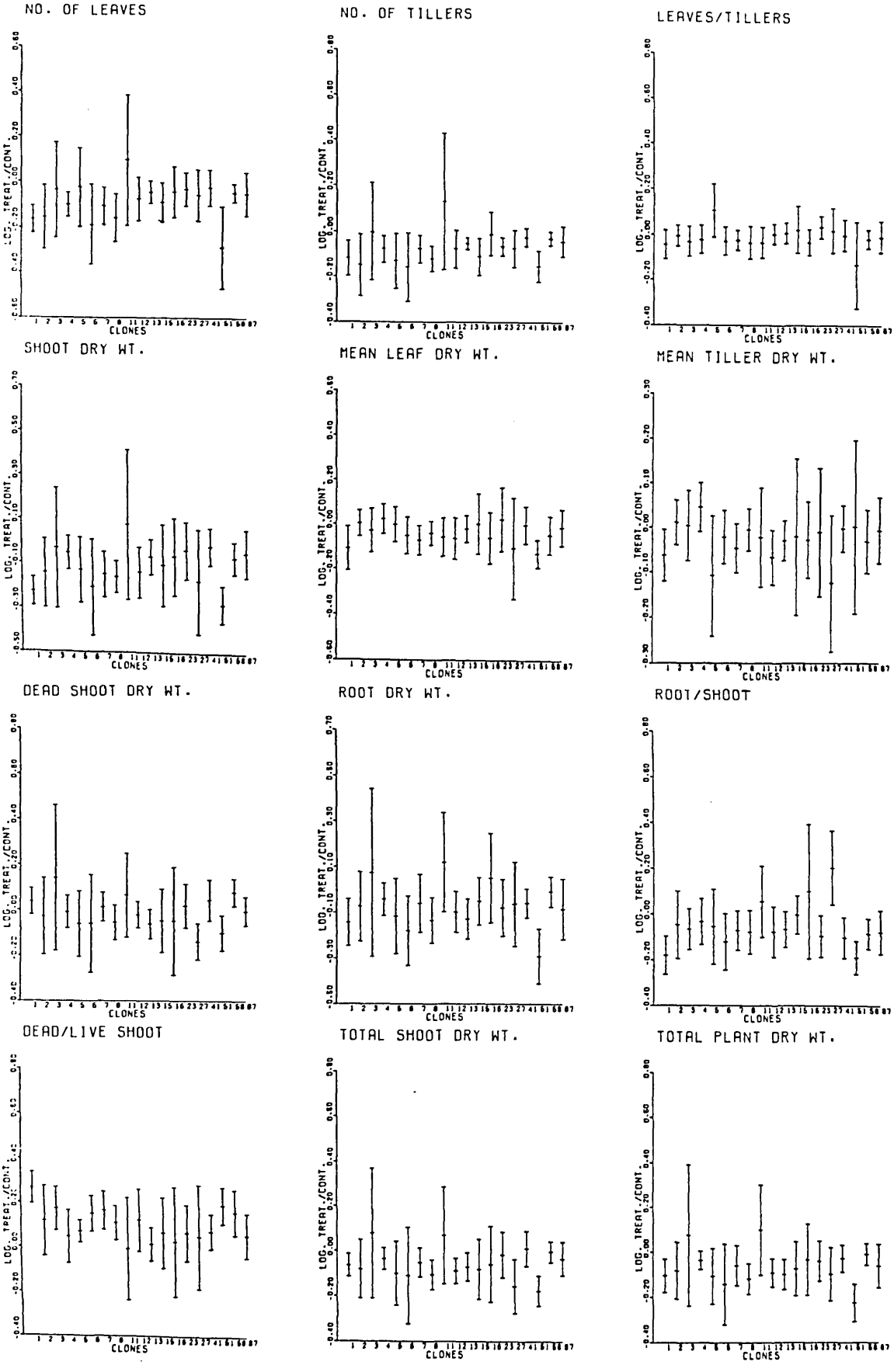
Differential response to the SO_2 fumigation between populations (Askern, Helmshore, S23 resistant, c.v. S23), was investigated. The only populations significantly differentially tolerant to chronic SO_2, were Askern and c.v. S23 (Table 7.5), the former suffering less SO_2 injury in shoots, senescence and root/live shoot ratio.

Inspection of the effects of SO_2 on different harvest parameters for individual clones (Fig. 7.2), shows that there were no clearly tolerant or non-tolerant clones. For almost all growth parameters there were some clones which revealed significant differences, but rarely for the same clones in more than one parameter, except where the parameters were related (e.g. Clone 51 exhibited tolerance in live shoot dry wt. and number of tillers).

Fig. 7.2 reveals that significant changes in growth, due to SO_2 treatment, can be described by 5 responses among all 19 clones:

1. A reduction in the number of tillers, expressed in the decrease of number of tillers, number of live leaves and live shoot dry wt., but no change in the number of live leaves per tiller.

Fig. 7.2 Growth of 19 Clones of Lolium perenne Subjected to a Mean of 336μgm^{-3} SO_2 for 130 days Compared to Growth in Clean Air.



Confidence limits at 95%

TABLE 7.5 Single Degree of Freedom Comparisons Between Populations of *L. perenne* for Chronic SO_2 Injury

Growth Parameters	% Change of Growth in SO_2 cf. Clean Air			
	Askern	Helmshore	C.V. S23	S23 Res
No. of Tillers	-1 a	-13 ab	-23 b	-16 ab
No. of Live Leaves	-2 a	-15 ab	-26 b	-20 ab
Live Shoot dry wt (g)	-9 a	-19 ab	-33 b	-29 ab
Dead Shoot dry wt (g)	-8	-2	-5	+1
Root dry wt (g)	-7	-20	-18	-6
$\frac{\text{No. Live Leaves}}{\text{No. Tillers}}$	+2	-3	-5	+1
Mean Live Leaf dry wt (g)	-2	0	-4	-15
Mean Tiller dry wt (g)	-5	+3	-5	-19
$\frac{\text{Dead shoot}}{\text{Live}}$	+9 a	+28 ab	+40 b	+15 ab
$\frac{\text{Root}}{\text{Live Shoot}}$	-5 a	-17 ab	-20 b	-8 ab
Total Shoot dry wt (g)	-8	-16	-20	-16
Total Plant dry wt (g)	-7	-11	-18	-13

Populations with different alphabetic symbols for the same parameter are significantly different at the 5% level.

2. A decrease in leaf size, expressed as a reduction in the mean live leaf dry wt. This is interesting since it represents a different form of response to chronic SO_2 fumigation. All clones showing a decrease in leaf size (1, 7, 51, 58) also exhibited a reduction in tiller number, suggesting that this may be a secondary mechanism.

3. A reduction of root dry wt. and root/live shoot ratio occurred in 2 clones (1, 51).

4. In one clone (27) there was an increase in root/live shoot ratio, but this had a very small live shoot dry wt., and little significance can be attached to this in the absence of further experimental evidence.

5. Almost half the clones show an increase in senescence, reflected in the dead shoot/live shoot ratio (1, 3, 5, 6, 7, 8, 51, 58).

Each clone had its own combination of the 5 responses to SO_2, which suggests that within a population, there may be several different forms of response and adaptation to selection pressure from SO_2. Such a system of adaptation may partly explain the conflicting and confusing results of different workers (Bell, 1982). On the other hand, it is possible that there is only one mechanism, but it is expressed differently, depending on fumigation conditions, SO_2 concentration and tolerance of plant material.

The differing responses of the clones make it difficult to select those clones which have tolerant phenotypes. It can be said that clones 11, 16 and 87 showed no reduction due to treatment, and that clone 27 only showed an increase in root/live shoot ratio. Clone 11 had poor

growth throughout the experiment, and is therefore not reliable in its behaviour. However, clones 16, 27 and 87 are the most likely to have phenotypes tolerant to chronic levels of SO_2.

7.5 *General Discussion*

The plant material used for fumigations in this chapter was selected using data from previous experiments. The lack of agreement with the findings of Ayazloo (1979) demonstrates the large error attached to measurements of acute and chronic SO_2 injury, thus highlighting the need for a very high level of replication, when attempting to quantify the degree of sensitivity of genotypes.

The most successful of the three screening experiments in identifying a range of tolerant phenotypes was the acute fumigation (Section 7.3). This revealed clones with low, medium and high tolerances, using only 6 replicates compared with 10 in the second chronic SO_2 fumigation. This suggests that variability is much lower for acute injury than chronic injury. This seems probable when considering the lack of environmental control, the length of fumigation and the characters measured in a chronic fumigation compared to an acute fumigation.

Neither of the chronic fumigations enabled the clear separation of tolerant and non-tolerant clones. The evidence suggests that greater replication is needed and further control of the chamber environments to lower variability, if differences between genotypes are to be exposed. However, an increase in replicates would introduce logistic problems in an already large experiment.

CHAPTER 8

AN INVESTIGATION OF REMOTE SENSING TECHNIQUES FOR ESTIMATING CHRONIC SO_2 TOLERANCE IN *LOLIUM PERENNE*

8.1 *Introduction*

Remote sensing of pollution damage on plants is based on optical measurements associated with the injury mechanism. There are several measurable characteristics: i) thermal emission, measured using infra red photography, gives information regarding the short term changes in the physiological activity of the plant, ii) chlorophyll fluorescence, which relates to the activity of photosystem 2(PS2) in photosynthesis, iii) reflectance, used to detect long term changes of a phenological nature, being less subject to rapid fluctuations than thermal emissions. In the following experiments the use of fluorescence and reflectance measurements was investigated, for prediction of the level of chronic SO_2 tolerance in clones of *L. perenne*.

8.1.1 *Fluorescence*

Fluorescence is one of several competing processes by which excess absorbed photon energy, in chloroplasts, is dissipated. Other forms of energy loss are, by heat production or transfer to other chlorophyll molecules.

The two photosystems (Fig. 8.1) of photosynthesis have separate "light harvesting systems" which absorb light at peaks of 700 nm (PS1) and 680 nm (PS2), such that the two systems have different action spectra (Fig. 8.2). It is important to note that light wavelengths above 700 nm can only be used by PS1, and that at around 450 nm to 500 nm there are major differences in action spectra due to the

Fig. 8.1 'Z Scheme' of Electron Flow in Photosynthesis, Showing the Involvement of the Two Photoreactions.

Thornber and Barber (1979)

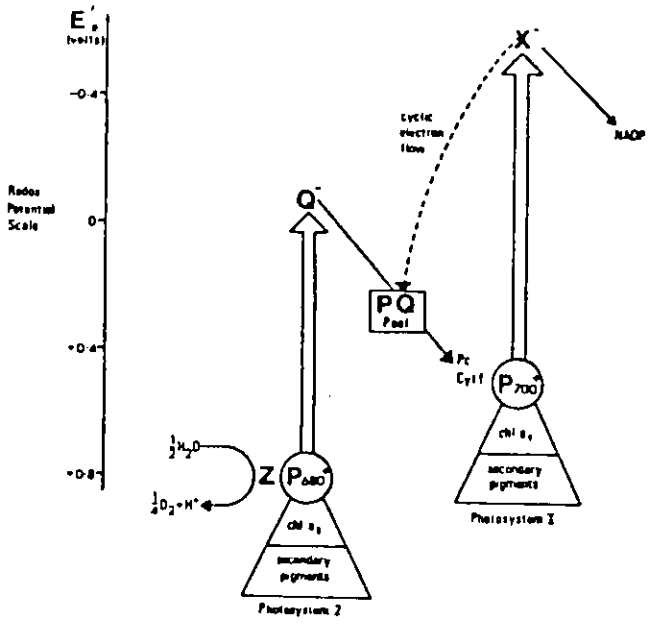
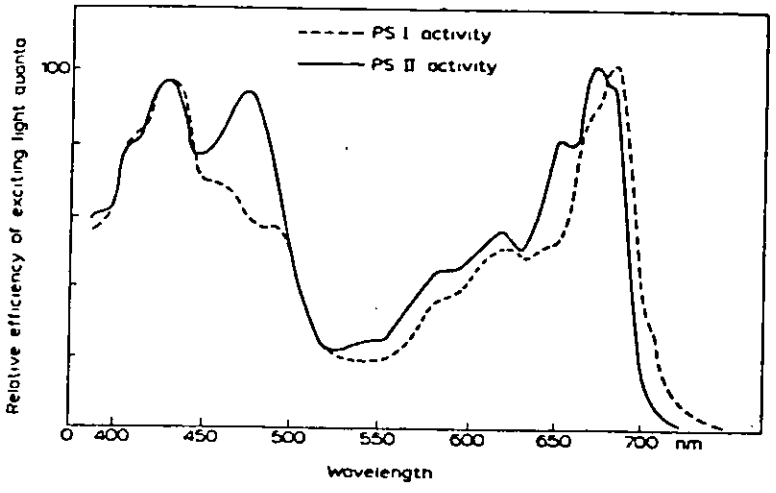


Fig. 8.2 Action Spectra for Photosystem 1 and 2.

Thornber and Barber (1979)



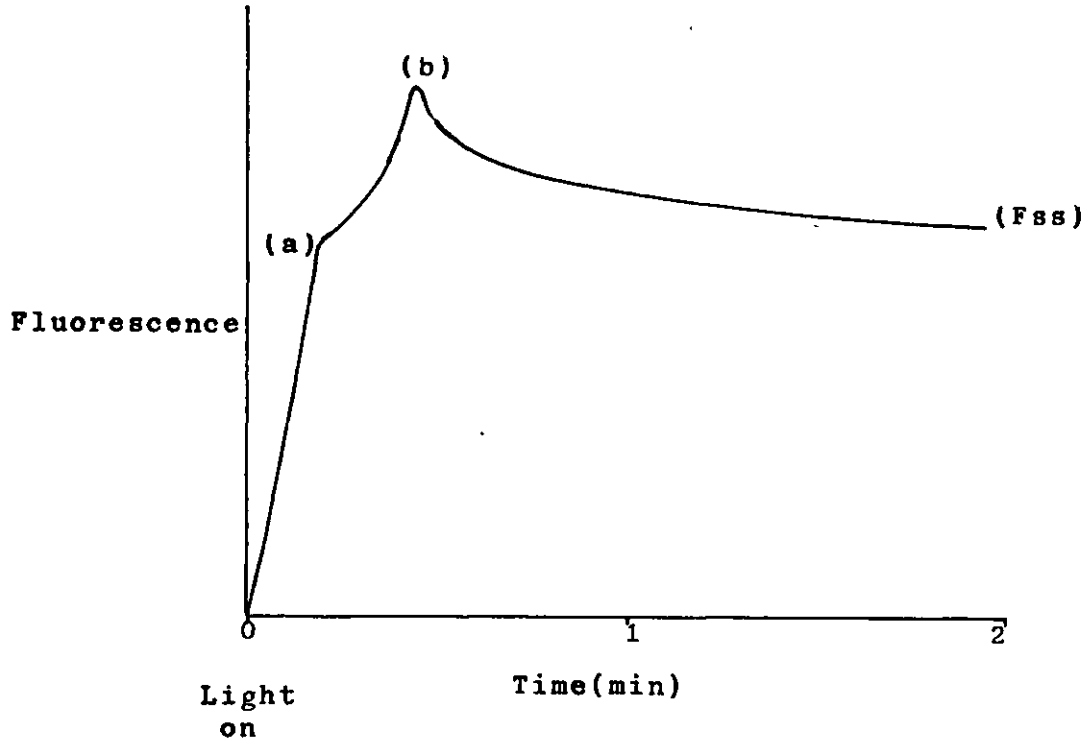
association of chlorophyll b and carotenoids with PS2.

Fluorescence is mainly associated with PS2, but depends on the ability of PS1 to accept electrons. When illuminated a plant gives an immediate response of fluorescence. At this point there are a maximum number of P680 molecules available, therefore the fluorescence is minimal to the pigment system. As the P680 molecules are used up, the fluorescence rapidly increases to a peak level, followed by a decrease to an equilibrium steady state (Fss), as a result of secondary electron flow along the electron transport pathway (Fig. 8.3). These changes of fluorescence, during the period a few seconds after illumination, are known as the Kautsky effect (Kautsky and Hirsch, 1931).

In light predominantly absorbed by PS1 there is little fluorescence. If PS1 light is removed, leaving predominantly PS2 light, there is a great deal of fluorescence, since P680 remains in a reduced state. The fluorescence in PS2 light does not remain high, but decreases to a new equilibrium (Fss_2). This is due to the "Spill Over" mechanism, which optimises the photochemical energy conversion for PS1 to PS2, probably by increased rate of transfer to non-fluorescing chlorophyll (Barber, 1976).

In previous work, on the fluorescence of plants subjected to SO_2 pollution (Arndt, 1972, 1974), measurements were made of the Kautsky effect which showed only the additive effects of the two photosystems. In the present experiment, the interactions of the two photosystems were observed, by using two selected wavelengths of light (PS1 and PS2 light) to excite chlorophyll fluorescence (Barber and Horler, 1980).

Fig. 8.3 The Kautsky Effect as Observed for *L. perenne*



(a) Minimal fluorescence of pigment system.

(b) Maximum variable fluorescence.

(Fss) Equilibrium steady state.

8.1.2 *Reflectance*

Reflectance spectra measured from a leaf surface depend on the optical and physical properties of the leaf (Fig. 8.4; Table 8.1). Where there is high absorption, such as by pigments or water, the reflectance is generally low (Gates, Keegan, Schleter and Weidner, 1965). In the absence of absorption the reflectance is dependant on leaf internal structure: light entering the leaf is critically internally reflected at the cell walls, where refraction indices change from 1.33 (liquid water) to 1.00 (air) (Willslatter and Stoll, 1913). Reflectance may be reduced over parts of the spectrum by scattering, other pigments, and internal structures of a similar size to the wavelengths of spectra (e.g. mitochondria, starch granules and microbodies). Thus it is evident that changes in certain aspects of leaf structure could be measured indirectly, using reflectance.

The majority of research in the field of remote sensing, has concentrated on the detection of acute pollution injury on plants (Omasa et al, 1980a, b; Wert, 1969; Murtha, 1976). Relatively little work has been carried out with chronic levels of pollutants, one exception being the use of infra red photography on a large scale outdoor fumigation experiment (Taylor, Leininger and Osberg, 1979). This showed negative correlations between image density and SO_2 treatment, using concentrations below 300 μgm^{-3}

The present work involves the measurement of reflectance and fluorescence of *L. perenne* plants after a chronic fumigation, with the aim of using remote sensing techniques as a possible quick method of screening for chronic SO_2 tolerance.

Fig. 8.4 Spectral Reflectance Curve of *Mimulus cardinalis* (Gates et al, 1965)

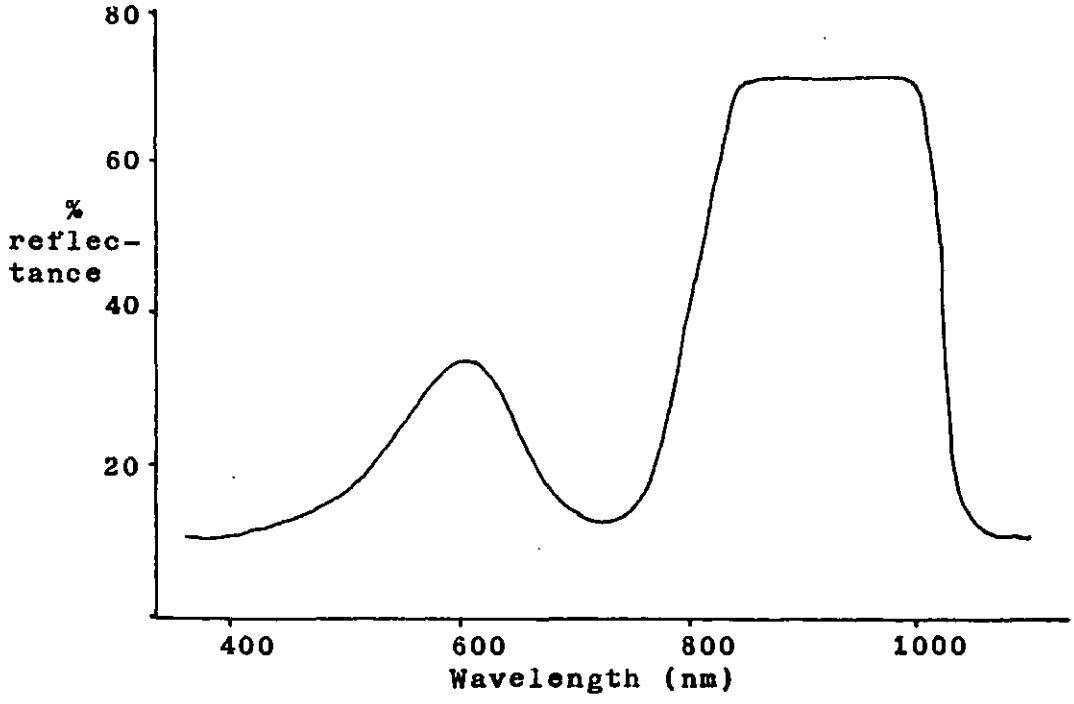


Table 8.1 Leaf Absorption Spectra

< 500nm	Blue, absorption by most pigments, low reflectance.
500nm - 600nm	Green, high reflectance.
600nm - 700nm	Red, strong absorption by chlorophylls a and b, low reflectance.
700nm - 1200nm	Plateau of high reflectance.
> 1200nm	Water absorption curve giving low reflectance with occasional peaks.

8.2 *An Investigation of the Relationship between Chlorophyll Fluorescence and Chronic SO_2 tolerance*

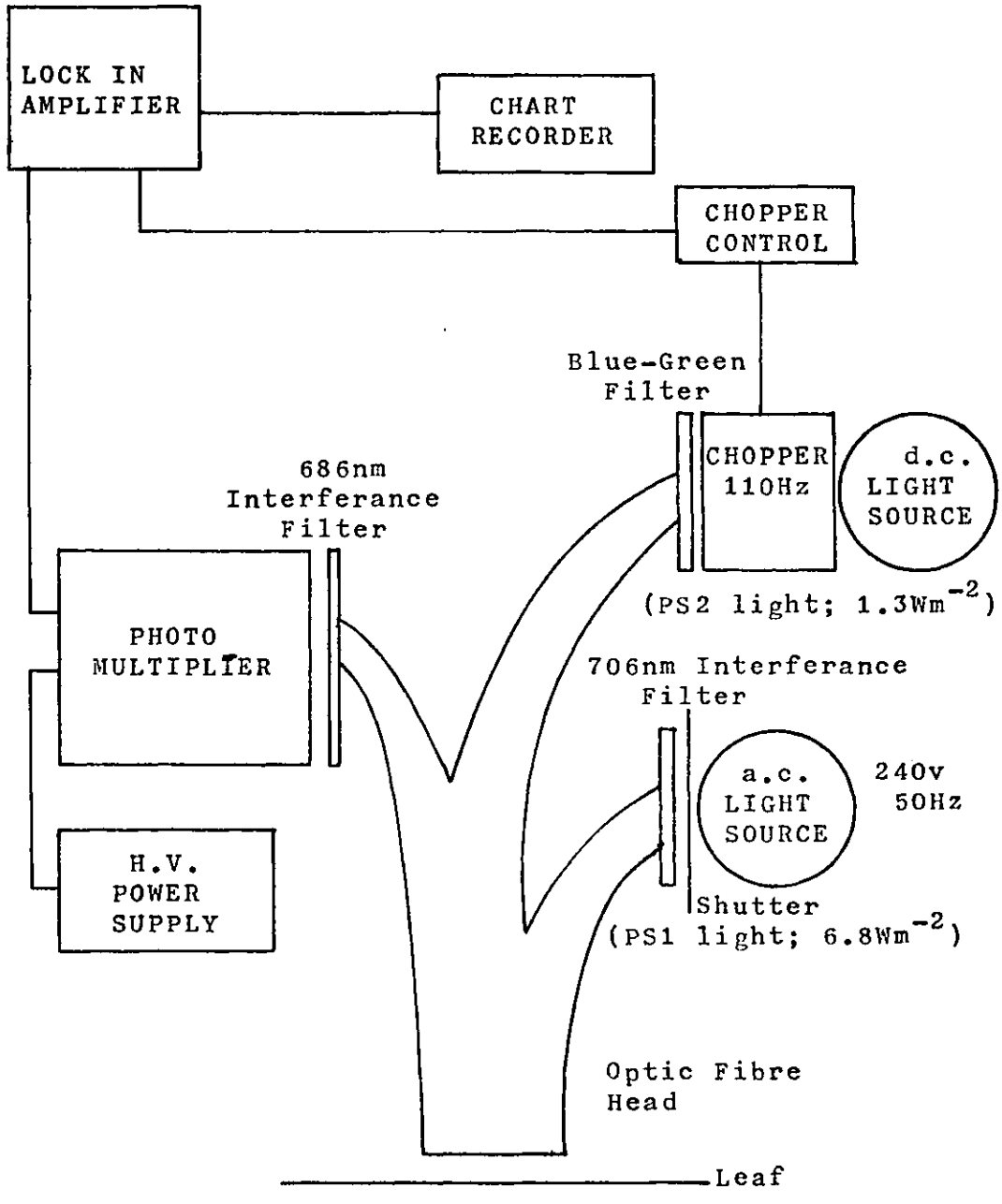
8.2.1 *Method*

In Section 7.3, 19 selected clones of *Lolium perenne*, replicated 10 times, were subjected to a mean concentration of 336 μgm^{-3} SO_2 for 130 days (17/4/80 - 25/7/80).

Before harvesting, the fluorescence of the second youngest leaf, of a randomly chosen tiller, of each replicate was measured, using the dual beam method (Bonaventura and Myers, 1969) adapted by Barber and Horler (1981). Each leaf was chopped into segments before being placed, with the abaxial surface downwards, on a piece of black sticky adhesive tape, arranged to give a continuous area of green leaf of approximately 1 cm^2. An optic fibre head was positioned in contact with the adaxial surface of the arranged leaf segments, such that its surface area was totally covered by the latter.

The optic fibre was divided into three branches, two of which supplied excitation light, and one which recorded emissions (Fig. 8.5). Continuous PS1 light was provided by a tungsten filament source, filtered through a Balzer B-40 706 nm interference filter (12 mm HPBW at half the peak transmission) giving an intensity of 6.8 Wm^{-2} at the optic fibre head. Modulated PS2 light at an intensity of 1.3 Wm^{-2} was supplied by a tungsten filament source, filtered through a 4 mm thick Balzer B 18 filter plus 2 mm of BG 38, which provides a wide blue-green illumination. To modulate the light, a vibrating chopper at a frequency of 110 Hz was used. Fluorescence from the modulated light was measured by feeding a signal from the chopper into a lock-in amplifier, at a time constant of 1 second. Fluorescence was measured

Fig. 8.5 Apparatus for Dual Beam Excitation of Chlorophyll Fluorescence



(Barber and Horler, 1980)

at 686 nm through a Balzer B-40 interference filter (11nm, H.P.B.W.). By operating a shutter on the PS1 light source, it was possible to measure fluorescence of PS2 in the presence and absence of PS1 light.

The fluorescence curves were recorded on a chart recorder. Each reading involved 2 min with both lights on (PS1 + PS2) followed by 3 min. with only PS2 light. This gave a characteristic pattern shown in Fig. 8.6.

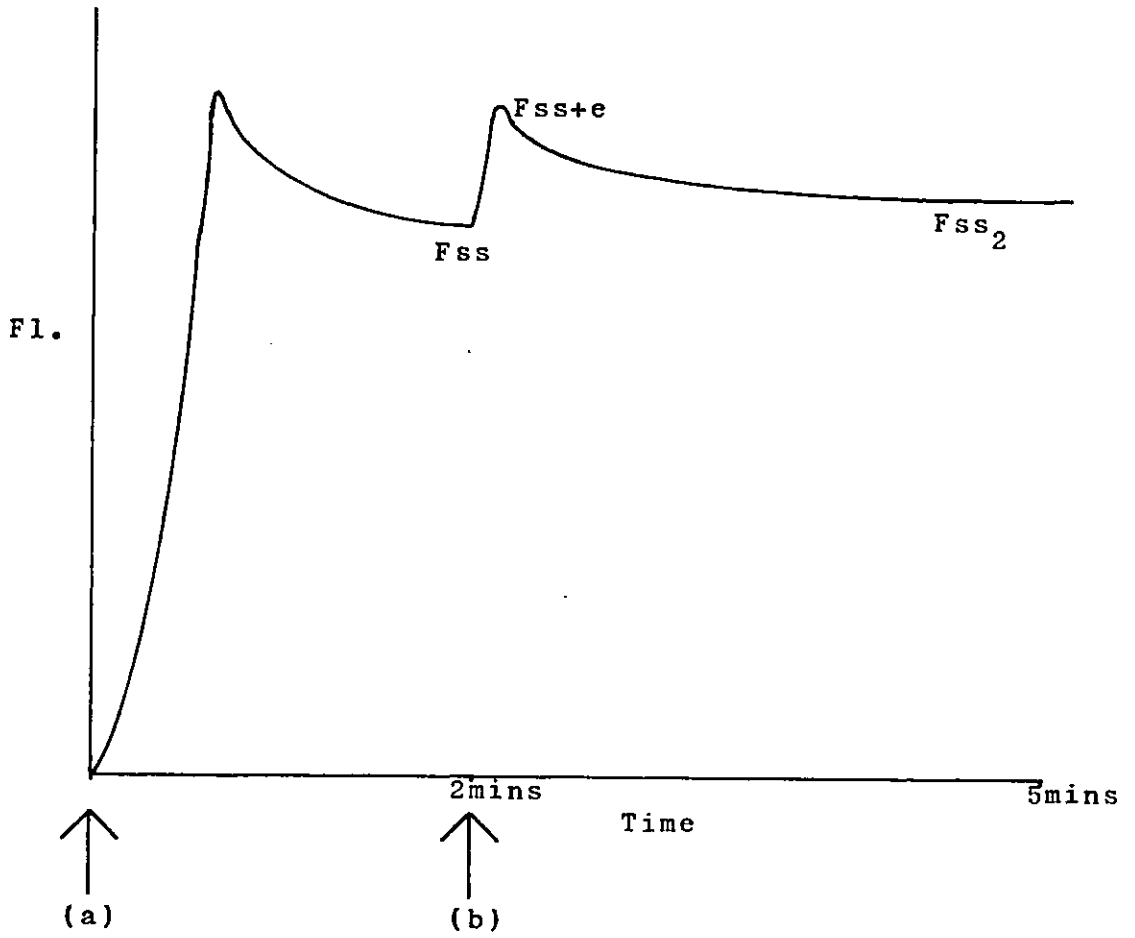
Most plants were subjected to this procedure after about 1 hr in the dark, but due to unavoidable interruptions some plants were analysed in daylight. Plants were investigated in a random order, to avoid bias in the results.

The data collected from the chart recorder were the PS1 + PS2 steady state level (Fss), the PS2 steady state level (Fss_2), and the fluorescence peak level after exclusion of PS1 light (Fss + e).

8.2.2 *Results and discussion*

Table 8.2 shows the results of the analysis of the fluorescence measurements (Appendix 3), revealing several significant differences attributable to gas treatment. In general, SO_2 treatment caused a decrease in fluorescence intensity. This is similar to the effect on algae of SO_3^{2-} concentrations above 10 μM found by Arndt, (1974). There were no changes due to SO_2 fumigation evident in the value of either e or e/Fss+e, showing that the treatment did not affect the degree of fluorescence enhancement in relation to the steady state fluorescence in white light. However there was a significant decrease in the "spill over" effect expressed by $(Fss+e - Fss_2) / (Fss+e)$. The decrease of the Fss_2 level was proportionally less than the decrease in

Fig. 8.6 Typical Fluorescence Curve from Dual Beam Method.



(a) PS1+PS2 light on

(b) PS1 light off

TABLE 8.2 Analysis of Fluorescence Measurements of Single Leaves of *L. perenne* after Chronic SO_2 Fumigation

FLUORESCENCE PARAMETERS	TREAT	FLUORESCENCE	SIGNIFICANCE OF SOURCE OF VARIATION		
			GAS TREATMENTS	CLONES	INTERACTION
Fss	C SO_2	57.86 55.29	**	**	N.S.
e	C SO_2	7.09 6.78	N.S.	**	N.S.
Fss_2	C SO_2	59.47 57.55	*	**	N.S.
e/Fss+e	C SO_2	0.1071 0.1059	N.S.	**	N.S.
$\frac{Fss+e-Fss_2}{Fss+e}$	C SO_2	0.0811 0.0688	*	**	N.S.
Fss/Fss_2	C SO_2	0.9739 0.9621	*	**	N.S.

* = $p < 0.05$ ** = $p < 0.01$

Fss. This suggests that two effects of the treatment are taking place: firstly an overall decrease of fluorescence and secondly an inhibition of the "spill over" mechanism.

All parameters showed highly significant differential fluorescence between phenotypes. However, there were no significant gas treatment x clones interactions, thus providing no evidence that individual clone fluorescence was affected differentially by the SO_2. This tends to agree with the findings of the growth analysis, performed after this fumigation, in which SO_2 caused reductions in the root and shoot dry wts, but no detectable differential response between phenotypes.

Single degree of freedom comparisons of fluorescence between populations (Askern, Helmshore, S23, S23 res.) were made for all combinations for both treatment and control fluorescence parameters, but no differences were revealed.

The relationship of leaf fluorescence to chronic SO_2 tolerance is of major interest for the prediction of the latter without fumigation. Thus, correlations were made of the clone means, using all the fluorescence parameters (Fss, Fss_2, e, e/Fss+e, $(Fss+e-Fss_2)/(Fss+e)$, Fss/Fss_2) for the control and treatment plants separately as the x variables and chronic injury for all growth parameters as the y variables (Table 8.3). Eight significant correlations were detected between chronic injury and control plant fluorescence, while only 3 were found for the treatment plant fluorescence of which two involved the same parameters as the control plant fluorescence (Fss/Fss_2 α dead shoot dry wt; Fss α root/shoot-ratio). Further examination of Table 8.3, reveals that almost all the significant correlations are related and can be summarised: as

TABLE 8.3 Coefficients of Correlation Between Parameters of Chronic SO_2 Injury and Fluorescence

GROWTH IN SO_2 cf CLEAN AIR	CONTROL						SO_2 TREATMENT					
	Fss	e	Fss_2	$\frac{e}{Fss+e}$	$\frac{Fss+e-Fss_2}{Fss+e}$	$\frac{Fss}{Fss_2}$	Fss	e	Fss_2	$\frac{e}{Fss+e}$	$\frac{Fss+e-Fss_2}{Fss+e}$	$\frac{Fss}{Fss_2}$
No. of Live leaves	0.058	0.070	0.078	0.054	-0.060	-0.068	0.206	0.258	0.171	0.238	0.260	0.047
No. of Tillers	0.185	0.064	0.147	-0.135	-0.112	0.009	0.252	0.276	0.217	0.240	0.258	0.031
Liveshoot Dry Wt.	0.080	-0.111	0.010	-0.135	0.034	0.114	0.214	0.220	0.122	0.197	0.276	0.178
Dead Shoot Dry Wt.	-0.011	-0.282	-0.328	-0.258	0.618 **	0.606 **	-0.073	-0.058	-0.294	-0.016	0.226	0.541 *
Root Dry Wt.	0.128	0.179	0.198	0.141	-0.133	-0.166	0.264	0.156	0.258	0.126	0.116	0.014
No Live Leaves per Tiller	0.243	-0.218	0.148	-0.321	-0.109	0.118	0.140	0.110	0.130	0.074	0.075	-0.013
Mean Leaf Dry Wt.	-0.203	-0.104	-0.276	-0.018	0.298	0.225	-0.058	-0.109	-0.181	-0.082	0.048	0.313
Mean Tiller Dry Wt.	0.191	-0.451 *	-0.032	-0.561 **	0.108	0.424	0.112	-0.054	-0.030	-0.073	0.066	0.318
$\frac{\text{Dead Shoot}}{\text{Live Shoot}}$	-0.207	-0.123	-0.352	-0.054	0.546 *	0.423	0.394	-0.263	-0.474 *	-0.190	-0.070	0.270
$\frac{\text{Root}}{\text{Live Shoot}}$	0.223	0.388	0.469 *	0.320	-0.595 **	-0.610 **	0.386	0.166	0.527	0.100	-0.078	-0.389
Total Shoot Dry Wt.	-0.002	-0.111	-0.090	-0.115	0.180	0.211	0.124	0.109	-0.003	0.110	0.236	0.291
Total Plant Dry Wt.	-0.082	-0.241	-0.259	-0.220	0.403	0.431	-0.037	0.126	-0.202	0.149	0.330	0.408

* = $p < 0.05$ ** = $p < 0.01$

senescence increases and root/shoot ratio decreases as a result of chronic SO_2 fumigation, the fluorescence parameters $(Fss+e - Fss_2)/(Fss+e)$ and Fss/Fss_2 increase, but Fss_2 decreases. Two of the significant correlations could not be described by this relationship, viz. between the reduction in mean tiller dry wt., and e and e/Fss+e of the plants grown in clean air. This only occurred in one treatment and there was no further evidence to substantiate it. Therefore, assuming an increase in senescence or a decrease in root/shoot ratio to be symptomatic of chronic SO_2 injury, it would seem that chronic SO_2 tolerance may have a negative relationship with the ability of the plant to balance the photosystems in predominantly PS2 light, that is the functioning of the "spill over" mechanism.

To assess the extent of this relationship, regression analyses were carried out between the relevant parameters (Table 8.4). The "best fit" relationship was given by SO_2 effects on root/shoot ratio against Fss/Fss_2 for control plant: this only accounts for 34% of the variation and would therefore not be a highly reliable method of predicting relative susceptibility to chronic SO_2.

There were no significant differences in the regression slopes between control and SO_2 treatment fluorescences (Table 8.4), thus providing no evidence of a differential effect of chronic SO_2 on fluorescence, between tolerant and susceptible phenotypes.

8.3 *An Investigation of the Relationship Between Reflectance and Chronic SO_2 tolerance*

8.3.1 *Method*

At the same time as fluorescence measurements were made on plants

TABLE 8.4 Regressions of Fluorescence Against Growth in SO_2 Treatment cf Growth in Control

y FLUORESCENCE CHARACTER		x GROWTH RESPONSE SO_2 CHARACTER	EQUATION FOR FLUORESCENCE α GROWTH RESPONSE	SIG. SLOPE FROM 0	SIG. CONT -SO_2 SLOPES	R^2 %
Fss_2	CONT	ROOT/SHOOT	y = 60.7 + 24.4x	*	N.S.	17.4
	SO_2		y = 59.0 + 27.4x	*		23.6
$\frac{Fss+e-Fss_2}{Fss+e}$	CONT		y = 0.0746 - 0.125x	*	N.S.	26.1
	SO_2		y = 0.0652 - 0.0720x	N.S.		4.8
$\frac{Fss}{Fss_2}$	CONT		y = 0.963 - 0.213x	**	N.S.	33.6
	SO_2		y = 0.956 - 0.126x	N.S.		9.2
Fss_2	CONT	DEADSHOOT / LIVESHOOT (SENESCENCE)	y = 61.8 - 23.8x	N.S.	N.S.	7.3
	SO_2		y = 60.7 - 32.0x	*		17.9
$\frac{Fss+e-Fss_2}{Fss+e}$	CONT		y = 0.0658 + 0.157x	*	N.S.	23.9
	SO_2		y = 0.0571 + 0.122x	N.S.		12.1
$\frac{Fss}{Fss_2}$	CONT		y = 0.956 + 0.186x	N.S.	N.S.	12.0
	SO_2		y = 0.952 + 0.108x	N.S.		0.7

from the experiment detailed in Section 7.3, reflectance was also recorded. The second leaf of a randomly chosen tiller, from each replicate, was removed for screening. The reflectance was recorded from the adaxial surface, using spectrophotometers equipped with integrating spheres. For wavelengths up to 900 nm a Perkin Elmer model 544 spectrophotometer was used and for longer wavelengths a Beckman DK2A. Ba SO_4 was used for standardisation of reflectance measurements. In all cases the readings were taken as quickly as possible, after the leaf segments were cut from the plants.

Percentage reflectances were recorded at 5 wavelengths, chosen from parts of the spectrum which show important characteristics of the leaf. These wavelengths were:

460 nm Absorption band of chlorophylls and carotenoids, giving a minimal reflectance.

545 nm Peak reflectance of the green band, between blue and red absorption bands.

675 nm Red absorption maximum for chlorophyll, giving low reflectance.

800 nm Point on reflectance plateau in near infra-red, where neither pigments nor water absorb.

1650 nm Reflectance peak between two water absorption bands.

8.3.2 *Results and Discussion*

An analysis of variance on the data (Appendix 3) shows a significant effect of the SO_2 treatment on leaf reflectance at 545 nm and 800 nm (Table 8.5). Both these wavelengths are in areas of the spectrum where absorption is relatively low and reflectance is dependant on leaf internal structure. Thus there is evidence of changes in leaf

structure, as a result of SO_2 fumigation. The reflectance was decreased at 800 nm, but increased at 545 nm showing opposite effects for different parts of the spectrum.

TABLE 8.5 Analysis of Hemispherical Reflectance of Single Leaves of *L. perenne* after Chronic SO_2 Fumigation

WAVELENGTH nm	TREAT	% REFLECTANCE	SIGNIFICANCE OF SOURCE OF VARIATION		
			GAS TREATMENTS	CLONES	INTERACTION
460	C SO_2	16.02 16.06	N.S.	**	N.S.
545	C SO_2	32.65 31.99	**	**	N.S.
675	C SO_2	14.94 15.05	N.S.	**	N.S.
800	C SO_2	69.50 69.07	*	N.S.	N.S.
1650	C SO_2	35.11 35.28	N.S.	**	**

* = $p < 0.05$ ** = $p < 0.01$

There were significant differences between all wavelengths except 800 nm, which possibly reflects the variability of chlorophyll and water content between genotypes, while leaf structure is relatively uniform. Only one interaction of clones with treatment was observed (at 1650 nm) but in this case there was no significant SO_2 treatment effect.

Single degree of freedom comparisons were made between all combinations of the four populations (Askern, Helmshore, S23, S23 res),

TABLE 8.6 Coefficients of Correlation Between Parameters of Chronic SO_2 Injury and Reflectance

Growth in SO_2 Treatment cf. Clean Air	CONTROL					SO_2 TREATMENT				
	460nm	545nm	675nm	800nm	1650nm	460nm	545nm	675nm	800nm	1650nm
No of Leaves	-0.019	0.196	0.078	-0.528 *	-0.402	0.047	0.128	0.080	-0.394	-0.089
No of Tillers	0.001	0.209	0.128	-0.263	-0.258	0.081	0.179	0.078	-0.008	0.134
Live Shoot Dry Wt.	0.065	0.242	0.136	-0.292	-0.325	0.133	0.150	0.052	-0.125	0.145
Dead Shoot Dry Wt.	0.136	0.098	0.118	-0.249	-0.299	0.100	-0.004	-0.051	-0.213	0.254
Root Dry Wt.	0.145	0.237	0.255	-0.185	-0.115	0.179	0.177	0.216	0.087	0.096
No. Leaves per Tiller	0.026	0.075	0.108	0.321	0.142	0.069	0.125	0.015	0.577 **	0.373
Mean Leaf Dry Wt.	0.136	0.085	0.028	0.076	-0.153	0.119	-0.046	-0.045	-0.243	0.033
Mean Tiller Dry Wt.	0.150	0.155	0.137	0.260	0.008	0.179	0.087	0.025	0.370	0.411
Dead Shoot / Live Shoot	0.051	-0.197	-0.050	0.112	0.108	-0.066	-0.184	-0.111	-0.053	0.066
Root / Live Shoot	0.019	0.175	0.149	-0.389	-0.079	0.020	0.110	0.104	-0.299	-0.213
Total Shoot Dry Wt.	0.145	0.245	0.193	-0.313	-0.281	0.167	0.133	0.072	-0.113	0.199
Total Plant Dry Wt.	0.117	0.188	0.141	-0.348	-0.356	0.132	0.061	0.002	-0.219	0.247

* = $P > 0.05$ ** = $P > 0.01$

but no significant differences were found.

As with fluorescence, reflectance readings (control and treatment) were correlated against growth in SO_2, compared with growth in the control for clone means (Table 8.6).

Only two significant correlations were observed: for the effects of SO_2 on number of leaves against control reflectance at 800 nm ($r = -0.528$) and number of leaves per tiller against SO_2 treatment reflectance at 800 nm ($r = 0.577$). However, regression analysis of the curves showed no significant differences of the slope from 0 and accounted for only a maximum of 1.4% of the variation (Table 8.7).

Thus it must be concluded that under the conditions of the fumigation there was no substantial relationship between chronic SO_2 tolerance and leaf reflectance, and that reflectance probably has no value for predicting SO_2 tolerance in *Lolium perenne*.

TABLE 8.7 Regressions of Reflectance Against Growth Response to Chronic SO_2 Concentrations

y Reflectance	x Growth Response SO_2 Character	Equation for Reflectance α Growth Response	Significance of slope from 0	R^2 %
800 nm CONT	NO LEAVES	$y = 69.3 - 1.9100x$	N.S.	1.4
800 nm TREAT	NO LEAVES	$y = 69.1 - 0.0663x$	N.S.	0
800 nm CONT	LEAVES/TILLER	$y = 69.4 - 1.44$	N.S.	0
800 nm TREAT	LEAVES/TILLER	$y = 69.1 + 0.929$	N.S.	0

8.4 *Conclusions*

The remote sensing measurements of plants from Section 7.4, has revealed several interesting aspects of chronic SO_2 effects on *Lolium perenne*. It would be of further interest to consider how these relate

to biochemical, physiological and morphological functions of the plant.

Fluorescence is linked with photosynthesis, changes of which have been suggested as the first sign of SO_2 injury (Showman, 1972; Hill, 1974). In the present experiments there was an overall decrease in fluorescence, attributable to the prolonged fumigation with chronic concentrations of SO_2. On a biochemical level this would imply an increased efficiency of photosynthesis in oxidising P680 molecules. Arndt (1974) has argued that a decrease in fluorescence is the result of increased electron flow in the photosystem Z scheme. Though it has been reported that photosynthesis undergoes an initial stimulation, due to SO_2 fumigation (Puckett, Richardson, Flora and Nieboer, 1974; Hallgren and Huss, 1975; Hill 1974; Bull and Mansfield, 1974) after prolonged fumigation, as in the present case, or increased concentrations of SO_2, photosynthesis is inhibited (Bull and Mansfield, 1974). This is supported by a large body of biochemical evidence. Noack, Wehner and Griessmeyer, (1929) and Griessmeyer (1930) showed irreversible bonding with "chloroplast iron" in ferredoxin and cytochrome in the presence of SO_3^{2-}, and Nieboer, Richardson, Puckett and Tomassini (1976) have supported this, reporting that SO_2 and its oxidation products interfere with electron flow in the photosystems. It is also generally believed that SO_2 competes with HCO_3 for active sites on a range of enzymes of the Calvin cycle such as a glucan phosphorylase, phosphoenol-pyruvate carboxylase (Mansfield and Freer Smith, 1981), and in particular ribulose diphosphate carboxylase (Ziegler, 1972; Black and Unsworth, 1979). These effects have been observed for both acute and chronic fumigations. This evidence would tend to suggest, that the effect of the prolonged fumigations with chronic SO_2 levels would be to cause a decrease in photosynthesis, by inhibition of the metabolic

pathways. Such a blockage would cause an increase in fluorescence, rather than the decrease observed. Thus evidence from previous work leads to the conclusion, that the decrease observed in fluorescence was not due to acceleration of electron flow.

Fluorescence may be reduced if there is a decrease in reaction centres or primary donor modules. Shimazaki and Sugahara (1980) have observed that the quinone (Q) pool size was smaller, but not the rate of its photoreduction after treatment with 5000 μgm^{-3} SO_2 for 4 hrs. Decreases in photon energy absorbed per unit leaf area, may be related to loss of chlorophyll or disturbance of chloroplasts.

Reductions of chlorophyll content have been observed for several angiosperm species subjected to chronic SO_2 concentrations (Bell et al, 1979). However, results from the present reflectance measurements at 460 nm and 675 nm suggested, that no significant change in chlorophyll content per unit leaf area had occurred.

Disturbance of chloroplast ultrastructure, due to moderate levels of SO_2, has been reported for a range of angiosperms (Wellburn, Majernik and Wellburn, 1972; Malhotra, 1976; Godzik and Sassen, 1974; Mlodzianowski and Bialobok, 1977). Generally, at low SO_2 concentrations, reversible swelling of the thykaloids occurs, followed by disruption and disintegration of chloroplasts at higher SO_2 levels. It has already been concluded from reflectance spectra at 545 nm and 300 nm that structural changes within the leaf, may have taken place. This provides circumstantial support for possible SO_2 induced changes having occurred in chloroplast structure in the present work.

The second fluorescence response observed, due to SO_2 treatment,

was a reduction of the "spill over" effect. This is a reduction of the plant's ability to convert excess PS2 energy to PS1 and suggests a biochemical effect of the treatment. This might be an inhibition of the "spill over" mechanism itself or a blockage at a later reaction of the metabolic pathway, such as those previously described.

The main effects of chronic SO_2 on reflectance were an increase at 545 nm and a decrease at 800 nm, both suggesting structural disturbance in the leaf, but in opposite directions. It is possible that structural changes in the leaf caused greater internal scattering at 800 nm and less at 500 nm. If for example, structures of less than 800 nm in size swelled to 800 nm an increase in scattering may result.

It is of interest to compare conclusions drawn from the remote sensing measurements, with those from the plant growth experiment (Section 7.4). It was shown, that the SO_2 treatment had caused reductions of live shoot dry wt. (23%), due to decreased tiller production (17%), a decrease in root dry wt. and an increase in scenescence (24%). The overall effects of the pollutant on fluorescence (Fss 5%, Fss/Fss_2 1%) and reflectance (545 nm 0.66%, 800 nm 0.43%) were relatively small, suggesting that SO_2 acts principally on growth of the whole plant rather than the functioning of leaves per unit area.

A relationship between fluorescence and chronic SO_2 injury accounting for 33% of the variation, was demonstrated (Fss/Fss_2 α root/live shoot ratio). Although, it did not provide a sufficiently reliable relationship, on which to base predictions of tolerance. However, this does provide a case for further investigation of the technique, with the aim of defining the relationship more precisely. This relationship implies that chronic tolerant phenotypes of *L. perenne* have a less

efficient "spill over" mechanism. This is unlikely to be of competetive advantage and may thus be a compensatory adaptive feature.

Conditions under which a weak "spill over" mechanism may be a disadvantage would occur, where the R : FR (ratio of photons in 10 nm bandwidths centred on 660 nm and 730 nm) was high. R : FR is at a maximum when the sun has an angle of incidence to the earths' surface of $\geqslant 10^{o}$ in a clear sky (Smith and Morgan, 1981). However, under these conditions the intensity of PS1 would not be limiting and therefore it is unlikely that "spill over" would be important. Daylight can be divided into incident (I - light) and diffuse (D - light). R : FR is greater in D - light, which is the only light available to plants when out of the direct line of the sun. It is not clear whether such differences in daylight R : FR would be of ecological or competitive importance in relation to the "spill over" mechanism.

CHAPTER 9

DISCUSSION OF THE EFFECTS OF SO_2 ON GRASSES

The primary aim of the present work was to investigate the development of intraspecific tolerance of grasses to SO_2: considered in Chapter 10. However, this generated, incidentally, a considerable amount of information on the effects of SO_2 in general, which is discussed in this chapter.

9.1 *Chronic SO_2 Injury*

The simultaneous chronic SO_2 fumigation of the Philips Park species, enabled comparisons to be made of interspecific tolerance. The following order of increasing tolerance, using the OS populations, was based on total shoot dry wts., the differences between *Festuca rubra, Lolium perenne* and *Phleum pratense* being significant: *F. rubra* < *L. perenne/Poa pratensis / L. multiflorum/Phleum pratense.*

Only two previous examples of simultaneous chronic fumigation of several species have been published (Ashenden, 1979; Ashenden and Williams, 1980; Ayazloo and Bell, 1981). These were reviewed by Bell (1982), who noted the following order of increasing tolerance, based on total shoot growth: *Holcus lanatus* < *Dactylis glomerata/Phleum pratense/ Poa pratensis* < *F. rubra/L. multiflorum/L. perenne.*

The two rankings show several differences: Bell (1982) classified *F. rubra* as a relatively tolerant species, while *Phleum pratense* was susceptible, but in the present study these tolerances were reversed. It is evident that these rankings must be studied more closely.

Table 9.1 summarises the results of previous simultaneous chronic

SO_2 fumigations of several grass species (Ashenden, 1979; Ashenden and Williams, 1980; Ayazloo and Bell, 1981) and the Philips Park results. There is no complete agreement for the order of chronic tolerance over the three fumigations for any of the growth parameters recorded. Using statistically significant differences between species from the Philips Park results, at least 3 of the growth parameters (live shoot dry wt., total plant dry wt., and root dry wt), showed disagreements in the order of chronic tolerance, when compared to the previous investigations. Furthermore, the rankings are not consistant within fumigations for different growth parameters, or between populations of the same species. Each worker employed material originating from different sources (Table 9.1). Some of the differences observed between rankings, may be due to differential susceptibility of cultivars and populations. Other causes for such differences could have been in methods of screening or variation in environmental factors such as light or temperature, which may modify the effects of SO_2 on different growth parameters (Davies, 1980; Jones and Mansfield, 1981).

In spite of the difficulties of defining an order of chronic SO_2 tolerance, a few species showed fairly consistent trends. It seems that *D. glomerata* was generally non-tolerant, while *L. perenne* showed at least an intermediate tolerance. The classification of the latter is interesting, because it has commonly been considered the most susceptible of common grass species (Bell, 1982). Since the effects of chronic SO_2 on British grasslands have often been estimated on the basis of the response of this species to SO_2 (O.E.C.D., 1981), it would seem that these effects may be significantly greater than previously thought.

TABLE 9.1 Summary of Growth Analyses from Fumigations Involving Several Grass Species

Species	Population Source	% Change of Growth in SO_2 c.f. Clean Air + Rankings				
		Live Shoot	Root	Dead/Live Shoot	Total Shoot	Total Plant
Original Seed Population						
F. rubra	c.v. "Engina"	-62[1] a	-53[1] a	+27[5] a	-61[1] a	-57[1] a
L. multiflorum	c.v. "RVP"	-20[4] b	-32[3] b	+44[2] ab	-15[4] bc	-22[4] c
L. perenne	c.v. "S23"	-31[2] b	-28[5] b	+35[3] ab	-27[2] b	-27[3] bc
Phleum pratense	c.v. "S50"	-20[4] b	-31[4] b	+95[1] b	- 7[5] c	-21[5] c
Poa pratensis	c.v. "Baron"	-22[3] b	-42[2] ab	+29[4] ab	-20[5] bc	-34[2] b
Philips Park Population 1980						
F. rubra	P.P.	-50[1]	-54[2]	+40[4]	-50[1]	-52[1]
L. multiflorum	P.P.	-45[2]	-36[3]	+33[5]	-41[2]	-40[3]
L. perenne	P.P.	-13[4]	-12[5]	+53[3]	- 6[4]	- 9[4]
Phleum pratense	P.P.	- 4[5]	-18[4]	+79[1]	- 4[5]	-10[5]
Poa pratensis	P.P.	-39[3]	-57[1]	+74[2]	-32[3]	-48[2]
Ayazloo and Bell, (1981)						
D. glomerata	Askern	-50[1]	-50[2]	+426[1]	-26[2]	-37[2]
F. rubra	Askern	-21[4]	-40[3]	+288[2]	-18[3]	-31[3]
Holcus lanatus	Askern	-49[2]	-58[1]	+73[4]	-44[1]	-54[1]
L. perenne	Askern	-27[3]	- 5[4]	+146[3]	- 8[4]	- 9[4]

Continued

TABLE 9.1 (Continued)

Species	Population Source	% Change of Growth in SO_2 c.f. Clean Air + Rankings				
		Live Shoot	Root	Dead/Live Shoot	Total Shoot	Total Plant
Ashenden, (1979); Ashenden and Williams, (1980).						
D. glomerata	c.v. "S37"	-28^{2}	-37^{3}		-44^{1}	-41^{3}
L. multiflorum	c.v. "Milano"	-28^{2}	$+7^{4}$		-14^{4}	-5^{4}
Phleum pratense	c.v. "Eskimo"	-25^{4}	-58^{1}		-36^{3}	-51^{1}
Poa pratensis	c.v. "Monopoly"	-39^{1}	-54^{2}		-39^{2}	-46^{2}

Numeric superscripts indicate tolerance rankings of species for growth parameters within experiments.

The largest ranking is the most tolerant.

Different alphabetic symbols for the O.S. population indicate significant differences between rankings.

Being of major agricultural importance, *L. perenne* has been the subject of more investigations of chronic SO_2 effects than any other grass species. Bell (1982) attempted to define a dose-response relationship, with chronic SO_2 concentrations, for spaced *L. perenne* plants. He plotted the logarithm of the ratio of the dry weights from the control/SO_2 treatments, divided by time, against the mean SO_2 concentrations given in fumigation experiments. This normalised the effect of time, which otherwise progressively increases the difference in productivity between treatments. The resulting dose-response curve was not significant, but more recently Mansfield and Freer-Smith (1982) have determined a significant regression coefficient by removing fumigations of less than 40 and greater than 160 days duration. Bell (1983) has proposed some possible justifications for these omissions:

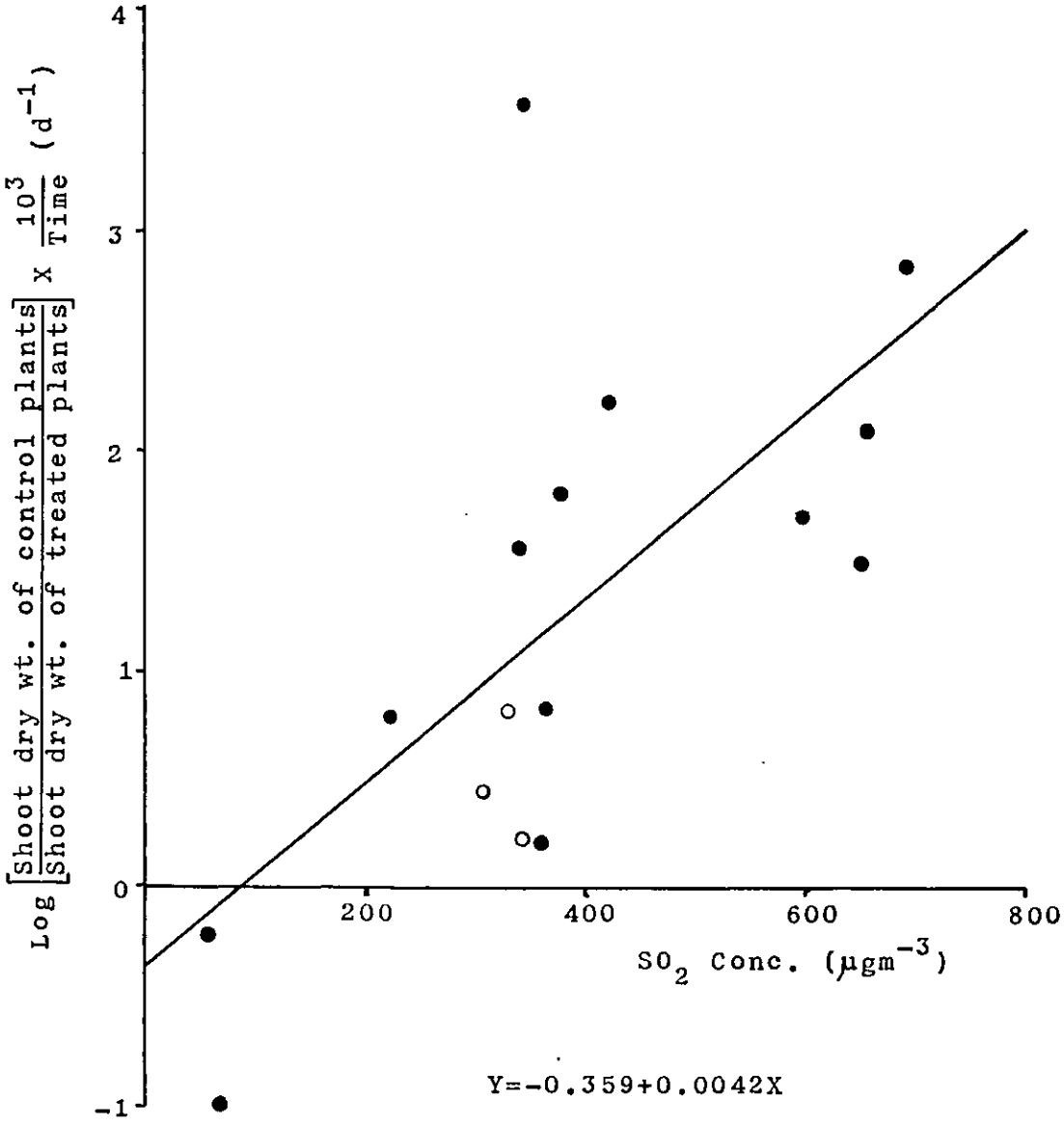
1. There is some evidence that young plants are particularly susceptible to chronic SO_2 injury (Crittenden and Read, 1979; Bell et al, 1979). This has been confirmed recently by Whitmore and Mansfield (1983), who have reported greater sensitivity of newly emerged seedlings than 42 day old plants to a chronic SO_2 fumigation.

2. Two recent investigations have shown marked changes in the nature of response to SO_2 after fumigations of greater than 160 days (Whitmore and Freer Smith, 1982; Colvill, Bell, Roberts and Bradshaw, 1982). They found that the deleterious effects of SO_2 occurred predominantly in the winter and early spring, and that there were few, if any effects during the summer with a certain degree of recovery in some cases. However, it is not clear whether this is due to phenological or seasonal effects.

Means for the present chronic fumigations of *L. perenne* did not differ significantly from the regression line of Mansfield and Freer-Smith (1982). Thus there is general agreement of the dose-response result of *L. perenne* observed in the present work with that of previous investigations. The three means from these fumigations were closely grouped, showing the fumigations to have been relatively consistant in effect. A new regression line was calculated, incorporating these points (Fig 9.1): this gave a curve of $y = 0.0359 + 0.0042x$ ($P < 0.01$), accounting for 44.6% of variation. The estimated threshold level of SO_2 with 95% confidence limits to cause injury was 86 ± 116 μgm^{-3}. Little importance can be attached to this however, due to the enormous error, probably a reflection of both environmental and genotypic influences. However, it leaves little doubt that concentrations of SO_2 greater than 200 μgm^{-3} are likely to cause chronic injury to spaced *L. perenne* plants.

The effects of chronic SO_2 fumigation on the growth of *L. perenne* are summarised in Table 9.2. All of the % changes in the live shoot dry wt. compared with the control were decreases, three of which were significant, while the remaining example was from screening experiment 1 (Section 7.2) in which the overall effect of the SO_2 treatment was low. There is a certain amount of conflicting evidence concerning how this reduction occurs: some reports show that a reduced live shoot dry wt. is accompanied by a corresponding decrease in the number of tillers (Bell and Clough, 1973; Bell et al., 1979), while others show substantial reductions in live shoot growth, without change in tiller number (Ashenden and Mansfield, 1978; Horsman et al, 1979). There was one example of a reduction of number of tillers and another of a decrease in the mean live leaf dry wt., in the present work. In all fumigations

Fig. 9.1 Dose Response Curve for Reductions in Shoot Dry wt. After Chronic SO_2 Fumigation of L. perenne.



○ Data from present expts.

● Data from Horsman et al (1978, 1979), Bell et al (1979), Ayazloo and Bell (1981).

TABLE 9.2: Summary of the % change in Growth of Grass Species in Chronic SO_2 Fumigations Compared to Growth in Clean Air

Species	Fumigation	No. of live leaves	No. of Tillers	Live Shoot dry wt.	Dead Shoot dry wt.	Root dry wt.	No. of live leaves per tiller	Mean live leaf dry wt.	Mean tiller dry wt	Dead/ Live Shoot	Root/ Live Shoot	Total Shoot dry wt.	Total Plant dry wt.
L. perenne	Screening 1 (Section 7.2) 354 $\mu gm^{-3}SO_2$ 140d	-	-	-8	+23*	+13*	-	-	-	+35	+23*	0	-5
	Screening 2 (Section 7.4) 336 $\mu gm^{-3}SO_2$ 130d	-18*	-18*	-23*	-4	-17*	-2	-9	-6	+24*	-15*	-15*	-13*
	O.S. Pop. 317 $\mu gm^{-3}SO_2$ 123d	-13	-9	-31*	-8	-28*	+3	-18	-20	+35*	+19	-27*	-27*
	P.P. Pop. 1979	-4	-10	-24*	-2	-22	-8	-27*	-18	+38*	+13	-20	-19*
	1980	-1	+7	-13	-31*	-12	+8	-8	-15	+53*	-2	-6	-9
F. rubra	Askern 299 $\mu gm^{-3}SO_2$ 139d	-8*	-6	-12*	-5	-14*	-2	-3	-5	+11	0	-8*	-11*
	O.S. Pop. 317 $\mu gm^{-3}SO_2$ 123d	-35*	-34*	-62*	-49	-53*	-7	-41*	-46*	+27	+31	-61*	-57*
L. multiflorum	O.S. Pop. 317 $\mu gm^{-3}SO_2$ 123d	-22*	-13	-20*	+8	-32*	+8	+8	-4	+44*	-9	-15*	-22*
Poa pratensis	O.S. Pop. 317 $\mu gm^{-3}SO_2$ 123d	-15	-11	-22	+4	-42*	+7	+5	-3	+28	-26*	-20	-34*
Phleum pratense	O.S. Pop. 317 $\mu gm^{-3}SO_2$ 123d	-29*	-18	-20	+95*	-31*	+19*	+10	-8	+95*	+7	-7	-21*

* = $P < 0.05$

the changes in growth of these two parameters were negative. Thus it is apparent that reduction in shoot growth of *L. perenne*, due to chronic SO_2 fumigation, involves decreases in the initiation of tillers and the mean dry wt. of live leaves on occasions. Furthermore the proportion by which each parameter contributes to the loss of live shoot dry wt. is variable.

There are three major causes of tiller inhibition and leaf size reductions: low light intensity, low temperature and nutrient deficiency. The first two environmental factors are associated with a reduced carbohydrate production reflecting effects on net photosynthesis (Langer, 1972). Considering the known effects of SO_2 on photosynthesis (Thomas and Hill, 1937; Ziegler, 1972; Bull and Mansfield, 1974) it is not unreasonable to propose that at least some of the growth effects observed, following chronic SO_2 fumigation, may be due to reduced photosynthesis. Crittenden and Read (1977) have used a similar argument, pointing out that reductions of tiller number and mean tiller wt. caused by chronic SO_2 levels are similar to those caused by shading. Furthermore Bell et al (1979) found significant reductions of relative growth rate (RGR) in the early and final stages, of a 173 day fumigation with 43 μgm^{-3} SO_2. The effect on RGR was proportional to fluctuations in the net assimilation rate (NAR), but not on leaf area ratio (LAR), which suggests an effect on photosynthesis or respiration. The reductions of fluorescence of plants from screening experiment 2 (ref Section 8.2) also indicate an effect of moderate levels of SO_2 on photosynthesis.

Relatively little attention has been paid to the effects of SO_2 on roots, although various cases have occurred in which fumigation has caused significantly greater effects on these than on the shoots

(Bell, 1982). Both the 2nd screening experiment (Section 7.4) and the O.S. population from Philips Park (Section 3.3) showed significant decreases in root dry wt., but only the former showed a decrease in the root/live shoot ratio. Reductions in root/shoot ratio have been related to increases in leaf area ratio (LAR) and specific leaf area (Bell et al, 1979). These observations may have been the result of a change in assimilate composition or a reduction of its availability to the roots (Jones and Mansfield, 1982b). In screening experiment 1 however, there was an increase in root growth and root/live shoot ratio, a combination which has not been previously reported for chronic SO_2 fumigations on grasses, although the latter has been found by Bell et al (1979) and the former by Ayazloo and Bell (1981). Possible reasons for this are given as the effects of drought, or nutrient deficiency, enhanced by SO_2 fumigation (see Section 7.2).

Increases of senescence occurred in all fumigations of *L. perenne*, but results in several previous publications have shown reductions of shoot dry wt. without changes in senescence (Bell et al, 1979; Horsman et al, 1979). Thus it would seem that increased senescence is not a pre-requisite for growth reduction by SO_2. Ayazloo and Bell (1981) have suggested that senescence is not a principal factor, since dead leaf dry wt. accounts for a relatively small proportion of the total dry wt.; this is also borne out by the present study (Tables 3.3; 7.2; 7.4). However, it must be concluded that increased senescence can be one of the mechanisms contributing to chronic SO_2 injury in *L. perenne*.

F. rubra was fumigated with chronic concentrations of SO_2, on 2 occasions, causing decreased yields. In both cases these were the result of approximately equal reductions in root and live shoot dry wts.

giving no significant changes in root/live shoot ratio (Table 9.2). Live shoot dry wt. reductions could be accounted for by decreases in number of tillers and mean live leaf dry wt. as for *L. perenne*. A significant increase of senescence was only detected for the Askern fumigation. These findings were contrary to the only previous reports of a chronic fumigation of *F. rubra* (Ayazloo and Bell, 1981), in which although the roots exhibited a relatively similar degree of growth reduction (-39%), the change in senescence was much larger (+180%). This is interesting when it is considered, that the bred cultivar, used in both the Philips Park fumigation and one of Ayazloo and Bell's (1981) fumigations was 'Engina', and that the same experimental methods were employed. Thus the main differences between the two investigations were SO_2 dose (482 μgm^{-3} SO_2 for 105 days, Ayazloo and Bell, 1981; 299 μgm^{-3} SO_2 for 139 days, Philips Park) and the contemporary environmental conditions. The effect of either of these factors may have caused an increase in senescence, without further affecting the roots or the shoots.

A further 3 species were subjected to chronic SO_2 fumigation: *Poa pratensis*, *Phleum pratense* and *L. multiflorum*, which all showed decreases in yield (Table 9.2). *L. multiflorum* was reduced in live shoot dry wt. (-20%), which is in agreement with Ashenden and Williams (1980), but there was also a reduction in the root dry wt. (-32%) and increased senescence (+44%). *Poa pratensis* only exhibited significant growth changes in the root dry wt (-42%) and root/live shoot ratio (-26%), whereas Ashenden (1979) also showed reductions of shoot-dry wt. (-39%). Likewise *Phleum pratense*, for which there were reductions of root dry wt (31%) and an increase of senescence, (+95%) did not show the same effects of SO_2 as in the previous studies: Ashenden and

Williams (1980) and Davies (1980), observed decreases in both live shoot dry wt. and the number of tillers.

The relationship between plant growth and chronic injury was investigated by regression of the growth of plants in clean air (x axis) against their growth in SO_2 (y-axis) (Tables 9.3-6). Regressions for the second screening experiment and the Askern transect were made for all plants in the fumigations, while those for Philips Park were made on the individual populations and those for screening experiment 1 between the means for each clone.

It was found that all regression slopes for the two screening experiments and the Askern transect were significantly less than 1 and most (excepting live shoot dry wt. and senescence for screening experiment 1), were significantly greater than 0 (Tables 9.3-5). This evidence suggests, that the individuals with the largest dry wts. in clean air suffered the greatest damage as a result of SO_2 fumigation.

The Philips Park data (9.6) reveal few regression slopes which are not significantly less than 1, although these relationships are based on only 30 values. None of the gradients was significantly greater than 1, nor were many greater than 0. Those curves with a slope of more than 0 and less than 1 were almost all original seed populations, the only Philips Park examples being: senescence of *L. multiflorum* and root/live shoot ratio of *L. perenne*. It was also evident that the slopes of the original seed populations were always steeper than the Philips Park populations, except in 4 cases: senescence of *L. multiflorum* and *L. perenne*, live shoot dry wt. of *L. multiflorum* and root/live shoot ratio for *F. rubra*. These observations tend to agree with those for the other chronic fumigations, that larger plants

TABLE 9.3: Regressions of Clonal Mean Growth in SO_2 Against Growth in Clean Air for Screening Experiment 1 (after log transformation)

Growth Parameter	b	S.E. of b	Sig of 1 - b	Sig. of 0 - b
Live Shoot dry wt.	0.0886	0.0818	***	N.S.
Dead/Live Shoot	0.0357	0.1010	***	N.S.
Root dry wt.	0.342	0.0826	***	***
Root/Live Shoot	0.591	0.1030	***	***
Total Plant dry wt.	0.214	0.0743	***	**

** = P < 0.01 *** = P < 0.001

TABLE 9.4: Regressions of Growth in SO_2 Against Growth in Clean Air for Screening Experiment 2 (After log transformation)

Growth Parameter	b	S.E. of b	Sig. of 1 - b	Sig. of 0 - b
No. of Tillers	0.384	0.0532	***	***
Mean Live Leaf dry wt.	0.653	0.0644	***	***
Live Shoot dry wt.	0.518	0.0558	***	***
Dead/Live Shoot	0.667	0.0595	***	***
Root dry wt.	0.434	0.0497	***	***
Root/Live Shoot	0.412	0.0470	***	***
Total dry wt.	0.488	0.0514	***	***

*** = $P < 0.001$

TABLE 9.5: Regressions of Growth in SO_2 Against Growth in Clean Air for Chronic Fumigation of the Askern Populations (after log transformation).

Growth Parameter	b	S.E. of b	Sig. of 1 - b	Sig. of 0 - b
No. of Tillers	0.452	0.0590	***	***
Mean Live Leaf Dry wt.	0.698	0.0525	***	***
Live Shoot Dry wt.	0.257	0.0621	***	***
Dead/Live Shoot	0.373	0.0758	***	***
Root Dry wt.	0.208	0.0559	***	***
Root/Live Shoot	0.233	0.0630	***	***
Total Plant Dry wt.	0.226	0.0572	***	***

*** = $P < 0.001$

TABLE 9.6: Regressions of Growth in SO_2 Against Growth in Clean Air for the Philips Park Populations (after log transformation).

No. of Tillers

Species	Pop.	b	S.E. of b	Sig. of 1 - b	Sig. of 0 - b
Poa pratensis	O.S.	0.351	0.267	*	N.S.
	P.P.	0.198	0.321	*	N.S.
Phleum pratense	O.S.	0.604	0.182	*	**
	P.P.	-0.252	0.219	***	N.S.
Festuca rubra	O.S.	0.600	0.287	N.S.	*
	P.P.	0.047	0.232	***	N.S.
Lolium multiflorum	O.S.	0.472	0.296	N.S.	N.S.
	P.P.	-0.239	0.311	*	N.S.
Lolium perenne	O.S.	0.277	0.254	**	N.S.
	79	0.029	0.165	***	N.S.
	80	-0.148	0.157	***	N.S.

Mean Live Leaf dry wt.

Poa pratensis	O.S.	0.072	0.303	**	N.S.
	P.P.	0.049	0.260	***	N.S.
Phleum pratense	O.S.	0.477	0.089	***	***
	P.P.	0.159	0.220	***	N.S.
Festuca rubra	O.S.	0.693	0.182	N.S.	***
	P.P.	0.147	0.223	***	N.S.
Lolium multiflorum	O.S.	1.180	0.287	N.S.	***
	P.P.	0.859	0.471	N.S.	N.S.
Lolium perenne	O.S.	1.220	0.220	N.S.	***
	79	0.529	0.296	N.S.	N.S.
	80	0.190	0.261	**	N.S.

Continued

TABLE 9.6: (Continued)

Live Shoot dry wt.

Species	Pop.	b	S.E. of b	Sig. of 1 - b	Sig. of 0 - b
Poa pratensis	O.S.	0.057	0.211	***	N.S.
	P.P.	-0.119	0.139	***	N.S.
Phleum pratense	O.S.	0.625	0.105	***	***
	P.P.	0.232	0.233	**	N.S.
Festuca rubra	O.S.	0.522	0.361	N.S.	N.S.
	P.P.	0.078	0.208	***	N.S.
Lolium multiflorum	O.S.	0.046	0.489	N.S.	N.S.
	P.P.	0.096	0.458	N.S.	N.S.
Lolium perenne	O.S.	0.790	0.281	N.S.	**
	79	-0.140	0.265	***	N.S.
	80	-0.058	0.177	***	N.S.

Dead/Live Shoot

Poa pratensis	O.S.	0.301	0.144	***	N.S.
	P.P.	-0.314	0.323	***	N.S.
Phleum pratense	O.S.	0.418	0.168	**	*
	P.P.	0.068	0.215	***	N.S.
Festuca rubra	O.S.	0.031	0.168	***	N.S.
	P.P.	-0.058	0.135	***	N.S.
Lolium multiflorum	O.S.	0.089	0.357	*	N.S.
	P.P.	0.674	0.314	N.S.	*
Lolium perenne	O.S.	0.138	0.149	***	N.S.
	79	-0.026	0.172	***	N.S.
	80	0.336	0.169	***	N.S.

Root dry wt.

Poa pratensis	O.S.	-0.171	0.183	***	N.S.
	P.P.	-0.001	0.161	***	N.S.
Phleum pratense	O.S.	0.262	0.133	***	N.S.
	P.P.	0.261	0.321	*	N.S.

Continued

TABLE 9.6: (Continued)

Root dry wt.

Species	Pop.	b.	S.E. of b	Sig. of 1 - b	Sig. of 0 - b
Festuca rubra	O.S.	0.876	0.524	N.S.	N.S.
	P.P.	0.150	0.340	*	N.S.
Lolium multiflorum	O.S.	0.373	0.379	N.S.	N.S.
	P.P.	-0.578	0.300	N.S.	N.S.
Lolium perenne	O.S.	0.308	0.214	**	N.S.
	79	0.251	0.183	***	N.S.
	80	0.193	0.177	***	N.S.

Root/Live Shoot

Poa pratensis	O.S.	-0.108	0.189	***	N.S.
	P.P.	-0.220	0.150	***	N.S.
Phleum pratense	O.S.	1.040	0.350	N.S.	**
	P.P.	-0.087	0.344	**	N.S.
Festuca rubra	O.S.	-0.290	0.544	*	N.S.
	P.P.	0.023	0.599	N.S.	N.S.
Lolium multiflorum	O.S.	0.888	0.330	N.S.	*
	P.P.	0.679	0.539	N.S.	N.S.
Lolium perenne	O.S.	1.040	0.254	N.S.	***
	79	-0.598	0.206	***	**
	80	-0.685	0.297	***	*

Total Plant dry wt.

Poa pratensis	O.S.	-0.135	0.192	***	N.S.
	P.P.	-0.382	0.163	***	N.S.
Phleum pratense	O.S.	0.373	0.112	***	**
	P.P.	0.258	0.300	*	N.S.
Festuca rubra	O.S.	0.817	0.411	N.S.	N.S.
	P.P.	0.103	0.254	***	N.S.
Lolium multiflorum	O.S.	0.029	0.447	*	N.S.
	P.P.	-0.362	0.376	***	N.S.

Continued.

TABLE 9.6: (Continued)

Total Plant dry wt.

Species	Pop.	b	S.E. of b	Sig. of 1 - b	Sig. of 0 - b
Lolium perenne	O.S.	0.413	0.259	*	N.S.
	79	0.330	0.229	**	N.S.
	80	-0.049	0.162	***	N.S.

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

are probably more sensitive to chronic SO_2 injury. Furthermore they suggest that this effect is greater in the Philips Park population than the original seed. This decrease in gradient of the Philips Park population may be due to the elimination of small, susceptible phenotypes by selection. In some cases (e.g. *L. multiflorum* live shoot dry wt) the slope was not significantly different from 0 or 1. This suggested the SO_2 treatment had caused disruption of the growth of the population in a way which was not related to growth in clean air.

There are several possible explanations for the occurrence of the relationship of chronic SO_2 injury with plant growth:

1. There may be some genetic/physiological linkage between SO_2 tolerance and growth. In this case the reduced growth of the tolerant phenotypes could be considered as one of the 'costs' of tolerance (ref. Chapter 10).

2. Larger plants are more likely to become pot bound. There is some evidence that this constraint on root growth may modify the response to SO_2 in promoting senescence (Davies, 1980b).

3. Faster growing and therefore larger plants may be more sensitive to SO_2. However, the present published evidence suggests

that slowly growing plants are more susceptible to chronic injury (Davies, 1980).

4. Large plants may offer shelter to the smaller phenotypes from wind and air turbulence resulting in an increased ra of the latter, thus increasing their resistance to SO_2 uptake.

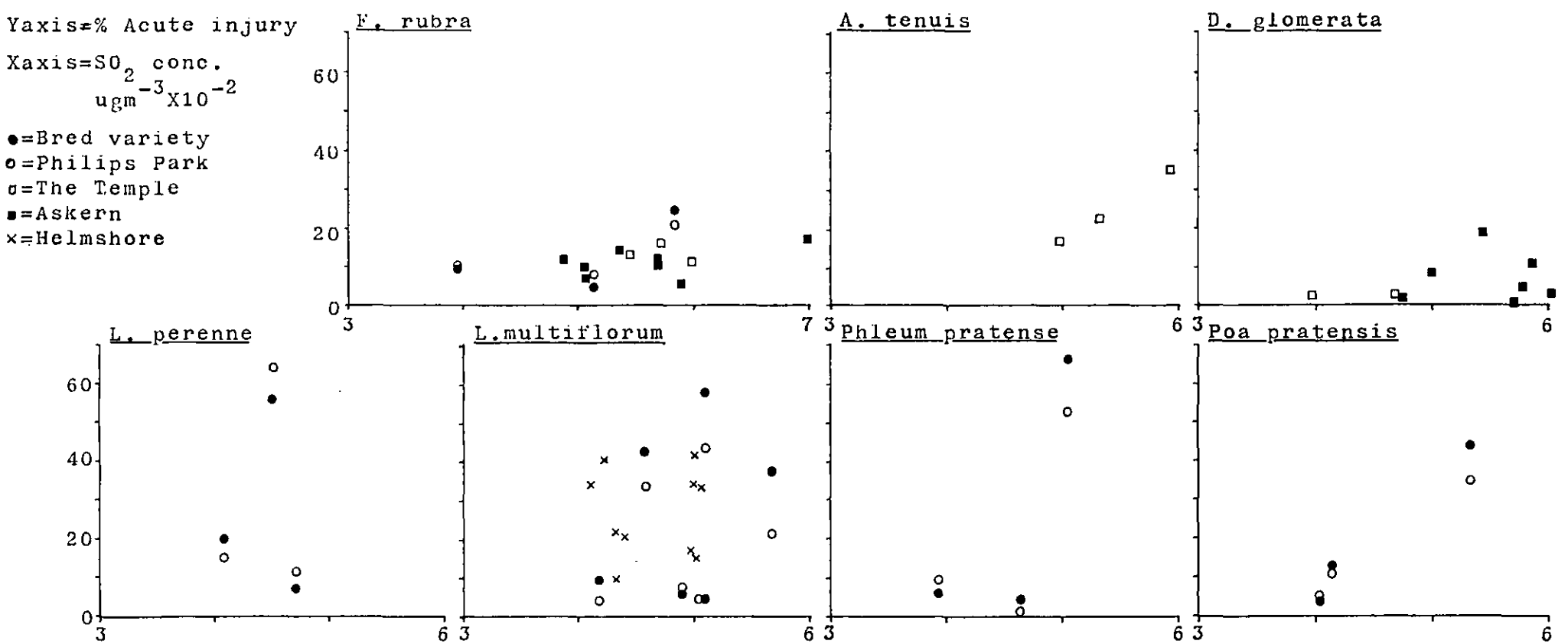
9.2 *Acute SO_2 Injury*

In order to detect any trends of dose-response and study interpecific acute SO_2 tolerance, the mean % acute injury of each fumigation, was plotted against SO_2 concentration for each species (Fig. 9.2).

Only two species exhibited any form of relationship, *Agrostis tenuis* and *Poa pratensis*, but in both cases this was only based on 3 fumigations and could not be considered reliable. The difficulty of classifying the relative tolerance of individual species was largely due to the variability of the mean response to individual SO_2 concentrations. For example, *L. perenne* cv. S23 fumigated on one occasion with 4500 μgm^{-3} SO_2 suffered a 43% leaf length injury, but on another occasion with 5000 μgm^{-3} SO_2 only 5% was injured. Since acute SO_2 experiments are carried out in an environmentally controlled chamber, these differences are probably the result of differences in sensitivity caused by environmental conditions in the greenhouse prior to fumigation.

F. rubra and *D. glomerata* were relatively tolerant to acute SO_2, showing a maximum injury of less than 25%, while *L. perenne, L. multiflorum* and *Phleum pratense* suffered as much as 60% leaf length injury. It should be noted that the *D. glomerata* fumigations did not include any non-tolerant populations or cultivars and in these cases may be more tolerant. The high tolerance of *F. rubra* contrasts with the

Fig. 9.2 Dose Response of Populations to Acute SO_2 Fumigation for Each Species.



findings of Brennan and Halisky (1970), who found that the cultivar "Pennlawn" was the most sensitive to acute SO_2 injury, of 11 turfgrass cultivars screened with similar SO_2 concentrations (5148 μgm^{-3} SO_2 6 hrs). However, the present results are in agreement with Ayazloo et al (1980), who also employed cv. "Engina", in which *F. rubra* was the most tolerant of 6 turfgrass cultivars fumigated with 5320 $\pm$ 140 μgm^{-3} SO_2 for 6 hrs. The classification of *Phleum pratense* and *L. multiflorum* is in agreement with Ayazloo et al (1980), but *L. perenne* has been classified as intermediate (Ayazloo et al 1980) or even highly tolerant (Brennan and Halisky, 1970). However, in the case of the latter, the commercial cultivars "Manhatten" and "Lamora" were used, which are known to be relatively tolerant.

Poa pratensis has previously been considered a species of intermediate acute tolerance on the basis of fumigations of "Deta" and "Merion" cultivars (Benedict and Breen, 1955; Brennan and Halisky, 1970), but on the basis of cv. "Baron", as highly tolerant (Ayazloo et al, 1980). In the present fumigations acute injury as great as 45% was observed, so this species can only be classified as intermediate in tolerance.

One further species, *A. tenuis*, was fumigated on 3 occasions using The Temple populations. These generally suffered greater injury than the *F. rubra* and *D. glomerata* populations, indicating this species to be probably low to intermediate in tolerance.

The collation of these observations provides a tentative order of increasing acute tolerance: *L. multiflorum/Phleum pratense/L. perenne* < *Poa pratensis, (A. tenuis?)* < *(D. glomerata?)/F. rubra* . This generally

agrees with the order of tolerance shown by Ayazloo et al (1980), the differences being that *L. perenne* and *Poa pratensis* were more tolerant in the present work: *Phleum pratense* < *L. multiflorum* < *L. perenne* < *Poa pratensis/F. rubra.*

The results of Brennan and Halisky (1970) showed far less agreement with the present work than the studies by Ayazloo et al (1980). This may be attributable to the use of different cultivars and emphasises the importance of intraspecific differences. It may also be significant, that they used different fumigation procedures.

9.3 *The Relationship of Acute and Chronic SO_2 Injury*

In much of the early and even in some recent work (West, McLaughlin and Shugart, 1980) on the effects of SO_2 on plants it has been assumed, that assessment of visible injury could be used to predict growth responses. Thus plants were generally subjected to acute SO_2 fumigations when screening for SO_2 sensitivity in the field. However, recently it has been shown, in several grass species, that there is a complete lack of correlation, between chronic and acute SO_2 injury (Horsman et al, 1979; Ayazloo and Bell, 1981). Table 9.7 gives the correlation coefficients between % acute injury and % reduction of total plant dry wt. in SO_2 compared with clean air for screening experiment 1, the Askern transect and the Philips Park species: none of these were significant. This suggests that the reaction of plants to high and low concentrations of SO_2 may be controlled by independent mechanisms.

The "accidental" acute exposure of the Philips Park populations (Section 3.3.2) during chronic fumigation, provided further evidence of the lack of relationship between chronic and acute injury: response

TABLE 9.7: Correlation Coefficients Between the Levels of Sensitivity to Acute and Chronic SO_2 Injury for Several Grass Species.

Experiment	Species	r	n
Screening 1	*L. perenne*	0.015	93
Askern Acute 1	*F. rubra*	-0.002	230
Acute 2	*F. rubra*	0.013	230
Philips Park	*Poa pratensis*	0.053	54
	Phleum pratense	0.042	50
	F. rubra	0.109	43
	L. multiflorum	0.087	41
	L. perenne	0.046	76

to chronic fumigation did not correlate significantly with acute fumigation, even though this involved the same plants (Fig. 3.1). The difference in the rates at which chronic and acute SO_2 tolerance developed in the *L. perenne* population at Philips Park is further evidence of independent mechanisms.

There is no correlation between the two types of SO_2 injury on an interspecific basis. *F. rubra* was the most tolerant to acute levels of SO_2, but is the most sensitive to chronic. *L. multiflorum* and *Phleum pratense* were both highly sensitive to acute fumigations, but tolerant to chronic. This is consistent with the observations of Ayazloo and Bell (1981) who showed similar reversals in the order of SO_2 tolerances of species.

The results clearly demonstrate the danger of using fumigations with high concentrations of SO_2, to predict the effects of the moderate levels which are believed to be reducing plant growth in polluted areas of Britain (Crittenden and Read, 1978, 1979; Bell et al, 1979) and for the identification of cultivars suitable for growth in these areas.

Chronic fumigations are time consuming and involve substantial effort, while the use of high SO_2 concentrations allows the assessment of a large amount of material in a relatively short time. The problem is accentuated by the greater within genotype variability of chronic injury compared to acute injury, so that a large number of replicates must be employed in order to detect differences. This difference in variability may be partially due to the nature of the two injury parameters (leaf length and dry weights of plant parts), but it must also

be due to the method of assessment. Chronic fumigations may last over several months compared to only a few hours for acute, thus allowing more time for variability to be expressed. Furthermore acute fumigations are often carried out under environmentally controlled conditions, while the conditions in a chronic fumigation chamber are only partially controlled in most cases, including all examples in the present work. Therefore it is evident that there is a necessity for a rapid and accurate method of estimating the susceptibility of plants to chronic SO_2 injury.

The investigation of fluorescence and reflectance in respect to chronic fumigation showed one possible line for further research. Although reflectance showed no close relationship with chronic injury, a significant regression, accounting for over 30% of variation, was obtained for the parameters of fluorescence, which is related to the transfer of energy between photosystems, and the root/live shoot ratio. However, the employment of this to predict chronic SO_2 susceptibility needs an improved definition of the relationship before it can be considered to be reliable.

9.4 *Conclusions*

It is well known that atmospheric SO_2 pollution may cause damage to grass species (Bleasdale, 1973; Crittenden and Read, 1978, 1979) and it is predicted, that this may result in economic loss both to agriculture and amenity grassland management. The aim of most fumigation experiments is primarily to define the relationships of this gas with plant growth. In much of this work, the present being no exception, it has been difficult to establish such relationships because of the variability of response, which is highly sensitive

to environmental and genotypic differences. However, it is evident that SO_2 acts by causing reductions in leaf size, tiller initiation and root growth and increases senescence. Each of these parameters appears to vary, to some extent, independently with environmental conditions and genotype. As information has accrued, some relationships have been established. A dose-response curve has been constructed, which has enabled the determination of a threshold concentration, although at present this has a very large error value. This error must be a reflection of environmental and genotypic influence. Chronic SO_2 injury appears to be associated with inherent growth rate and fluorescence, but there is increasing evidence that it is not related to acute injury and that there are thus independent mechanisms for the two types of injury.

Of major importance in the study of SO_2 effects is the threshold level of SO_2 injury and its economic importance. Further work is needed to define this constant more precisely and to obtain similar values for other species. The difficulty of establishing the economic importance of SO_2 pollution arises from extrapolating laboratory conditions to the field. It is hoped that open-air fumigation systems may solve some of these problems although they will need close environmental monitoring and every fumigation will be unique. In the present experiments the concentrations of SO_2 used were generally higher than those in the field. These were designed to cause differential effects between tolerant and non-tolerant phenotypes. Thus the effects observed might be expected to be higher than those in the field. However, there is a certain amount of evidence that the effects of SO_2 are more severe in the field than in chamber experiments using similar concentrations, because of the presence of other gases, fluctuations in gas levels and adverse environmental conditions (Davies, 1979; Jones and Mansfield, 1981; Mansfield

and Freer-Smith, 1981).

CHAPTER 10

DISCUSSION OF SO_2 TOLERANCE

It is well known that any environmental factor which has adverse effects on plant growth, may exert selection pressure. It is also evident that localised variation of these factors may promote the process of population differentiation, by acting on their inherent genetic variability. Tolerance to SO_2 pollution provides a useful example of this process, involving two separate adaptive mechanisms: acute and chronic SO_2 injury tolerance.

In the present study, high acute SO_2 tolerance was demonstrated at all field sites and in four grass species: *Phleum pratense*, *Lolium perenne*, *Agrostis tenuis* and *Festuca rubra*. Tolerance to chronic SO_2 concentrations in *L. perenne* was observed at Philips Park only. Both *L. perenne* and *F. rubra* have been shown previously to have the ability to develop differential intraspecific SO_2 tolerance (Horsman et al, 1979; Ayazloo and Bell, 1981). These results contribute to the increasing amount of evidence which suggests that SO_2 tolerance is widespread in grasslands in polluted areas of the U.K. (Bell and Clough, 1973; Bell and Mudd, 1976; Horsman et al, 1979; Ayazloo and Bell, 1981).

Certain populations from polluted sites, showed no indications of differential tolerance: *Dactylis glomerata* at The Temple (acute injury); *F. rubra* and *L. multiflorum* at Philips Park (acute injury); *F. rubra* and *Phleum pratense* at Philips Park (chronic injury). Some possible explanations for this are:

1. These populations may not have possessed the necessary genetic variability, as seems to be the case for heavy metal tolerance in all populations of *L. perenne*. However, tolerance to acute and chronic SO_2 injury has been shown in other populations of *D. glomerata* and *F. rubra* (Ayazloo and Bell, 1981).

2. Some species or bred cultivars may be innately tolerant to the prevailing ambient SO_2 concentrations, at their sites of collection, in which case SO_2 injury would not be an important selective factor. Possible examples in the present work are: *F. rubra* c.v. "Engina" at Philips Park and *D. glomerata* at The Temple.

3. It is possible that insufficient time has elapsed for detectable differential SO_2 tolerance to evolve, especially in the case of Philips Park.

4. In the case of very small or very large amounts of injury being produced by the SO_2 fumigation, the differential tolerance may have been obscured: this could possibly be the case for *F. rubra* at Philips Park, which suffered 60% growth reduction after chronic fumigation. However, it should be noted that differential acute tolerance was detectable, between the OS and PP populations of *L. perenne* with 10 and 4% leaf length injury respectively in one case and, 59% and 43% leaf length injury in another.

A large variation of response, to acute and chronic SO_2 fumigation, was observed in non-tolerant populations, indicating the probable presence of tolerant genotypes. Further evidence of this was provided by the development of tolerance in the Philips Park populations of *Phleum pratense* and *L. perenne*. These populations

were grown from non-tolerant commercially available seed, under a management regime which permitted only negligible further genetic input, suggesting that tolerant genes were already present in the seed. Thus it seems that non-tolerant grass populations may have the genetic variability to develop SO_2 tolerance under selection pressure by the pollutant, without an influx of tolerant genes.

The speed with which a genetically controlled character spreads within a population has been of interest to evolution ecologists for some time. Probably the best documented character in this respect, is heavy metal tolerance. It has been shown that the genes for such tolerance may exist in a non-tolerant grass population at a rate of 1-2% (Walley et al, 1974; Gartside and McNeilly, 1974) and that a heavy metal contaminated soil, can become colonised within a period of 5 years (Wu et al, 1975). It might be expected that evolution of heavy metal tolerance would be faster than that of SO_2 tolerance, considering that the levels of heavy metals in soils at many contaminated sites tend to be lethal to non-tolerant genotypes, while the selection pressure imposed by SO_2 pollution stress is unlikely to cause mortalities directly and may not be continuous, especially at acute concentrations.

Previous investigations of acute and chronic SO_2 tolerance, have revealed both forms of tolerance in *L. perenne* plants from a lawn established 17 years earlier. In the present work the *L. perenne* population at Philips Park developed tolerance to acute and chronic SO_2 within $3\frac{1}{2}$ and $4\frac{1}{2}$ years, respectively, while *Phleum pratense* was shown to develop acute tolerance only, after $4\frac{1}{2}$ years. Two estimates of the rate of evolution of acute SO_2 tolerance were obtained from

the site histories : 30 years for *A. tenuis* at The Temple and 50 years for *F. rubra* at Askern, the latter also having been demonstrated by Ayazloo and Bell (1981).

It is evident from the Philips Park work, that chronic and acute SO_2 tolerance may develop at different rates in the same species, and furthermore, that this rate varies between species. The rate of selective progress for any character in any species, will depend on its genetic qualities, the contribution of non-heritable agents to the progeny variation and the intensity of selection.

The availability of genetic variability for a character in a population, limits the extent of possible adaptation to the relevant selection pressure. This probably accounts for the lack of resistance to heavy metals and herbicides in most species. However, intra-specific differences in tolerance to SO_2 and other gaseous pollutants seems to be common in most species (Roose, Bradshaw and Roberts, 1982). Genetic variability can affect the degree to which stress causes differences in fitness between individuals, and thus may affect the rate of selection. *L. perenne* c.v. S23 is well known for its wide variability, due to its origin from a large number of standard genotypes, unlike most modern cultivars which are based on only a few. This heterogeneity may explain, to some extent, the rapidity with which the *L. perenne* population at Philips Park, has adapted to the local ambient SO_2 concentrations.

The effectiveness of selection will, to some extent, increase with decreasing numbers of segregating genes (Mather and Jinks, 1971).

The genetic analysis carried out on 5 clones from Helmshore suggested that acute SO_2 tolerance in *L. perenne* was controlled mainly by only a few genes, which is therefore consistent with a rapid development of tolerance, as at Philips Park. However, the diallel analysis also indicated a large dominance component towards sensitivity to acute SO_2 injury, which would tend to decrease the rate of selection. This is more likely to be important in plants in which the genetic composition of the population is changed by recombination every year. SO_2 tolerance exhibits a large non-heritable component of variation. This will tend to cause the phenotypic expressions of tolerance within a population to overlap, thus reducing the efficacy of selection to distinguish between genotypes: the continuous range of degree of SO_2 injury observed in most populations may be a reflection of this.

Gene flow may increase or decrease the rate of development of tolerance within a population, by the influx of tolerant or non-tolerant genes, respectively. In general, it is unlikely that gene flow will retard the development of SO_2 tolerance, because SO_2 pollution tends to cover fairly large areas with diffuse boundaries, whereas gene flow only occurs at high densities over distances of a few metres. Gene flow, however, may contribute towards the spread of SO_2 tolerance: at Philips Park this may have occurred from adjacent lawns, which are known to be tolerant to acute and chronic SO_2 injury (Ayazloo and Bell, 1981); at Askern the prevailing westerly wind would tend to promote the flow of tolerant genes along the transect, away from the Coalite factory.

Sexual reproduction is necessary for the process of gene flow and the mode of inheritance to influence the rate of evolution. The

difficulty of seedling establishment in a closed sward and/or the low degree of flowering under a mowing regime, suggest that the mode of inheritance and gene flow play only a small, possibly negligible role at most of the sites investigated. This will tend to limit the genetic variability to that available when the plants were established and thus restrict evolutionary processes to those mediated by growth and survival of genotypes.

Probably the most important factor determining the rate of evolution, is the intensity of selection. In the case of SO_2 pollution this must ultimately depend on its toxicity to the organism. This has been shown to vary between and within species, at all field sites in the present and previous studies (Ayazloo, Bell and Laycock, 1980; Ayazloo and Bell, 1981). Concentration and duration of exposure are primary in determining toxicity, especially for SO_2 pollution, in which the magnitude of these parameters specifies the form of injury: chronic or acute. The Askern cline shows the adaptive response of grass populations subject to a range of SO_2 concentrations, which are assumed to be decreasing with distance, from a point source. This resulted in a clear clinal pattern of acute SO_2 tolerance for *F. rubra:* however, acute tolerance was not shown for *D. glomerata,* nor was chronic SO_2 tolerance clearly demonstrated in *F. rubra.* The steepest point of the cline was between the populations closest to the source and therefore presumably, subject to the greatest stress from SO_2 pollution. If the populations are all of a similar age (50 yrs), this would suggest that evolution has been more rapid in the populations subjected to the greatest selection pressure.

It must be noted, that the highest SO_2 concentration will not

necessarily give the greatest selection intensity. If injury is too small or great, there may be no selective advantage. The greatest intensity of selection will occur, where the differences in fitness, between tolerant and non-tolerant phenotypes, are maximised.

Competition is a factor which may increase the intensity of selection and therefore the rate of evolution. Most of the sites investigated had good ground cover and since their ambient SO_2 concentrations are not lethal to non-tolerant phenotypes, the establishment of tolerant populations must involve a significant amount of competition.

Competition between individuals is known to increase in intensity with increasing similarity, as a result of greater demands made on the same resources. Therefore it is likely, that where species diversity is small, competition is high. The Philips Park plots were sown as monocultures. It is likely that intraspecific competition was intense under these conditions and may have contributed to the rapid selection of chronic or acute SO_2 tolerance, in populations of *L. perenne* and *Phleum pratense*.

The wide range of injury response to acute and chronic SO_2 fumigations, observed in tolerant populations, seems to be typical of wild populations from polluted areas (Taylor, 1978; Horsman et al, 1979; Ayazloo and Bell, 1981). Much of this variation may be attributed to non-heritable sources, but the clear separation of clones on the Wr/Vr regression (Fig. 6.1a), indicates that in the Helmshore population of *L. perenne*, at least some of the variation was accountable to genetic differences. Thus there is evidence of the survival of relatively non-tolerant genotypes in an acute tolerant population, in SO_2 polluted

environment. This heterogeneity may be expected if the populations are still actively developing SO_2 tolerance, which is very likely in the case of Philips Park.

The maintenance of apparently disadvantageous phenotypes in the tolerant populations, may be explained by the relative fitness of individuals. Wu et al (1975) reported considerable variation of copper tolerance in *Agrostis stolonifera* populations growing on contaminated soil, for which one explanation was niche diversity. This may occur where the habitat is heterogeneous and each plant is adapted to its own micro-habitat. However, this seems unlikely on the apparently homogeneous, experimentally prepared plots of Philips Park. It is also possible that plants of differing phenotypes may survive as a result of reduced competition, due to different demands on the environment (Hayward, 1970). An example of this is the development of tolerance to acute and chronic SO_2 injury in different genotypes of the same population, evident in the non significant acute/chronic SO_2 injury correlations of *L. perenne* at the Philips Park plots and also shown elsewhere for several species by Ayazloo and Bell (1981).

An alternative explanation for the presence of phenotypes non-tolerant to SO_2 injury in a tolerant population is frequency dependence, which may arise when tolerant phenotypes suffer more from competition between their own tillers, than from non-tolerant phenotypes. As stated previously, competition tends to be more fierce between increasingly similar individuals, due to demands on the same resources. Therefore as tolerant phenotypes become more frequent, they tend to dissipate much of their competitive fitness through internal competition.

Relationships have been demonstrated between plant growth (ref Section 9.1), leaf fluorescence (ref Section 8.2) and chronic SO_2 injury, suggesting that chronic tolerant plants tend to be smaller, have a reduced overall fluorescence and reduced ability to transfer electrons between the photosystems (the "Spill Over" effect). It was suggested that such relationships if defined more precisely, would be of value for the rapid detection of tolerant and non-tolerant phenotypes, without prolonged fumigation with moderate concentrations of SO_2. The existence of these relationships raises several interesting points, assuming that they are genetically controlled:-

1. The reduction of "Spill over" efficiency and yield in chronic SO_2 tolerant plants may be a cost factor. Bell (1983) has presented evidence showing that a low growth rate of a population is not a prerequisite for SO_2 tolerance, for example: the growth of a chronic tolerant population of *Phleum bertolonii* from a polluted area originating from a bred cultivar was greater than the growth of a non-tolerant sample of the same cultivar, in clean air. However, this does not necessarily imply that chronic SO_2 tolerance does not involve a cost in growth. The relationship of plant growth and chronic SO_2 tolerance can be considered in a simple model in which only 4 phenotypes are possible: small, tolerant; large, non-tolerant; small, non-tolerant; large, tolerant. The existence of the former two phenotypes in a grass population is justified by the significant regressions of plant growth against chronic SO_2 injury (Table 9.3.6). It is not unreasonable to assume that in a non-tolerant population small, non-tolerant phenotypes may also occur, but that large, tolerant phenotypes are rare or non-existent. Under stress from SO_2, it could be hypothesised, that the small, non-tolerant phenotypes are likely to be selected out. This will result in a

simultaneous increase in the average growth and tolerance of the population. It would also cause a reduction of slope of the regression as observed between the PP and OS populations (Table 9.6). A decrease in the average growth of the population may occur, if the ambient SO_2 concentrations cause differential fitness between the large, non-tolerant phenotypes and small, tolerant phenotypes, with the subsequent elimination of the former. Thus the present evidence supporting this hypothesis suggests there is a cost, in plant growth, of chronic SO_2 tolerance, but that this is not necessarily expressed in the population means.

2. The existence of large, non-tolerant phenotypes and small, tolerant phenotypes together in a population, suggests that they are competitive under the local environmental conditions.

Similar arguments to those above could be made for leaf fluorescence, although the competitive importance of the physiological or biochemical disturbances that this reflects is unknown.

The detection of SO_2 tolerant populations, has so far been restricted to polluted areas. This suggests that genes for SO_2 tolerance have not spread in unpolluted areas, possibly because the phenotypes are at a selective disadvantage. Assuming plant growth and possibly "spill over" to influence fitness, their relationships with chronic SO_2 tolerance provide indirect evidence that *L. perenne* tolerant to chronic SO_2 injury, is less fit in clean air than non-tolerant genotypes of the same species.

During the past 20 years a steady decline has taken place in the levels of ambient SO_2 in the United Kingdom (ref Chapter 1). This reduction is evident in the National Survey reports for 3 of the sampling

sites used in the present experiments (ref Section 2.3): The Temple, Helmshore and Philips Park. Askern has not shown this decrease, because it is influenced primarily by a single low level point source, whose emissions have remained fairly constant. At Philips Park it was observed, that SO_2 tolerance disappeared in the *L. perenne* population between 1980 and 1981. It was considered that this might be a result of the reversal of selection due to the local decrease in ambient SO_2 concentrations. If this is so, it would be of great interest to investigate sites such as Helmshore, where pollution levels are now very low, but where tolerant populations have been found in the recent past (i.e. 1975 Ayazloo and Bell, 1981).

There are many examples of relatively rapid evolution of tolerance to pollutants which cause severe damage (Antonovics, Bradshaw and Turner, 1971; Ryan, 1970). However, the speed of development of chronic SO_2 tolerance in *L. perenne* at Philips Park is remarkable, when considering the relatively small effects of moderate SO_2 concentrations on plant growth. It is generally considered that acute SO_2 concentrations rarely occur in Britain and the majority of research is currently concerned with chronic SO_2 effects. The present evidence shows, that *L. perenne* and *Phleum pratense* populations from Philips Park have developed acute SO_2 tolerance more rapidly than chronic SO_2 tolerance. It follows that the nature of the ambient SO_2 pollution at Philips Park has in recent years, been sufficiently debilitating to commercial cultivars of *L. perenne* or *Phleum pratense* to cause the selection of tolerant populations. Acute tolerance was detected at all the other sites investigated in the present work: The Temple, Askern and Helmshore. This evidence indicates that both acute and chronic

injury may be widespread in herbage and sports grasses, in polluted areas. Contemporary reports of visible SO_2 injury in populations of grasses in polluted areas are virtually unknown. One possible explanation is that "subliminal" acute injury may occur, in which an acute SO_2 incident is not severe enough to cause the visible symptoms associated with acute injury, but produces sufficient biochemical or cytological disturbance to be selective. There are a great many reports describing the biochemical and cytological effects of acute SO_2 concentrations on plants (Ziegler, 1973, 1975; Horsman and Wellburn, 1976; Malhotra and Hocking, 1976). In many cases these could conceivably occur without the manifestation of necroses on the foliage. More recently Ayazloo, Garsed and Bell (1982) have shown acute SO_2 concentrations to cause increased membrane permeability to K^+, while solutions of sulphite of a greater concentration than $10^{-2.5}M$ caused a reduction of $^{14}CO_2$ fixation in leaf segments. However, it is not known whether these effects are large enough, at SO_2 concentrations below the threshold for visible symptoms, to be of a selective disadvantage. "Subliminal" acute injury may be linked to recent findings, demonstrating that ambient SO_2 levels cause a lowering of resistance to frost (Baker et al, 1982; Davison and Bailey, 1982). Frost, after SO_2 treatment, caused senescence at the tips of leaves, giving these a waterlogged translucent appearance. It was considered that these effects may greatly increase the effects of selection in response to SO_2.

The evidence of the genetic nature of acute SO_2 tolerance of *L. perenne* at Helmshore indicates that breeding for tolerance is possible. The low narrow sense heritabilities of acute tolerance, would

make this process relatively slow, but it may be possible to define some physiological characteristic, such as fluorescence, which may be more efficient for selecting genotypes. Furthermore it is evident that selection for tolerance may select incidentally for smaller plants, and efforts would have to be made to avoid a decline in yield. The only published report of a breeding programme for SO_2 tolerance is for *Pinus strobus* (Thor and Gall, 1978). However, plant breeders, working in polluted areas will inadvertantly select for tolerant cultivars, as seems to have occurred in the case of *L. perenne* c.v. "Manhatten" a cultivar based on clones from New York.

The work at Philips Park provides evidence, that for some species the breeding of tolerant cultivars may not be necessary. The rapid development of SO_2 tolerance in response to selection by ambient SO_2, may ameliorate the deleterious effects of the gas. However, the experiment was designed to find the minimum time for differential tolerance to occur and not the point at which the mortality and reduction in growth of non-tolerant clones, may be compensated by the growth of the survivors. It would be of considerable interest to conduct further research to establish this time. The maintenance of the "ceiling yield" (Harper, 1977) during the evolution of SO_2 tolerance would be of importance for agriculture and amenity grassland management.

SUMMARY

1. The development of SO_2 tolerance was investigated in several economically important grass species at 4 polluted sites in the U.K. The tolerance of grass populations to SO_2 was determined by comparison of growth in 300 - 350 μgm^{-3} SO_2 for several weeks with growth in clean air for chronic SO_2 injury, and measurement of % leaf length injured after exposure to 4000 - 5500 μgm^{-3} SO_2 for 6 hrs, for acute SO_2 injury.

2. The minimum time for the development of SO_2 tolerance was investigated at Philips Park, Manchester. Samples of *Lolium perenne*, *L. multiflorum*, *Festuca rubra*, *Poa pratensis* and *Phleum pratense* were collected annually, from plots sown in 1975 and compared for SO_2 tolerance with samples grown from the original seed which was sown. Acute tolerance was demonstrated in *L. perenne* and *Phleum pratense* after only 3½ years and 4½ years, respectively; chronic tolerance was evident in *L. perenne* after 4½ years.

3. The long term development of acute SO_2 tolerance in *F. rubra*, *Agrostis tenuis* and *Dactylis glomerata* was studied in a range of lawns of 1 to 200 years of age at The Temple, London. An evolutionary series was demonstrated in which the degree of acute tolerance of *F. rubra* populations increased with the age of lawn, presumably as a result of the length of exposure to the polluted ambient air. Similar temporal clines were not observed for *D. glomerata* and *A. tenuis*, although the latter exhibited differential tolerance between lawns.

4. The effect of a range of SO_2 concentrations on populations of

F. rubra and *D. glomerata* was investigated at 8 sampling sites along a transect east of a point source of SO_2, at Askern, Yorkshire. A distinct cline of increasing acute tolerance with proximity to the pollution source was evident in *F. rubra*, but not *D. glomerata*. A cline of chronic SO_2 tolerance was not detected. Comparisons between clinal patterns of plant growth and SO_2 injury were made, but few trends were evident.

5. The genetic control of acute SO_2 tolerance of *L. perenne* in 4 clones from Helmshore in Lancashire and the S23 res clone was examined, using a half diallel analysis. The interpretation of the results warranted a certain degree of caution, due to the small number of clones employed. However, it was found that acute SO_2 tolerance is heritable, with a significant additive component, but primarily controlled by only a few genes, with dominance towards sensitivity. Estimates of heritability were made with 95% confidence limits: narrow sense = 22.38% $\pm$ 8.99%; broadsense = 85.04% $\pm$ 4.97%.

6. Attempts were made to isolate tolerant and non tolerant phenotypes of *L. perenne*. Nineteen clones from Helmshore, replicated 5 times, showed no differential tolerance to chronic SO_2 injury, although high, low and intermediate acute tolerant phenotypes were detected. In a second chronic fumigation, in which 19 clones from 4 populations (Askern, Helmshore, c.v. S23, S23 res) were replicated 10 times, it was still not possible to distinguish phenotypes clearly.

7. The relationships between chronic SO_2 injury of *L. perenne* and two physiological characteristics measured by remote sensing techniques, were investigated with the view to establishing a possible quick

screening method for SO_2 tolerance. It was found that SO_2 treatment caused reductions in overall fluorescence, "Spill Over" fluorescence and reflectance at 800 nm, but an increase in reflectance at 545 nm. The changes in reflectance suggested structural changes in the leaf. The effects on fluorescence were discussed in relation to the effects of SO_2 on photosynthesis. The relationship FSS/FSS_2 = 0.963 - 0.213 (root/shoot) suggested that chronic tolerant plants have a less effective "spill over" mechanism. It was suggested that this relationship would need to be defined more accurately before use in detecting chronic tolerant phenotypes.

8. Information collected incidentally on the effects of SO_2 on grasses, during this study is discussed. Discrepancies were found in the rankings of chronic SO_2 tolerance of different species fumigated simultaneously: within the present work and also in comparison with previous results in the literature. *L. perenne* was found to be more tolerant to chronic injury than was generally believed previously. This suggests that chronic SO_2 injury may be more widespread and severe in the field than has been predicted on the basis of *L. perenne* being the grass species most sensitive to SO_2. Chronic SO_2 fumigation caused reductions in mean live leaf dry wt, tiller initiation, root dry wt. and increases in senescence. These responses varied in magnitude between species, populations, consecutive fumigations and the experiments of different research groups. A relationship indicating larger plants to be more susceptible to chronic injury was demonstrated. The following order of increasing acute SO_2 tolerance from the present study generally agreed with that proposed by Ayazloo, Bell and Laycock (1980): *L. multiflorum / Phleum pratense / L. perenne < Poa pratensis / Agrostis tenuis < Dactylis glomerata / Festuca rubra*. The present

work provided further evidence of the lack of correlation between acute and chronic injury, thus supporting the case for two independent injury mechanisms.

9. The present work contributes to the growing body of data indicating that SO_2 tolerance is widespread in the U.K. The development of SO_2 tolerance in polluted areas suggests that the local ambient levels of SO_2 are sufficiently debilitating to cause chronic and acute injury. Acute SO_2 injury has not often been observed in the field. Therefore the occurrence of 'subliminal' acute injury was proposed. The effects of genetic variability, gene flow, mode of inheritance, selection and competition on the rate of SO_2 evolution are discussed in the light of the present findings. The cost of SO_2 tolerance is considered, in view of the relationships of chronic injury with plant growth and fluorescence. Genetic analysis of acute SO_2 tolerance in *L. perenne* indicated the possibilities for breeding for acute tolerance. However, the rapid rate of evolution of tolerance observed at Philips Park, suggested that some species may be able to adapt naturally to local ambient SO_2 pollution.

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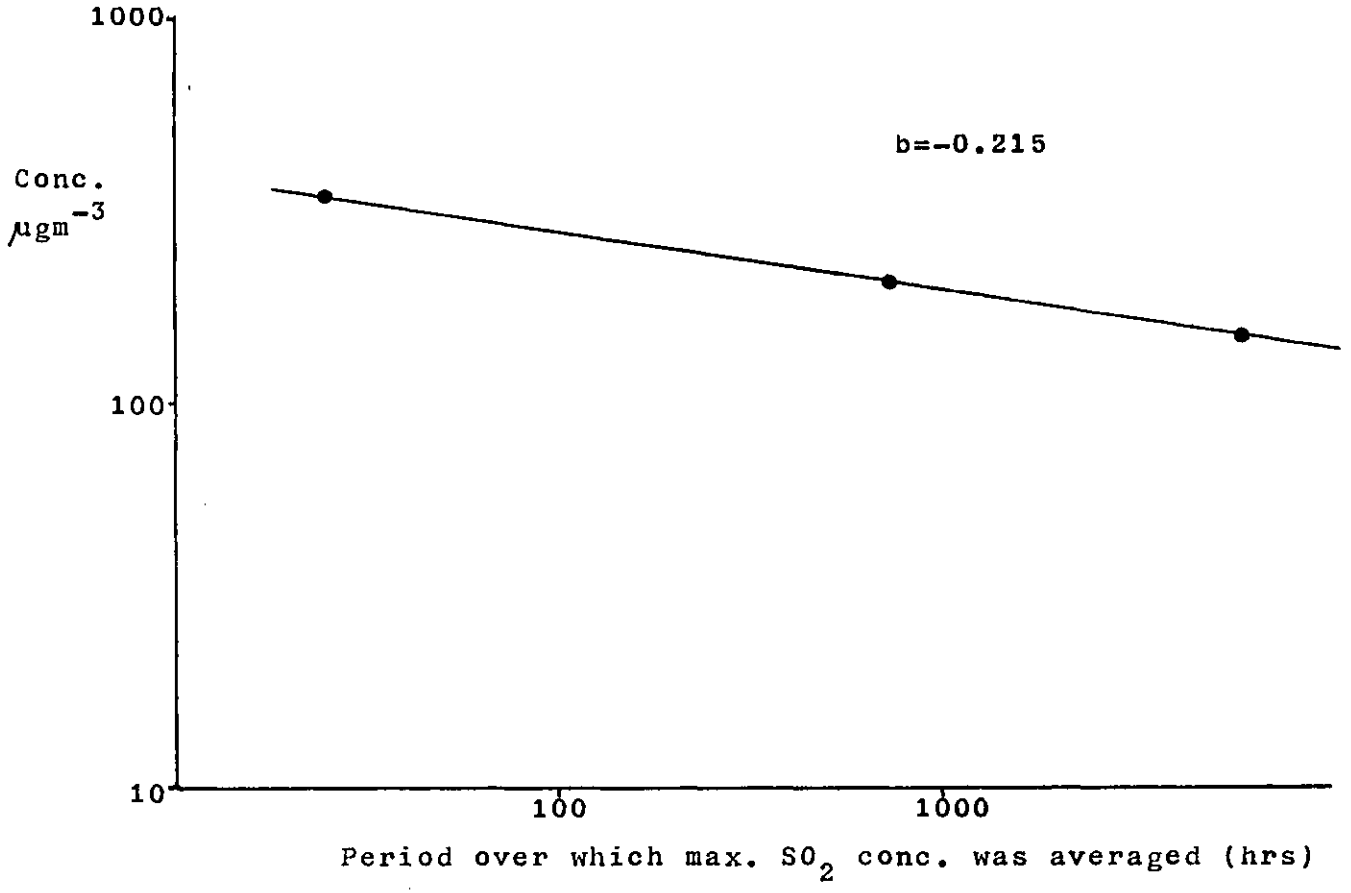
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Appendix 1 Regression of SO_2 Concentration Against Averaging Time for SO_2 Pollution Characteristics at Philips Park, as Employed in Section 2.3.3.



APPENDIX 2

Assessment of Acute Injury

In previous acute fumigations (Ayazloo and Bell 1981) the acute SO_2 injury was measured as the total % leaf length injury for every leaf produced over the period of 3 weeks growth after potting. This was found to be very time consuming and in the present experiments only the first three fully grown leaves were used, others being removed before fumigation. Two experiments were carried out to investigate whether this method gave similar results to the original method.

Comparison of Acute Injury Scoring Systems.

Method

Seventy five *Lolium perenne* plants from the Helmshore sample, were subjected to an acute fumigation at 4984 μgm^{-3} SO_2 for 6 hrs (ref Section 2.6.2), with all leaves and tillers intact. Three days after fumigation, the plants were scored for % acute injury on every leaf and tiller. Percentage leaf injury for the whole plant was compared with % leaf length injury of the original tiller.

Results and Discussion

A paired t-test ($t = 0.5603$) indicated no evidence of any significant deviation in measurement of acute injury between the two methods.

This demonstrates that both methods of measurement provide similar results. However, it must be noted that the plants were not cut before fumigation as they normally were, which may affect their performance during and after fumigation. Also there was a smaller total leaf area in the chamber which may affect the absorption of SO_2. It must be noted that the test was carried out for *L. perenne*, only and that it has been assumed, that these conclusions are valid for the other grass species investigated.

An Investigation of the Relationship Between Leaf Age of *Lolium perenne* plants and Acute SO_2 Sensitivity

It was decided to investigate the sensitivities to acute SO_2 fumigation of a range of ages of *L. perenne* leaves. This was in order to establish the possible effects of leaf age on the method of acute injury assessment.

Method

To ascertain the age of each leaf on each plant, the day on which the junction between the leaf blade and leaf sheath appeared was recorded as a convenient measure and termed the "sheaving date". This measure was chosen for two reasons: firstly it could be obtained quickly and without disturbance to the plant; secondly that this stage in the leaf cycle is the point at which the blade becomes fully exposed to the open air and thus to fumigation. In some cases the sheaf of the inner leaf did not grow as tall as the sheath covering it, causing difficulty when recording the leaf age. In these cases the leaf was excluded from statistical analysis.

Thirty individual tillers of each of the 1979 Philips Park population sample and original seed were potted with all fully grown leaves removed. On the following day and successive days any newly "sheathed" leaves were recorded between 09.00 and 10.00 hrs. Thus the age of each leaf was known. This procedure was continued until almost all the tillers had some senescent leaves and at least 3 live leaves.

On the 32nd day (16/10/80) after potting the plants, the lengths of senescent and "unsheathed" leaves were measured. The plants were then subjected to an acute fumigation (Section 2.6.2) of 5083 μgm^{-3} SO_2 for 6 hrs.

Results and Discussion

Comparisons between the percentage acute injury of the Philips Park population (43.3%) and original seed (58.5) showed the P.P. population to suffer significantly less injury (Table A2.1). This is the third repetition of the fumigation of the 1979P.P.population of *L. perenne* which showed the development of acute SO_2 tolerance.

A histogram with data from both populations of the frequency of new leaves per 3 days showed a rapid increase in the first week of the experiment with a gradual decline to a constant level after 4 weeks (Fig. A2,1). The final 3 days of the experiment after acute SO_2 fumigation showed a slight increase in the "sheaving" rate, possibly as a result of the SO_2 exposure or the environmental conditions of the fumigation chamber, although it is possible that this is a random fluctuation.

Throughout the experiment the frequency of "sheaving" was consistantly higher in the O.S. population than the P.P. population (Fig.A2.1) This was supported by a significantly greater mean rate of leaf production per 3 days in the O.S. population (10.5) than the P.P. population (8.0), but there was no difference in leaf lengths (Table A2.1). This suggests that the non-tolerant population has a faster more competitive growth rate than the tolerant plants when grown in clean air. The ratios of % leaf length injury to leaf production rate for P.P. and O.S. are almost identical (0.54 and 0.55 respectively). However, correlation of rate of leaf production with acute sensitivity to SO_2 was not signifcant for either population (P.P. $r = 0.030$, O.S. $r = -0.146$). Therefore the rate of leaf production does not seem to influence SO_2 sensitivity.

The main aim of the experiment was to establish the existence of any relationship between leaf age and acute SO_2 sensitivity. The regression

Fig.1 Rate of Leaf Production of O.S. and P.P. Populations.

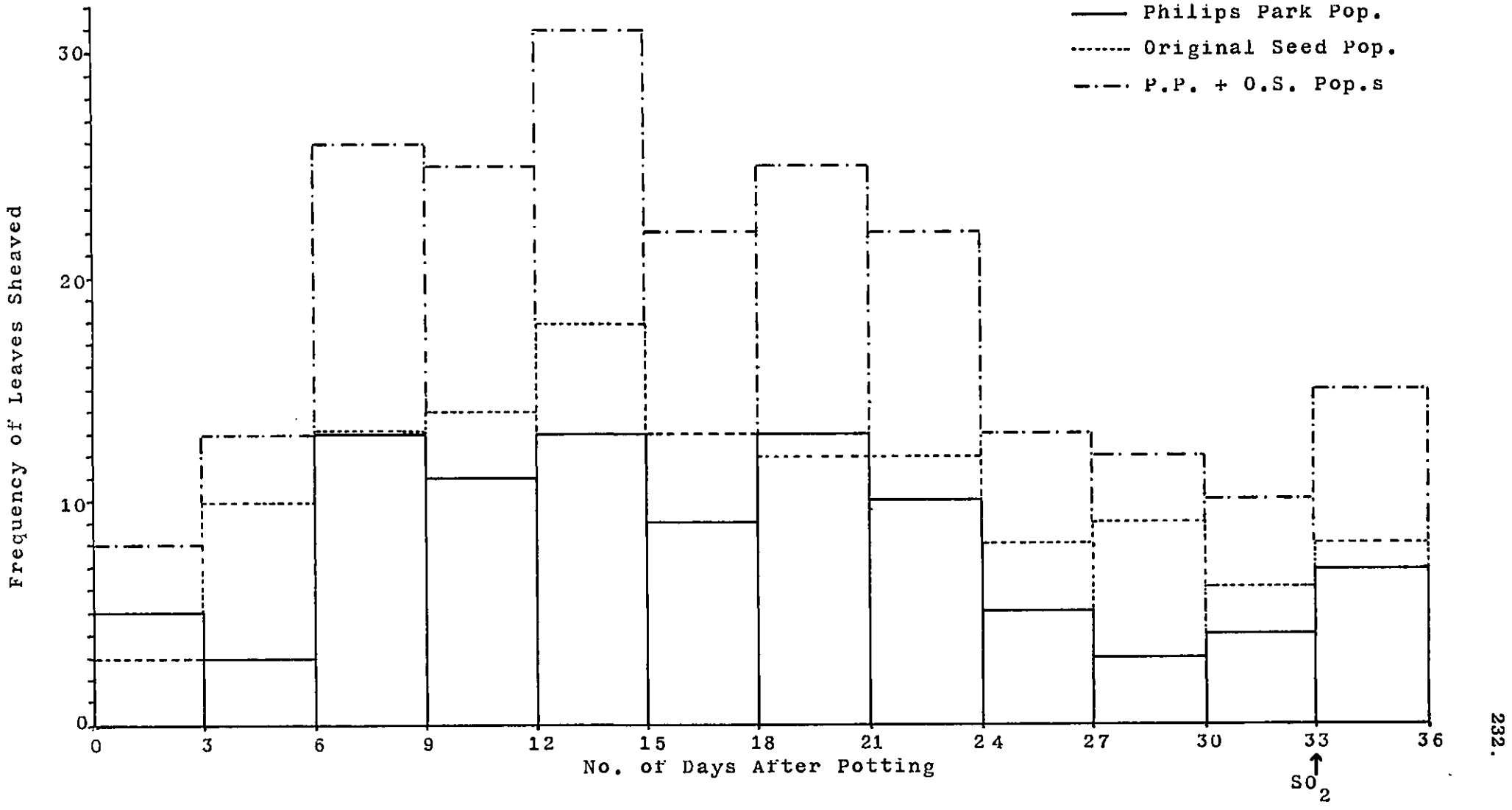


TABLE A2.1 Comparisons of Original Seed and Philips Park Populations

Characters	O.S.	P.P.	Level of Significance
% Leaf Length Injury	58.5	43.3	**
Mean leaf length	148.1	138.9	N.S.
No. of leaves produced per 3 days	10.5	8.0	**

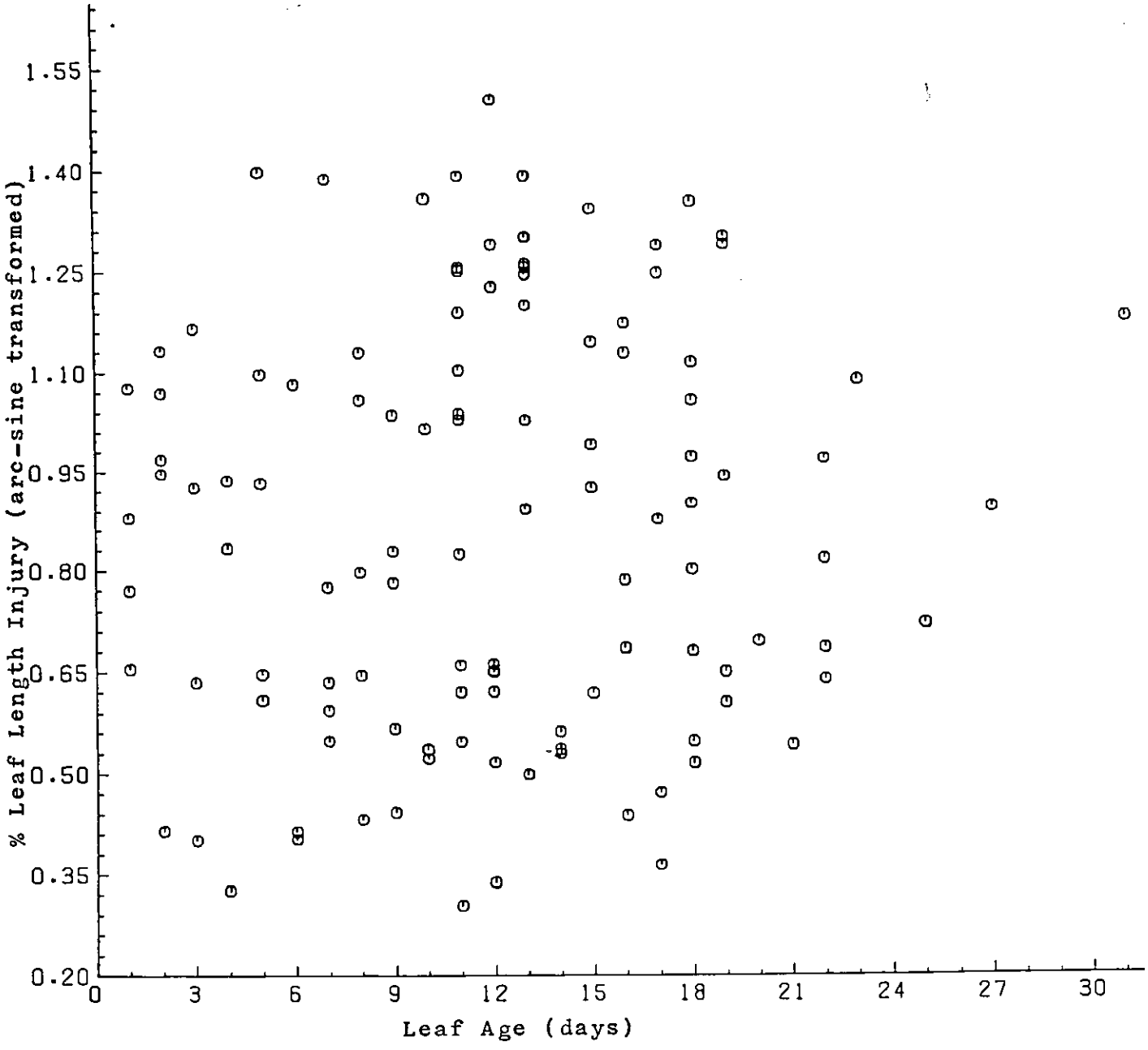
** = $P < 0.01$

line gradient was not significant from 0 ($y = 0.872 + 0.0062x$) and the two characters showed a random relationship with an R^2 of 0.0% (Fig. A2.2).

Thus it seems that there is no relationship between leaf age and acute SO_2 sensitivity in *L. perenne*, contrary to the findings of previous workers on other species: Craker and Starbuck (1973) showed that middle aged leaves of tobacco were more sensitive to acute SO_2 than young or old leaves. Similarly Smith and Davis (1977) have demonstrated that young needles of *Pinus sylvestris* are more resistant than old needles, which in turn are more resistant than intermediate.

Thus it is concluded that acute SO_2 has a similar degree of effect over most of the life cycle of a single leaf of *L. perenne* in both tolerant and non-tolerant populations and that assessment of acute SO_2 injury is not dependant on the age of leaves sampled.

Fig. 2 Relationship of Acute SO_2 Injury with Leaf Age.



Y=0.872+0.006X

b is n.s. from 0, t=1.0, df=65

Appendix 3. Mean Fluorescence Values of Leaves of *L. perenne* after Chronic Fumigation (Ref: Section 3.2).

Clone	Fss	e	Fss_2	e/Fss+e	$\frac{Fss+e-Fss_2}{Fss+e}$	Fss/Fss_2
Plants Grown in Clean Air:						
1	56.5	6.1	56.8	0.0952	0.0860	0.9920
2	54.0	6.3	54.5	0.1044	0.0929	0.9887
3	55.5	6.7	53.8	0.1050	0.1264	1.0272
4	56.3	6.7	58.1	0.1042	0.0710	0.9668
5	58.0	5.6	58.8	0.0855	0.0725	0.9880
6	52.2	4.9	51.4	0.0854	0.0966	1.0138
7	53.5	6.6	55.1	0.1097	0.0787	0.9682
8	57.8	7.3	60.6	0.1090	0.0675	0.9575
11	55.2	5.3	56.5	0.0881	0.0668	0.9779
12	62.7	6.6	63.4	0.0956	0.0838	0.9884
13	53.5	5.5	55.1	0.0906	0.0606	0.9697
15	62.0	6.5	64.6	0.0940	0.0571	0.9622
16	61.3	9.5	67.8	0.1305	0.040	0.9054
27	61.7	10.5	67.8	0.1470	0.0624	0.9103
41	63.6	8.1	64.5	0.1117	0.0966	0.9839
51	58.5	8.5	61.2	0.1260	0.0841	0.9565
58	58.2	8.1	59.2	0.1209	0.1046	0.9832
87	54.0	9.0	57.3	0.1368	0.0806	0.9421
S23	64.8	6.9	63.4	0.0952	0.1123	1.0215
Plants Grown in SO_2						
1	50.4	6.1	52.5	0.0952	0.0681	0.9591
2	57.6	5.3	56.7	0.1044	0.0895	1.0131
3	54.8	7.0	54.0	0.1050	0.1193	1.0135
4	55.5	6.4	58.5	0.1042	0.0542	0.9520
5	56.7	5.4	59.3	0.854	0.0419	0.9543
6	49.4	4.8	49.8	0.0889	0.0769	0.9892
7	46.1	5.7	48.6	0.1082	0.0588	0.9486
8	51.5	6.4	53.7	0.1055	0.0663	0.9591
11	57.7	5.5	57.9	0.0850	0.0825	0.9978
12	63.4	7.0	64.1	0.0987	0.0855	0.9876
13	52.8	4.2	55.2	0.0725	0.0319	0.9582

Appendix 3. (Continued)

Clone	Fee	e	Fss_2	e/Fss+e	$\frac{Fss+e-Fss_2}{Fss+e}$	Fss/Fss_2
Plants Grown in SO_2 (cont).						
15	58.5	7.3	61.9	0.1106	0.0573	0.9455
16	61.8	9.2	67.7	0.1265	0.0457	0.9154
27	56.6	9.6	62.9	0.1398	0.0458	0.9027
41	52.7	8.6	56.8	0.1361	0.0683	0.9288
51	57.5	8.3	60.8	0.1196	0.0688	0.9467
58	55.8	7.8	57.7	0.1143	0.0856	0.9682
87	54.4	8.6	57.6	0.1303	0.0779	0.9469
S23	57.3	5.6	57.7	0.0899	0.0827	0.9933

Appendix 3 (Continued) Mean % Reflectance of Leaves of Lolium perenne After Chronic Fumigation (Section 8.3)

Clone	Reflectance				
	460 nm	545 nm	675 nm	800 nm	1650 nm
	Control				
1	16.42	32.05	15.25	69.53	36.48
2	15.52	30.66	14.55	69.53	35.70
3	16.03	33.29	14.69	69.06	34.73
4	16.53	33.06	15.04	70.89	36.33
5	16.25	33.56	15.25	70.20	35.02
6	15.93	32.62	15.19	69.58	36.38
7	15.74	31.49	14.52	69.50	32.87
8	16.40	31.71	15.08	70.22	35.93
11	15.98	33.34	15.22	68.24	34.23
12	15.39	31.36	14.41	70.12	36.32
13	15.01	31.49	14.25	69.19	34.22
15	17.02	35.54	15.94	69.35	35.86
16	16.89	36.61	16.09	69.15	33.64
27	15.55	31.23	14.40	69.61	36.75
41	16.41	33.42	15.16	68.71	33.18
51	15.88	32.75	14.53	68.89	34.11
58	16.19	32.95	15.44	69.73	34.39
87	14.59	28.82	13.48	69.51	36.49
S23*	16.59	34.47	15.41	69.48	34.55
Mean	16.02	32.65	14.94	69.50	35.11
	SO_2 Treatment				
1	16.37	31.59	15.50	68.77	36.43
2	15.47	29.99	14.46	69.65	35.44
3	16.15	32.46	14.65	69.35	35.84
4	16.46	32.26	14.93	70.16	35.82
5	16.44	34.27	15.39	70.30	35.85
6	16.08	31.26	15.28	68.97	35.27
7	15.54	29.87	14.53	68.74	36.28
8	16.07	31.02	14.90	68.69	34.30
11	15.87	31.95	14.98	66.93	34.46
12	15.59	32.49	15.11	69.10	35.82
13	15.04	30.58	14.27	68.64	34.81
15	16.90	34.44	15.78	69.09	35.79
16	17.78	37.37	18.00	69.43	34.90
27	15.40	30.20	14.27	70.22	35.94
41	16.32	32.15	15.06	69.32	32.96
51	15.82	32.29	14.72	67.88	34.50
58	15.99	32.12	14.89	69.12	35.86
87	15.10	28.91	13.96	68.79	34.04
S23*	16.70	32.60	15.29	69.09	36.07
Mean	16.06	31.99	15.05	69.07	35.28

Appendix 4. Growth of Plants in First Screening Experiment

(Section 7.2)

Clones	Growth Parameters		
	Live Shoot dry wt (g)	Dead Shoot dry wt (g)	Root dry wt (g)
-Growth in Clean Air			
10	2.02	0.56	1.30
17	2.55	1.07	2.56
19	2.88	1.18	1.90
27	3.93	1.07	1.70
35	4.39	1.32	2.44
37	3.50	1.43	2.41
41	2.76	1.29	3.34
42	3.66	1.89	2.00
47	4.14	1.91	4.10
50	3.82	1.22	2.49
51	2.15	0.84	1.23
58	2.66	1.21	2.44
61	3.98	1.53	3.20
66	3.01	1.22	2.33
71	3.05	1.18	1.76
75	3.51	1.09	2.82
86	3.10	0.95	1.89
87	2.17	1.12	2.52
89	2.88	0.71	1.82
-Growth in SO_2			
10	1.82	0.79	1.18
17	2.20	1.33	2.29
19	2.44	1.41	1.85
27	4.24	1.51	1.83
35	3.51	2.05	3.92
37	3.76	1.92	2.60
41	3.05	1.67	4.11
42	2.51	1.82	1.52
47	3.05	2.16	4.00
50	3.23	1.48	2.58

Appendix 4. (Continued)

Clones	Growth Parameters: Live Shoot dry wt (g)	Dead Shoot dry wt (g)	Root dry wt (g)
-Growth in SO_2(cont.)			
51	3.38	1.07	2.00
58	3.11	1.86	2.99
61	3.29	1.90	3.59
66	2.62	1.33	2.87
71	2.47	1.34	1.99
75	2.75	1.28	3.11
86	2.06	0.73	1.51
87	2.86	1.30	4.21
89	2.86	1.04	2.02

Appendix 4. Growth of Plants in Second Screening Experiment (Section 7.4). (Continued)

Clones	GROWTH PARAMETERS				
	No. Tillers	No. Lvs.	Live Lf. Wt.	Dead Lf. Wt.	Root Wt
	Control				
1	44.3	123.0	1.44	1.69	3.21
2	39.6	116.2	1.13	1.36	2.19
3	40.5	121.5	0.98	1.70	2.07
4	36.6	110.9	1.62	1.84	3.60
5	55.5	154.4	1.92	1.66	1.75
6	42.0	98.7	1.28	1.94	2.29
7	32.3	86.8	1.04	1.44	2.72
8	53.9	148.8	1.26	1.11	2.10
11	15.2	44.2	0.29	0.86	0.77
12	34.7	118.6	1.68	1.23	2.11
13	26.2	90.3	1.36	1.27	2.64
15	33.3	96.9	1.10	1.52	1.12
16	38.9	130.2	1.28	1.31	0.92
27	40.8	129.4	2.07	1.86	1.22
41	49.7	159.5	1.65	1.43	3.60
51	52.6	157.5	1.27	1.37	2.34
58	43.8	134.2	1.57	1.32	3.13
87	41.4	151.8	1.29	1.20	2.49
S23*	53.7	132.0	2.13	2.16	2.59
Mean	40.8	121.3	1.39	1.49	2.26
	SO_2 Treatment				
1	33.7	84.8	0.89	1.86	2.28
2	27.7	80.7	0.80	1.20	1.72
3	35.8	102.6	0.81	1.90	1.97
4	30.4	87.4	1.37	1.80	3.39
5	40.7	142.6	1.40	1.41	1.46
6	28.8	61.8	0.73	1.64	1.51
7	26.5	67.9	0.74	1.47	2.35
8	39.9	102.1	0.86	0.98	1.54
11	22.3	55.8	0.38	0.99	1.05
12	29.3	100.6	1.25	1.17	1.69
13	23.2	80.7	1.17	1.09	1.95
15	25.8	77.2	0.85	1.41	1.04
16	38.5	121.2	1.12	1.31	1.01
27	34.3	109.2	1.39	1.36	1.35
41	47.0	148.2	1.57	1.61	3.07
51	37.2	90.4	0.68	1.10	1.25
58	40.8	121.2	1.31	1.56	2.98
87	37.9	132.7	1.12	1.17	1.96
S23*	46.2	121.0	1.88	2.20	2.13
Mean	34.0	99.4	1.07	1.43	1.88

Appendix 5. Enzyme Staining Solution Recipes for Starch Gel Electrophoresis. (Section 3.4.2)

G.O.T.

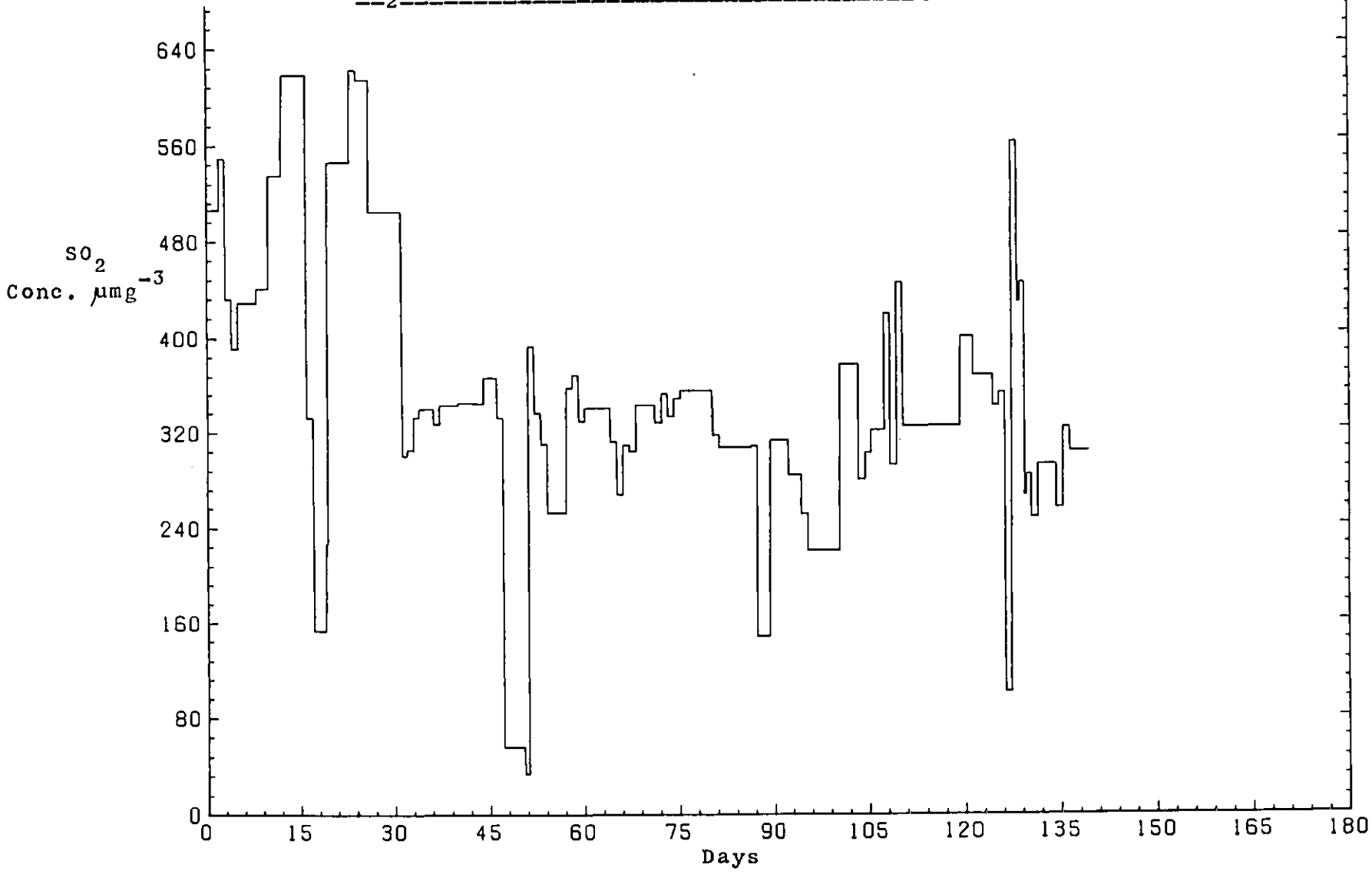
L. aspartic acid	100 mg
α- ketoglutaric acid	100 mg
Fast - Blue B.B.	150 mg
Pyridoxal-5-Phosphate	5 mg
0.1M TRIS/HCl 0.5N pH 7.5	100 ml

P.G.I.

Fructose-6-Phosphate	15 mg
N.A.D.P.	7 mg
Methyl Thiozoyle Blue (M.T.T.)	15 mg
Phenosine Methosulphate	20 units
1M $MgCl_2$	1 ml
TRIS/HCl 0.1M pH 7.5	100 ml

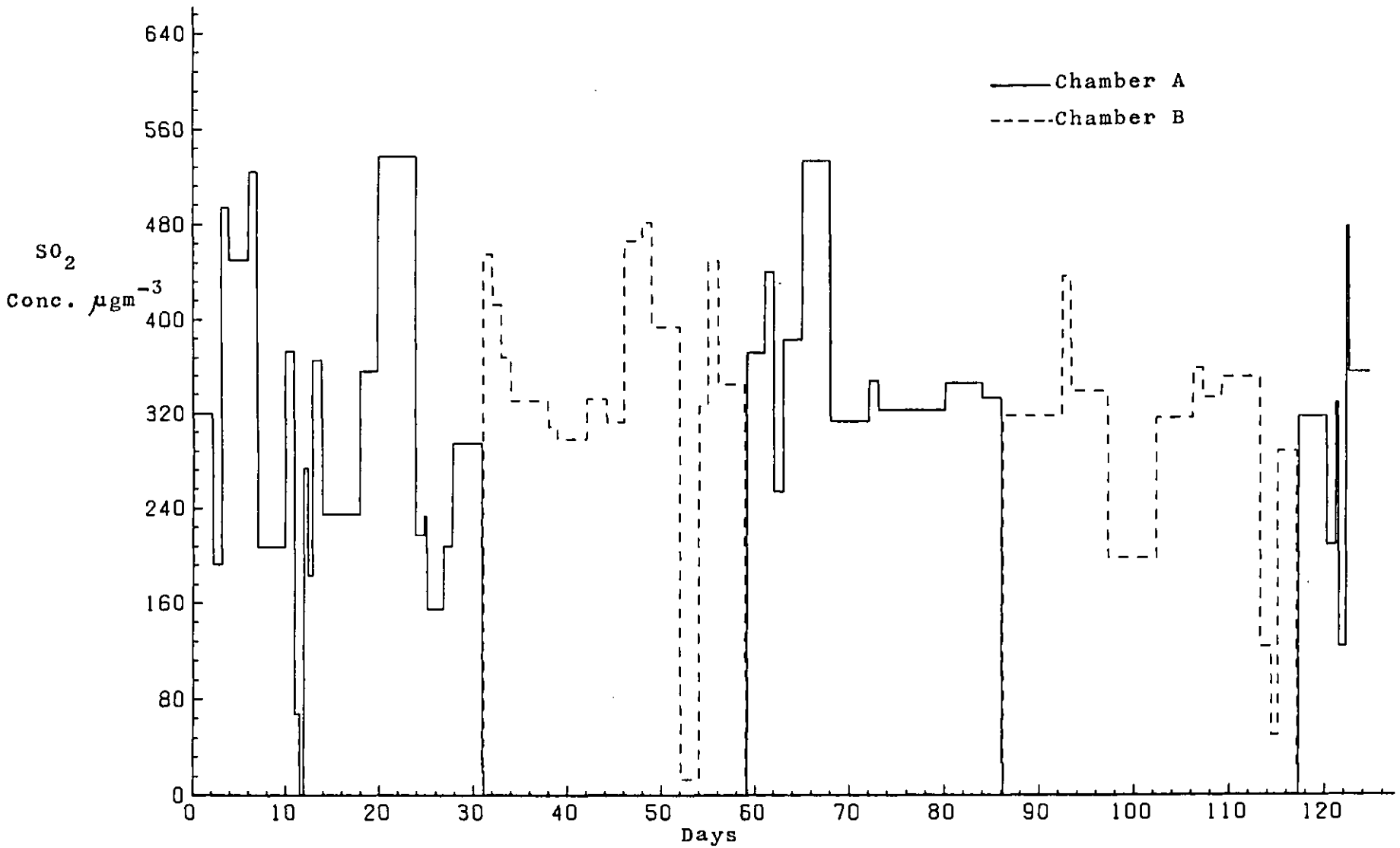
Appendix 6

SO_2 Concentrations During Chronic Fumigation in Section 7.2



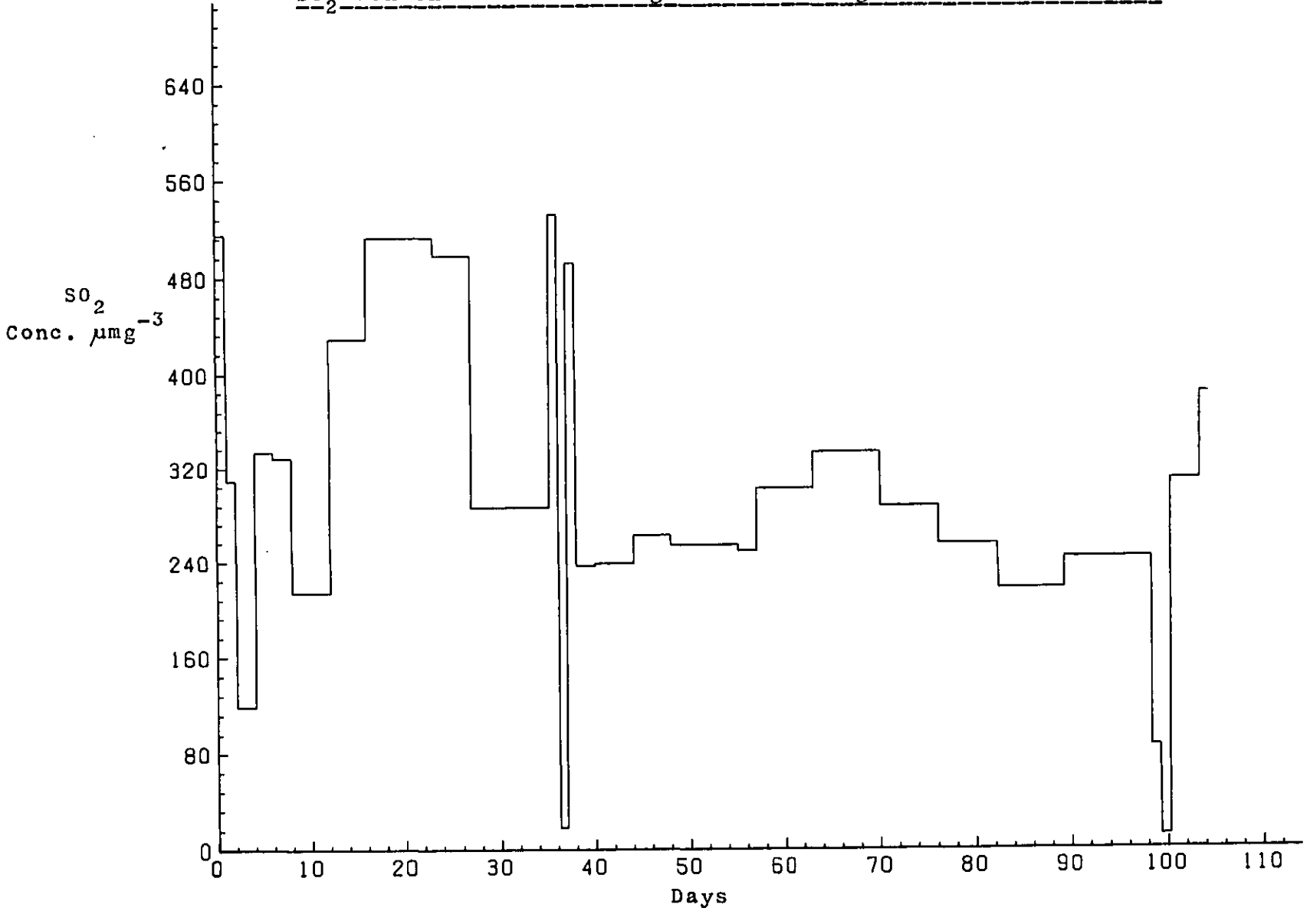
Appendix 6

SO_2 Concentrations During Chronic Fumigation in Section 3.3



Appendix 6

SO_2 Concentrations During Chronic Fumigation in Section 5.3



Appendix 6

SO_2 Concentrations During Chronic Fumigation in Section 7.4

