COMPETITIVE INTERACTIONS IN THE PARASITISM OF CEREALS BY *CLAVICEPS PURPUREA*

A Thesis Presented by
Dorothy Janet Swan, B.Sc.

November, 1980

From the Department of Biochemistry,
Imperial College of Science and Technology,
London SW7

ABSTRACT

Competitive interactions have been shown to occur throughout the life of Claviceps purpurea. These interactions may be intraspecific or may be between pathogen and host.

Throughout germination, developing stromata have to compete with each other for sclerotial nutrient reserves. Sclerotia labelled with a pulse of ^{14}C at a particular growth stage, and later allowed to germinate, showed that substrates for stromatal growth are mainly derived locally. However, some nutrients may be obtained from adjacent regions, possibly as the local source becomes depleted. Surprisingly, stromata rarely developed from sclerotial tissues which developed after exposure to ^{14}C. Using the same technique of pulse labelling, the acropetal growth of ergot sclerotia was confirmed. Further, the relative incorporation of ^{14}C into primary (lipids) and secondary (alkaloids) metabolites showed that biosynthesis of the latter occurred in tissues which had already become sclerotial.

When a sclerotium consists of two strains, its germinative ability does not necessarily reflect the proportion of the strains. Where a poorly-germinating strain exerts a disproportionately dominant effect this interaction may be due to an inhibitory factor.

Spores of C.purpurea have to compete with pollen for entry into a receptive ovary. Epidemiological experiments with male sterile wheat have confirmed that the potential for ergot infection is inversely proportional to seed set.

There is no evidence for competition between ergot strains during infection, provided that they are inoculated simultaneously. However, there may be a dramatic antagonistic effect, detrimental to both host and pathogen(s), if a second strain is introduced into an infected floret about one week after the ovary is initially infected.

As an ergot develops it has to compete with seeds and any other sclerotia for nutrients. In wheat plants, whose growth was not nutrient-limited, seed quality was not impaired by ergot infection. However, in impoverished conditions the presence of developing ergots apparently assisted the plant in yielding heavier seeds. Radio-isotope experiments have demonstrated, for the first time, that sphacelial and sclerotial tissues can fix carbon dioxide.

CONTENTS

		Page
ABSTRACT		2
CONTENTS		3
INDEX OF TABLES		6
INDEX OF FIGURES		8
1.	INTRODUCTION	
1.1	Host susceptibility and resistance	12
1.2	Control of ergot disease	14
1.3	Toxicity and pharmacology	17
1.4	Sclerotial dormancy and germination	17
1.5	The infection process	19
1.6	Honeydew formation	20
1.7	Honeydew composition	20
1.8	Differentiation and sclerotial development	21
1.9	Sclerotial metabolism	22
1.10	Competition between seeds and ergots within an ear	23
1.11	Carbon assimilation by C.purpurea	24
1.12	Carbon dioxide fixation by fungi	25
2.	GENERAL METHODS	
2.1	Growth of cereals in field plots	27
2.2	Growth of cereals in open plots at the Chelsea Physic Garden	27
2.3	Growth of cereals in the glasshouse	27
2.4	Storage of harvested material	28
2.5	Storage and preparation of inoculum	28
2.6	Inoculation of cereals	28
2.7	Supply of $^{14}CO_2$ to intact plants and excised ears	29
2.8	Supply of $^{14}CO_2$ to isolated parasitic tissues	33
2.9	Detection and assay of radiolabelled compounds	33
2.9.1	Autoradiography	33
2.9.2	Elution from paper chromatograms	33
2.9.3	Scintillation counting	33
2.9.4	Tissue oxidation	34
2.10	Perchloric acid extraction of tissues	34
2.11	Serial extraction of tissues	34
2.12	Quantitative extraction of lipids	35
2.13	Quantitative extraction of alkaloids	36
2.13.1	Batch extraction	36
2.13.2	Extraction of individual sclerotia	36
2.14	Quantitative estimation of sclerotial alkaloid content	36
2.15	Qualitative analysis of sclerotial alkaloids	36
2.16	Separation of carbohydrates from amino acids by ion exchange	37
2.17	Separation of amino acid mixtures by two-dimensional paper chromatography	37
2.18	Separation of carbohydrate mixtures by paper chromatography	39

Page

2.19 Identification and estimation of carbohydrates by gas-liquid chromatography - - - - - - - - - - - - - 39
2.20 Supply of ^{14}C glucose to stromata - - - - - - - - - 40
2.21 Extraction of polyols from stromata - - - - - - - - 41
2.22 Preparation of a spore trapping surface - - - - - - 41
2.23 Analysis of spore trap tapes - - - - - - - - - - - - 41

3. SELECTION OF C.PURPUREA STRAINS FOR USE IN GERMINATION EXPERIMENTS

3.1 Experimental - 42
3.2 Results - 45

4. INFLUENCE OF SOME PLANT HORMONES ON SCLEROTIAL GERMINATION

4.1 Experimental - 46
4.2 Results and discussion - - - - - - - - - - - - - - - - 46

5. INFLUENCE OF A COLD PERIOD ON THE GERMINATION OF SCLEROTIA FROM PHRAGMITES COMMUNIS AND LOLIUM PERENNE

5.1 Experimental - 49
5.2 Results - 49

6. GERMINATION STUDIES ON ^{14}C-LABELLED SCLEROTIA

6.1 Experimental - experiment 1 - - - - - - - - - - - - - 51
6.2 Results and discussion - experiment 1 - - - - - - - - 51
6.3 Experimental - experiment 2 - - - - - - - - - - - - - 56
6.4 Results and discussion - experiment 2 - - - - - - - - 58

7. THE UPTAKE OF GLUCOSE BY EXCISED STROMATA

7.1 Experimental - 73
7.2 Results and discussion - - - - - - - - - - - - - - - - 73

8. PERIODICITY OF ASCOSPORE EJECTION BY ERGOTS IN THE FIELD

8.1 Experimental - 76

8.1.1 1975 - 76
8.1.2 1976 - 76

8.2 Results and discussion - - - - - - - - - - - - - - - - 78

9. COMPETITION BETWEEN STRAINS OF C.PURPUREA IN VIVO

9.1 Experimental - experiment 1 - - - - - - - - - - - - - 85
9.2 Results - experiment 1 - - - - - - - - - - - - - - - - 85
9.3 Experimental - experiment 2 - - - - - - - - - - - - - 93
9.4 Results - experiment 2 - - - - - - - - - - - - - - - - 93
9.5 Discussion - 97

10. COMPETITION FOR NUTRIENTS BETWEEN SEEDS AND ERGOTS

10.1 Experimental - Spring wheat - - - - - - - - - - - - - 101
10.2 Results and discussion - - - - - - - - - - - - - - - - 101
10.3 Experimental - Winter wheat - - - - - - - - - - - - - 116

Page

10.4 Results and discussion - - - - - - - - - - - - - - - 116
10.5 Experimental - Glasshouse - - - - - - - - - - - - - - 122
10.6 Results and discussion - - - - - - - - - - - - - - - 126

11. THE EFFECT OF ERGOTS ON THE QUALITY OF ADJACENT SEED

11.1 Experimental - 135
11.2 Results and discussion - - - - - - - - - - - - - - - 135
11.3 Experimental - 144
11.4 Results and discussion - - - - - - - - - - - - - - - 144

12. CARBON DIOXIDE FIXATION BY SPHACELIA AND SCLEROTIA OF C.PURPUREA

12.1 Experimental - 149
12.2 Results and discussion - - - - - - - - - - - - - - - 153

13. INFLUENCE OF SEED SET ON SECONDARY ERGOT INFECTION OF MALE STERILE WHEAT

13.1 Experimental - 164
13.2 Results and discussion - - - - - - - - - - - - - - - 167

14. GENERAL DISCUSSION - 173

15. REFERENCES - 178

16. APPENDICES-STATISTICS - - - - - - - - - - - - - - - - - -

I Student's t-tests - - - - - - - - - - - - - - - - - - 183
II Correlation coefficients - - - - - - - - - - - - - - - 186
III Analysis of variance - - - - - - - - - - - - - - - - 187
IV Kolmogorov-Smirnov two sample test - - - - - - - - - 192

17. ACKNOWLEDGEMENTS - 195

INDEX OF TABLES

		Page
Table 1	Origin of strains and percentage germination in strain selection experiment	43
Table 2	Concentration of total and assimilated/synthesised carbohydrates in stromata supplied basally with ^{14}C glucose	74
Table 3	Spore combinations used for mixed strain inoculations a - experiment 1 b - experiment 2	 86 92
Table 4	Germination of sclerotia of mixed and single strain origin	87
Table 5	Alkaloid content of sclerotia resulting from single and mixed strain inoculations a - experiment 1 b - experiment 2	 89 89
Table 6	Alkaloid spectra of sclerotia resulting from single and mixed strain inoculations	90
Table 7	Comparison of seed and ergot production in wheat ears inoculated with strain B and/or strain E of C.purpurea	95
Table 8	Alkaloid distribution within single and mixed strain sclerotia	96
Table 9	The percentage of ergots in each group of ears used in the spring wheat nutrient competition experiment	103
Table 10	The lipid content of selected groups of ergot in the spring wheat nutrient competition experiment	115
Table 11	The percentage of ergots in each group of ears used in the winter wheat nutrient competition experiment	117
Table 12	Summary of treatment groups in the glasshouse nutrient competition experiment	125
Table 13	Variation in seed weight, seed number and ergot weight with position of ergots in an ear	138
Table 14	Summary of treatments used in carbon dioxide fixation experiments	150
Table 15	The distribution of ^{14}C in successive extractions of ergots from ears supplied with $^{14}CO_2$	156
Table 16	The distribution of ^{14}C in successive extractions of seeds from ears supplied with $^{14}CO_2$	157
Table 17	Distribution of fixed ^{14}C between amino acids and carbohydrates	161

Page

Table 18 Analysis of the labelled amino acids and carbohydrates in the low molecular weight fraction of ergots from ears pulsed with $^{14}CO_2$ - - - - - - - - - - - 162

Table 19 Specific activity of carbohydrates in ergots from ears supplied with $^{14}CO_2$ for five hours - - - - - - - 163

INDEX OF FIGURES

		Page
Figure 1	The inoculation of spikelets - - - - - - - - - - - - -	29
Figure 2	Design for inoculating 20% or 80% of spikelets - - -	30
Figure 3	Apparatus for the supply of $^{14}CO_2$ to cereal ears - -	31
Figure 4	Chamber used when supplying $^{14}CO_2$ to isolated parasitic tissues - - - - - - - - - - - - - - - - -	32
Figure 5	Serial arrangement of ion exchange columns for separating amino acids from carbohydrates - - - - -	38
Figure 6	The supply of ^{14}C-glucose to stromata - - - - - - -	40
Figure 7	Germination of strain 330 (grown on rye) in relation to the number of days at room temperature following 7 weeks at 4°C -	44
Figure 8	The formation of 'normal' and 'secondary' stromata by strain 330 of C.purpurea - - - - - - - - - - - -	45
Figure 9	Mean percentage germination of Phragmites sclerotia treated with plant hormones - - - - - - - - - - - -	47
Figure 10	The production of stromata by sclerotia of Phragmites communis and Lolium perenne after incubation at 4°C for 0, 2, 4 or 6 weeks - - - - - - - - - - -	50
Figure 11	Division of a labelled sclerotium into eight segments prior to analysis - - - - - - - - - - - - - - -	52
Figure 12	Distribution of ^{14}C in the PCA-soluble fractions of sclerotia pulsed with $^{14}CO_2$ during different stages of growth -	53
Figure 13	Distribution of ^{14}C in the insoluble fractions of sclerotia pulsed with $^{14}CO_2$ during different stages of growth -	54
Figure 14	Distribution of ^{14}C in the lipid fraction of sclerotia pulsed with $^{14}CO_2$ during growth - - - - -	55
Figure 15	Modified GM tube for examining the distribution of ^{14}C activity along a sclerotium - - - - - - - - - -	57
Figure 16	Division of a labelled sclerotium into segments for lipid and alkaloid analysis - - - - - - - - - - - -	57
Figure 17	Uptake of $^{14}CO_2$ by wheat ears inoculated with C.purpurea 14, 21, 36 or 38 days previously - - - -	59
Figure 18	The distribution of ^{14}C in sclerotia and stromata a) supplied with $^{14}CO_2$ 14 days after ear inoculation- b) supplied with $^{14}CO_2$ 21 days after ear inoculation- c) supplied with $^{14}CO_2$ 38 days after ear inoculation- d) supplied with $^{14}CO_2$ 14 and 36 days after ear inoculation -	61 63 64 66

Page

Figure 19 The distribution of ^{14}C-labelled lipids and alkaloids in sclerotial segments - - - - - - - - - - - - 68

Figure 20 The distribution of stromata along sclerotia in relation to the peak of ^{14}C activity within each sclerotium - 69

Figure 21 The distribution of stromata along sclerotia not exposed to $^{14}CO_2$ - 70

Figure 22 The distribution of stromata along sclerotia pulsed with $^{14}CO_2$ at different stages of growth, compared with unlabelled controls - standardised data - - - - - 71

Figure 23 Plot layout for ascospore ejection experiments
a) 1975 - 77
b) 1976 - 77

Figure 24 Diurnal variation in the release of ascospores
a) 1975 - 79
b) 1976 - 80

Figure 25 Variations in the time of peak ascospore discharge - - 81

Figure 26 The germination of ergots in the 1975 ascospore ejection experiment - - - - - - - - - - - - - - - - - - 82
a) Percentage germination of ergots buried beneath the soil surface
b) Percentage germination of ergots on soil surface
c) Total percentage germination

Figure 27 Percentage germination of sclerotia of single and mixed strain origin - - - - - - - - - - - - - - - - - - 88

Figure 28 Variation in the percentage dry matter of ergots and spring wheat seeds during development
a) + 0N - 104
b) + 50N - 105
c) +100N - 106

Figure 29 Variation in seed number/control spikelet and ergot number/inoculated spikelet - - - - - - - - - - - - - - - 107

Figure 30 Variation in the mean dry weight of spring wheat seeds during development
a) + 0N - 109
b) + 50N - 110
c) +100N - 111

Figure 31 Variation in the mean dry weight of ergots during development
a) + 0N - 112
b) + 50N - 113
c) +100N - 114

Page

Figure 32 Variation in the percentage dry matter of ergots and winter wheat seeds during development
a) Low nitrogen - 118
b) Normal nitrogen - - - - - - - - - - - - - - - - - - 119

Figure 33 Variation in the mean dry weight of winter wheat seeds with time and soil nitrogen
a) Low nitrogen - 120
b) Normal nitrogen - - - - - - - - - - - - - - - - - - 120

Figure 34 Variation in the mean dry weight of ergots with time and soil nitrogen
a) Low nitrogen - 121
b) Normal nitrogen - - - - - - - - - - - - - - - - - - 121

Figure 35 The position of a pot on the glasshouse bench at weekly intervals during the nutrition experiment - - 123

Figure 36 Variation in seed number/uninoculated spikelet with nutrient treatment (glasshouse experiment) - - - - - 128

Figure 37 Variation in mean seed weight with nutrient treatment (glasshouse experiment) - - - - - - - - - - - - - - - 129

Figure 38 Variation in mean ergot weight with nutrient treatment (glasshouse experiment) - - - - - - - - - - - - - - - 130

Figure 39 Variation in ergot number/inoculated spikelet (glasshouse experiment) - - - - - - - - - - - - - - - - - - - 131

Figure 40 Inter-relationship between mean seed weight and ergot weight/spikelet (glasshouse experiment)
a) Low base - 132
b) Low base + Liquinure - - - - - - - - - - - - - - - - 132
c) Half base - 133
d) Half base + Liquinure - - - - - - - - - - - - - - - 133
e) Full base - 134
f) Full base + Liquinure - - - - - - - - - - - - - - - 134

Figure 41 Field design of an experiment to assess the influence of ergot on adjacent seeds - - - - - - - - - - - - - 136

Figure 42 Inoculation patterns used to assess variation in the influence of ergot on adjacent seeds according to the position of ergots in the ear - - - - - - - - - - 137

Figure 43 Variation of mean seed weight with total ergot weight in individual ears - - - - - - - - - - - - - - - - - 145

Figure 44 Variation of mean seed weight with mean ergot weight in individual ears - - - - - - - - - - - - - - - - - 146

Figure 45 Variation of mean seed weight with the number of ergots/spikelet within individual ears - - - - - - - 147

Figure 46 Preparation of excised ears for feeding with $^{14}CO_2$ - - 151

Page

Figure 47 The depletion of $^{14}CO_2$ supplied to infected rye ears and isolated parasitic tissues in an enclosed system - 154

Figure 48 Plot layout for experiment to examine the influence of seed set on secondary ergot infection of male sterile wheat - 165

Figure 49 Sample composition at harvest in experiment to examine the influence of seed set on secondary ergot infection of male sterile wheat - - - - - - - - 166

Figure 50 Variation of final ear density within plots - - - - 168

Figure 51 Variation of seed set and secondary ergot infection with distance from the pollinator - - - - - - - - - 169

Figure 52 Competitive interactions during parasitic and non-parasitic stages of C.purpurea - - - - - - - - - 174

Figure 53 Competitive interactions between fungus and fungus and between fungus and seed during the parasitism of cereals by C.purpurea - - - - - - - - - - - - - - - 175

1. INTRODUCTION

1.1 Host susceptibility and resistance

The single most important factor governing the period of susceptibility of potential host ovaries to Claviceps Purpurea (Fr) Tul. infection is probably the pollination mechanism of the host. Self-fertilised flowers are vulnerable for a much shorter time than those of cross-pollinated plants. Whereas the glumes of self-pollinated cereals and grasses generally remain closed until fertilisation has been achieved, and may then only open for a few hours, the glumes of cross-pollinated plants gape before anthesis and remain open until pollination has been effected. It is during this period of gaping that the plant is vulnerable to ergot infection since spore access is easiest. The duration of gaping is consequently a major factor governing the susceptibility of a host plant. To achieve hybrid seed production male sterile lines are necessarily open flowering in order that cross-pollination can be effected. It is this characteristic of hybrid seed production that makes ergot infection a more important threat to hybrid cereals than it is every likely to be to conventional (self-fertilised) wheats and barleys (Puranik and Mathre 1971). This is also why ergot is much more prevalent on rye and triticale (two naturally cross-pollinated cereals) than other cereals (Hulse 1974; Kiss 1974).

Grasses which have a protogynous flowering mechanism, such as Alopecurus myosuroides and A.pratensis are vulnerable to C.purpurea infection since they are effectively male sterile during the period between ovary and pollen maturation (Mantle et al 1977).

The speed with which pollination is achieved is important because the susceptibility of an ovary declines after it has been fertilised (Puranik and Mathre 1971, Shaw 1975, Cunfer et al 1975). Puranik and Mathre (1971) reported that the susceptibility of fertilised ovaries of male sterile barley began to decrease within 4 days of fertilisation and no infection was achieved in ovaries inoculated with C.purpurea 9 days after fertilisation. In contrast, unfertilised ovaries showed no decline in susceptibility until 10 days after anthesis and could be infected up to 14 days post-anthesis. Shaw (1975) reported that unfertilised ovaries of male sterile wheat (cv. Capitole) were readily infected by C.purpurea for 6-10 days after 'anthesis', depending on environmental conditions. Thereafter, susceptibility rapidly declined until by 10-20 days male sterile ovaries did not support infection.

The susceptibility of self-pollinated cultivars started to decline within 24 hours of anthesis.

The mechanism of pollination-induced resistance is unclear but Mathre (1978) postulated that changes in hormone levels, which occur in recently fertilised tissues, may prevent spore germination or hyphal penetration.

Darlington et al (1976) recorded an increased ergot susceptibility of fertile barley cultivars infected by barley stripe mosaic virus (BSMV) as compared to virus-free plants. Furthermore, the level of enhanced ergot susceptibility was directly related to the degree of virulence of the BSMV strain. It was inferred that increased ergot susceptibility occurred because BSMV infection caused partial male sterility.

Shaw (1975) indicated that environmental conditions can affect the duration of the susceptible period. There was a negative correlation between infection of cms Capitole and temperature, but the variations in relative humidity recorded in the crop had little effect. Shaw also demonstrated that the rate of parasitic development of C.purpurea is dependant on the ambient temperature, and quoted two extremes; a 6 day lag between inoculation and the onset of honeydew production in a warm glasshouse and 13 days in the field during cool weather. Irrespective of the time scale, no honeydew was observed until the wheat ears had entered the phase of declining susceptibility.

A greater proportion of ergots initiated during the period of declining susceptibility abort at the sphacelial stage, compared to earlier formed sclerotia (Shaw 1975), and more seeds may be half infected or have a watersoaked appearance. Late infection of florets produces less ergots than usual per unit weight of grain, and therefore reduces the potential toxic hazards, but the problems of separating partly infected and healthy seeds are compounded.

Uniformity of flowering and pollination is an important feature of cereals if the risk of ergot spreading within a crop is to be minimised. Since honeydew may be produced within 6 days of infection (Shaw 1975) it is desirable that the period between the first and last ears flowering should not exceed 5 days. Any florets which open after this time may, if suitable conditions prevail, be vulnerable to ergot infection. Cereal varieties which produce very few late tillers are therefore

generally less likely to be heavily infected with ergot.

Although C.purpurea can colonise a wide range of grasses it is recognised that some species are far more important than others in the aetiology of ergot disease of cereals. Loveless (1971), and more recently Mantle and Shaw (1977), have demonstrated the existence of host restriction among strains of C.purpurea. By combining their host restriction data with information on the flowering time of grasses, Mantle and Shaw (1977) proposed that Alopecurus myosuroides and A.pratensis may be important in the aetiology of ergot disease, whereas Lolium perenne, Dactylis glomerata, Holcus lanatus and Arrhenatherum elatius are probably of little importance. The involvement of A.myosuroides has been further implicated by an observed association between blackgrass infestation and the occurrence of ergot in wheat ears (Mantle et al 1977). Although honeydew from late flowering ergot-infected grasses is unlikely to be a direct source of inoculum for cereals, the grass sclerotia which develop may contribute to the aetiology by releasing ascospores in the following spring. Shaw (1975) suggested that in general the lowest levels of infection are encountered when both crop and headland are weed-free; if suitable weed grasses are present the fungus may pass through one or two hosts and thus a significant inoculum may build up prior to cereal anthesis.

The possibility of the existence of cereal cultivars resistant to ergot infection has received considerable attention during the last decade. Although Platford and Bernier (1970) described the wheat varieties Kenya Farmer and Carleton as resistant, additional studies by Ratanopas and Bernier (1972) and Shaw (1975) showed these claims to be premature. Bernier (1978) reported that of 292 accessions, representing 23 Triticum species, inoculated with a range of ergot isolates, only one completely failed to develop sclerotia. Infection of this T.zhukovskyi accession has since been achieved using a British isolate of C.purpurea (P.G.Mantle - personal communication), but the number of sclerotia formed was very low and only one of the three ergot strains tested was infective.

1.2 Control of ergot disease

The importance of sowing ergot-free seed cannot be overstressed. The quality requirement for intervention purchase in the UK during

1979/80 was less than 0.05% ergot by weight for all wheat and barley. This figure is reduced if other impurities (including shrivelled and sprouted grain) are present as the total may not exceed 12% by weight (10% for breadmaking wheat): since the amount of impurities affects the value of the grain there is a considerable financial incentive for farmers to minimise ergot infection within their crops. The Canada Grain Act specifies that rye seed containing >0.33% ergot is to be classified as ergoty and that the highest grades of rye intended for milling should be free from ergot (Sosulski and Bernier 1975). The most efficient method of separating ergots from grain utilises their different specific gravities (Amos 1973): when placed in a brine solution the grain sinks whereas over 95% of the ergots will float.

The role of certain weed grasses in the aetiology of ergot disease has been discussed in section 1.1; it is evident that exclusion of these potential intermediate hosts from a crop area reduces the risk of infection. In situations where total grass exclusion is not possible, control may be achieved by spraying the headland grasses with growth retardants, eg. maleic hydrazide at 8 lbs/acre, to suppress heading (Campbell and Friesen 1959), although this is often impractical both in physical and financial terms.

With the increasing popularity of minimum cultivation techniques such as direct drilling, the control of ergot by deep ploughing is less widely practised, although this remains the most efficient method of control. Stubble burning is also effective but in some areas of the world, such as Oregon USA, this practice has been outlawed thus eliminating it as a possible method of control (Hardison 1975).

The use of chemicals to control ergot has recently received an increased amount of attention and some promising compounds have been described (Puranik and Mathre 1971; Hardison 1972, 1975). However even if fungicides for ascocarp suppression are available, a suitable method of application has to be found. Ergots which are completely buried in soil or plant litter may germinate but would be very inaccessible for chemical treatment.

Puranik and Mathre (1971) reported that an application of benomyl to seed beds failed to reduce the level of ergot infection in a crop. However, the application of this systemic fungicide to ears of male sterile

barley gave a 56% reduction in the number of sclerotia produced, although only some of the benomyl penetrated ovary tissues thus limiting its fungicidal effect.

Hardison (1972, 1975) screened many systemic and protectant fungicides for their ability to suppress ascocarp formation and found that the majority failed to provide adequate suppression at reasonable dosages. Complete, or a high degree of, control was obtained using a single application of 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone (BAY MEB 6447) at 1 mg/92 cm^2 soil surface (≡1 lb/acre), triarimol at 1 mg/92 cm^2, cadmium chloride at 2 mg/92 cm^2, α-(2-chlorophenyl)-α-cyclohexyl-5-pyrimidine methanol (EL-279) at 2 mg/92 cm^2 and benomyl at 6 mg/92 cm^2. Of these chemicals, cadmium chloride is the cheapest, effective compound, but at present is not approved in the UK or USA for use as a fungicide.

The possibility of controlling ergot disease by biological methods has been the subject of several studies. Futtrell and Webster (1966) reported that ergot honeydew of male sterile sorghum in Nigeria was colonised by Fusarium spp. during the rainy season, and sclerotia failed to develop. In contrast, during the dry season no honeydew colonisation was recorded and sclerotial development was normal. Similar observations have been reported for Paspalum ergots in Florida (Simpson and West 1952). Cunfer (1976b) demonstrated the occurrence of a factor(s) in honeydew which inhibits the germination of fungal conidia thus preventing many saprophytic fungi from colonising the honeydew. It was suggested that a means of overcoming this inhibitory factor(s) would be potentially useful in the biocontrol of C.purpurea. It has also been shown (Mower et al 1975; Cunfer 1976a) that raising the water potential of honeydew, for example by overhead watering, encourages colonisation by Fusarium spp. Although honeydew colonisation undoubtedly offers some control of sclerotium development, there is a risk of grain spoilage by the following factors (alone or in combination); the controlling micro-organisms, the physical conditions necessary for colonisation, other micro-organisms whose growth might also be encouraged by the aforementioned physical conditions. The possibility of using biocontrol to suppress ascocarp formation of buried sclerotia has surprisingly received little attention, although the biodegradation of sclerotia in soil is well documented (see section 1.4).

1.3 Toxicity and pharmacology

Symptoms of toxicity among animals which have ingested ergot, either as a result of grazing infected pasture grasses or of eating contaminated grain, are wide ranging. Peripheral circulation, fertility, lactation and general behaviour may be adversely affected. Although the sclerotial alkaloids are undoubtedly a major cause of the clinical potency of ergot, the possibility of other toxic constituents should not be overlooked. There is reason to believe that much of the bovine abortion attributed originally to ergot ingestion was probably due to some other agent such as Brucella abortus or Aspergillus fumigatus (Mantle 1969a).

Although many cases of human ergotism have been documented (Barger 1931; Bové 1970) there is now fortunately little risk of this affliction providing that the recommended grain cleaning procedures, prior to milling, are adhered to. Several ergot alkaloids are in fact used theropeutically especially in the relief of migraine headaches and during childbirth (Bové 1970).

1.4 Sclerotial dormancy and germination

Sclerotia may be introduced into the ground when they fall from the ears of wild grasses in the autumn or from cultivated grasses and cereals prior to their harvest. Alternatively they may be introduced, if, and when, contaminated grass or cereal seed is sown in either autumn or spring.

The period of survival of sclerotia in the field apparently varies with the climate. Ergots introduced into the soil during the late summer or autumn in Great Britain clearly survive until the following spring (Jenkinson 1958; Shaw 1975). However, in the southeastern United States C.purpurea sclerotia rarely survive until the following spring since they are rapidly colonised by micro-organisms (Cunfer and Seckinger 1977). In addition, soil insects and mites are often found in association with the rotten sclerotia. Mitchell and Cooke (1968) reported that the viability of sclerotia declined with time when they were stored wet or dry, the decline being faster at higher temperatures. They assumed it unlikely that sclerotia could survive in a viable form for a second winter if germination was not achieved during the first year.

Sclerotia lie dormant in the soil until the spring. The precise conditions necessary for germination remain unclear although it is generally accepted that exposure to temperatures around $0^{o}C$ - $10^{o}C$ for at least 6-8 weeks is a pre-requisite for most strains of C.purpurea (Kirchhoff 1929; Mitchell and Cooke 1968; Mantle 1969b). However this requirement is not universal and C.purpurea sclerotia have, on occasion, germinated without any apparent cold treatment Cooke and Mitchell 1970). Other conditions conducive to sclerotial germination have been less thoroughly investigated but it has been suggested that alternate periods of freezing and thawing (Brentzel 1927), a rest period, and certain humidity regimes are beneficial or necessary. The possible substitution of a period of cold by treatment with indole acetic acid (Cooke and Mitchell 1970) remains unconfirmed.

Once any pre-requirements have been fulfilled, sclerotia germinate most freely at temperatures between $10^{o}C$ and $25^{o}C$. Germination is less readily achieved above $20^{o}C$ (Mitchell and Cooke 1968).

During germination many complex metabolic changes take place with lipids providing the principal carbon source (Mitchell and Cooke 1968). Corbett et al (1975) have suggested that developing stromata obtain nutrients primarily from the sclerotial tissue immediately beneath the point of stromatal exertion. Very high concentrations of polyols, especially mannitol, were recorded by Corbett et al (1975) in developing stromata. Cooke and Mitchell (1970), having detected mannitol in sclerotia, offered a putative pathway for its production from glucose via phosphorylated intermediates.

Once the germination process has been initiated stromata are produced over a period of several weeks. Cooke and Mitchell (1967) demonstrated that, in laboratory experiments, the periodic removal of developing stromata greatly increased the period of stromata production.

Ascospores are formed within the perithecial cavities of the capitula and are forcibly ejected when suitable conditions prevail. Ascospore discharge commences approximately 7 days after the stromata emerge and may be released over a period of several weeks (Cooke and Mitchell 1967; Shaw 1975). Discharge ceases if the stromata become desiccated but may recommence after rain (Shaw 1975). Shaw (1975) also showed that sclerotia germinating in conditions of water stress respond to watering both in terms of an increased proportion of

sclerotia germinating and an increased number of capitula germinated per sclerotium.

1.5 The infection process

Infection of a host ovary may follow germination of an ascospore or conidium within the floral cavity. The path of infection has received attention for over a century (Kuhn 1863; Kirchhoff 1929; Campbell 1958), but the most detailed and comprehensive investigations are those recently reported by Shaw and Mantle (1980a) and Luttrell (1980) for C.purpurea, and Luttrell (1977) for C.paspali.

Although the colonisation process is thought to be the same for C.purpurea in different cereals and grasses, the timing of events may vary with the host as well as with environmental conditions (Shaw 1979).

In contrast to C.paspali infection of Dallisgrass, in which system infection has only been observed through the stigma and style (Luttrell 1977), C.purpurea may invade host tissues through the stigma or directly through the ovary wall (Shaw and Mantle, 1980a). After entering a host via the stigma an invading C.purpurea hypha takes the path of least resistance through the soft transmitting tissue (which normally facilitates the passage of a pollen tube) and the outer integument before penetrating the ovule (Shaw and Mantle, 1980a).

The electron micrographs of Shaw (1979) indicated that hyphal invasion is intercellular. Invading hyphae cause extensive enzymic degradation of the middle lamellae and a general weakening of the host cell walls thus facilitating their colonisation of the tissue.

Following invasion of the ovule, hyphal proliferation causes a rapid destruction of the ovary and formation of a sphacelial body. Sporulation commences 5-6 days after infection (Campbell 1958; Shaw 1979) and honeydew production about a day later (Shaw 1979).

Although complete destruction of the ovary is the normal course of events, partial colonisation followed by the development of a structure comprising both a sclerotium and a normal grain has been reported (Mantle 1972). The seed portion of this association was viable inspite of there being no conducting tissue, other than the plectenchymatous sclerotium, connecting the developing seed with the

plants' nutrient supply. Fungal colonisation did not proceed beyond the outer integument and it was this author's contention that at least partial development of the grain probably occurred after detachment from the rachilla.

1.6 Honeydew formation

The release of honeydew from florets parasitised by C.purpurea commences about seven days after infection (Shaw 1979). Although honeydew synthesis has in the past been attributed to disease-induced host enzymes (Campbell 1959), the more recent cross-inoculation work of Mower and Hancock (1975a) suggested that fungal enzymes are responsible for this process. Sucrose, drawn from the host, is converted by fungal enzymes, particularly β-D-fructofuranosidase (Dickerson 1972), to a mixture of monosaccharides, disaccharides and oligosaccharides within the diseased floral cavities. It was argued (Mower and Hancock 1975b) that this conversion causes a sucrose deficit within the fungal tissues and elicits further movement of sucrose from plant to fungal tissues. The water potential of the sphacelial tissue was considerably lower than that of the host on account of the utilisation of sucrose and, as a consequence, the plant sap was drawn into the floral cavitiy. This movement was demonstrated by greater translocation rates in the culms of grasses infected by C.purpurea than in healthy plants.

Shaw (1979) argued that although a sucrose deficit most probably contributes to the maintenance of honeydew flow it is unlikely to be the initial cause of release. Shaw postulated that fungal β-glucanase may be important in the initiation of honeydew release by causing degradation of the callose in the phloem tubes, thus enabling continuous and unimpeded flow. It was also suggested that the contents of the vascular tissues around the core of cells in the rachilla continually flood the plate of cells at the host/parasite interface. This would greatly increase the area of fungal cells which can absorb nutrients supplied by the host.

1.7 Honeydew composition

The sugar composition of honeydew was analysed in detail by Mower and Hancock (1975a) and includes hexoses (predominantly glucose and fructose), alditols, fructofuranosyl-glucoses and fructofuranosyl-alditols. The qualitative composition is almost constant in C.purpurea honeydew from different origins but the quantitative composition varies

with age of infection.

Nisbet (1975) proposed that the breakdown of host-supplied sucrose is achieved by a fructosyl transferase system (sucrose → glucose + oligosaccharides) but, under conditions of high fungal energy demand or high free sugar concentration, may invoke sucrose hydrolysis (sucrose → glucose + fructose). His experiments also demonstrated the ability of sclerotial hyphae to hydrolyse sucrose.

Although honeydew usually contains more than 50% w/v free sugars it also contains a small amount of ethanol (<1% v/v) and is rich in amino acids (Nisbet 1975). Nisbet (1975) compared the amino acid content of honeydew with that of a naturally released viscous guttation fluid from uninfected cereals. The concentration of amino acids was markedly less in the ergot honeydew, presumably because of partial utilisation by this parasite. There was evidence of the preferential uptake of glutamine in the presence of glutamate. In general the basic amino acids predominated during the first few days of honeydew exudation, but with increasing age of the infection the acidic amino acids became predominant.

Sugars and amino acids are likely to satisfy carbon and nitrogen requirements but ergot parasitism also requires the supply of a range of biologically important cations (Na, Mg, Fe, Zn), anions (PO_4, SO_4) and possible vitamins and growth factors. The composition of honeydew is likely, therefore, to be very complex.

1.8 Differentiation and sclerotial development

Approximately 12-16 days after infection, depending on the fungal strain (Corbett et al 1974) and to a lesser extent on temperature and humidity, the fungus 'switches' from a sphacelial to a sclerotial growth form. The loosely arranged hyphae of the sphacelium become increasingly septate, wider, more bulbous, and pigmented (Ramstad and Gjerstad 1955; Shaw 1979). The lipid content of the cells starts to increase about two days before these gross changes become apparent; small lipid droplets coalesce and bulge into the vacuoles thus causing invagination of the tonoplast (Shaw and Mantle 1980a). Pigmentation is usually first seen at the sphacelium base (Nisbet 1975) but in some strains may be initiated at random within the sphacelium (Corbett et al 1974).

Shaw and Mantle (1980b) showed that the youngest absorbing hyphae at the base of a developing sclerotium are not themselves sclerotial. The lipid content of these cells is low and they contain many vacuoles. The zone of lipid-free cells extends distally for about 150μ, above which the hyphae become sclerotialised.

The anthers of the parasitised flower and the remains of the sphacelium often remain attached to the distal end of the developing sclerotium, and the aborted ovary may sometimes be found embedded in the sphacelial cap (Ramstad and Gjerstad 1955). The switch from sphacelial to sclerotial growth is accompanied by a rapid decline of both spore production and exudation of honeydew from the florets.

The volume of plectenchymatic sclerotial tissue increases, apparently by proximal hyphal growth (Corbett et al 1974), and after a few weeks the ergot sclerotium may protrude from the host floret. Shaw and Mantle (1980b) postulated that the increase in sclerotial length is achieved by hyphal tip extension in the zone of lipid-free cells, and that the dense plectenchymatous form results from hyphal branching and lipid deposition in tissues distal to this zone.

1.9 Sclerotial metabolism

After the onset of sclerotial growth the lipid content of the hyphae increases rapidly to about 30% w/w (Corbett et al 1974; Nisbet 1975). The proportion of ricinoleate (OH 18:1) rises steadily during sclerotial development (Corbett et al 1974) and is thought to be synthesised from linoleate (18:2) (Morris et al 1966; Nisbet 1975). The lipid concentration is highest in the basal 1 mm of a developing sclerotium thus suggesting that, as the mycelium is displaced further from the source of host nutrients, some of the fatty acids are oxidised (Nisbet 1975).

There is a lag of approximately 5 days between the first signs of sclerotial growth and the onset of alkaloid production (Corbett et al 1974). Nisbet (1975) reported that alkaloid synthesis continued for several days in sclerotial tissues which had already achieved their maximum lipid content. The distribution of alkaloids within a developing sclerotium contrasts with that of lipids and is usually highest at the distal end (Nisbet 1975), reflecting the respiration of lipid in this region. The mean alkaloid content of sclerotia varies

between strains and is usually within the range of 0.2-1.0% (Kybal 1963); at least one strain (12-2) is completely devoid of alkaloids (Corbett et al 1974).

The lower metabolic rate of sclerotial tissues, as compared to sphacelial tissues, is reflected in the decreasing concentration of RNA, malate and the major polyols (glycerol, arabinitol, mannitol) during the first few weeks of sclerotial development (Corbett et al 1974). The respiration studies of Nisbet et al (1977) indicated that fungal metabolism changed from carbohydrate utilisation during the sphacelial stage to lipid catabolism during sclerotial growth. They reasoned that the acropetal displacement of tissues during sclerotial growth cuts most of the mycelium off from the host nutrient supply thus enforcing the change to lipid respiration.

Corbett et al (1974) demonstrated that the spectrum of amino acids, and their relative abundance, fluctuated during sclerotial development in response to changes in free sugars which apparently varied with prevailing environmental conditions. The amino acid content of mature sclerotia was reported to be around 1% (w/w); the spectrum of alkaloids remained constant throughout sclerotial development.

From three to four weeks after infection until maturity the sclerotial content of protein, total carbohydrate, lipid, nucleic acid and alkaloid is almost constant, thus resulting in a sclerotium composed of homogeneous tissue (Corbett et al 1974).

1.10 Competition between seeds and ergots within an ear

During their studies of the parasitic metabolism of C.purpurea, Corbett et al (1974) compared the development of seeds from healthy and infected rye ears. The mean dry weight of sclerotia of one strain (12-2) exceeded that of normal seeds, and the seeds which developed in ears infected with this strain were less than half the weight of those from healthy ears. Although the sclerotia of a second strain (17-3) were lighter in weight than normal seeds, the weight of seeds from ears infected with strain 17-3 was 30% less than that from healthy ears. No differences in maturation rate were detected between groups of seeds and all were similar with respect to moisture content and composition (see over).

The protein content of sclerotia was twice that of rye seeds. Although the DNA content of the seeds was slightly greater than that of mature sclerotia, the RNA concentration was only about 5% of the sclerotial level since most of the seed RNA is in the embryo. The DNA content of seeds from infected ears was lower than in those from healthy ears, and the RNA levels suggested that the development of seeds in infected rye ears was retarded.

The total carbohydrate and free amino acid concentrations were similar in seeds from healthy and infected ears. However the relative proportions of amino acids showed considerable variations, the level of alanine being temporarily depressed during sclerotial development.

Pomeranz et al (1975) compared the amino acid composition of ergot sclerotia with that of seeds removed from ergot infected cereal and grass ears. In agreement with Corbett et al (1974) they recorded higher levels of Kjeldahl-nitrogen in sclerotia as compared with the seeds. Quantitative differences among the protein amino acids of ergots and seeds were recorded, and five unidentified amino acids were present in the sclerotia but not in the seeds.

1.11 Carbon assimilation by C.purpurea

The directional flow of metabolites to fungal parasites within infected plants has been demonstrated for a wide range of pathogenic systems (Yarwood and Jacobsen 1955, Smith et al 1969). Dickerson et al (1976) designed a system for studying $^{14}CO_2$ fixation by ergot-infected rye ears and found that most of the fixed carbon appeared in the fungal tissue rather than in adjacent uninfected seeds. The fixed carbon reached the developing sclerotia mainly as sucrose.

With respect to carbon assimilation, Dickerson et al (1976) showed that young developing sclerotia were more metabolically active than older ergots. Also the mycelium at the base of a developing sclerotium was shown to be the most active, probably on account of the more readily available supply of host nutrients.

Carbon fixed by the plant reaches developing ergots as sucrose and is cleaved by fungal sucrase to form oligosaccharides and free hexose which the parasite can assimilate (Dickerson 1972; Mower and Hancock 1975b); Dickerson et al 1976). Some of the hexose (glucose or fructose) is

converted within the developing sclerotium to mannitol via phosphorylated intermediates which are thought to include glucose-6-phosphate and fructose-6-phosphate (Cooke and Mitchell 1970; Dickerson et al 1976). The isotopic studies of Dickerson et al (1976) showed enrichment of the specific activity of mannitol over that of the hexose precursor, which led them to suggest that the pentose phosphate pathway may be involved.

Corbett et al (1975) reported that mannitol synthesis via phosphorylated intermediates also occurred when excised stromata were incubated in solutions of labelled soluble carbohydrates. The principal site of mannitol synthesis was within the capitulum and it was suggested that the rapid uptake of exogenously supplied sugars was passive. Very little exogenously supplied mannitol was metabolised.

1.12 Carbon dioxide fixation by fungi

Changes in carbon dioxide concentration affect the morphology and differentiation of many fungi (Yanagita 1963) and CO_2 may be an acceptable substrate as well as being a major excretion product. Although at elevated concentrations carbon dioxide stimulates the growth of some fungal species (MacCawley and Griffin 1969b) and inhibits the growth of others (Tabak and Cooke 1968; Griffin 1972), at less than 2.1 atmospheres it generally has no effect on the linear growth rate of fungi provided that conditions are aerobic (MacCawley and Griffin 1969a).

The first direct evidence that fungi can fix carbon dioxide came from the experiments of Foster et al (1941) who detected ^{14}C incorporation into submerged cultures of Rhizopus nigricans and Aspergillus niger when they were supplied with $^{14}CO_2$. Since then CO_2 fixation has been demonstrated more or less universally in fungi (Larmour and Marchant 1977). The extent of CO_2 fixation varies with species and conditions but Mirocha and DeVay (1971) suggested that a Fusarium sp. could obtain all its carbon from CO_2 fixation, although under these circumstances its growth was very slow. A large proportion of fixed carbon is usually incorporated into amino acids (Foster et al 1941; Lynch and Calvin 1952; Hartmann et al 1972). and the remainder is distributed among the other major cell components (Larmour and Marchant 1977). However, the metabolic products into which CO_2 is incorporated by a particular fungal species may vary with the growth stage: Yanagita (1963) reported that $^{14}CO_2$ was incorporated into an acid-soluble fraction by dormant conidia

of A.niger, whereas proteins and nucleic acids became heavily labelled in germinating conidia of that species.

Studies on carbon dioxide fixation by plant pathogenic fungi have concentrated on the rust organisms. Staples and Weinstein (1959) incubated rust uredospores (from bean and wheat leaves) in $NaH^{14}CO_3$ solution for one minute and demonstrated that ^{14}C was widely distributed among the intermediates of the disease cycle. Enhanced CO_2 fixation was recorded by Zaki and Mirocha (1965) only four days after bean leaves were inoculated with rust uredospores, and before any disease symptoms were visible. Seven days after inoculation, dark fixation by infected plants was 3.6 - 4.5 times greater than that of healthy controls. Between days five and seven, although the respiration rate of rust-infected tissue increased and photosynthesis remained steady, an increase in dry weight was recorded and attributed, at least in part, to enhanced CO_2 fixation in the dark. Stallknecht and Mirocha (1970, 1971) demonstrated that the rate of CO_2 fixation by uredospores of Uromyces phaseoli was greatest during the initial stages of germination and decreased rapidly during the first two hours after germination. The fixation rate was the same in the light as in the dark, and most of the fixed carbon was incorporated into proteins and polysaccharides. Soluble low molecular weight compounds, RNA and other macromolecules were also labelled: more ^{14}C was detected in the purine nucleotides of RNA than in the pyrimidines.

2. GENERAL METHODS

2.1 Growth of cereals in field plots

Several varieties of spring and winter wheat were grown in experimental plots at R.H.M. trial sites near High Wycombe, Buckinghamshire for harvest in 1975, 1976 and 1977. The plots (15.5m x 1.15m) were sown in blocks using an eight coulter seed drill and seed rates of 125 kg/ha (290 gm/plot) for winter wheat and 185 kg/ha (430 gm/plot) for spring wheat. Plots were incorporated into the crop rotation schemes at Model Farm, Pyrton (Map ref. SU696972), Grove Farm, Kingston Blount (Map ref. SU742982) and Hall Barn Farm near Beaconsfield (Map ref. SU943893). Cultivation and the application of agrochemicals was carried out as necessary by agronomists of the Lord Rank Research Centre. Netting was used to protect plots where bird damage could be expected. All experimental ears were harvested by hand.

2.2 Growth of cereals in open plots at the Chelsea Physic Garden

Tetraploid spring rye, cv. Fourex (Swedish Seed Association, Svalof, Sweden), and several varieties of spring wheat were grown in open plots (10m x 2m) at the Chelsea Physic Garden, London, for harvest in 1975, 1976 and 1977. Seed was hand sown in double drills, 0.5m apart, and developing plants were kept weed free by periodic hoeing. Agrochemicals were not used and soil fertility was maintained by application of compost. Ripening plants were netted to prevent bird attack. Mature ears were harvested by hand.

2.3 Growth of cereals in the glasshouse

In order to maintain a succession of cereals throughout the year, several varieties of spring wheat and a tetraploid spring rye, cv. Fourex, were grown in the glasshouse at the Chelsea Physic Garden. Plants were grown in five inch plastic pots containing John Innes potting compost no. 2 (5 plants/pot). A capillary bench provided continuous watering. A 21 hour day was sustained and daylight supplemented by 700W MBFR/V mercury vapour lights. A minimum temperature of 10°C was maintained by electric heating and excessively high temperatures were avoided by using a thermostatically controlled extractor fan. Insect pests were controlled by periodic fumigation with nicotine. No fungicides were used.

2.4 Storage of harvested material

Immediately after harvest any wet ears were dried in a stream of warm air before being stored at room temperature (c.20°C) in cloth or paper bags.

Threshed grain and ergots were stored at room temperature in cloth bags or loosely capped glass vials.

2.5 Storage and preparation of inoculum

Freshly collected honeydew was mixed with glycerol 10% (w/v) and pipetted into polypropylene ampoules (2 ml capacity, Sterilin), sealed, and frozen at -20°C prior to storage under liquid nitrogen.

When required, ampoules were removed from liquid nitrogen and quickly thawed by immersion in water at 30°C (Shaw 1975). Inoculum was prepared by diluting the honeydew with distilled water to give a minimum spore concentration of $1x10^6$ spores/ml.

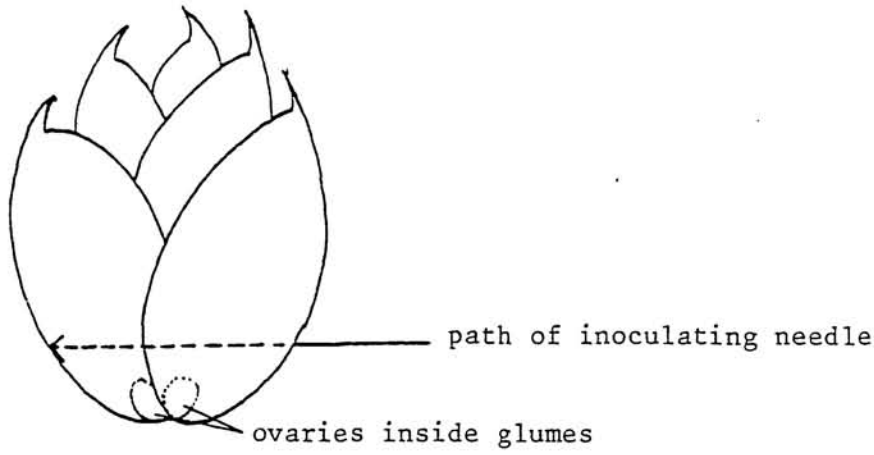
Only when parasitically produced (honeydew) spores were unavailable were spores from agar cultured C.purpurea used. The requisite strain was grown for 2-4 weeks on slopes of an asparagine/sucrose agar (Mantle and Tonolo1968). A spore suspension was prepared by shaking the slope with a small volume of distilled water: further dilutions were made, if necessary, to give a minimum spore concentration of $1x10^6$ spores/ml.

2.6 Inoculation of cereals

Plants were usually inoculated after the ear had emerged from the leaf sheath and, except when specifically stated, before anthesis ie. between growth stages 10.1 and 10.5 (Large 1954). Approximately 0.1 ml of inoculum was injected into each of the two basal florets of spikelets, as required by the experiment, using a hypodermic needle (25 gauge). The needle was inserted at a median level (figure 1) in order to avoid damaging the ovary.

For experiments which involved the comparison of heavily and lightly infected ears, approximately 80% and 20% respectively of the spikelets were inoculated using the regular pattern shown in figure 2. Inoculation of each ear was carried out acropetally.

The inoculation of spikelets Figure 1

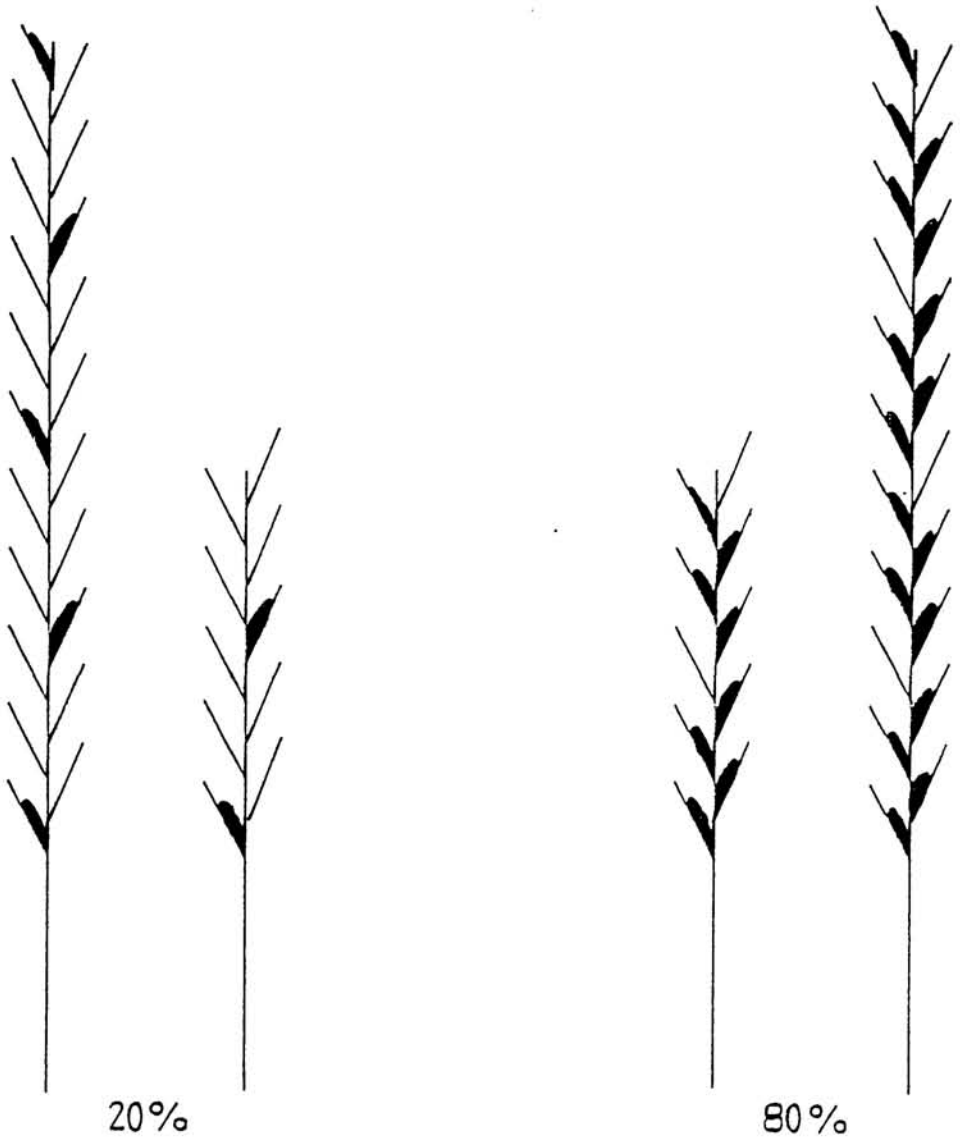


2.7 Supply of $^{14}CO_2$ to intact plants and excised ears

$^{14}CO_2$ was supplied to infected and healthy ears of rye using the apparatus described by Dickerson et al (1976). Briefly this involved generating $^{14}CO_2$ (c. 0.5m Ci) within an enclosed system, and pumping the radioactive gas round the circuit into which the rye ears were sealed (figure 3). The CO_2 concentration at the beginning of each experiment did not exceed about 2.4 times the background concentration in order to avoid atypical metabolism of either the host or parasite. CO_2 was supplied over a 5 hour period during which time actively photosynthesising ears reached gaseous equilibrium between assimilated and expired CO_2 (eg. figure 47).

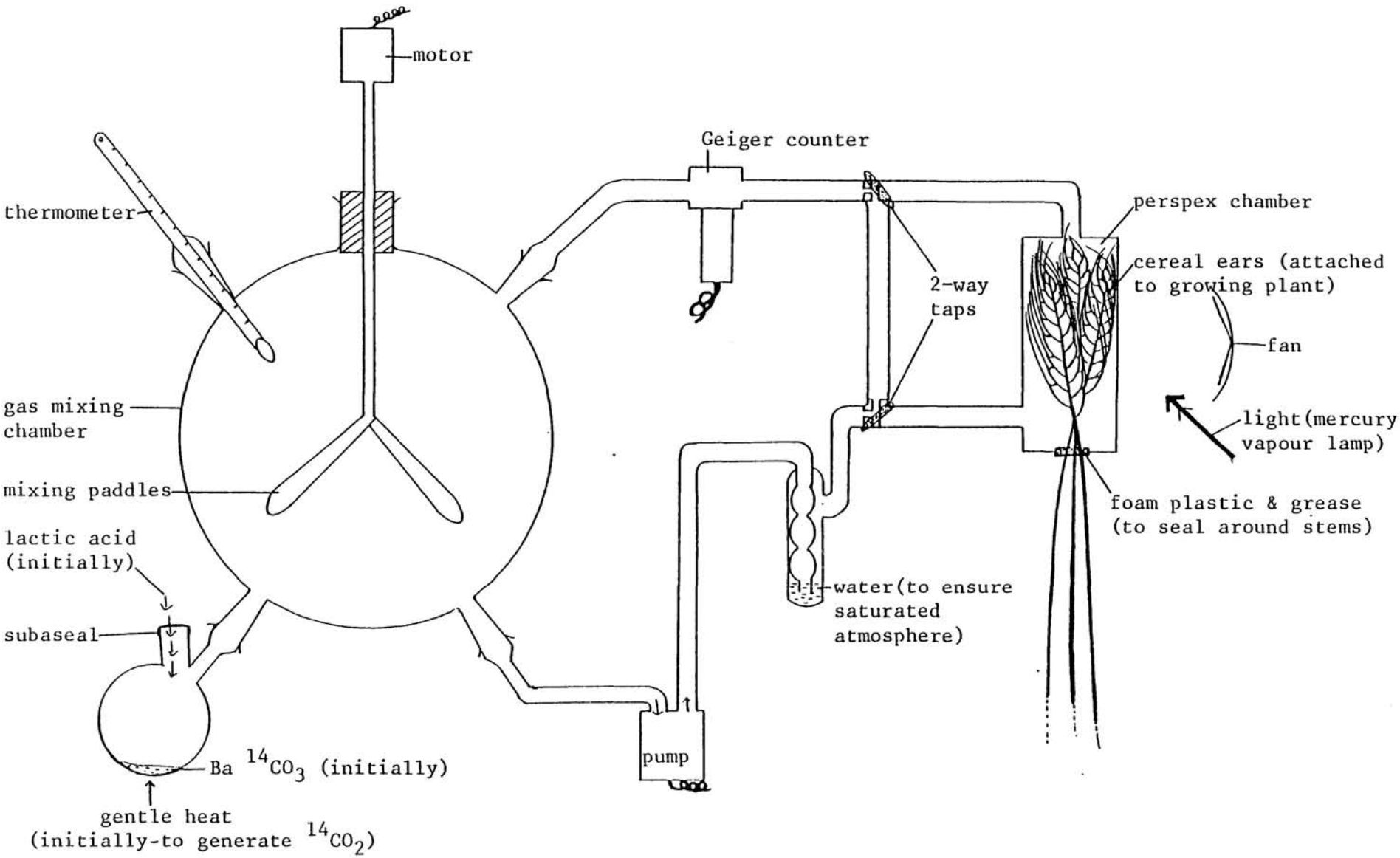
After feeding, the plants were either allowed to grow to maturity or the ears were immediately plunged into liquid nitrogen to terminate gaseous exchange. After 5 minutes submersion they were transferred to dry aluminium foil packets and stored at $-20^{\circ}C$ until required for analysis.

Design for inoculating 20% or 80% of spikelets Figure 2



Apparatus for the supply of $^{14}CO_2$ to cereal ears

Figure 3

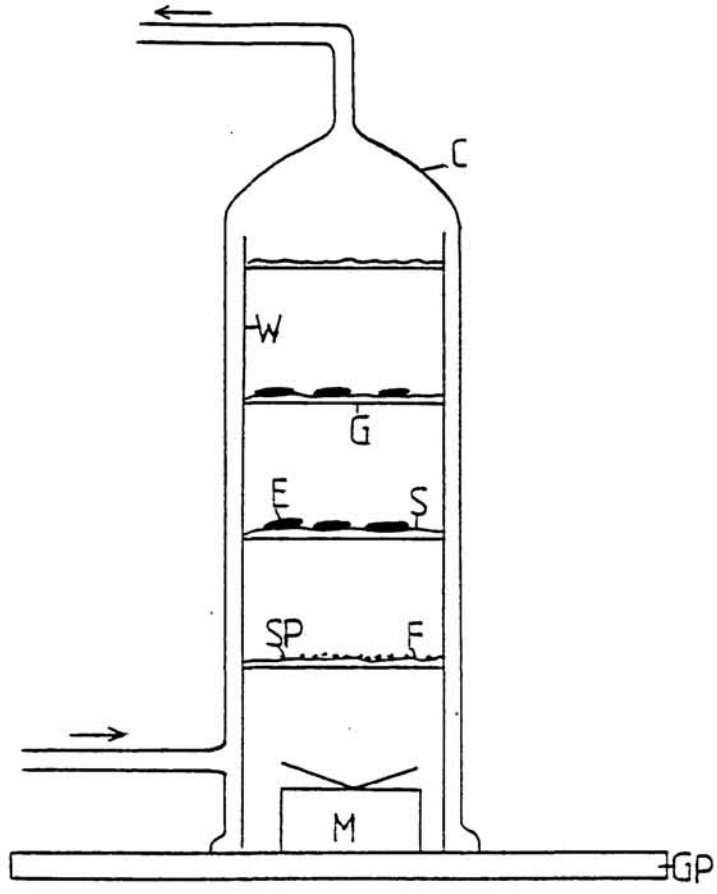


-ground glass joints were used wherever possible but a few of the connections were made with rubber tubing.

Figure 4

Chamber used when supplying $^{14}CO_2$ to isolated parasitic tissues

- substitutes for the perspex chamber in figure 3.



Arrows indicate direction of gas flow.

E = whole sclerotia

F = filter paper (moist)

G = nylon gauze

GP = glass plate

M = magnetic stirrer

C = chamber

S = stockinette (moist)

SP = sphacelial tissue or macerated sclerotia

W = wire supports

2.8 Supply of $^{14}CO_2$ to isolated parasitic tissues

Parasitic tissues (sphacelial or sclerotial) were excised from infected rye ears and all adhering flower parts were detached. This procedure was performed as rapidly as possible, and in order to maintain maximal metabolic activity, they were placed on moist filter paper in enclosed petri dishes until dissection of all the ears was complete. The apparatus and method described by Dickerson et al (1976) for supplying $^{14}CO_2$ to excised ears was used. The isolated parasitic tissues were supported in the round 'plant' chamber on nylon gauze platforms covered with damp stockinette for whole sclerotia or moist filter paper for smaller tissues (figure 4).

To terminate gaseous exchange, on removal from the chamber the tissues were dropped into 1-2 ml of liquid nitrogen in plastic tubes, which were supported in a liquid nitrogen bath. After about 5 minutes, when all the liquid nitrogen had evaporated from within the tubes, they were wrapped in foil and stored at $-20^{o}C$ pending analysis.

2.9 Detection and assay of radiolabelled compounds

2.9.1 Autoradiography

Paper chromatograms and thin layer silica gel chromatograms of radioactive (^{14}C) carbohydrates, amino acids or alkaloids were exposed for a suitable period to Kodirex No-screen X-ray film. During early experiments the autoradiograms were developed manually, but latterly an automatic processor (Kodak Industrial X-OMAT, model 3) was used.

2.9.2 Elution from paper chromatograms

In experiments where quantification of the isotope content of chromatographically separated compounds was required, the labelled areas were cut from the paper chromatogram following autoradiography. These paper strips were eluted with water and the eluate was evaporated to dryness using an infra-red lamp and fan. The dry compound was dissolved in a suitable solvent and an aliquot used for scintillation counting.

2.9.3 Scintillation counting

To determine the isotope content of solutions an aliquot was added to 5 or 10 ml of scintillation fluid and counted in a Beckman (LS230) counter. The scintillation fluid used for all experiments, except those involving tissue oxidation (section 2.10.4), contained 6g butyl PBD (5-[4-biphenyl]

-2-[4-t-butylphenyl]-1-oxa-3,4 diazole) and 50g naphthalene per litre of toluene. When aqueous samples were to be counted, sufficient 2-methoxyethanol was added to produce a single phase system.

Corrections for variable efficiencies were made after counting unlabelled samples, to which a known amount of ^{14}C hexadecane had been added. It was assumed that no ^{12}C-^{14}C exchange reactions occurred.

2.9.4 Tissue oxidation

Rapid determination of the isotope content (^{14}C) of undigested sclerotial and stromatal tissues was achieved using an Intertechnique sample oxidiser (model 4101) in which the $^{14}CO_2$ released during oxidation was automatically dissolved into 15 mls of a scintillation cocktail (400 mls toluene AR, 330 ml phenylethylamine [water white; scintillation grade], 220 mls methanol AR, 7g butyl PBD [scintillation grade], 50 ml water). Each sample was counted for 5 minutes in a Beckman scintillation counter (model LS230). The efficiency of the system was determined by oxidation and scintillation of paper standards impregnated with a known amount of ^{14}C.

2.10 Perchloric acid extraction of tissues

15-30 mg sclerotial tissue were ground, using a pestle and mortar, with 0.2 ml 1M perchloric acid (PCA) at 4°C, and washed into centrifuge tubes with a further 0.8 ml PCA. The mixture was incubated at 4°C for 3 hours. After centrifugation the supernatant was removed and three successive two hour extractions of the tissue were made using 1 ml 0.2M PCA at 4°C, 1 ml H_2O at 4°C and 2 ml H_2O at 100°C. All the extracts were combined and made up to a standard volume before being assayed for radioactivity (section 2.9).

2.11 Serial extraction of tissues

The procedure used was essentially that described by Dickerson et al (1970).

The tissues were briefly washed with water, homogenised and freeze dried before successive extraction as outlined below. An approximate ratio of 10 mls extractant to 1g d.wt fungal tissue or 0.5g seed tissue was used. The larger volumes of extractant for the seed tissues were necessary to lower the viscosity of the suspensions. In experiments where less than 1g fungal tissue or 0.5g seed tissue was available for

extraction, 10 mls of each extractant was used as this was the smallest volume that could be handled efficiently.

The successive extractions involved:

a) removal of low molecular weight compounds by steeping the tissues in 5% trichloroacetic acid (TCA) at 4°C for 20 minutes. During the first five minutes of this extraction, to ensure complete homogenisation, the tissues were ground in a pestle and mortar with one quarter of the extraction volume of TCA

b) removal of lipid (and some protein) using 75% ethanol (v/v in water) at 45°C for 30 minutes

c) removal of any remaining lipid using 75% ethanol (v/v in water) plus diethyl ether (1+1) at 45°C for 15 minutes

d) removal of nucleic acids using 5% TCA at 95°C for 30 minutes

e) hydrolysis of the insoluble residue (insoluble protein and polysaccharide). Prior to hydrolysis the residue was washed twice with ether to remove any remaining TCA and dried under vacuum over calcium chloride. Hydrolysis was achieved by adding 6M HCl (1 ml/10 mg sample) to the residue in tubes which were then sealed under vacuum and heated at 110°C for 18 hours. The hydrolysate was neutralised with sodium hydroxide.

In later experiments steps b & c in the above series of extractions were replaced by extraction at room temperature with chloroform/methanol 2:1 (v/v) (as detailed in section 2.12) to remove the lipids. This was preferred for the fungal tissues as it is a well established, exhaustive extraction method for sclerotial lipids.

2.12 Quantitative extraction of lipids

Finely milled or homogenised tissue was extracted three times with a 2:1 (v/v) mixture of chloroform and methanol. The extraction times were normally 18 hours, 3 hours and 3 hours and an approximate ratio of 40 mls extractant per 1g tissue was used. All three extracts were combined and filtered through a fat-free extraction thimble (Whatman) to remove any particulate matter. The lipids were partitioned into the chloroform by shaking out the chloroform/methanol extract with one fifth its volume of water. The methanol/water mixture was discarded after shaking with a little pure chloroform to remove any traces of lipid which may have been carried over. The chloroform extracts were combined and the solvent removed under reduced pressure. The crude lipid extract was purified by the addition of diethyl ether and

transferring the ether/lipid solution to a clean vessel. The ether was removed by evaporation leaving the pure lipids.

2.13 Quantitative extraction of alkaloids

2.13.1 Batch extraction

The method was similar to that described by Mantle (1967). Milled sclerotial tissue (1-3g) was made alkaline with aqueous sodium bicarbonate and exhaustively extracted with diethyl ether. The alkaloids were partitioned into aqueous tartaric acid (5% v/v) prior to quantification (section 2.14).

2.13.2 Extraction of individual sclerotia

Sclerotia (10-100 mg) were individually wrapped in strong paper (5x5 cm) and each packet was hammered to reduce the fungal tissue to a fine powder. The powdered tissue was weighed and mixed with about 1/10th its weight of sodium bicarbonate and sufficient water to form a stiff paste. It was extracted with 4 ml diethyl ether overnight in a sealed tube. The supernatant was pipetted off and, to ensure complete extraction, the tissue was treated with ether (1.5 ml) four more times, each of 1 hr duration. The ether extracts were combined and shaken out four times using a total volume of 3 ml aqueous tartaric acid (5% v/v).

2.14 Quantitative estimation of sclerotial alkaloid content

The procedure described by Mantle (1967) was essentially followed.

An aliquot of the tartaric acid extract of ergot (section 2.13) was diluted as appropriate and mixed with twice its volume of Van Urk reagent (1.25g 4-dimethyl amino benzaldehyde + 2 ml 5% $FeCl_3$ per litre 65% H_2SO_4). After 20 minutes the absorption of the blue colour was measured in an EEL colorimeter using a brown filter. The total alkaloid content was estimated from a standard curve for ergotamine and all results are therefore expressed as ergotamine.

2.15 Qualitative analysis of sclerotial alkaloids

Alkaloids were extracted as described in section 2.13. An aliquot of the tartaric acid extract was made alkaline (pH 8-9) with ammonium hydroxide and shaken out with an equal volume of chloroform. The chloroform extract was evaporated to a small volume before thin layer

chromatography on plastic plates precoated with silica gel G (Polygram Sil G, Camlab) with the relevant reference alkaloids. Separation was achieved using an ethyl acetate/ethanol/dimethyl formamide (19:0.1:1.9 by volume) solvent system (McLaughlin et al 1964) except when otherwise stated. The plates were examined under UV light (350 nm) and the blue fluorescent areas noted. The plates were then sprayed with 5% para-dimethyl amino benzaldehyde in concentrated hydrochloric acid to confirm the identity of lysergic acid derivatives by the characteristic blue/purple Ehrlich reaction.

2.16 Separation of carbohydrates from amino acids by ion exchange

Aqueous mixtures containing sugars and amino acids were rendered mildly acidic by the addition of HCl prior to their separation by ion exchange. The amino acids were removed by passage through a column of Amberlite IR 120 (H^+) cation exchange resin; the carbohydrates were exchanged by passing the cation-free eluate through a column of weak anion exchange resin (Amberlite IR 45; OH^-). The two columns were linked in series as shown in figure 5. Prior to elution the columns were separated; the amino acids were eluted with 2M NH_4OH and the carbohydrates with water. The eluates were reduced to the required volume, or to dryness, using a rotary film evaporator.

2.17 Separation of amino acid mixtures by two dimensional paper chromatography

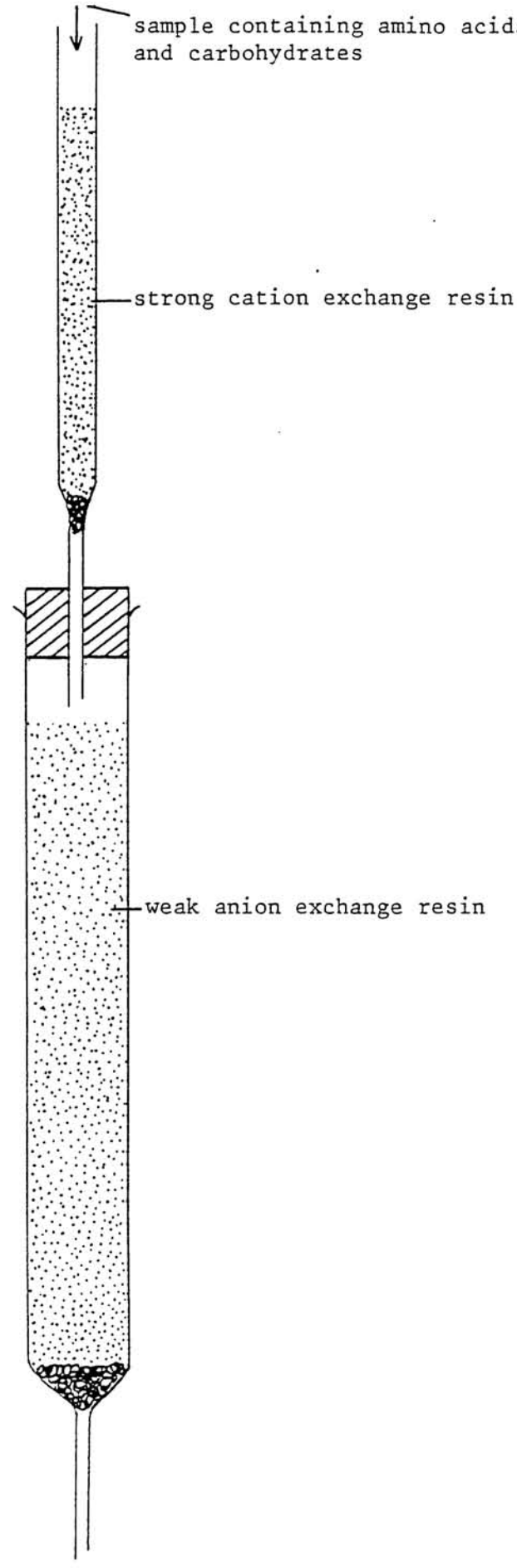
Each aqueous amino acid mixture was loaded onto one corner of a 10" x 10" piece of Whatman no. 3MM chromatography paper. Chromatography, in the ascending mode, was carried out for 16 hours using a butanol/acetic acid/water (120:30:50 by volume) solvent system. After drying at room temperature the papers were rotated through 90° and run (ascending) for 16 hours in a phenol/ammonia (200:1 v/v) mixture.

The chromatograms were dipped in ether to remove phenolic residues and developed by spraying with a solution of ninhydrin (0.2% w/v) in 95% ethanol (to which a few drops of 2:4, 2:5 lutidine had been added) followed by heating at $60^\circ C$ for a few minutes.

Individual amino acids were identified by reference to standards run on the same occasion.

Figure 5

Serial arrangement of ion exchange columns for separating amino acids from carbohydrates



2.18 Separation of carbohydrate mixtures by paper chromatography

Carbohydrate mixtures containing both sugars and polyols were separated on Whatman's 3MM paper using a methyl-ethyl-ketone/water saturated with boric acid/glacial acetic acid (9:1:1 by volume) solvent system in the descending mode for 20 hours.

The dry chromatograms were pulled through acidic methanol (methanol/acetic acid, 100:1 v/v) and dried at 100°C. This process was repeated five times to decompose the borate complexes. Detection was achieved using the two-step method essentially described by Trevelyan et al (1950) which involved quickly dipping the chromatogram in a silver nitrate solution (1 ml saturated $AgNO_3$/100 ml acetone; sufficient water added to dissolve the white precipitate). The carbohydrates were located by pulling the chromatograms through a trough containing ethanolic sodium hydroxide ([1g NaOH + 2 ml H_2O] + 200 ml ethanol) and allowing them to dry. This process was repeated three or more times until all the spots were clearly visible.

Sugars and polyols were identified by reference to standards chromatographed under the same conditions.

2.19 Identification and estimation of carbohydrates by gas-liquid chromatography

Volatile silyl derivatives of sugars and polyols were prepared by the method of Sweeley et al (1963). The carbohydrates were dissolved in anhydrous pyridine (5 mg/ml) by heating, in tightly capped polypropylene tubes, for four hours at 80-90°C. Hexamethyl disilazane (0.2 ml/1 ml pyridine) was added, followed by trimethylchlorsilane (0.1 ml/1 ml pyridine) and the mixture was incubated at 80°C for a further 5 minutes prior to centrifugation (8,000g, 2 minutes) to remove the precipitate.

1-7 µl of the supernantant were injected into a Pye 104 gas chromatograph fitted with a flame ionisation detector and a 5 ft column containing SE52 (3% or 5% w/w) on Chromasorb W. The column temperature was held at 130°C for 4 minutes after injection and then increased at 6°C/minute to 260°C. The programme was completed by a 10 minute isothermal period at this maximum temperature.

The carbohydrates were identified by comparing both the retention times

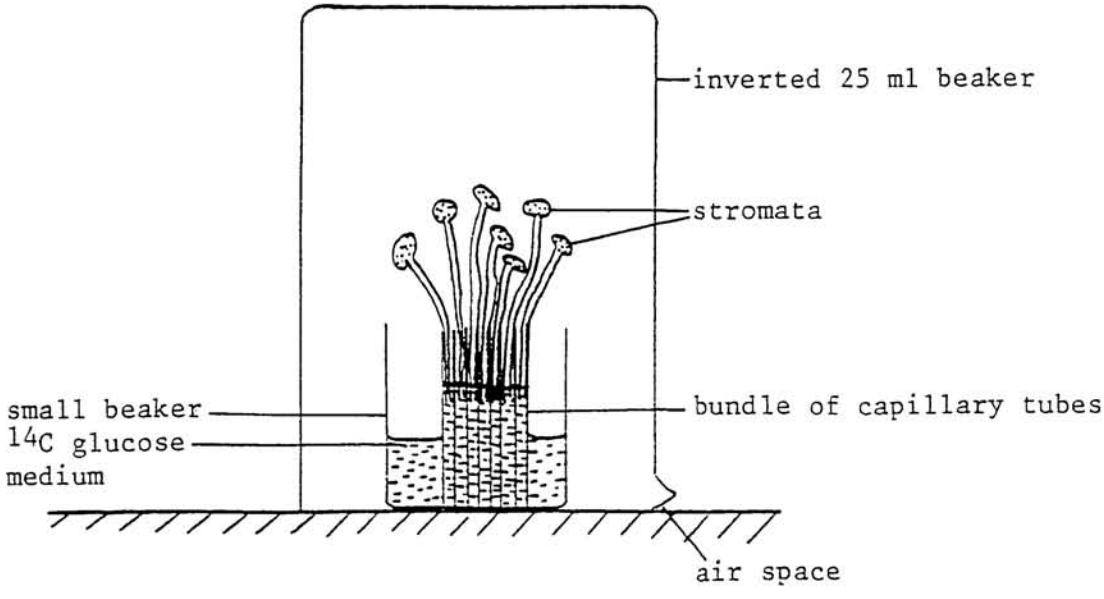
and elution temperatures with those of similarly derived standards. Concentrations were determined by integration of the peak areas and reference to standards of known concentration.

2.20 Supply of ^{14}C glucose to stromata

Bundles of 30 capillary tubes, 20 mm long and 1 mm internal diameter, were stood in small glass vessels (20 mm high and 16 mm wide) containing 2 ml of the incubating medium. The ^{14}C glucose medium used was that described by Corbett et al (1975) for the submerged incubation of stromatal tissues (7.5% (w/v) glucose in 2 mM sodium phosphate buffer at pH 7.0; 7.5 µCi ^{14}C/ml medium). Aureomycin (0.05 mg/ml) was included in this medium to minimise bacterial growth and all glassware was sterilised before use.

Stromata were excised from sclerotia 1-2 mm above the base of the stipes using a sharp scalpel. Each stipe was immediately inserted into one of the capillary tubes so that its cut end was just submerged in the glucose medium. The incubation period was timed from the midpoint of the excision period for that group (excision of each group took about 10 minutes). In order to maintain a steady, but not excessive, state of water tension during incubation each vessel was covered by an inverted 25 ml beaker with a lip (figure 6). Incubation was terminated by removing the stromata from the capillary tubes and washing them with 2 mM sodium phosphate buffer at pH 7.0.

The supply of ^{14}C-glucose to stromata Figure 6



2.21 Extraction of polyols from stromatal tissues

Polyols were extracted from stromata by incubating the macerated tissues in 80% ethanol (v/v in water) for 30 minutes, using 2 ml ethanol per 50-100 mg tissue (f.wt.). The polyol extract was removed from the macerate by centrifugation at 37,000g for 10 minutes at 4°C. The process was repeated twice more to ensure complete extraction.

2.22 Preparation of a spore trapping surface

A length of Melinex strip was attached round the 7 day drum of a Burkard Volumetric Recording Spore Trap, with a piece of double-sided adhesive tape, so that the two ends abutted but did not overlap.

A 10% solution of Gelvatol (Shawinigan Ltd., London SE1) was prepared as follows. Gelvatol (35g, grade 40-20) was added to 100 mls distilled water and allowed to stand overnight. Glycerol (50 ml) was added and the mixture warmed slightly to give a homogeneous solution in which phenol (2g) was then dissolved.

A thin film of the Gelvatol solution was applied to the Melinex strip. When the Gelvatol film was dry it was overlaid with a thin, even coating of Vaseline mixture (10% paraffin wax in Vaseline, by weight).

2.23 Analysis of spore trap tapes

At 7 day intervals the Vaseline-coated tape was removed from the spore trap and dissected into 48 mm lengths, each of which represented a period of 24 hours. The tapes were stained with 0.1% lactophenol blue and mounted on microscope slides. A thin film of Vaseline was injected with a fine hypodermic needle under the edge of each coverslip to prevent desiccation of the tape before the spores were counted.

The segmented tapes were examined under the microscope using 400 x magnification. Traverses across the width of the tape were made at 4 mm (≡ 2 hr) intervals and the *C.purpurea* ascospores in five fields of view/traverse, were counted.

3. SELECTION OF C.PURPUREA STRAIN FOR USE IN GERMINATION EXPERIMENTS

3.1 Experimental

Preliminary experiments involving the germination of C.purpurea sclerotia showed that germination was not always achieved and success was not predictable. To reduce the uncertainty which this introduced into experiments involving germination, an experiment was conducted to identify the most reliable strain(s).

Honeydew from 8 strains of C.purpurea was removed from liquid nitrogen and, after suitable dilution, used to inoculate 5-8 ears of greenhouse-grown wheat (cvs Maris Dove and Sappo) and Fourex rye (section 2.6). The origins of the strains used are summarised in table 1. The mature ears were harvested in July and the ergots were stored dry at room temperature after excision from the host. Ergots from the two wheat cultivars were combined.

100 wheat ergots and 50 rye ergots of each strain were rinsed in water containing Tween 80 (1 drop Tween 80 in 100 mls water) to wet the surface thoroughly. They were then surface sterilised in 0.1% mercuric chloride for 5 minutes and rinsed 5 times with sterile distilled water.

Trays containing one inch of moist sterile sand were prepared and after imbibing sterile water for one hour the ergots were spread over the sand surface so that none were touching. They were pressed lightly into the sand and each tray was covered loosely with a polythene sheet. The ergots were incubated at $4^{\circ}C$ for 7 weeks and then at room temperature for 7 weeks. Sterile distilled water was added to the trays as necessary to keep the sand moist throughout all phases of incubation. The ergots were examined periodically and the percentage germination and number of stromata/sclerotium scored for each strain. Any sclerotia which became heavily contaminated by other microorganisms were discarded to reduce the risk of infecting adjacent ergots.

Table 1

Origin of strains and percentage germination in strain selection experiment

Strain no.	Original host	Percentage germination		Sclerotial yield on wheat and rye[a]
		wheat	rye	
6	Dactylis glomerata	0	0	Good
17	Barley	0	0	Good
25B	Wheat	7	0	Good
44	Alopecurus myosuroides	1	0	Good
82	Rye	>70	>70	Good
330	Alopecurus myosuroides	>90	>70	Good
5/1	Phragmites communis	1	0	Poor
4/1	Lolium perenne	1	0	Poor

(a) see Shaw (1975).

Figure 7

Germination of strain 330 (grown on rye) in relation to the number of days at room temperature following 7 weeks at 4°C

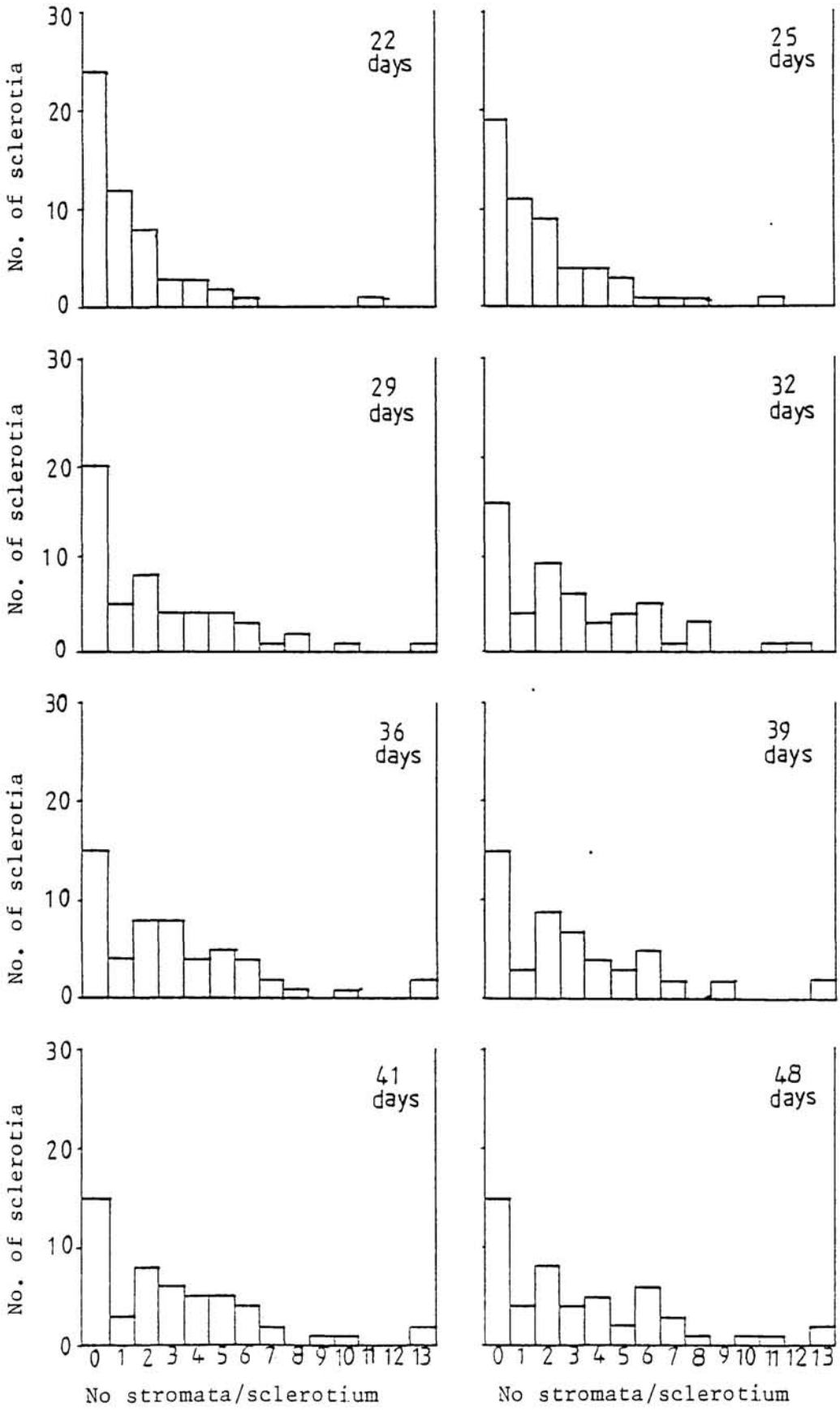
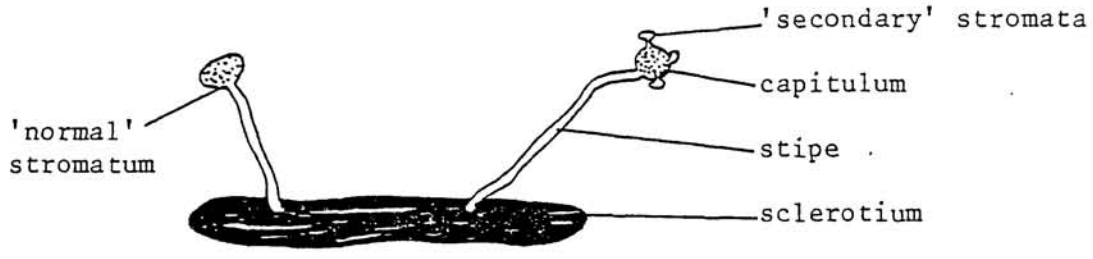


Figure 8

The formation of 'normal' and 'secondary' stromata by strain 330 of C.purpurea



3.2 Results

14 days after the trays of ergots were transferred from 4°C to room temperature, germination was observed in wheat and rye ergots of strains 82 and 330. Stromata production in these groups was prolific with up to 13 stromata arising from a single sclerotium (figure 7). After 5 weeks at room temperature the production of new stromata had apparently ceased and by 7 weeks most of these germinated sclerotia had a spongy texture due to the exhaustion of nutrient reserves. A high level of germination was only observed in these strains (table 1).

The differentiation of stromatal tissue into stipe and capitulum was well defined in strains 330 and 25B but was disorganised in about 20% of the stromata produced by strain 82. 5 weeks after germination started some old capitula of the strain 330 stromata produced extrusions of tissue which macroscopically had the appearance of secondary stromata although no perithecial cavities could be discerned (figure 8). Owing to the senescent state of the stromata by this stage no microscopic examination was made.

It was concluded from this experiment that although a high proportion of ergots of both strains 82 and 330 germinated, strain 330 produced better organised stromata except after the onset of senescence. On the basis of these results, strain 330 was used for most of the experiments which involved sclerotial germination.

4. INFLUENCE OF SOME PLANT HORMONES ON SCLEROTIAL GERMINATION

4.1 Experimental

Ergots were collected in December 1974 from Phragmites communis in Lincolnshire. One month later they were surface sterilised by steeping in 0.1% mercuric chloride for 5 minutes, and thoroughly washed using five changes of sterile distilled water. The sclerotia were divided into groups of twenty and soaked in one of the following test solutions for two hours: indole acetic acid (IAA), giberellic acid (GA_3), kinetin (CK) and indole-3-acetonitrile (IAN) at concentrations of 100, 10, 1, 0.1, 0.01 and 0.001 mg l^{-1}. Two groups of sclerotia were soaked in each test solution and seven groups were soaked in water to provide controls. Incubation chambers were prepared by lining 5 cm petri dishes with sterile cotton wool. One group of sclerotia was transferred to each chamber and the cotton wool was moistened with 3 ml of the appropriate test solution. The sclerotia were arranged so that none were touching.

The dishes were incubated at room temperature (c.20°C); sterile distilled water was added to each dish periodically to keep the sclerotia moist. After seven weeks the percentage of sclerotia which had germinated was recorded.

4.2 Results and discussion

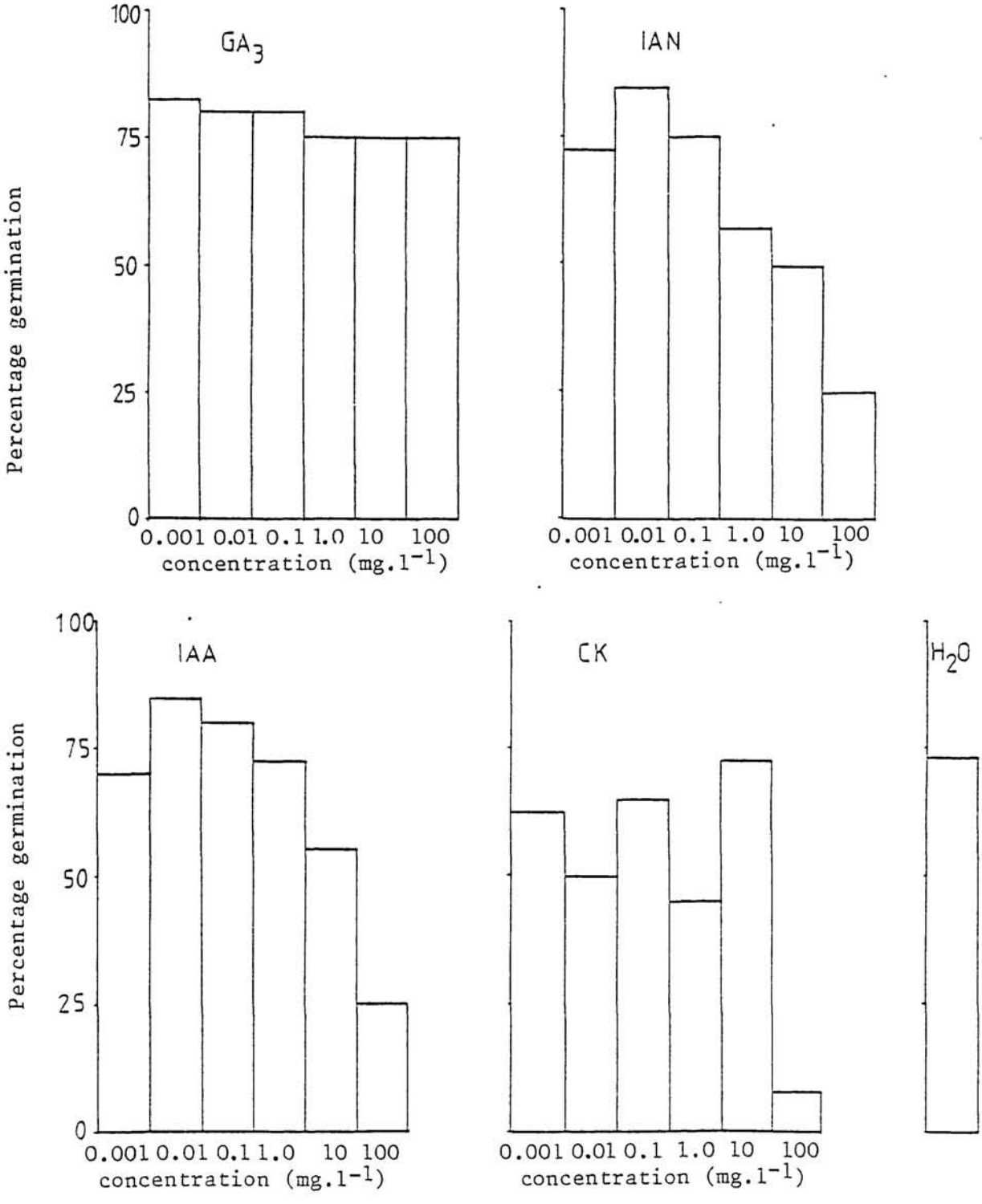
The mean percentage germination attained in each treatment group is recorded in figure 9. The sclerotia used in this experiment were collected in December so had probably been exposed to low temperatures while still attached to the Phragmites ears. This may account for the unexpected germination of the control sclerotia which had received neither hormone nor laboratory cold treatment. However, a requirement of exposure to low temperatures for initiating germination is not absolute for C.purpurea but varies considerably between sclerotia collected from different sources (Mitchell and Cooke, 1968). It is therefore possible that these Phragmites ergots did not need a cold treatment.

The germination of sclerotia was not affected by treatment with GA_3 at any of the concentrations used (figure 9).

The sclerotia treated with low concentrations of either IAA or IAN showed a similar percentage germination to the controls, but there was

Figure 9

Mean percentage germination of Phragmites sclerotia treated with plant hormones



a reduction in germination at higher concentrations of these hormones.

The germination response of sclerotia treated with CK appeared to be very variable, but there was a marked suppression of germination by the highest concentration. The solubility of CK in water is very low (c.50 mg l^{-1}): since the test solutions were prepared by dilution of a 100 mg l^{-1} solution/suspension, inaccuracy was inevitably introduced at this stage which may account for the observed variations.

The Molinia caerulea ergots studied by Cooke and Mitchell (1970) were similar to the Phragmites ergots used in this experiment in so far as they did not have an absolute requirement for a cold treatment. They also responded similarly to incubation with IAA and GA_3 within the concentration range used (0.01 to 10 mg l^{-1}).

5. INFLUENCE OF A COLD PERIOD ON THE GERMINATION OF SCLEROTIA FROM PHRAGMITES COMMUNIS AND LOLIUM PERENNE

5.1 Experimental

Sclerotia collected from Phragmites communis in December 1974 germinated without receiving a laboratory cold or hormone treatment (section 4.2). Since these ergots had been exposed to low temperatures while still attached to their host, an experiment was conducted to compare the germination capacity of sclerotia collected on 21.10.77 and 21.12.77 from the same roadside site in Lincolnshire from which sclerotia were obtained in December 1974. In addition, several ergotised ears of Lolium perenne were collected from this site on 21.12.77. The infected material were stored at room temperature until January 1978 when the sclerotia were removed and allowed to imbibe distilled water for one hour. 24 petri dishes were half filled with vermiculite which was moistened with distilled water and covered by a damp filter paper. 50 sclerotia were added to each dish and, in order to minimise contamination, arranged so that none were touching. The dishes were incubated for 0-6 weeks (figure 10) at $4^{o}C$, followed by 9 weeks at room temperature. Water was added as necessary to keep the sclerotia moist. The number of stromata per sclerotium was then scored.

5.2 Results

Germination commenced in each group of sclerotia approximately twelve days after they were transferred to room temperature. Germination in the different groups of ergots varied as shown in figure 10.

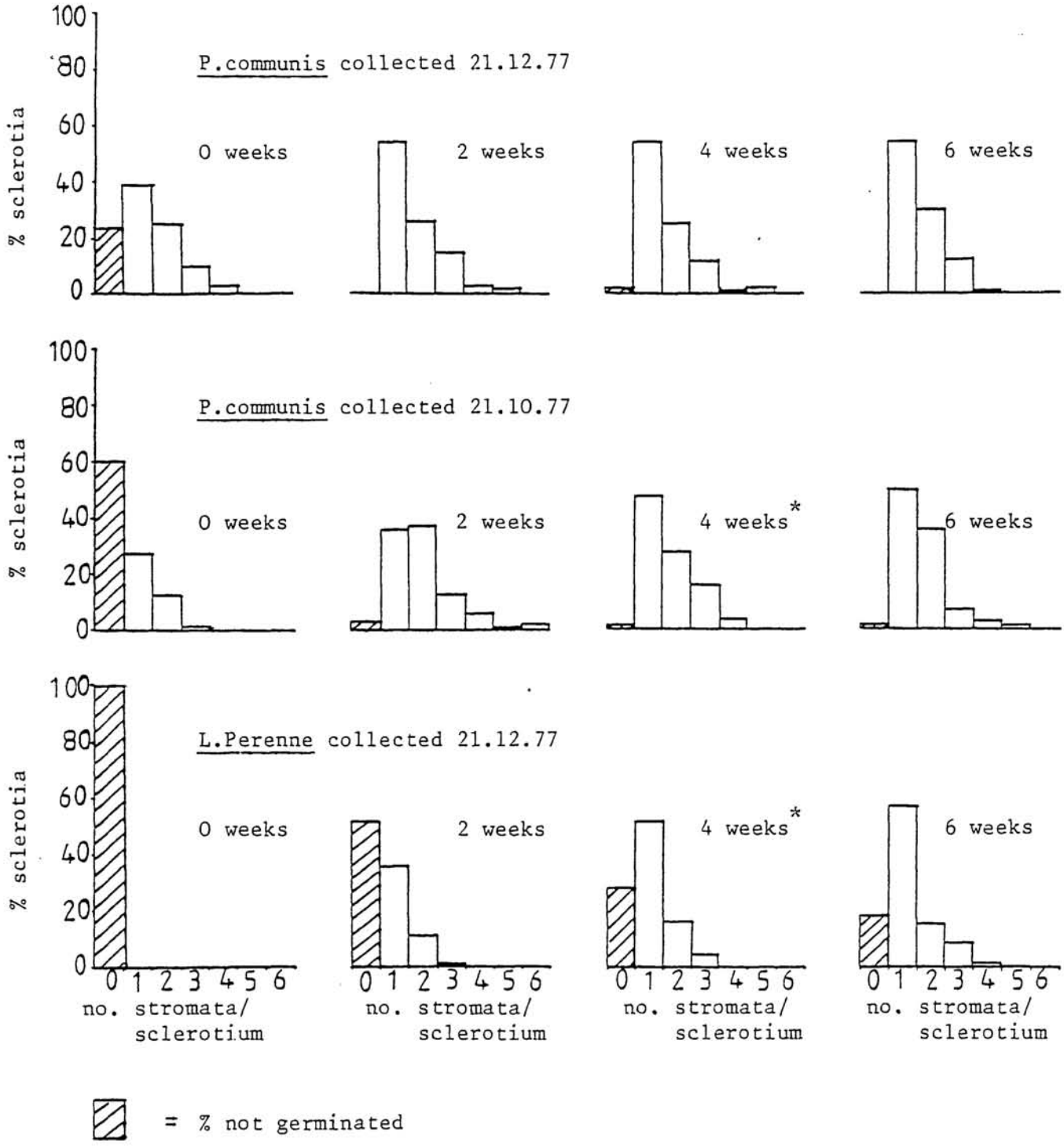
The Lolium perenne ergots, which were collected in late December, had an absolute requirement for additional cold treatment before they would germinate. The percentage germination improved progressively with increased cold treatment.

Neither the October- nor the December-collected P.communis sclerotia had an absolute requirement for additional exposure to low temperatures. However without cold treatment, 77% of the December-collected sclerotia germinated compared with 40% of those collected in October. It was notable that 2 weeks cold treatment was sufficient to enable more than 95% of the ergots, in both groups, to germinate. There was no apparent difference between treatment groups in the relative frequency of single and multiple stromata.

These results are in close agreement with the 74% germination of P.communis ergots collected in December 1974 (figure 9).

Figure 10

The production of stromata by sclerotia of Phragmites communis and Lolium perenne after incubation at 4°C for 0, 2, 4 or 6 weeks



All treatment group comprised 2 groups of 50 sclerotia, except those marked * for which only 1 group of 50 sclerotia were available.

6. GERMINATION STUDIES ON ^{14}C-LABELLED SCLEROTIA

6.1 Experimental - experiment 1

Fourex rye was grown in the glasshouse during Spring 1975 and approximately 80% of the spikelets were inoculated with strain 25B of C.purpurea prior to anthesis (section 2.6). The infected ears were divided into three groups and each group was exposed to an atmosphere of $^{14}CO_2$ (c.500μCi) for five hours, at twenty, twenty-five or thirty-three days after inoculation, using the methods described in section 2.7. Immediately after feeding (i.e. pulsing with $^{14}CO_2$) the plants were transferred to an open plot at the Chelsea Physic Garden and grown to maturity. All ears were harvested by hand and stored dry pending analysis.

Six ergots from each group were cut into eight segments, of approximately equal length, as shown in figure 11. The corresponding segments from each sclerotium were combined thus providing eight samples from each group. Each sample was extracted with perchloric acid (PCA) as described in section 2.10. The insoluble residue was dried in vacuo over calcium chloride and then hydrolysed using 1 ml 6M HCl/5 mg d.wt residue (section 2.11). An aliquot of each extract was assayed for radioactivity by scintillation counting (section 2.9.3).

To determine the extent to which ^{14}C had been incorporated into the sclerotial lipids, 3-4 ergots from each group were mixed together and segmented into eight samples as described above. Each sample was extracted by the method given in section 2.12, after grinding in a small volume of the chloroform/methanol extractant. The specific activity of the lipids was determined by scintillation counting (section 2.9.3).

6.2 Results and discussion - experiment 1

Analysis of the distribution of ^{14}C in ergots pulsed with $^{14}CO_2$ during this preliminary experiment demonstrated that although ^{14}C was incorporated throughout the sclerotia it was principally located in one region slightly distal to the sclerotium midpoint. This was shown for the PCA soluble fraction (figure 12), the hydrolysate of the non-PCA soluble residue (figure 13) and the lipids (figure 14) of sclerotia fed when 20, 25 or 33 days old. Since the growth rate of individual sclerotia appears to vary, and the sclerotia used for this

experiment were not all of the same length, the broad bands of ^{14}C activity could be the product of quite discrete bands within each sclerotium. Comparison of the distribution of ^{14}C in the PCA soluble fraction of sclerotia fed at 20 and 33 days suggests that there is a tendancy for displacement of the hot band towards the tip in some of the 20 day sclerotia, and towards the base in some of the sclerotia pulsed at 33 days (figure 12). There were no obvious differences between the distribution of ^{14}C in the PCA insoluble fraction of ergots pulsed at 20 and 25 days; this was probably due to the very short time interval (5 days) between these two feedings.

The limited number of ergots available for a lipid extraction necessitated combining segments from sclerotia fed at the three different ages. The very broad nature of the ^{14}C band in figure 14 reflects this procedure but, in common with the PCA-soluble and insoluble fractions, there was appreciably less ^{14}C towards the ends of the sclerotia. The peak of ^{14}C activity in each extraction is concentrated in the ergot tissues which were likely to have been growing most actively during the period of exposure to the $^{14}CO_2$-rich atmosphere, and a few days thereafter. These results are in agreement with those of Kobel and Sanglier (1979) who supplied ergot infected rye plants with labelled amino acids. They reported that labelled compounds were concentrated in that part of the sclerotium which was developing at the time of application.

Figure 11

Division of a labelled sclerotium into eight segments prior to analysis.

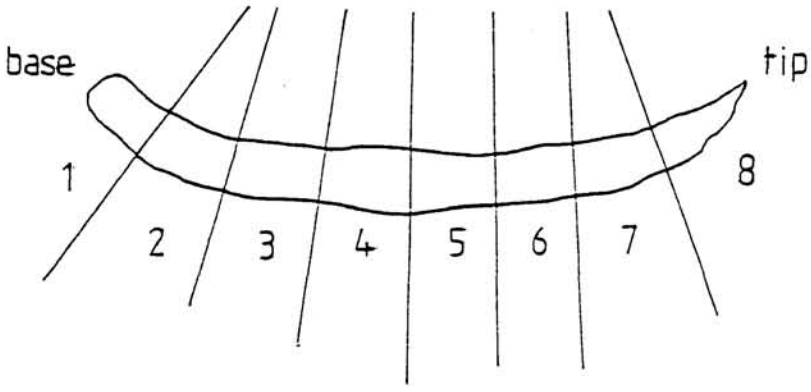


Figure 12

Distribution of ^{14}C in the PCA-soluble fractions of sclerotia pulsed with $^{14}CO_2$ during different stages of growth

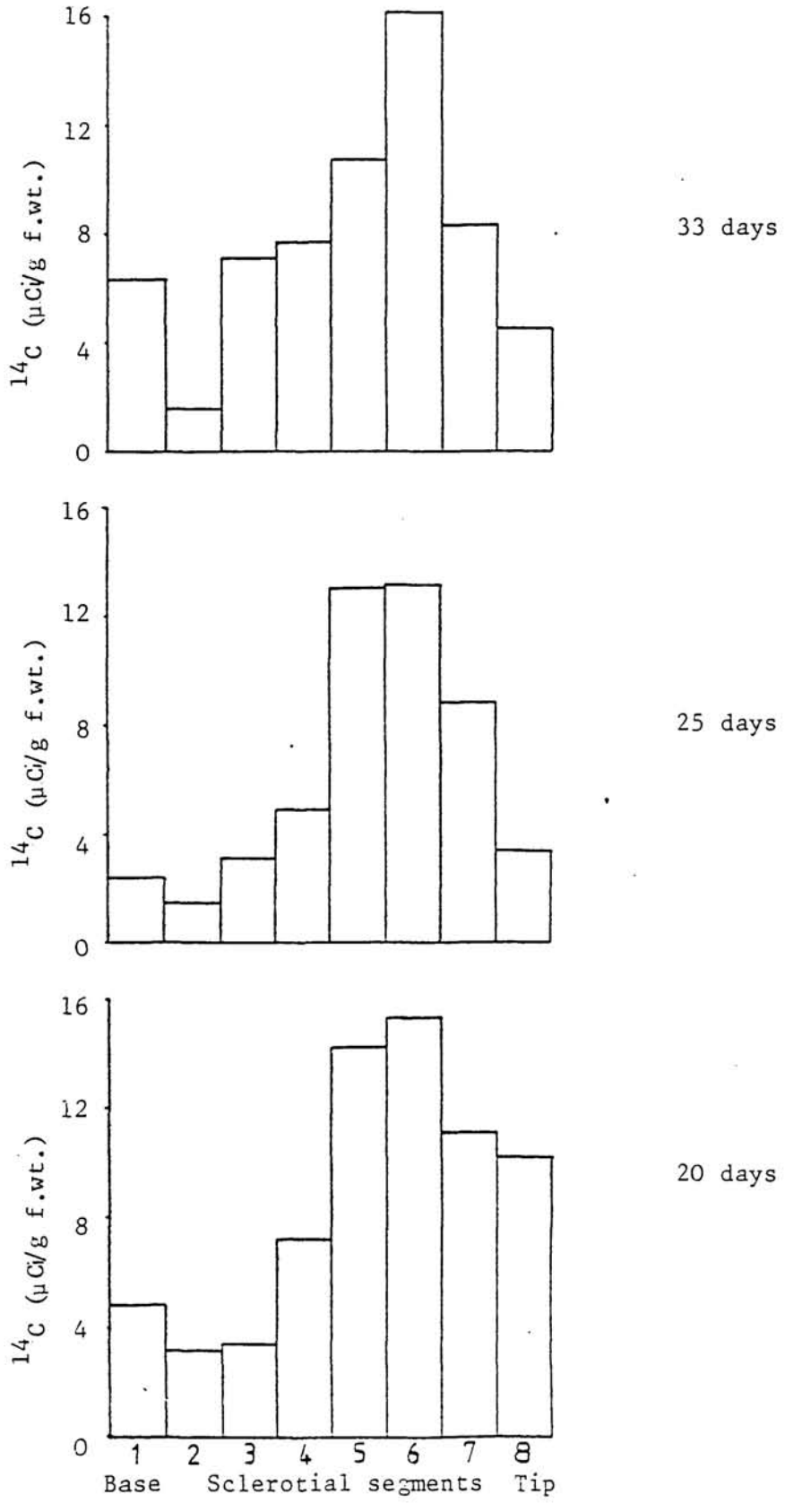


Figure 13

Distribution of ^{14}C in the insoluble fractions of sclerotia pulsed with $^{14}CO_2$ during different stages of growth

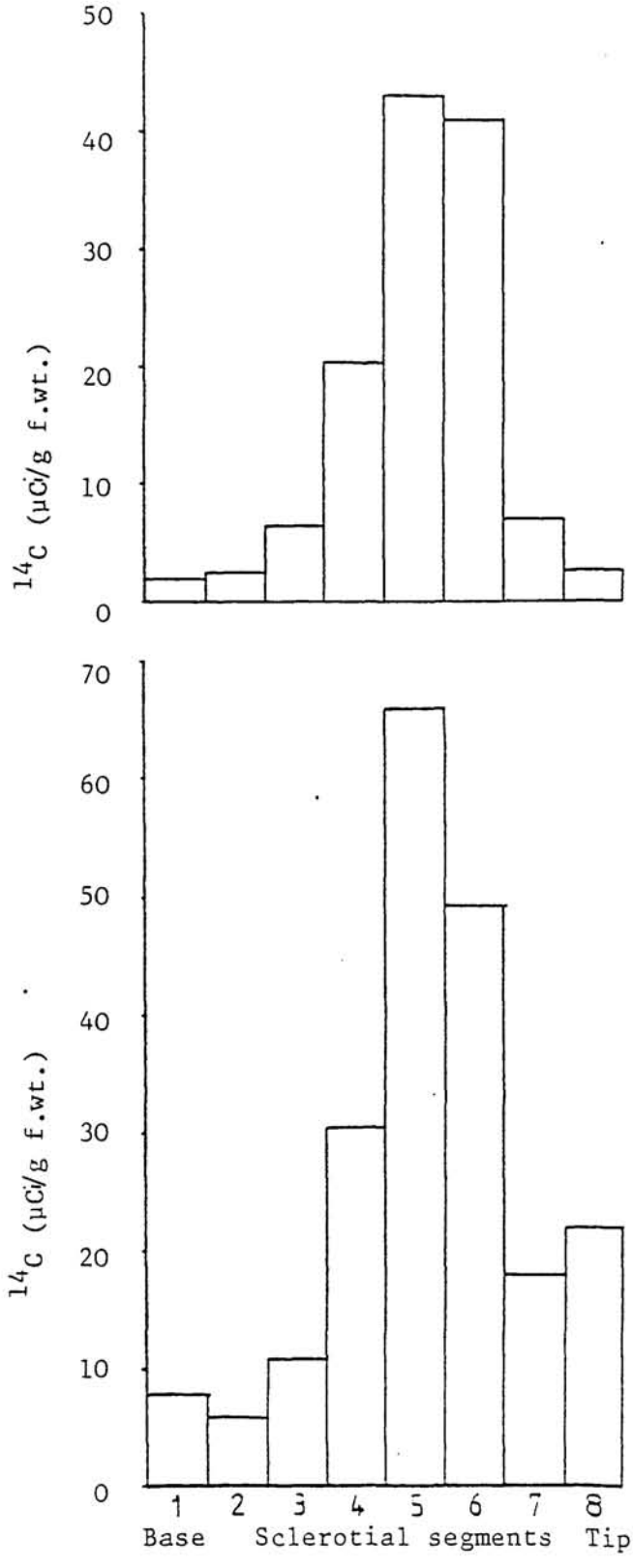
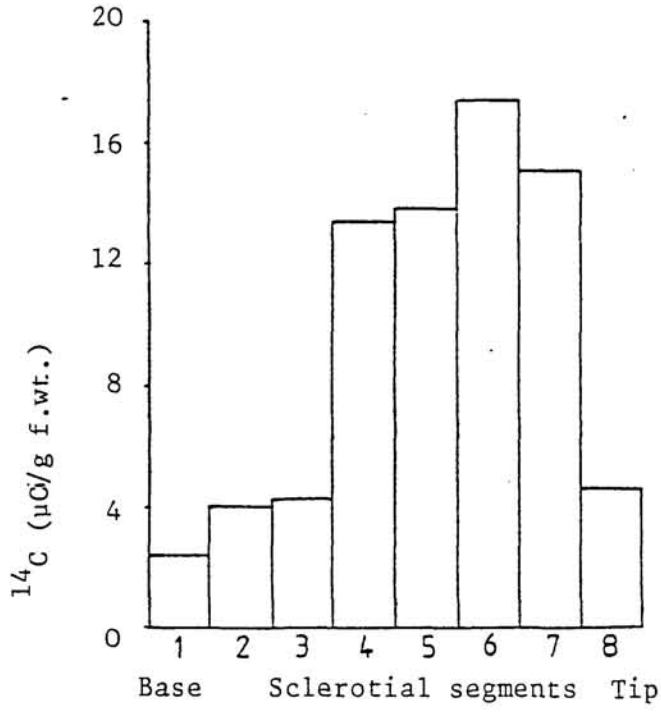


Figure 14

Distribution of ^{14}C in the lipid fraction of sclerotia pulsed with $^{14}CO_2$ during growth



20, 25 and 33 day old ergots combined before extraction

6.3 Experimental - experiment 2

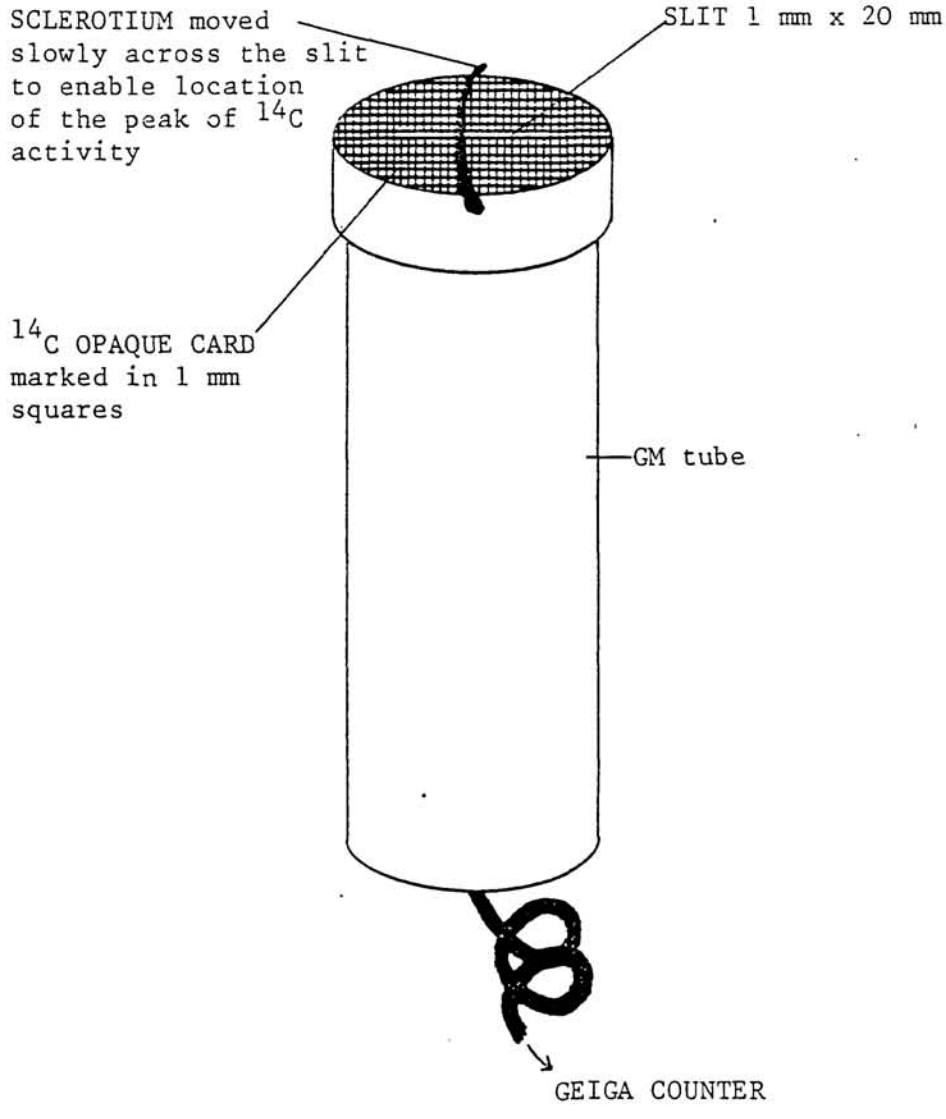
Fourex rye was grown in the glasshouse during 1977 and, prior to anthesis, 80% of the spikelets were inoculated with strain 330 of C.purpurea. Fourteen days after inoculation, once the transition from sphacelial to sclerotial growth had occurred in most of the ergots, a group of infected ears was given a five hour pulse of $^{14}CO_2$ as in experiment 1. Another group was similarly treated twenty-one days after inoculation, and a third group thirty-eight days after inoculation. Additionally a few of the ears fed fourteen days after inoculation were supplied with a second pulse of $^{14}CO_2$, twenty-two days later. A fifth group of ears were not given any ^{14}C treatment and therefore provided unlabelled sclerotia as controls. All the plants were returned to the glasshouse after feeding, and grown to maturity. The ears were harvested and threshed by hand.

Approximately one hundred ergots from each of the control, 14, 21 and 38 day treatment groups, and ten of the twice fed (14 + 36 days) sclerotia were set up for germination using moist vermiculite as the support medium. None of the sclerotia were surface sterilised. After incubation at $4^{o}C$ for seven weeks the ergots were transferred to room temperature (c.$20^{o}C$) and kept moist for a further ten weeks.

The window of a Geiga-Müller (GM) tube was masked with a piece of stiff ^{14}C-opaque card, marked in 1 mm squares, in which there was a slit 20 mm long and 1 mm wide (figure 15). The region of peak ^{14}C activity in individual ergots, was determined by scanning the length of each sclerotium with the modified GM tube, and recording the activity at 5 mm intervals. Fifteen to twenty-five sclerotia with clearly defined regions of ^{14}C activity in each of the 14, 21 and 38 day groups, and five of the twice-fed sclerotia, were selected for further experiment and were transferred to a separate tray of moist vermiculite. Mature stromata were periodically excised from these selected ergots and the

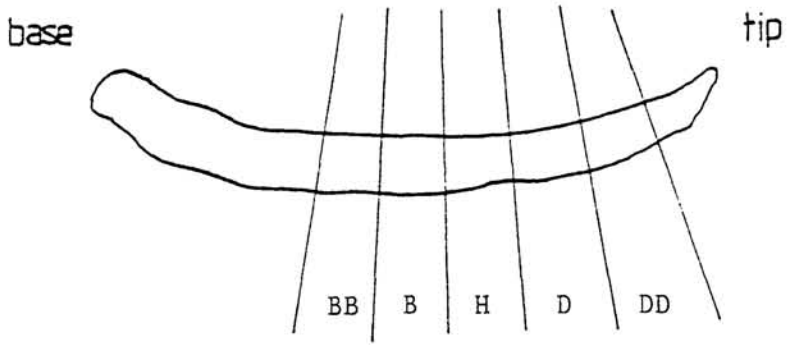
Figure 15

Modified GM tube for examining the distribution of ^{14}C activity along a sclerotium



Division of a labelled sclerotium into segments for lipid and alkaloid analysis.

Figure 16



H = hottest region

distance between their point of emergence and the sclerotium base was recorded. Stromata were not removed periodically from the unlabelled sclerotia but on completion of the incubation period at room temperature the point of exsertion of each stroma was recorded. The stromata were air dried and weighed prior to analysis of their ^{14}C content by tissue oxidation (section 2.9.4). Each excised stroma was analysed individually except when two or more arose from the same point.

Within each group of twelve ergots the ^{14}C content of at least six sclerotia which produced two or more stromata was determined. The region of peak ^{14}C activity (the 'hot' region) within each sclerotium was located using the modified GM tube, and a segment of tissue, approximately 2 mm long, was excised about this region. The rest of the sclerotium was cut into segments ranging in length from 2 mm, adjacent to the 'hot' region, to 5 mm near the sclerotium ends. The air-dry weight of each segment was determined and the ^{14}C content was measured by tissue oxidation (section 2.9.4).

A few sclerotia which had been stored dry, and had therefore not been through the germination process, were divided into eight, approximately equal length, segments and analysed for ^{14}C as described above.

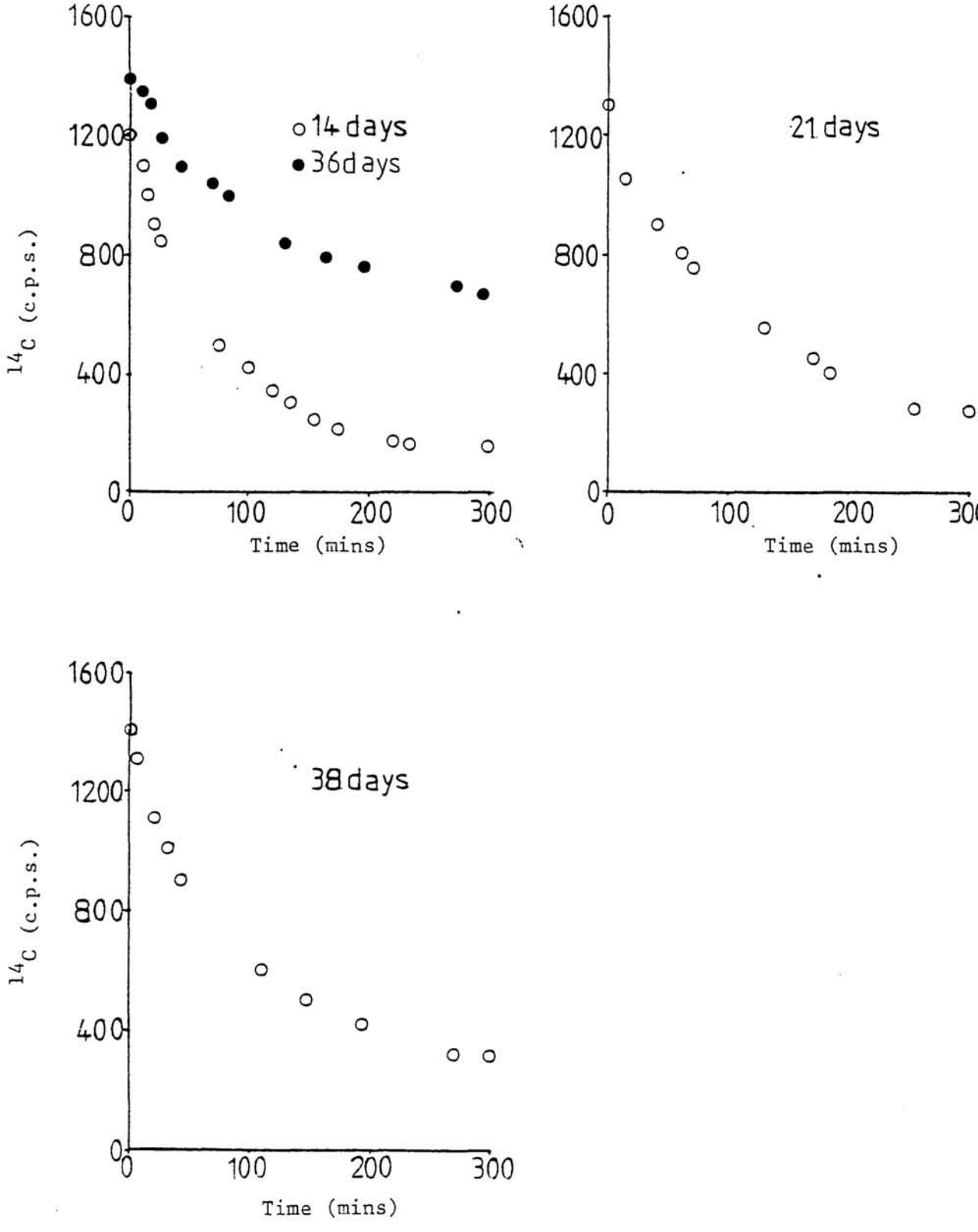
In order to compare the incorporation of ^{14}C into a primary (lipid) and secondary (alkaloid) metabolite, a group of seventeen sclerotia with distinct peaks of ^{14}C activity were selected. The hottest regions were located with the modified GM tube, and excised as 2 mm long segments. Four more 2 mm segments, close to the hot region, were excised from each sclerotium as shown in figure 16. Each group of segments was reduced to a fine powder and quantitatively extracted for alkaloids (section 2.13.1). After partitioning the alkaloids into tartaric acid the remaining ether was returned to the ergot residue and dried down. The lipid content of this residue was determined by extraction with chloroform/methanol (section 2.12). The isotope content of the lipids and the alkaloids was determined by scintillation counting (section 2.9.3).

6.4 Results and discussion - experiment 2

The ears fed at 14, 21 or 38 days after inoculation showed no signs of premature browning before feeding, and depletion of the supplied $^{14}CO_2$ at a similar rate (figure 17) by all three groups indicates that they

Figure 17

Uptake of $^{14}CO_2$ by wheat ears inoculated with *C.purpurea* 14, 21, 36 or 38 days previously



had comparable metabolic activities. The assimilation of $^{14}CO_2$ was slow in the twice fed ears when they were pulsed 36 days after inoculation. Several of these ears had browned prematurely, probably because they were exposed to abnormally high temperatures during the first feeding (see page 73) and therefore had less photosynthetic tissue for CO_2 fixation during their second treatment. The morphology of the mature ergots was similar in the controls and in all the treatments except for the twice fed group, most of which were shorter and thinner.

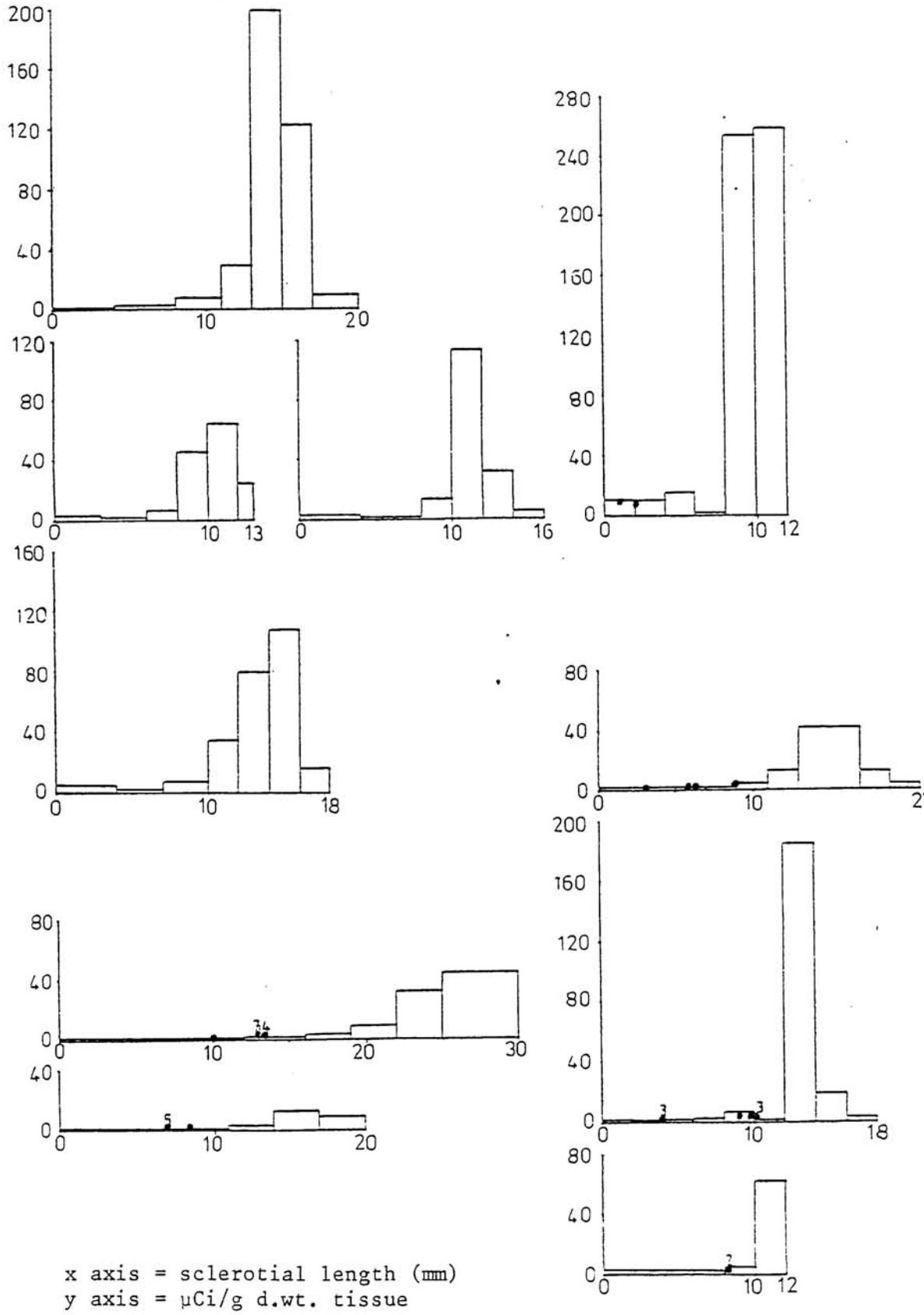
As predicted after the preliminary experiment (section 6.2), analysis of sclerotia by sample oxidation showed a localised concentration of ^{14}C within each sclerotium. This band of ^{14}C was evident irrespective of whether the sclerotium had germinated or had been stored dry (figure 18). Most of the translocation of ^{14}C from plant to ergot tissues would have occurred during the period of $^{14}CO_2$ supply and the few days thereafter, thus accounting for the peak of ^{14}C activity residing in the tissues which were growing at this time. The distribution of low levels of ^{14}C throughout the other sclerotial tissue may have been due to the limited movement of a small proportion of the ^{14}C from the area of peak activity. However, a slow trickle of ^{14}C supplied by the host during the remaining period of sclerotial growth could also have contributed to the ^{14}C content of the younger sclerotial tissues. The ^{14}C in the older tissues may be attributed, at least in part, to direct $^{14}CO_2$ fixation (see section 12.2) by the ergot tissues since these were already formed when the $^{14}CO_2$ was supplied. This was shown most clearly by some of the ergots pulsed at 38 days, in which a secondary terminal peak of ^{14}C activity was detected.

The absence of a terminal peak in some of the 38 day ergots may be associated with the method of segmentation. For example, any increase in the activity of the terminal 1 mm of tissue may have been undetected if the terminal 3 mm of tissue was oxidised as one segment. Alternatively it may reflect the size of the sclerotium at the time of feeding. It is proposed that this terminal concentration of ^{14}C in some of the 38 day ergots resulted from the ability of any exposed sclerotial tissues to fix carbon dioxide in a moist atmosphere. For example, any ergots which did not protrude beyond the tightly adhering glumes of the rye floret would have been shielded from the circulating $^{14}CO_2$ and hence would not have been able to fix as much ^{14}C into their tissues.

Figure 18

The distribution of ^{14}C in sclerotia and stromata

a) supplied with $^{14}CO_2$ 14 days after ear inoculation



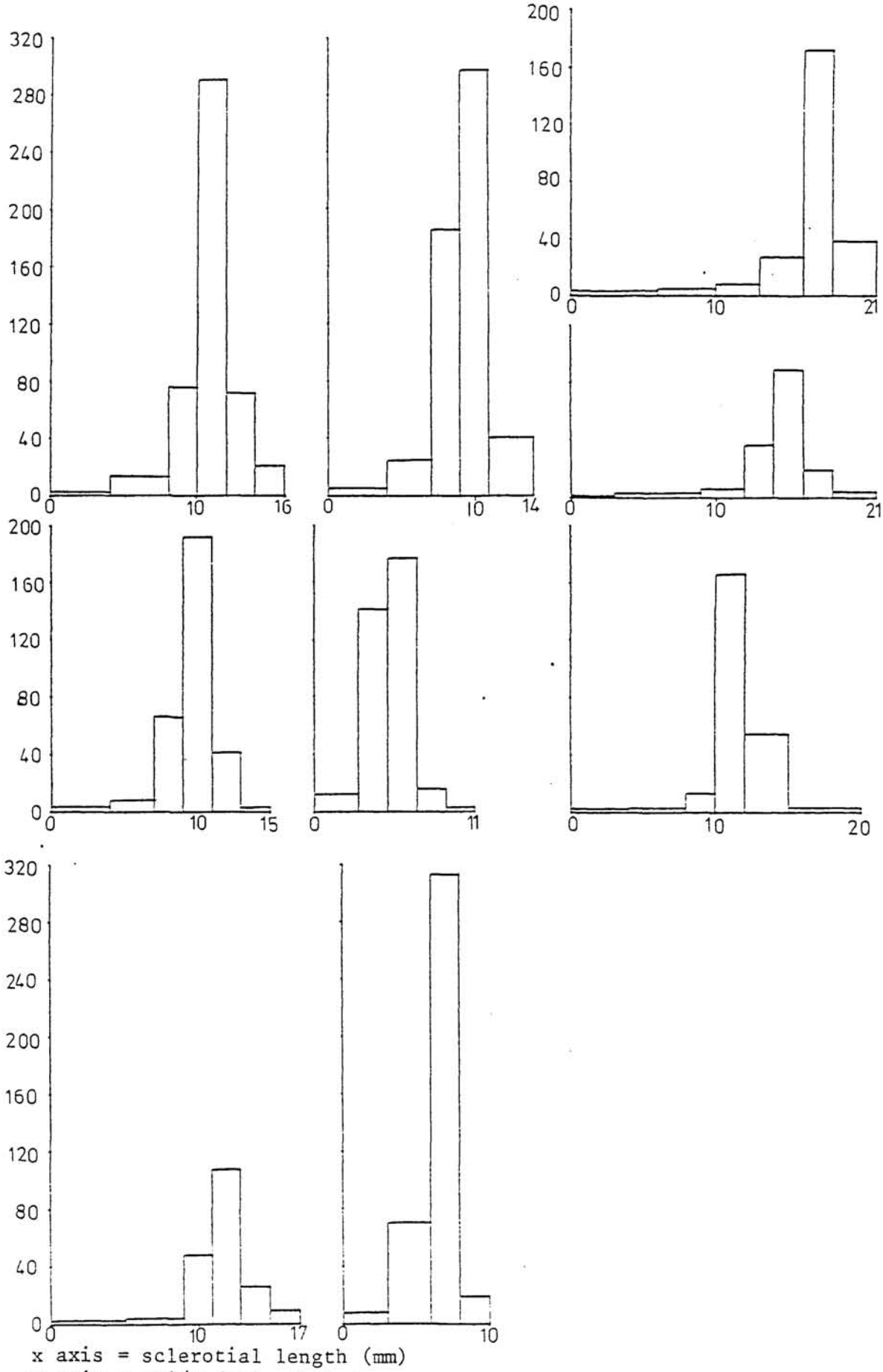
x axis = sclerotial length (mm)
y axis = µCi/g d.wt. tissue

● = stroma

^{5}● = 5 stromata produced in same region

Figure 18 cont'd

a) cont'd (14 days)



x axis = sclerotial length (mm)
y axis = µCi/g d.wt. tissue

No stromata were produced by these sclerotia

Figure 18 cont'd

b) supplied with $^{14}CO_2$ 21 days after ear inoculation

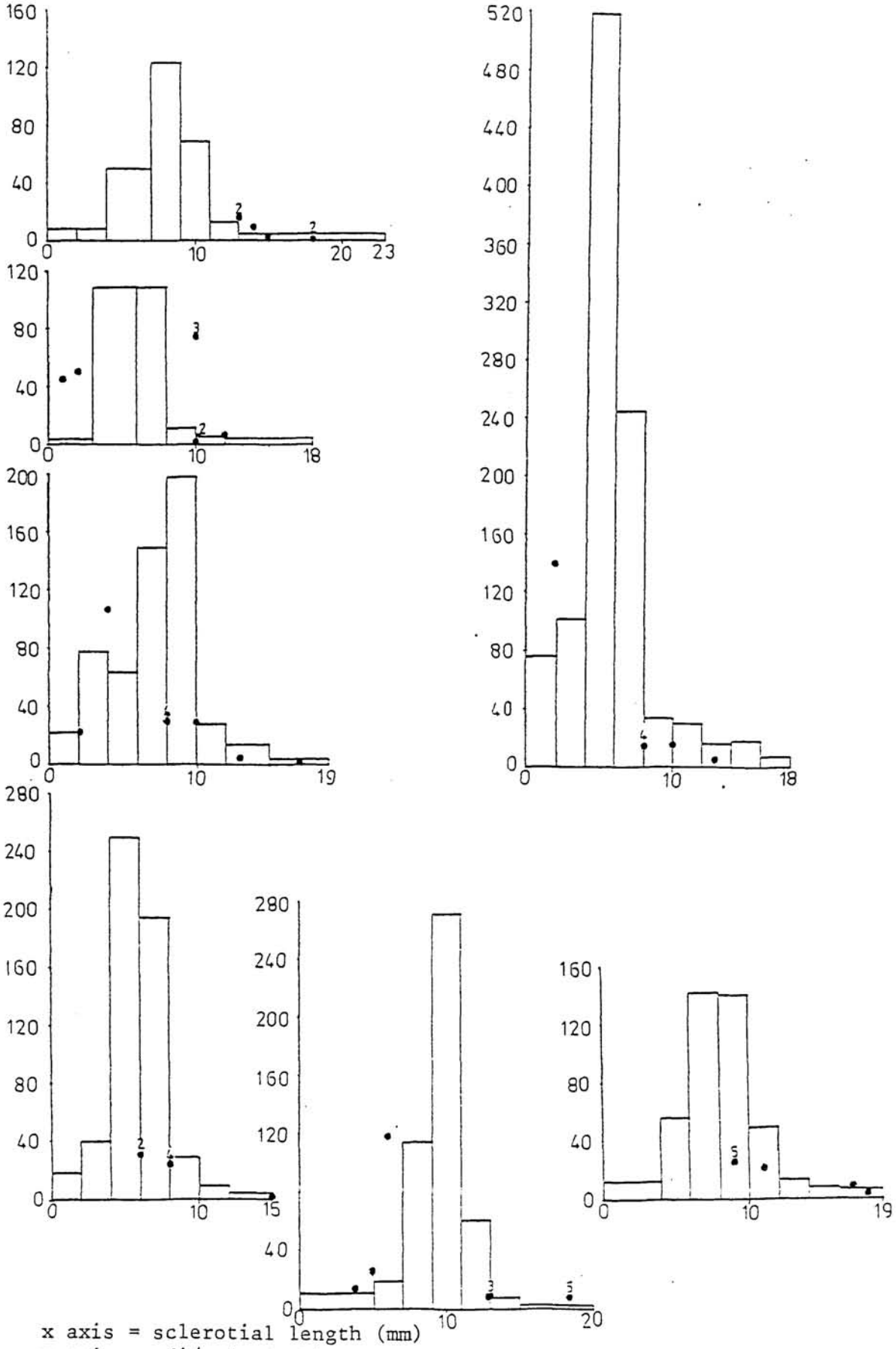
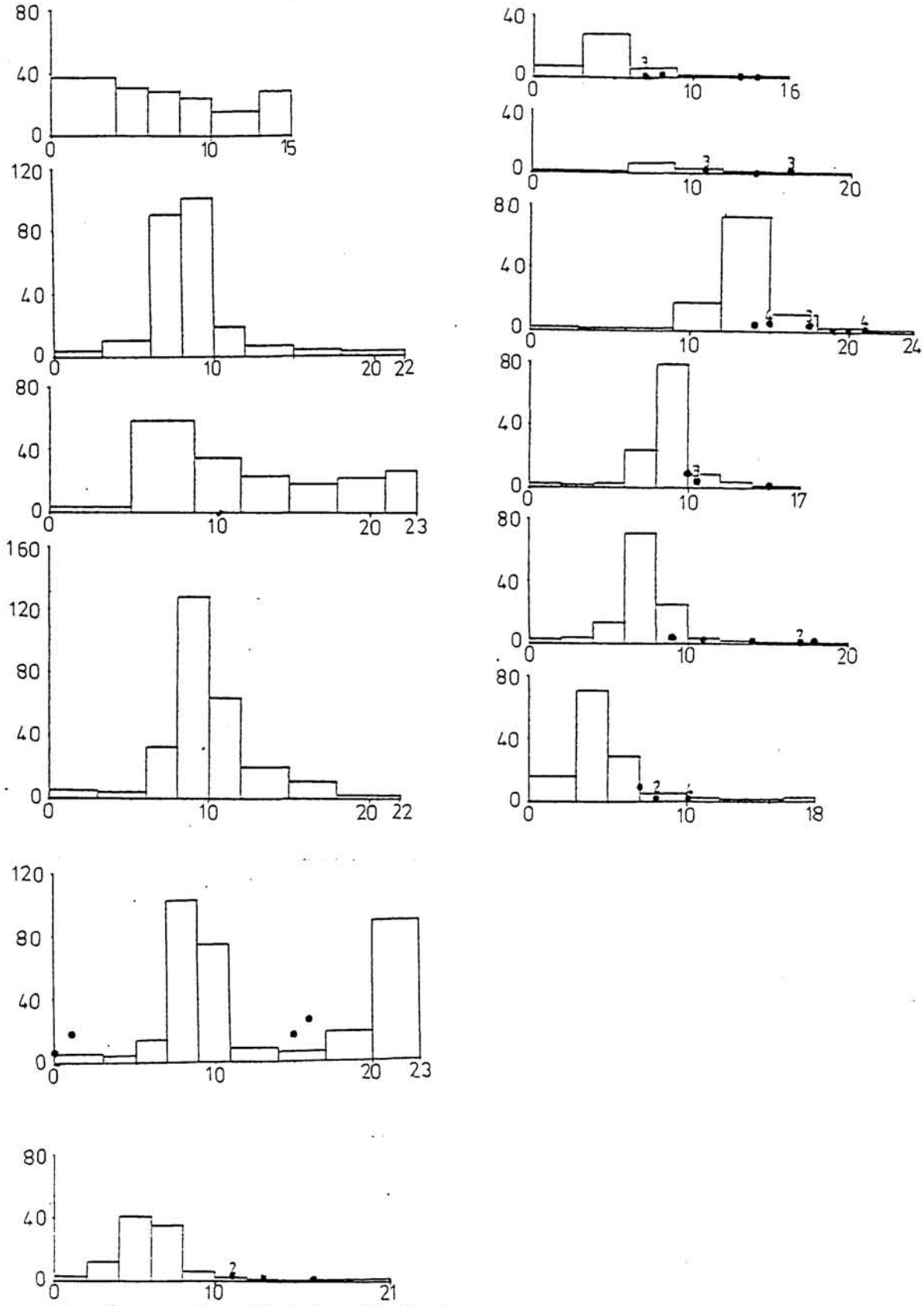


Figure 18 cont'd

c) supplied with $^{14}CO_2$ 38 days after ear inoculation



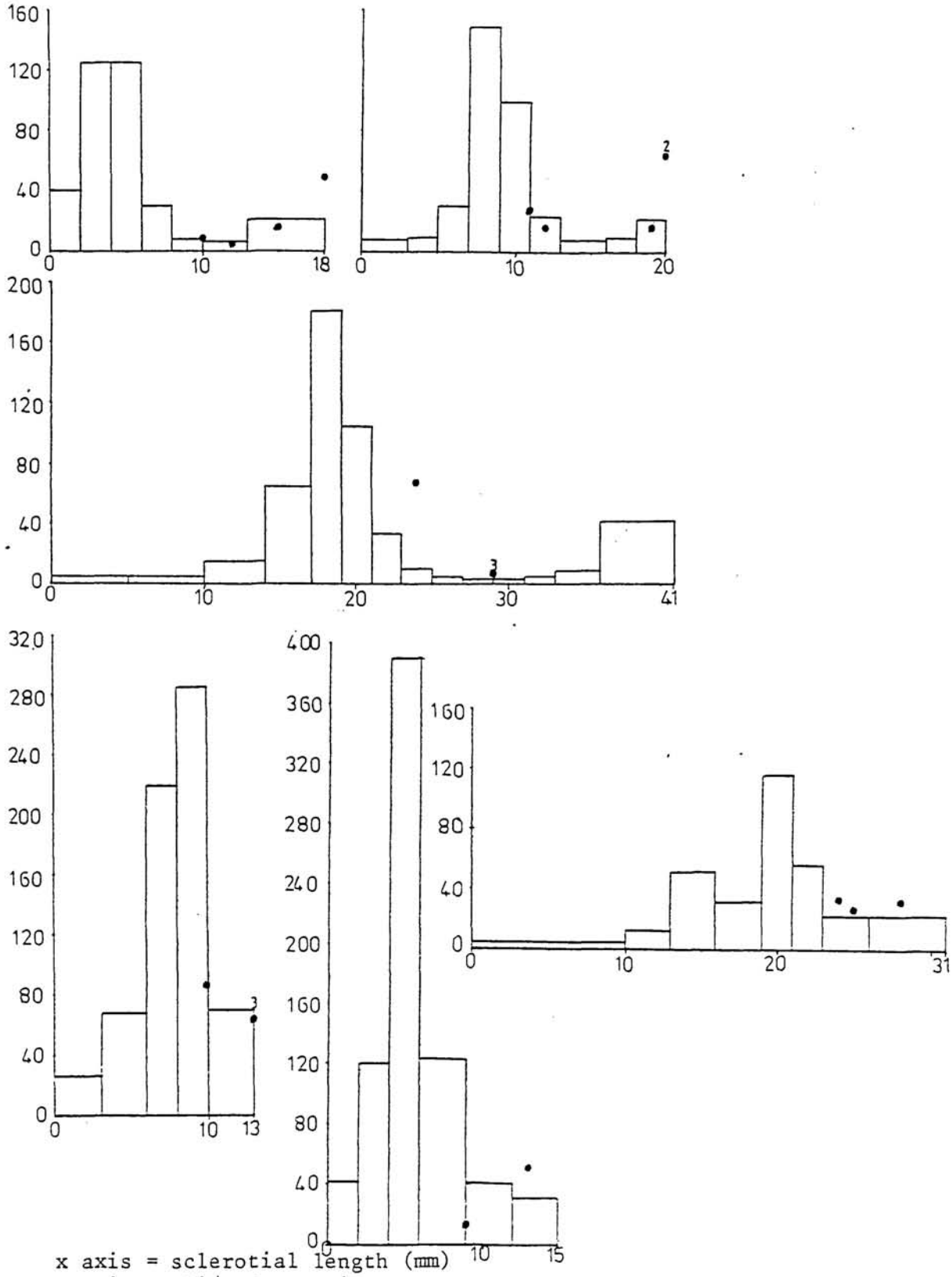
x axis = sclerotial length (mm)
y axis = µCi/g d.wt. tissue

● = stroma

$\overset{3}{\bullet}$ = 3 stromata produced in same region

Figure 18 cont'd

c) cont'd (38 days)



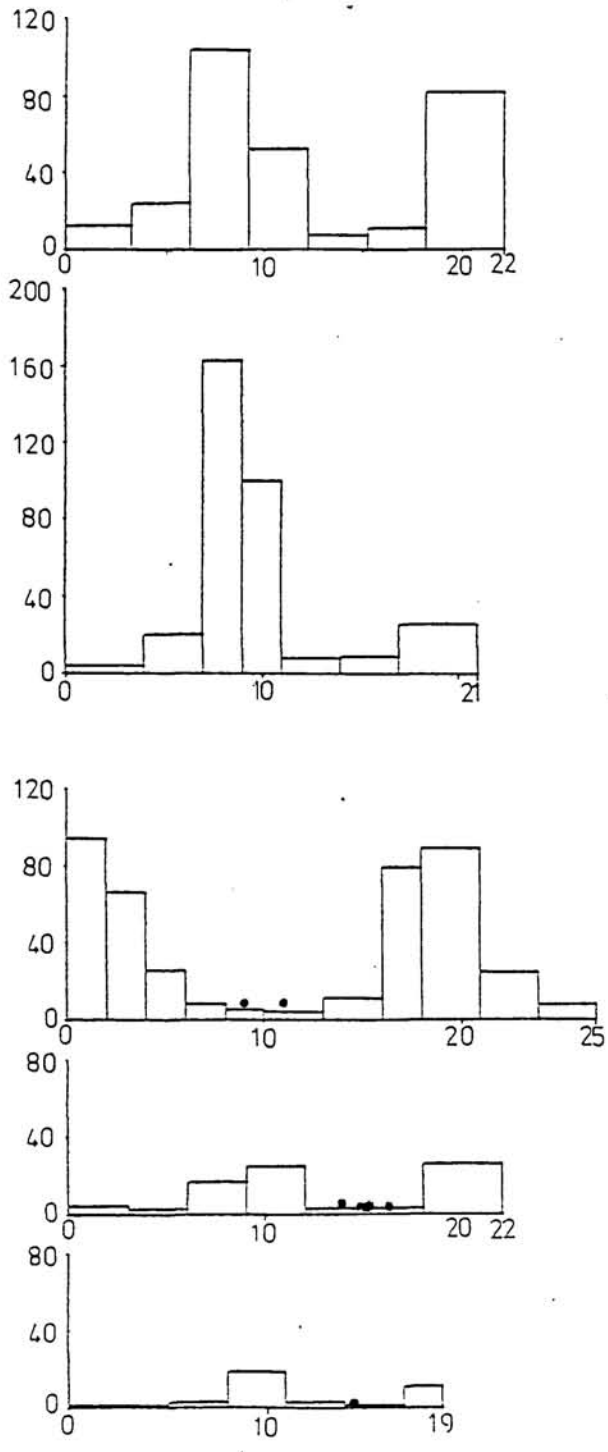
x axis = sclerotial length (mm)
y axis = µCi/g d.wt. tissue

● = stroma

^{2}● = 2 stromata produced in same region

Figure 18 cont'd

d) supplied with $^{14}CO_2$ 14 and 36 days after ear inoculation



x axis = sclerotial length (mm)
y axis = µCi/g d.wt. tissue

• = stroma

It is concluded from the relatively discrete bands of ^{14}C incorporated in the sclerotia that there is not a free flow of metabolites throughout the sclerotial tissues, although there is probably some limited translocation.

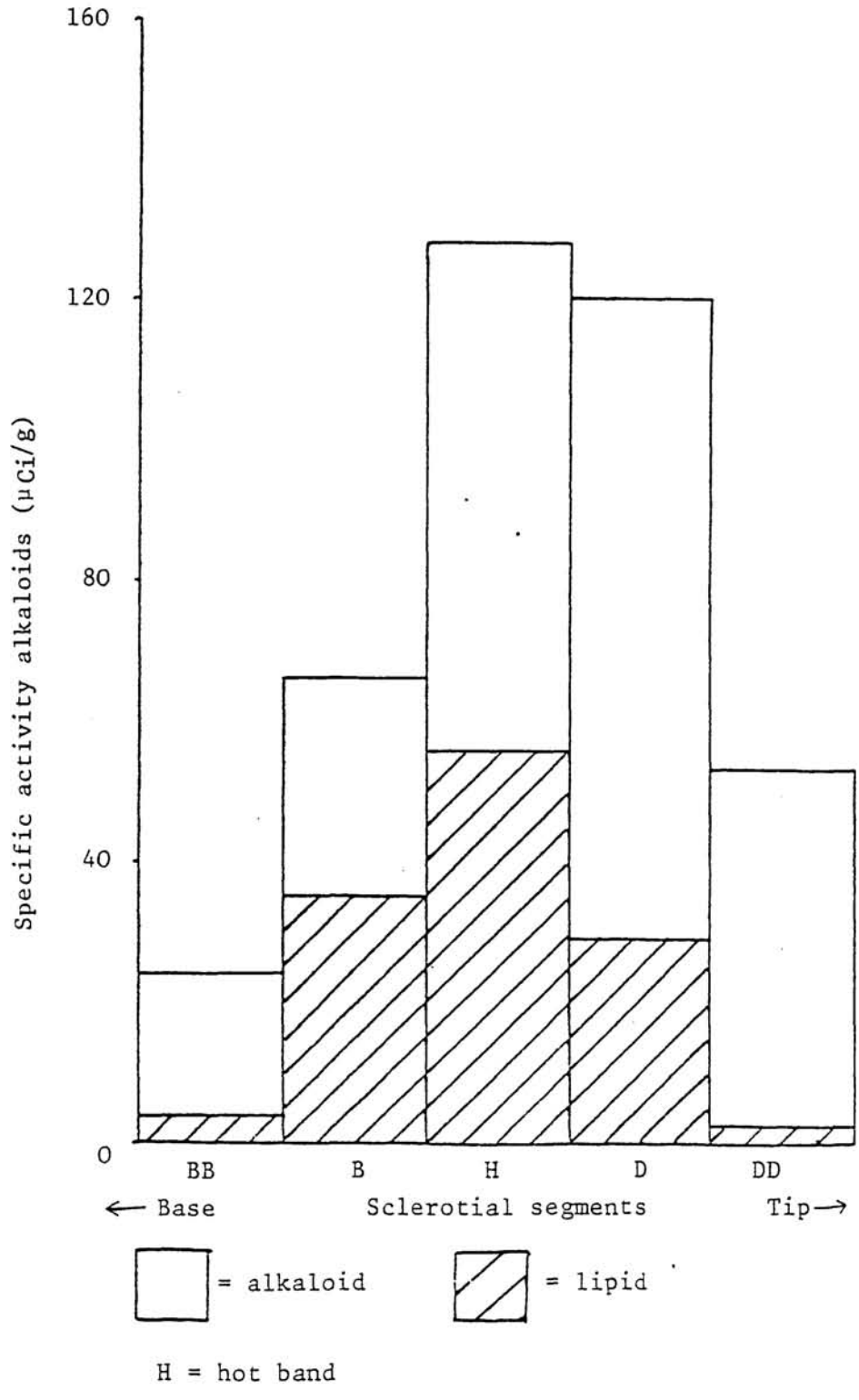
Twelve days after completion of the pre-germination cold treatment stromatal initials were first observed as they broke through the sclerotial cortex. In figure 20 the point of emergence of each stroma is related to the position of peak ^{14}C activity in the sclerotium. This peak of activity is closer to the sclerotium tip in the 14 day group than in those fed at 21 or 38 days, an observation which is consistent with the sclerotia of C.purpurea growing acropetally, new tissue being added only at the base.

Further evidence that growth is acropetal was obtained by comparing the relative distribution of a primary (lipid) and secondary (alkaloid) metabolite within a group of labelled sclerotia (figure 19). The highest specific activity of alkaloids was recorded in sclerotial tissue which was slightly distal to (ie. older than) that containing the highest lipid activity.

The point of origin of stromata produced by sclerotia which had not been exposed to $^{14}CO_2$, at any time during their growth, was apparently random (figure 21). This contrasts with the sclerotia pulsed at 21 or 38 days in which very few stromata arose from tissue younger than the region of peak ^{14}C activity (figure 20). The sclerotia from ears which were supplied with ^{14}C 14 days after inoculation produced no stromata from the tissue which was older than the region of peak activity, and a less than expected number from the tissue which was slightly younger than this region. In figure 22, the points of origin of stromata have been expressed in terms of percentage distance from the sclerotium base. (It is important to note that this figure illustrates only the distribution of stromata within each group; direct comparison of the total number of stromata produced by each group is not valid). Application of the Kolmogorov-Smirnov test (Siegel, 1956) to this standardised data shows (Appendix IV) that the distribution of stromata in all three groups of sclerotia which were pulsed with $^{14}CO_2$ is significantly different (P = at least 0.05) from the control sclerotia.

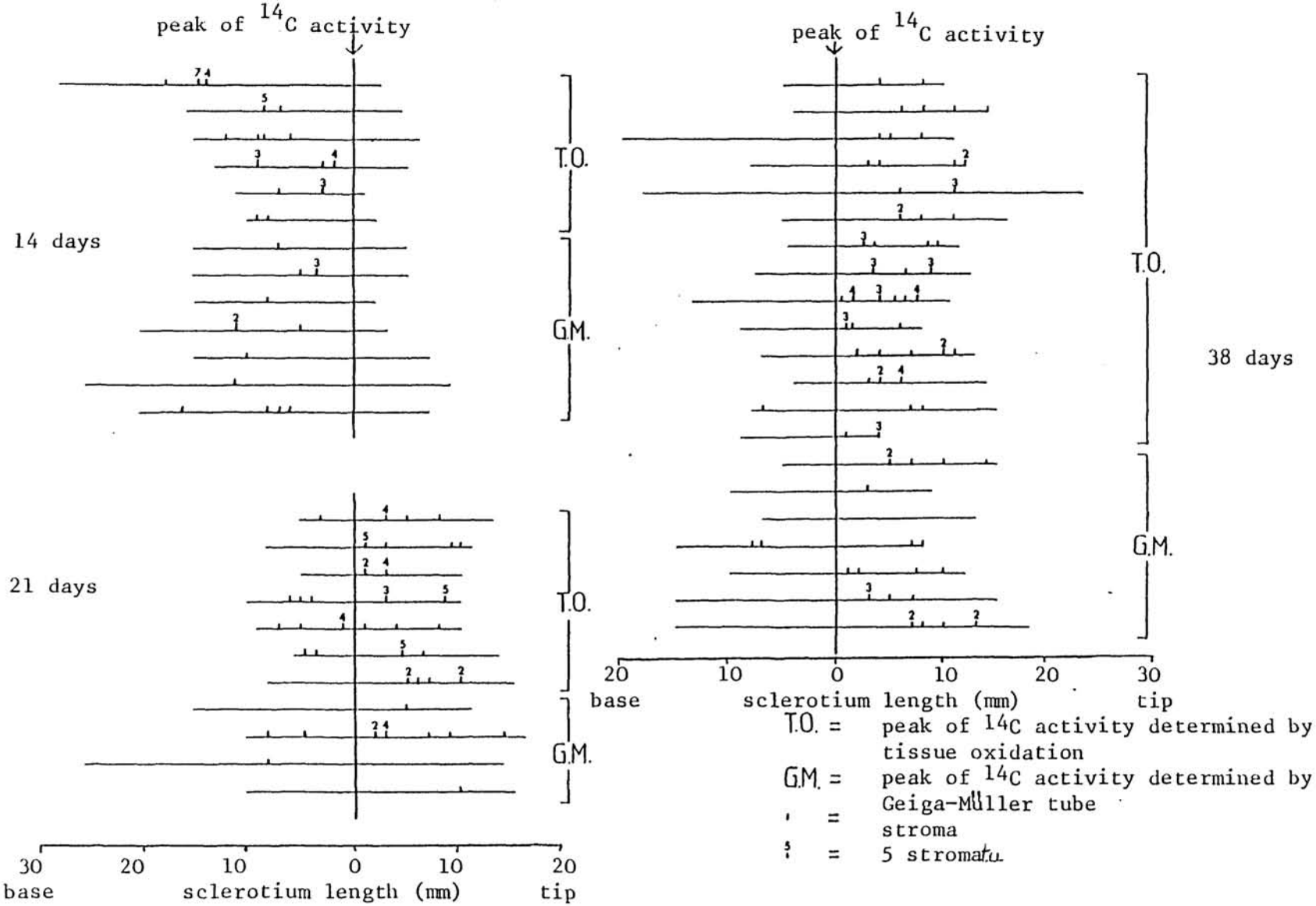
Figure 19

The distribution of ^{14}C-labelled lipids and alkaloids in sclerotial segments



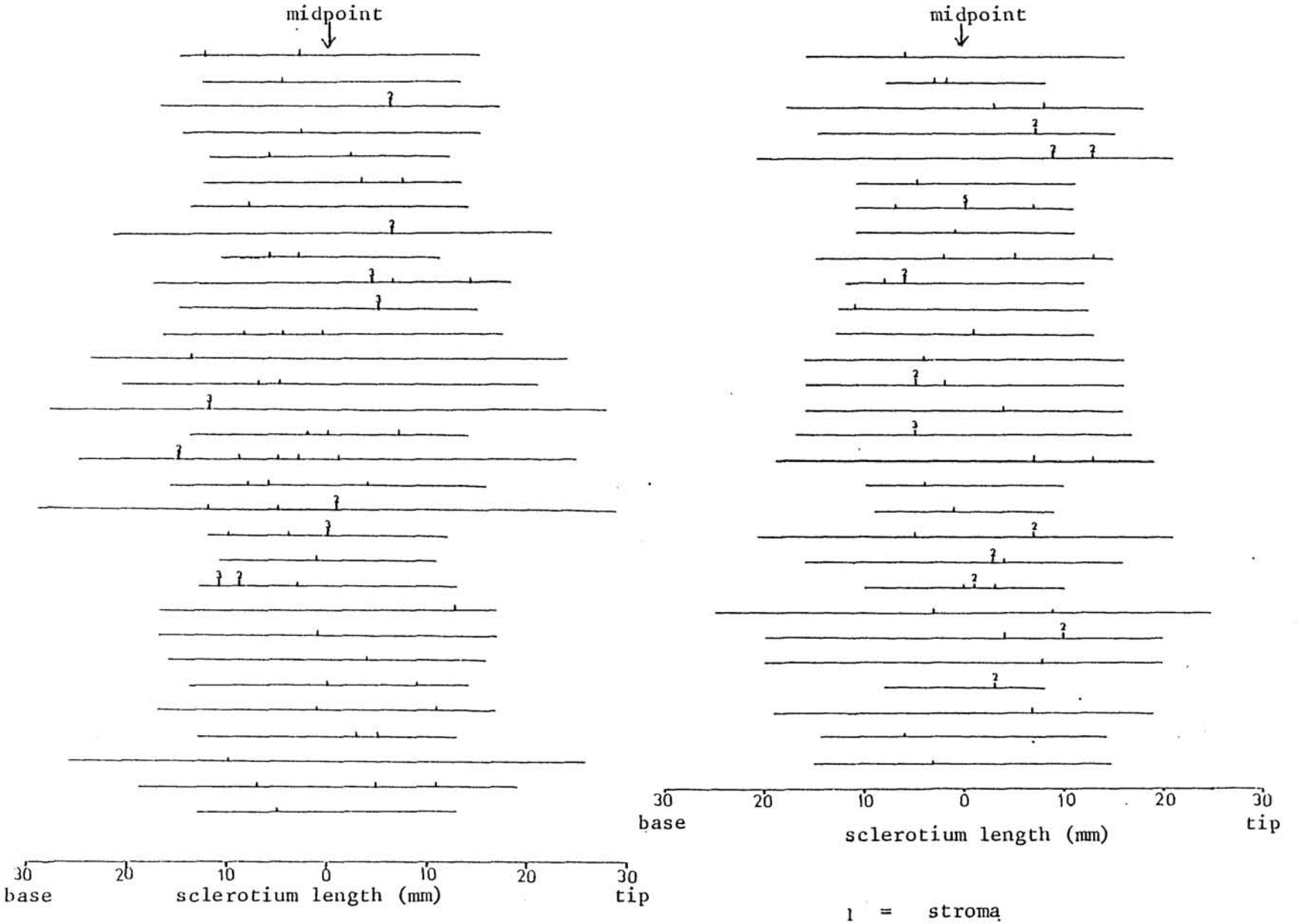
The distribution of stromata along sclerotia in relation to the peak of ^{14}C activity within each sclerotium

Figure 20



The distribution of stromata along sclerotia not exposed to $^{14}CO_2$

Figure 21



The distribution of stromata along sclerotia pulsed with $^{14}CO_2$ at different stages of growth, compared with unlabelled controls - standardised data

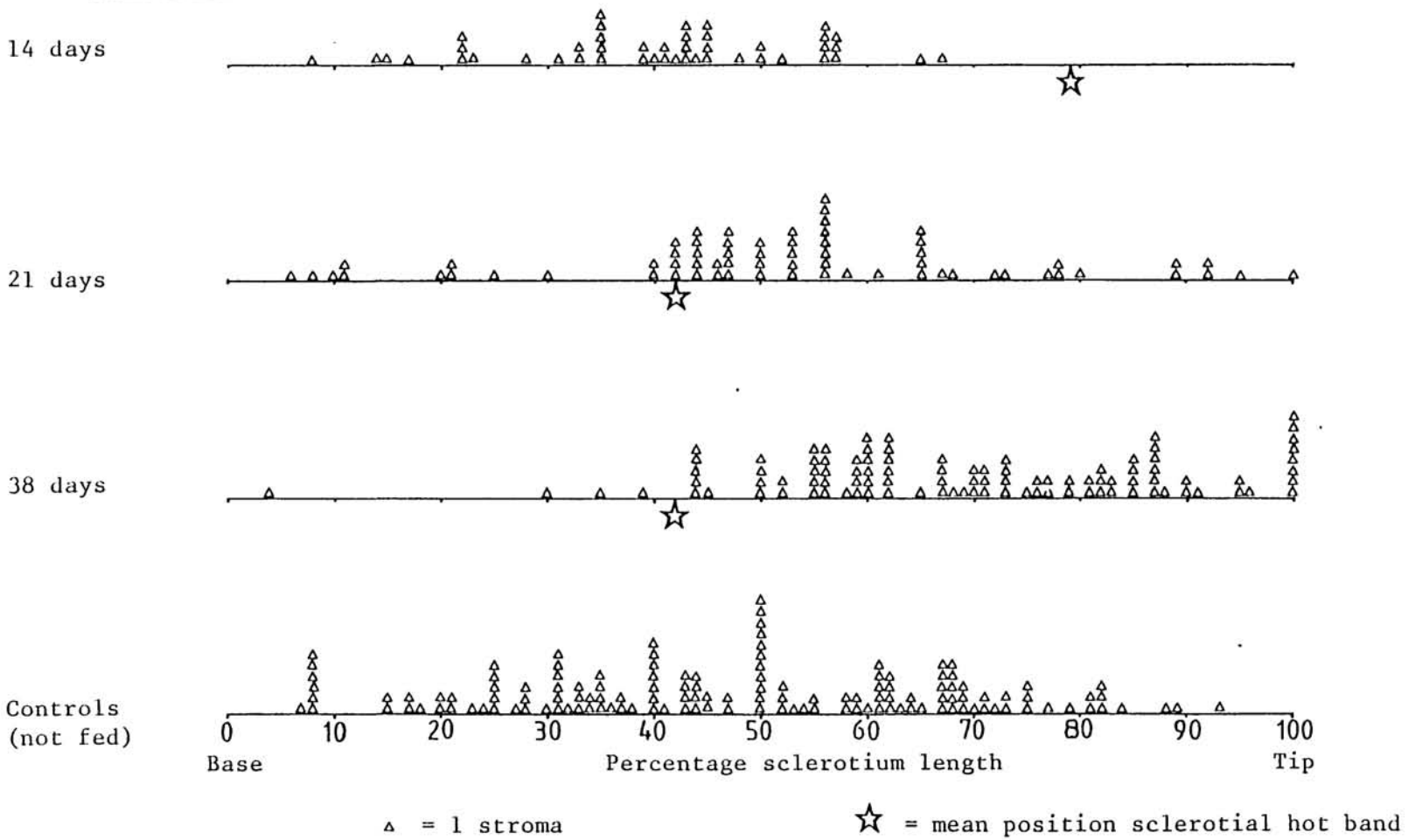


Figure 22

The reduced number of stromata produced by sclerotial tissues which were formed after exposure to $^{14}CO_2$ may reflect impaired host metabolism resulting from exposure to abnormally high temperatures during the period of $^{14}CO_2$ supply. These temperatures were caused by the high light intensity used during this period to ensure maximum photosynthetic activity of plant tissues. Within a week of treatment it was possible to identify the treated ears by their prematurely browned appearance. This must have impaired host metabolism and, therefore, would have resulted in reduced availability of nutrients, particularly carbon, to developing sclerotial tissues. Reduced stromata production may have been caused either by a change in the composition or quantity of available substrates in the subtending sclerotial tissues, or by reduced availability of a single compound vital for stromatal initiation.

The absence of stromata from the distal ends of the sclerotia fed at 14 days may reflect the delicate nature of this tissue at the time of feeding. Fungal metabolism may therefore have been impaired directly.

The stromata produced by many of the sclerotia which were pulsed with $^{14}CO_2$ at 21 or 38 days, increased in specific activity with increased proximity of their point of exsertion to the sclerotial hot band(s). In the 14 day sclerotia all the stromata had a very low specific activity and were subtended by sclerotial tissue of low activity; no stromata were produced within 2 mm of a hot band.

It is concluded that although substrates for stromatal growth are mainly derived locally, there is some evidence that nutrients may also be obtained from adjacent regions, possibly as the local source becomes depleted. This suggests that a component of sclerotial tissue which is important for stromatal initiation and development is in limited supply. Corbett et al (1975) concluded that stromatal utilisation of sclerotial lipids was a localised event, at least in Arrhenatherum ergots, and it seems probable that this also applies to ergots from other host plants. Nothing is known of the extent to which the abundant triglyceride oil reserves are utilised for stromatal formation, but it seems unlikely that they could be exhausted, even locally, by developing stromata. The ^{14}C bands were no more distinct in the ungerminated sclerotia, than in those which had been through the germination process, which experimentally supports this hypothesis.

7. THE UPTAKE OF GLUCOSE BY EXCISED STROMATA

7.1 Experimental

Strain 330 ergots, grown on Fourex rye, were germinated using John Innes potting compost as the support medium. The sclerotia were incubated at 4°C for 7 weeks and were then maintained at room temperature. Twelve days after the onset of germination, groups of thirty stromata were excised and supplied basally with ^{14}C glucose for 0, 1, 2, 5 or 22 hours as described in section 2.20. Immediately after incubation was terminated the capitulum and stipe of each stroma were separated. Each group of tissue was weighed, macerated in 2 ml 80% ethanol (v/v in water) and extracted for polyols (section 2.21). The ethanolic extracts were evaporated to dryness using an infra red lamp and fan, and separated into their component carbohydrates by paper chromatography (section 2.18). The chromatograms were autoradiographed (section 2.9.1) and each labelled spot was individually eluted from the paper with water (section 2.9.2), taken to dryness (I.R. lamp + fan) and assayed for ^{14}C by scintillation counting (section 2.9.3).

Attempts to decompose the borate complexes of the carbohydrates (by repeated solubilisation in, and evaporation of, acidified methanol) prior to quantification of the sugar component by GLC, were unsuccessful. In consequence the remaining crude polyol extracts were passed first through a strong cation (IR - 120H^+) and then a weak anion (IR - 4B OH^-) exchange resin (section 2.16). The anion exchange resin was eluted with water and each sample was dried by rotary evaporation. The carbohydrates were silylated and analysed by GLC (section 2.19).

7.2 Results and discussion

The concentrations of sugars and polyols in stipe and capitula tissues, as determined by ^{14}C scintillation counting and/or GLC are shown in table 2. The concentration of labelled glucose in the stipes increased during the first two hours of incubation but then decreased during the remaining experimental period. This contrasts with the pattern of glucose uptake by the capitula which rose steadily throughout the experimental period. The concentration of labelled fructose and mannitol in both capitula and stipes also increased steadily during the 22 hour period, indicating their synthesis from the assimilated ^{14}C glucose. The very low levels of labelled fructose suggest that it is either turned over very rapidly in stromatal tissues or that it is

Table 2

Concentration of total and assimilated/synthesised carbohydrates in stromata supplied basally with ^{14}C glucose

Incubation Time (hrs)	Carbohydrate	Assimilated/synthesised carbohydrate concentrations mg. g^{-1} f.wt.		Total carbohydrate concentrations mg. g^{-1} f.wt.	
		STIPES	CAPITULA	STIPES	CAPITULA
0	Glucose	0.0	0.0	1.88	3.68
1	"	4.3	1.1	2.03	12.75
2	"	7.6	1.6	2.39	6.70
5	"	7.0	3.2	4.82	-
22	"	3.2	5.9	4.84	2.52
0	Fructose	0.0	0.0	0.20	0.29
1	"	0.3	0.3	0.73	0.92
2	"	0.5	0.5	0.89	0.40
5	"	0.5	0.8	0.41	-
22	"	0.8	0.8	0.96	0.57
0	Mannitol	0.0	0.0	19.50	11.94
1	"	1.6	1.6	41.92	18.69
2	"	3.2	5.1	40.95	21.39
5	"	7.6	8.9	43.41	-
22	"	13.2	12.4	54.72	22.22
0	Trehalose	-	-	0.13	0.18
1	"	-	-	9.27	6.98
2	"	-	-	5.26	4.97
5	"	-	-	8.72	-
22	"	-	-	6.57	3.74
0	Arabinitol	-	-	Tr	0.52
1	"	-	-	ND	0.91
2	"	-	-	ND	0.69
5	"	-	-	ND	-
22	"	-	-	ND	0.40

ND = not detected

Tr = trace

only of minor metabolic significance.

Owing to technical difficulties with the GLC system, used to determine the total concentration of carbohydrates in the stromata, there was a delay of several months between preparing the ethanolic extract and silylating the carbohydrates. During this period the dried extracts were stored in vacuo over calcium chloride and sodium hydroxide, at $4^{o}C$ and should not therefore have been subject to microbial spoilage. Nevertheless several of the values for total carbohydrates are less than the calculated amount of ^{14}C glucose-derived carbohydrates. This inconsistency may have been a consequence of inefficient silylation and therefore the GLC analyses are unreliable. However, it is clear that large amounts of mannitol, and smaller amounts of trehalose, were present in the stromata.

Corbett et al (1975) showed that both capitula and stipe tissues rapidly, and probably passively, assimilated glucose when they were submerged in a buffered glucose medium. The current studies have demonstrated that glucose is also utilised by stromatal tissues when it is supplied through the stipe base only, and was metabolised to give similar concentrations of mannitol as found by Corbett et al (1975).

8. PERIODICITY OF ASCOSPORE EJECTION BY ERGOTS IN THE FIELD

8.1 Experimental

8.1.1 1975

Twenty-four holes (10 mm diameter) were punched in each of ten aluminium foil trays (200 x 120 mm, 30 mm deep). The trays were sunk level with the soil surface in a plot of male sterile wheat, variety W78, as shown in figure 23a, and filled to a depth of 20 mm with soil taken from the same experimental plot.

Wheat ergots, collected from several different localities during the previous year, were combined to provide a mixed population. During January 1975, six hundred of these ergots were distributed over the surface of the ten trays and lightly covered with soil.

The ergots were observed periodically and one week after the first germinating ergots were seen, a Burkard recording volumetric spore trap was positioned above one of the trays (no. 3 in figure 23a). The wind vane on the trap was locked in one position so that the trap orifice was always positioned immediately above the tray of ergots. The suction rate was adjusted to 10L air/min. The trapping surface used is described in section 2.22. At seven day intervals the trapping tape was renewed and the previous week's tape was analysed for ascospores (section 2.23).

Once germination had commenced the ergots were watered during dry weather so that ascospore ejection was not inhibited by water stress. Ascospore release was monitored for five weeks after which the number of germinated ergots in each tray was recorded.

8.1.2 1976

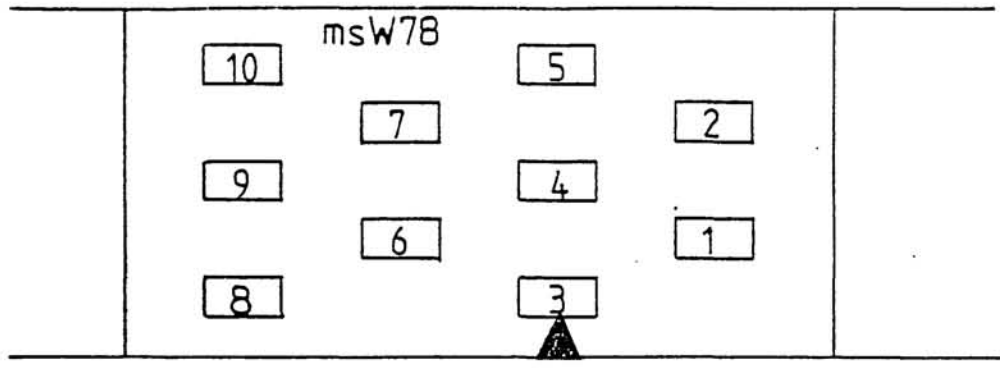
In order to compare the patterns of ascospore release during two consecutive years, the airspora above a tray of ergots was also sampled throughout June 1976. Although the experimental designs were essentially the same during both seasons a few modifications were made:

1. 100 ergots were sown in each of ten plastic plant pots (5" diameter) as shown in figure 23b.
2. The number of stromata above the soil surface in each plot was recorded on the last day of trapping.

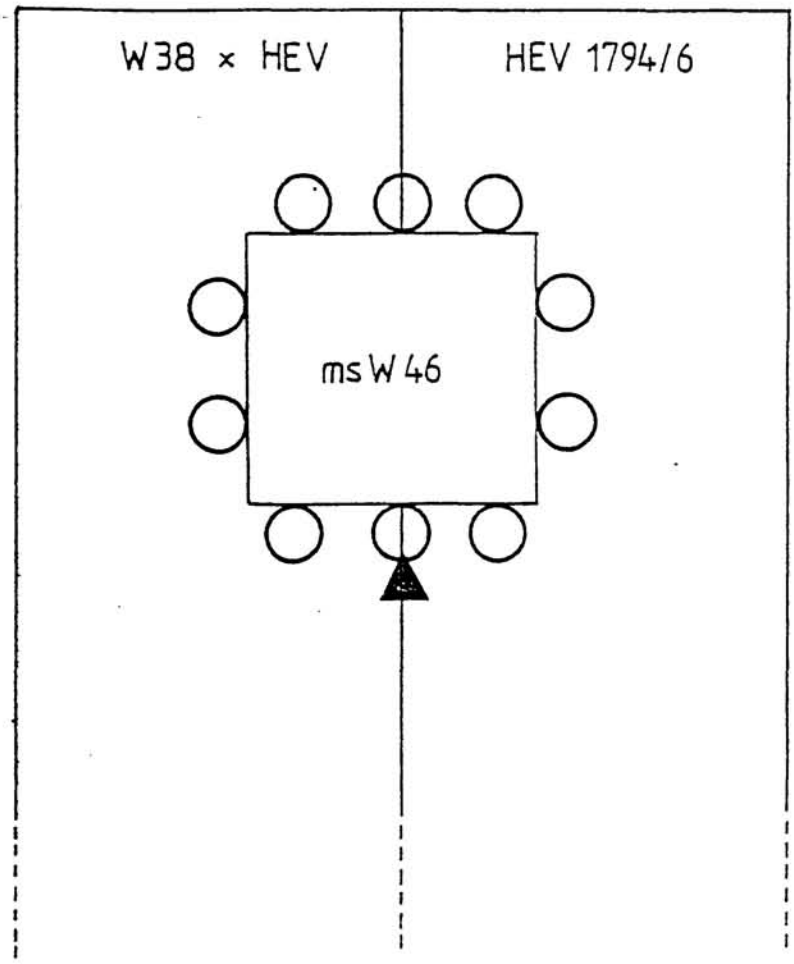
Figure 23

Plot layout for ascospore ejection experiments

a) 1975



b) 1976



= Burkard spore trap

= tray containing ergots

= pot containing ergots

3. The pots of ergots were not watered artificially at any time during the experiment.

4. Weather records for the trapping period were obtained. These values were recorded about ¾ mile from the site of the experiment at 09.00 hrs each day. In figure 24b some of these records have been superimposed on the spore trap results: the maximum temperature has been plotted at 14.00 hrs each day and the minimum temperature at 02.00 hrs. It is however recognised that fluctuations in the timing of both maximum and minimum temperatures would have occurred.

8.2 Results and discussion

Germination was first noticed in the 1975 experiment on June 8th in ergots which had fallen through the holes in the bottom of the foil trays. The small air spaces under the trays probably provided a suitably moist environment which, in contrast to the dry soil in and around the trays, favoured sclerotial germination. All the ergots which had fallen beneath the trays were replaced on the top of the appropriate tray and pressed into the soil surface. Germination of the remaining sclerotia in this experiment was first observed one week later and probably started in response to watering.

Fluctuations in the ascospore content of the air above tray no. 3 are shown for the period 17.6.75 to 22.7.75 in figure 24a. Although the most active periods of ascospore ejection were not always at the same time each day, they never occurred between noon and 6 pm when air temperatures would have been highest (figure 25). No rain fell during the three week trapping period prior to 8.7.75. This, together with high daytime temperatures, provided conditions generally considered to be unfavourable for sclerotial germination. Although no change in the periodicity of ascospore ejection was recorded in response to each application of water, it seems likely that it provided sufficient extra moisture to enable both the initiation and continuation of sclerotial germination.

Ascospore discharge during the fourth week of trapping (8.7.75-15.7.75) showed peaks of activity mainly during the late evening. This suggests that, in spite of the rain which fell at the beginning of this week, conditions during the evening were more favourable for ascospore

Figure 24a

Diurnal variation in the release of ascospores

1975 experiment
17.6.75-22.7.75

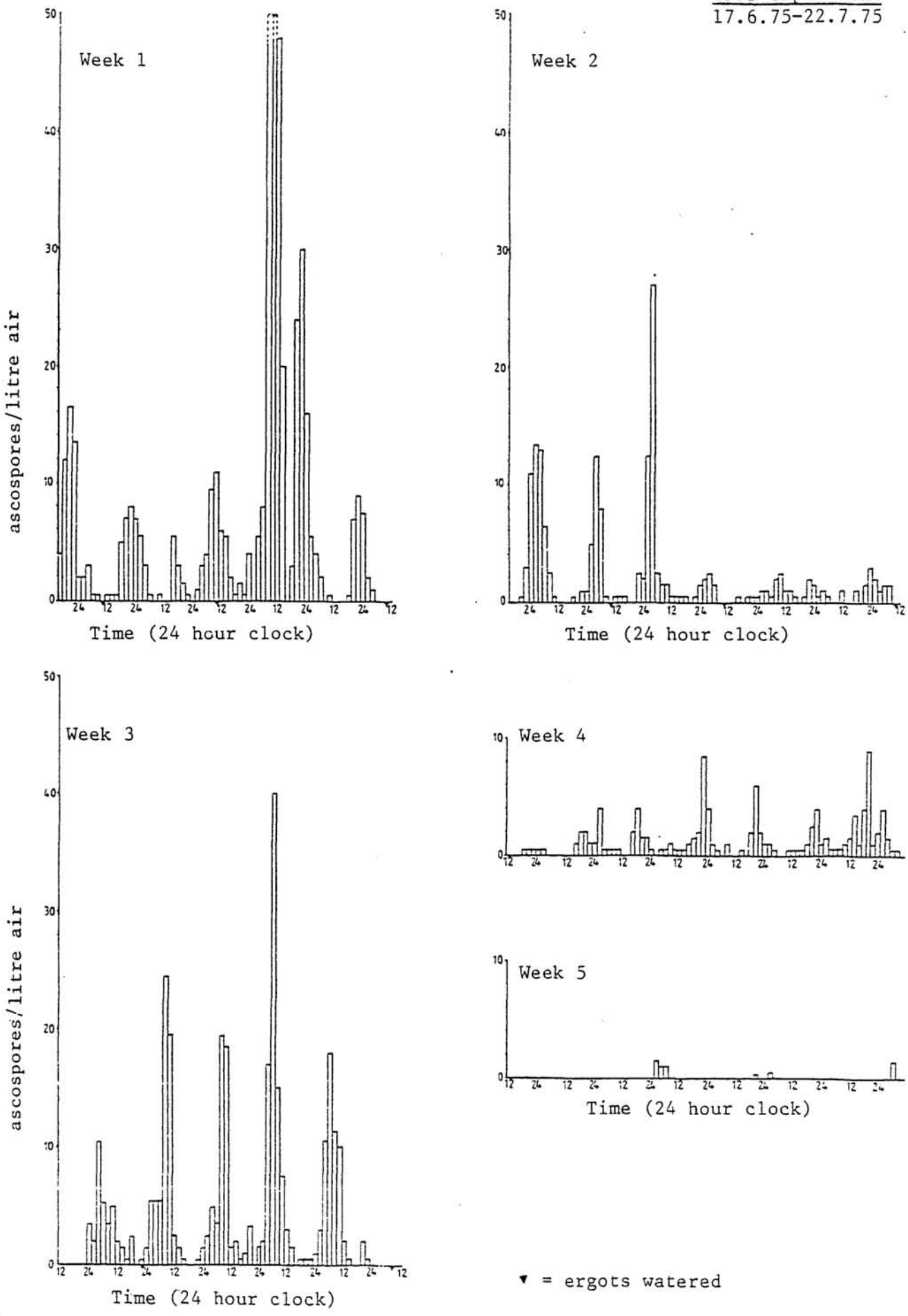


Figure 24b

Diurnal variation in the release of ascospores

1976 experiment

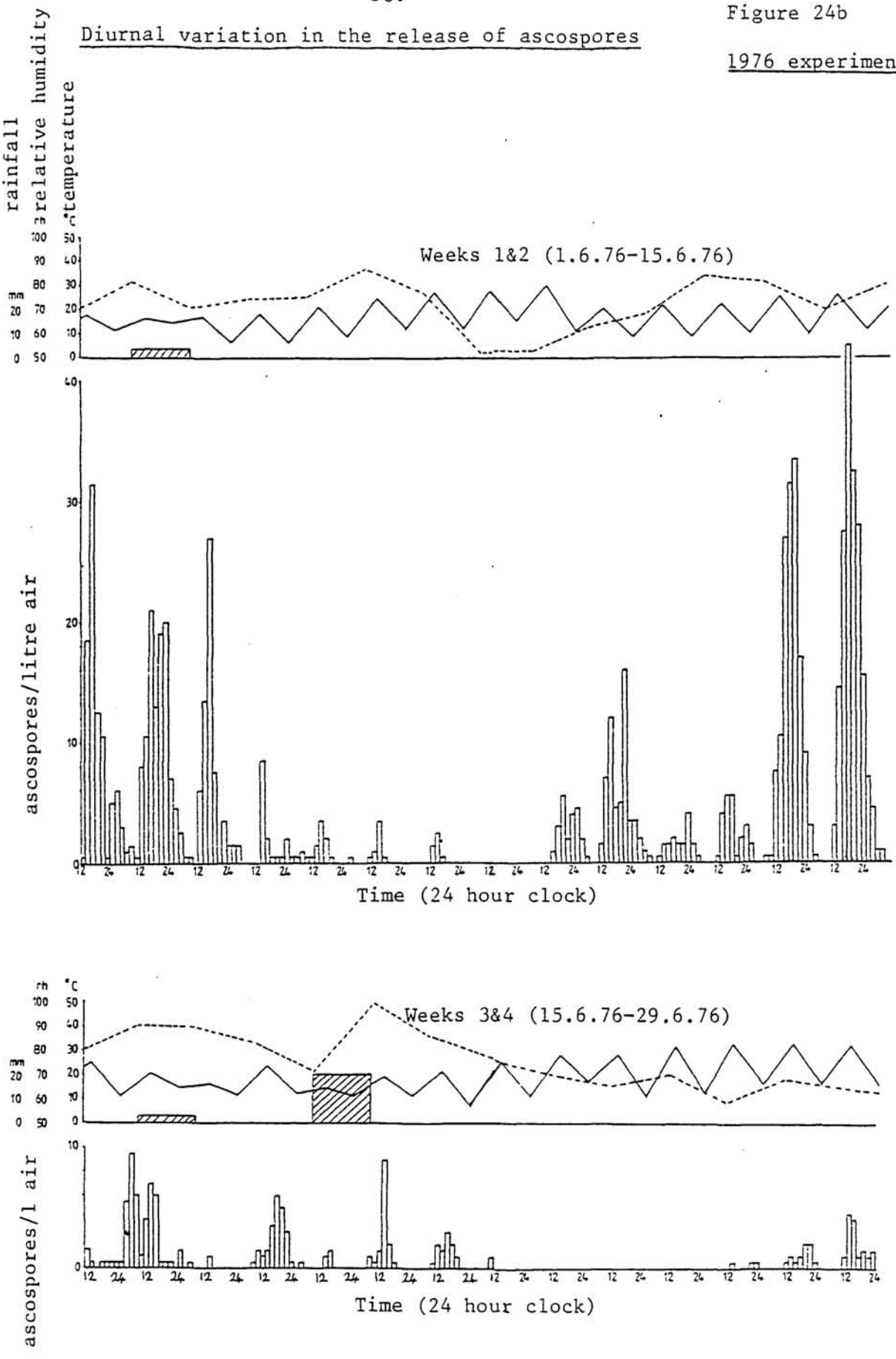


Figure 25

Variations in the time of peak ascospore discharge

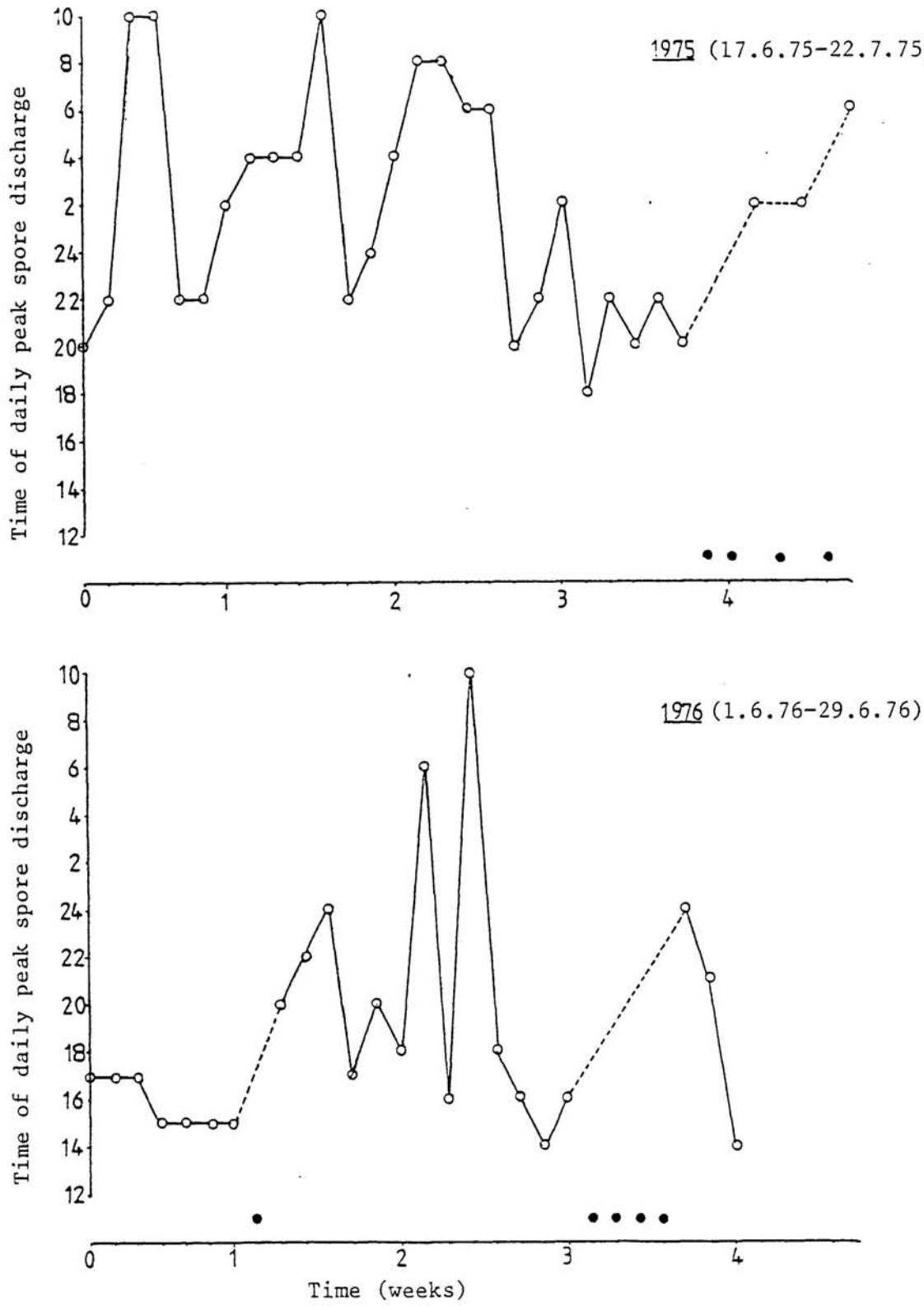
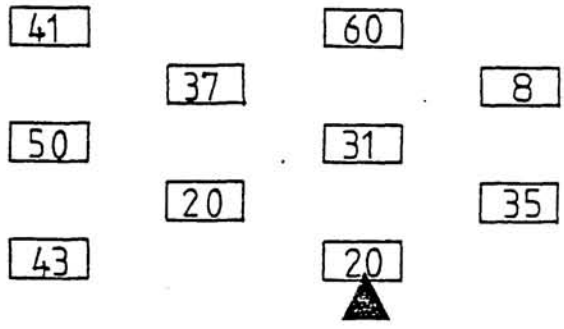


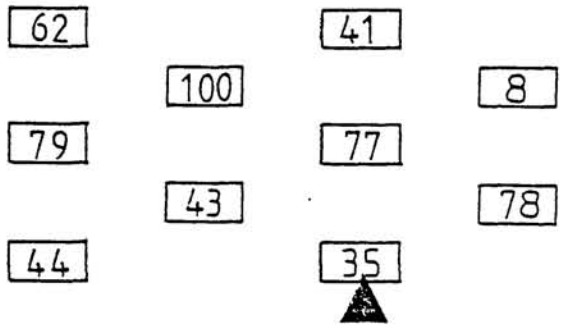
Figure 26

The germination of ergots in the 1975 ascospore ejection experiment

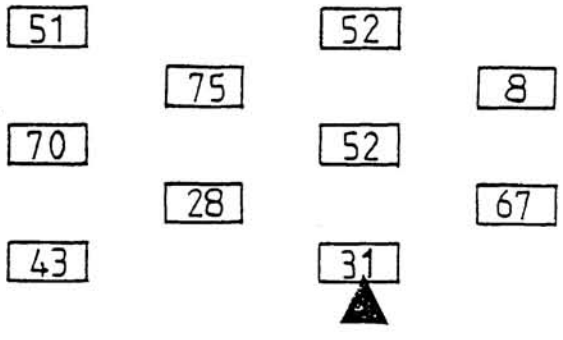
a) Percentage germination of ergots buried beneath soil surface



b) Percentage germination of ergots on soil surface



c) Total percentage germination



▲ = spore trap

discharge than they were during the day. This was probably associated with lower evening temperatures than in the preceding week.

Ascospore ejection was minimal during the following week (16.7.75-23.7.75), although moist conditions continued. The stromata were very shrivelled which was probably a consequence of them having ejected most of their ascospores.

The percentage of germinated sclerotia in each tray was very varied in 1975 (figure 26) but there was no apparent relationship between percentage germination and tray position. Germination of ergots on the soil surface showed greater variability than that of buried ergots. Nearly all the ergots which fell through the trays had started to germinate before being returned to the soil surface. It therefore seems likely that the percentage germination of ergots on the soil surface reflects the number of sclerotia which were washed through the drainage holes of each tray. Variation in the percentage germination of buried ergots probably reflects inconsistent microclimates in each tray.

Following cool damp weather at the end of May 1976, germination was first observed on 1st June and the spore trap was started. It can be seen from figure 24b that spore ejection was already well established by this time, although none of the stromata which were examined had elongated stipes. As in the 1975 experiment, diurnal fluctuations in spore release were recorded.

In 1975 the peak period of spore release oscillated between the evening and early morning (figure 25), the part of the day in which relative humidity at ground level within the crop would be expected to be maximal. A similar pattern of spore release seemed predominant in mid-June 1976, but in the preceding and subsequent weeks peak spore release occurred earlier in the day, mainly in the afternoon. The crude meteorological data does not offer any immediate explanation for spore release occurring in what is generally the warmest part of the day.

It is assumed that increasing temperature would be expected to:

a) increase spore release by increasing stromatal metabolic activity, and
b) decrease spore release by decreasing relative humidity and consequently increasing water stress on stromata.

It is concluded therefore that the timing of peak spore release results from the product of these, and possibly other, counteracting vectors, the relative influences of which are unknown. Increased temperature would be expected to increase metabolic activity, generating osmotic agents, such as glucose and mannitol which are possibly involved in ascospore discharge. Increased temperature would also promote the growth and maturation of asci within the perithecia. Stromatal water stress is determined not only by the relative humidity of the air, but also directly by soil moisture and/or indirectly by the influence of soil moisture on the reservoir of translocatable water in a sclerotium.

A water stress constraint on spore release may also cause an accumulation of mature asci, most of which will release their spores when the constraint is removed. The diurnal pulse of spores seen in these experiments may therefore be expected to consist partly of accumulated mature spores and does not necessarily only reflect surges in maturation.

9. COMPETITION BETWEEN STRAINS OF C.PURPUREA IN VIVO

9.1 Experimental - experiment 1

Four days prior to anthesis twenty groups of winter wheat (cv. Maris Huntsman), each containing ten uniformly sized ears, were selected and labelled. Four strains of Claviceps purpurea were chosen for their differing biochemical and morphological features. For each of the strains spore suspensions containing approximately $2x10^7$ and $4x10^4$ spores/ml were made by diluting honeydew (stored under liquid nitrogen) with distilled water. Combinations of equal volumes of these suspensions were prepared (table 3a) and inoculated into 75% of spikelets in each ear of the requisite treatment groups of plants. The ears were grown to maturity, harvested by hand and threshed by hand.

Three months after harvest one hundred ergots from each group were soaked in distilled water for twenty four hours and placed 2-4 mm beneath the surface of moist vermiculite in trays. They were incubated at 4^oC for seven weeks and then at room temperature (c 20^oC) until no further germination occurred. Once germination commenced the number of stromata/treatment group was scored periodically.

The total alkaloid content was determined using approximately one gram of ergot tissue, and the individual alkaloids were resolved by T.L.C.. Each sample was chromatographed in ethyl acetate/ethanol/dimethyl formamide 19:0.1:1.9 and 26:0.1:1.9 according, respectively, to whether the ergotamine group/ergotoxine group ratio was important or whether the components of the ergotoxine group needed to be resolved.

Twelve ergots were selected from strain combinations AB and AD, and three from each of the single strains A,B and D. The selected sclerotia were representative of the range of size yielded by each group. Alkaloid extractions were carried out separately on each of these sclerotia and the alkaloids were resolved by T.L.C. as usual.

9.2 Results

Nine days after inoculation honeydew exudation was prolific in all the inoculated ears except whenever strain B had been inoculated as the equal or dominant component (groups B,b,AB and aB).

The results of the sclerotial germination tests are summarised in table 4 and figure 27. The average of 35% sclerotia germinated for

Table 3a

Spore combinations used for mixed strain inoculations - experiment 1

Strain 1	Strain 2	Combination code
A	-	A
a	-	a
B	-	B
b	-	b
C	-	C
c	-	c
D	-	D
d	-	d
A	B	AB
A	b	Ab
a	B	aB
A	C	AC
A	c	Ac
a	C	aC
A	D	AD
A	d	Ad
a	D	aD
C	D	CD
C	d	Cd
c	D	cD

UPPER CASE denotes 2×10^7 spores/ml

Lower case denotes 4×10^4 spores/ml

Table 4

Germination of sclerotia of mixed and single strain origin

Combination code	No.stromata per 100 ergots			No.sclerotia (final) with stromata							% sclerotia germinated - final
	28 days	40 days	53 days	0	1	2	3	4	5	6	
	After incubation temp. raised to c.20°C										
A	29	49	61	69	19	3	5	0	3	1	31
a	12	34	61	61	24	7	4	2	1	1	39
B	0	1	2	98	2	0	0	0	0	0	2
b	1	1	1	99	1	0	0	0	0	0	1
C	2	19	52	70	17	8	3	1	0	1	30
c	6	38	52	71	13	10	5	1	0	0	29
D	2	2	2	99	0	1	0	0	0	0	1
d	1	2	1	99	1	0	0	0	0	0	1
AB	2	3	4	97	2	1	0	0	0	0	3
Ab	8	9	5	97	1	2	0	0	0	0	3
aB	3	5	16	94	1	3	0	1	1	0	6
AC	7	15	46	73	13	11	2	0	1	0	27
Ac	53	82	99	44	30	16	5	3	2	0	56
aC	2	10	19	86	10	2	1	1	0	0	14
AD	0	0	0	100	0	0	0	0	0	0	0
Ad	2	6 *	6	90	2	2	0	0	0	0	4
aD	2	4	4	98	0	2	0	0	0	0	2
CD	0	0	4	98	1	0	1	0	0	0	2
Cd	12	27	49	71	17	7	3	1	1	0	29
cD	1	5	7	95	3	2	0	0	0	0	5

* 6 moribund sclerotia were removed from this tray on day 40.

Figure 27

Percentage germination of sclerotia of single and mixed strain origin.

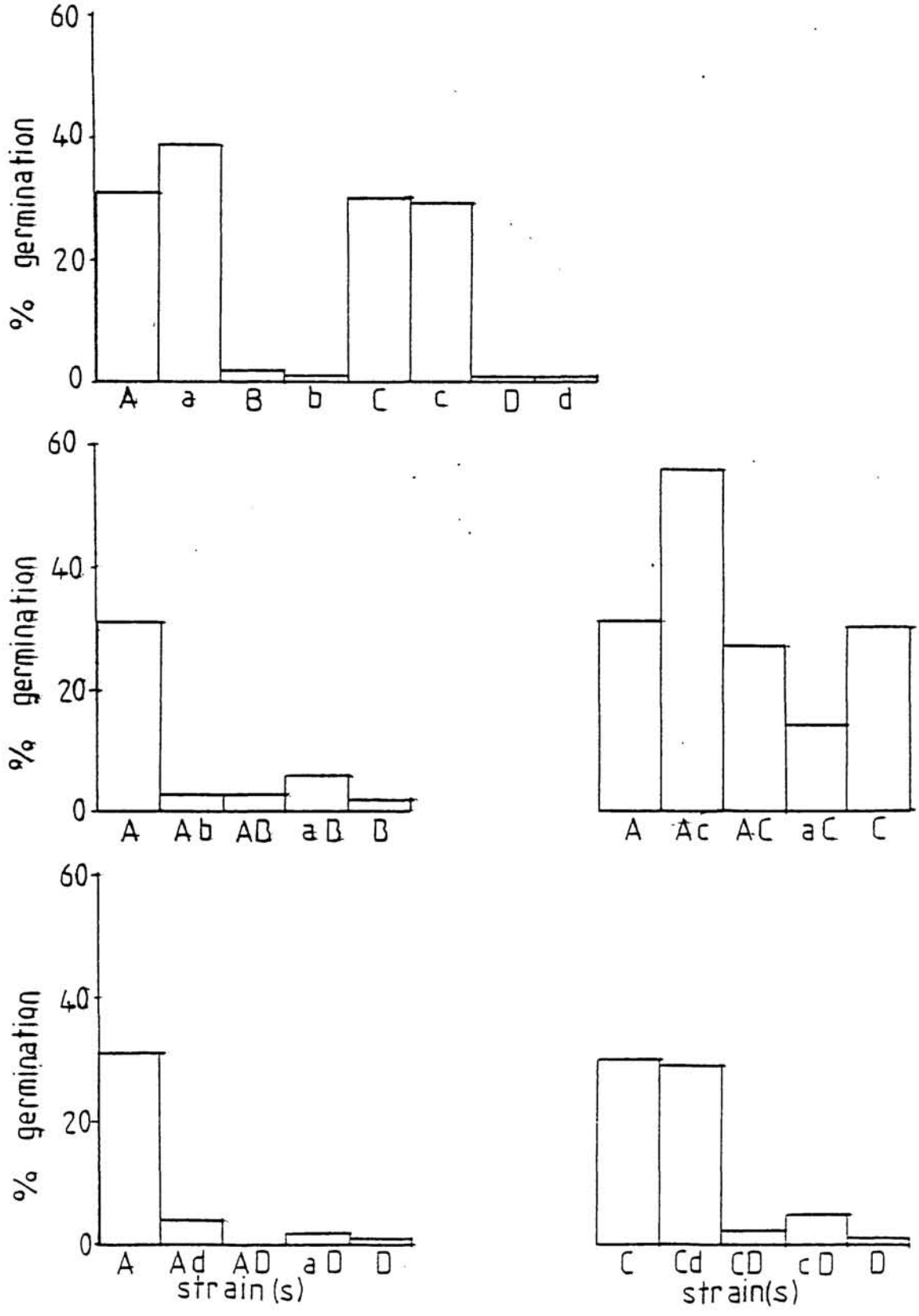


Table 5

Alkaloid content of sclerotia resulting from single and mixed strain inoculations

a) Experiment 1

Strain combination	Alkaloid content (% w/w)
A	0.26
B	0.00
C	0.41
D	0.40
AB	0.17
Ab	0.26
aB	0.03
AC	0.37
Ac	0.29
aC	0.35
AD	0.36
Ad	0.32
aD	0.39
CD	0.39
Cd	0.51
cD	0.29

b) Experiment 2

Strain combination	Alkaloid content (% w/w)
B	0.00
E	0.31
BE	0.15

Alkaloid spectra of sclerotia resulting from single and mixed strain inoculations

Table 6

	A&a	B&b	C&c	D&d	AB	Ab	aB	AC	Ac	aC	AD	Ad	aD	CD	Cd	cD
Alkaloids present	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Alkaloids absent		✓					✓									
Ergotoxine>ergotamine +ergosine	✓				✓	✓			✓			✓				
Ergotoxine<ergotamine +ergosine			✓					✓		✓				?	✓	TOO FAINT
Ergotoxine ergotamine +ergosine				✓							✓		✓	?		TOO FAINT
Ergocornine+ergokryptine >ergocristine	✓				✓	✓		✓	✓			✓				
Ergocornine+ergokryptine <ergocristine				✓							✓		✓	✓		
Ergotoxine group very faint			✓							✓					✓	✓
Parentage indicated by spectrum	A	B	C	D	A	A	B	A&C	A	C	A&D	A	D	C&D	C	TOO FAINT

strain A was lower than in previous experiments, and the stromata of strain C were not disorganised in the manner previously recorded. The results for the sclerotia derived from a single strain show a clear division into two groups:

i) strains C and A with a germination rate of 30-35%

ii) strains B and D with a germination rate of 1-2%

All the combinations containing strain B showed light contamination with Phycomycete hyphae which were apparent ten weeks after incubation commenced.

All the mature sclerotia were of similar shape except those in groups B,b and aB which were considerably shorter and broader.

The alkaloid content of each strain and strain mixture is given in table 5. The 26:0.1:1.9 ethyl acetate/ethanol/dimethyl formamide solvent gave good separation of the ergotamine group (ergotamine and ergosine) from the ergotoxine group (ergocornine, ergocristine, ergokryptine) but gave no separation within these groups. The best overall separation was achieved by the 19:0.1:1.9 mixture which resolved all the alkaloids present except ergocornine and ergokryptine. All four single strains have distinct alkaloid spectra without separating these two components so no attempt was made to achieve separation. The alkaloid spectra are summarised in table 6.

Since groups of sclerotia produced from mixed inoculum showed quantitative and qualitative alkaloid contents which suggested coexistence of the two strains, it was necessary to explore strain mixing within each ergot. So far, the results could be either the product of a heterogeneous group of single strain sclerotia or the mean of a group of sclerotia all of which contained both strains.

Consistent evidence of strain mixing within each sclerotium from an AB or AD combination could only be obtained by analysis of individual ergots. T.L.C. of extracts from individual sclerotia showed alkaloids to be present in all twelve ergots selected from the AB group and all had the alkaloid spectrum characteristic of strain A. Of the twelve ergots analysed from the AD group, one had ergocristine but no ergocornine or ergokryptine (as strain D) and another had ergocornine and/or ergokryptine but no ergocristine (as strain A). Ten had ergocristine and ergocornine and/or ergokryptine, strongly indicating the presence of both strains A and D.

It is concluded that:

a) where a non alkaloid-producing strain was inoculated in combination with an alkaloid-producing strain, the quantitative alkaloid content gave evidence of the existence of the former strain, the qualitative spectrum confirming the identity of the latter

b) where a mixture of alkaloid-producing strains was inoculated, characteristic features of the alkaloid spectrum of a strain on the chromatogram was evidence for the presence of this strain.

c) where an unequal mixture of alkaloid-producing strains was inoculated the presence of the minor component strain could be inferred from the quantitative assay except for combinations of strains C and D.

Table 3b

Strain combinations used in mixed strain inoculations - experiment 2

Combination code	Injected on Day 1	Injected on Day 8
B	B	-
E	E	-
BE	B+E	-
B-E	B	E
E-B	E	B

9.3 Experimental - experiment 2

Five groups of approximately twenty uniformly sized ears of winter wheat (cv. Maris Hunstman), in a plot adjacent to that used for experiment 1, were inoculated four days prior to anthesis. The inoculum contained approximately 2×10^7 honeydew-derived spores/ml and consisted of strains B and E of C.purpurea alone or in combination. The treatment groups are summarised in table 3b. Five central spikelets on one side of each ear were inoculated.

A group of ears inoculated either with strain B or E was inoculated again, seven days later, with the other strain. (The pathogenicity of this later inoculum was confirmed by injection into pre-anthesis ears in the same plot). When the second inoculations were made care was taken to superimpose the strains by inoculating through the original needle wounds. The plants were grown to maturity and harvested by hand. Ears from each of the treatment groups were matched for size and the number and weight of ergots and seeds was recorded for each ear. The quality of the seeds from ears inoculated with strain E only and with strain E followed by strain B, was compared with that of seeds from uninoculated ears, using a seedling emergence test. For this test the seeds were sown in an open plot at the Chelsea Physic Garden during November, and the number of emerged seedlings was recorded nine weeks later.

The alkaloid content of individual, or representative groups of, ergots was analysed to a) identify co-existence of strains in ergots from mixed strain inoculum and to determine the extent of this within the sclerotium, and b) to identify the strain(s) of ergots yielded by double inoculated ears. In order to determine the extent of strain mixing within ergots and to ensure separate analysis of older and younger tissues, the central portion (approximately $\frac{1}{3}$th total length) was excised from each sclerotium and discarded. All traces of sphacelial tissues were removed from the ergot tips. The alkaloid content of the tip and base of each sclerotium was determined separately.

9.4 Results

Ears inoculated with either strain B or strain E yielded ergots which differed not only in their abundance but also in their morphology. By comparison with strain E, strain B apparently released less honeydew and yielded fewer sclerotia although it was almost as efficient in

preventing seed development (table 7).

Inoculation with the strain mixture BE yielded ergots which, in abundance and alkaloid content were intermediate between those of strain B or E alone (tables 5b & 7). This is consistent with the results of experiment 1. Analysis of the older and younger parts of these ergots of mixed origin showed that not only was alkaloid present in each sclerotium but that the greater proportion was found in the younger (proximal) tissues (table 8). The same was found in pure strain E sclerotia. This shows that active growth of the alkaloid-producing strain E persisted at least throughout most of the growth period of the sclerotium. Additionally the lower overall alkaloid content of the ergots resulted from mixed inoculum is consistent with the non-alkaloid producing strain B also being present. Parasitism of host tissues by strain B is characterised by development of more spacelial tissue (Corbett et al 1974) and larger mature ergots (table 7) than are produced by strain E. The above results suggest that, in spite of this difference in spacelial and sclerotial growth rate, when strains B and E are simultaneously inoculated into a floret they both contribute to the growing sclerotial body during most, if not all, of its development.

Where strain B was inoculated first, and would be expected to yield about 3 sclerotia in each ear, the addition of strain E, one week later, reduced by half the number of ergots (table 7). Since the identity of these 19 ergots was uncertain they were analysed individually for total alkaloid content. Fifteen were devoid of alkaloid so were considered to be pure strain B, and one contained 0.39% alkaloid which at least indicates a predominance of strain E. Three had low alkaloid contents (0.01, 0.08, 0.08%) indicating the presence of strain E but suggesting that strain B may also have been present. It is concluded that strain E reduced sclerotia production by the already established strain B by approximately 50%. However, there is insufficient evidence that it could supplant strain B and produce a strain E sclerotium in a floret either where strain B would have yielded a sclerotium or where the pathogen would have aborted. The one ergot which appeared to be typical strain E may have developed in a previously uninoculated terminal floret.

When inoculation with strain E was followed one week later by the addition of strain B the majority of infections aborted at the spacelial stage. Instead of about 12 ergots developing in each ear, as was

Table 7

Comparison of seed and ergot production in wheat ears inoculated with strain B and/or strain E of C. purpurea

	Controls (not inoculated)				B		E				BE	B-E	E-B
	C												
No. of ears, matched* between treatment groups	16	16	17	31	15	16	16	16	17	74	31	11	16
Mean seed no./ear	53.8	41.6	47.7	46.9	34.7	34.3	30.3	23.0	29.9	27.7	29.4	34.8	31.4
Mean seed wt. (mg)	54.5	55.4	55.2	53.6	55.2	54.0	55.4	52.9	55.7	53.5	59.2	56.2	55.0
Mean ergot no./ear	-	-	-	-	3.3	2.0	13.2	11.5	12.0	16.2	8.9	1.5	0.5
Mean ergot wt. (mg)	-	-	-	-	66.7	58.8	41.3	36.4	40.0	41.3	28.5	84.5	77.7
Mean no. seeds + ergots per ear	53.8	41.6	47.7	46.9	38.0	36.3	43.5	34.5	41.9	43.9	38.3	36.3	31.9
Percentage of control florets yielding seed and ergots	100	100	100	100	70.6	87.3	80.8	82.9	87.8	-	81.7	67.5	59.3
Percentage seedling emergence in field test	88.8	-	-	-	-	-	94.5	-	-	-	-	-	83.0
Matching code (page 93, line 14)	★	☆	○	✪	★	☆	★	☆	○	●	✪	★	★

Table 8

Alkaloid distribution within single and mixed strain sclerotia

Combination no.	Alkaloid content % w/w		Tip: Base Ratio
	Tip	Base	
BE	0.07	0.10	1 : 1.4
"	0.05	0.15	1 : 3.0
"	0.08	0.16	1 : 2.0
"	0.03	0.11	1 : 3.7
"	0.02	0.12	1 : 6.0
"	0.12	0.16	1 : 1.3
"	0.03	0.13	1 : 4.3
"	0.08	0.18	1 : 2.2
E	0.08	0.21	1 : 2.6
"	0.18	0.25	1 : 1.4
"	0.04	0.20	1 : 5.0
"	0.26	0.32	1 : 1.2
"	0.39	0.48	1 : 1.2
"	0.43	0.55	1 : 1.3
"	0.31	0.37	1 : 1.2
"	0.43	0.53	1 : 1.2

recorded for strain E alone, each double inoculated ear yielded, on average, only 0.5 ergots (table 7). One to two weeks after the superimposition of strain B rapid and complete death of the floral parts of the inoculated spikelets occurred. Necrosis was confined to inoculated spikelets and these later became colonised by saprophytic fungi. The high alkaloid content (0.43 - 0.66%) of all seven sclerotia in this group indicates that they were pure strain E since individual analysis of seven pure strain E ergots showed an alkaloid range of 0.22 - 0.52%. This suggests that they developed in terminal florets to which strain B had not been introduced.

Apparently the addition of strain B spores to an established strain E infection completely eliminated sclerotium production by strain E. It is not clear whether this was an interaction solely at the fungal level or whether it was caused by nutrient deprivation resulting from necrosis of the infected spikelets. Irrespective of the cause of abortion of strain E infection it is notable that strain B was unable to become an established parasite and form sclerotia.

The mean seed weight was remarkably constant and was not affected by the presence of ergots (table 7). However, the percentage seedling emergence was significantly greater in seeds from ears infected with strain E than in seeds from healthy ears (appendix Iiia). It is postulated that the flow of honeydew from infected ears pulled nutrients past the developing seeds which consequently accumulated additional reserves (see also section 10.6). This could, in turn, result in enhanced seedling emergence. In contrast, seeds from ears inoculated with strain E followed by strain B (E-B) showed a significantly reduced percentage seedling emergence. It seems most probable that the dramatic necrosis of the floral tissue of the inoculated spikelets impared grain development in the uninoculated spikelets, possibly by reducing the level of local photosynthetic activity.

9.5 Discussion

Although the amount of honeydew released from a floret inoculated with C.purpurea may be used as a preliminary guide to the identity of the strain(s) involved, its viscosity makes it difficult to quantify, and this parameter is too variable to be of more than minor significance. Similarly, sclerotial morphology may be of use in preliminary identification of certain populations of ergots, but this feature can only be

subjectively assessed, and is too unreliable for use on individual sclerotia.

In the present studies, ergots from mixed inoculum, whose alkaloid content indicated the presence of both strains A and B, germinated poorly irrespective of the extent of each strain's presence. Thus, even when strain A, characterised by relatively high levels of germination, was in considerable excess of strain B, a poorly germinating strain, a very low percentage of the ergots germinated. Although it might be argued that the Phycomycete contamination of the sclerotia containing strain B was responsible for the low germination rates, there was no such contamination of ergots composed of strains A and D, whose germination rates were also consistently low.

The low germination of ergots originating from the mixed inocula CD and cD is consistent with the above germination results. In contrast, ergots of Cd origin germinated to the same extent (c.30%) as recorded for strain C alone. Strains C and D have both the same total alkaloid content and insufficiently distinct alkaloid spectra to allow certain identification of small amounts of strain D in a Cd mixture. Therefore it is possible that of the small group (100) of ergots set up for germination few, if any, contained strain D and consequently the group would be expected to behave as pure strain C sclerotia.

It is concluded that the germinative ability of mixed strain ergots is not simply a reflection of the proportion of the two single strains estimated to be present on the basis of the alkaloid evidence. There seems to be a disproportionately strong influence of a poorly germinating strain.

Ergots containing both strains A and C show a range (14-56%) of germinative capacity inspite of ergots of both the single strains germinating to the same (c.30%) extent (figure 27). Most probably this is due to experimental variation between samples, but in the absence of replicated treatments no firm inferences can be drawn.

Sclerotia germinated over a period of approximately four weeks and the final numbers of stromata/germinated sclerotium (table 4) show little variation between groups. The majority of germinated ergots produced only one or two stromata although more were produced by ergots in the groups in which the higher proportions of sclerotia germinated.

The cause of varied germination potential, apparently inherent in different strains of C.purpurea, is not understood. From the present results it is possible to postulate the presence in some strains of a factor inhibitory or antagonistic to germination, which can exert its influence even when present in only a portion of the sclerotial mycelium (eg. in ergots of the Ac and Ad groups where, according to the alkaloid content, the poorly germinating strain is in the minority).

The alkaloid content of ergots has, for many years, been used as a firm basis for distinguishing species of Claviceps. More recently Shaw (1975) demonstrated the value of alkaloid spectra in distinguishing host-restricted strains of C.purpurea. In the present experiments, both the quantitative and qualitative alkaloid content of ergots have been shown to be valuable parameters in determining the identity of sclerotia of mixed strain origin.

In all cases where spore suspensions containing an equal amount of two different strains were used to infect host plants, the qualitative and quantitative alkaloid contents of the resulting sclerotia were consistent with the conclusion that the two strains co-existed within the majority of sclerotia throughout most, if not all, of their development. Furthermore, the proportions of each strain in a group of ergots reflected the proportion of spores of the two strains in the original inoculum. It is hardly surprising that the proportions of spores in the inoculum are not exactly reflected by the apparent strain proportions in the sclerotium, since the alkaloid content of individual sclerotia composed of the same single strain shows considerable variation (table 8).

It is to be expected that many of the viable spores in an inoculum introduced into the floral cavity can contribute to the colonisation of the ovary. The present evidence that a strain in a 500:1 minority formed at least part of some sclerotia supports this contention.

The reduction in the expected sclerotial number (table 7) observed after a second strain was superimposed onto a C.purpurea infection initiated seven days previously, can only be attributed to an antagonistic interaction between the two strains. Since this dramatic effect was unexpected no microscopical studies were made on infected tissues. However, for such antagonism to be exhibited it seems likely that the spores of the second strain must have germinated thus enabling a hyphal

metabolite to initiate the interaction. The mean number of ergots/ear resulting from simultaneous inoculation of a host with strains B and E was intermediate between the values obtained when strains B and E are inoculated alone. This implies that antagonism is a feature of sequentially spaced inoculations and does not become evident when these two strains co-exist from inoculation onwards. Consequently strain B cannot simply have been producing a non-specific fungitoxic compound, but the external evidence of reaction (chlorosis of spikelets within one week of the second inoculation) and the absence even of miniature ergots in most of the double inoculated florets, suggests that the antagonism occurred within the space of a few days only.

Shaw (1979) demonstrated that glucanase probably plays an important role in the infection process of C.purpurea. Any interuption of glucanase release may result in callose formation at the host/parasite interface and hence deprive the developing ergot of essential nutrients and lead to abortion of the infection. It could be that the superimposition of a particular second strain on an established infection produces a substance which disrupts glucanase production by the established strain and consequently causes its abortion.

Almost all the sclerotia which developed in twice inoculated ears were, on the basis of their alkaloid content, composed only of the first inoculated strain. It has already been suggested that some or all of these ergots may have developed in terminal florets to which the second strain was not added. However, it is also possible that in these few cases the extent of ovary colonisation was more advanced at the time of introduction of the second strain, thus possibly reducing the importance of any interuption in glucanase production.

The occurrence of rogue sclerotia, particularly in the E-B sequence, is only a minor complicating feature but it might have been better to have restricted the experiment solely to the two basal florets of each spikelet in which inoculation could have been guaranteed.

During the discussion of these results the occurrence of factors inhibitory to the normal metabolic processes of C.purpurea have twice been postulated, namely in parasitic sphacelial hyphae and in mature ergots. Identification of any inhibitory compounds would be welcome since they might be of use as chemicals capable of controlling ergot in cereal crops since the targets are both critical and accessible phases of the life cycle.

10. COMPETITION FOR NUTRIENTS BETWEEN SEEDS AND ERGOTS

10.1 Experimental - Spring wheat

Three adjacent experimental plots to which 60 units (75 kg/ha) of nitrogen as Nitram (ammonium nitrate) had been applied were sown with Sicco spring wheat during March 1977. Three weeks before anthesis a top dressing of 50 units of nitrogen (62.5 kg/ha) was added to one of the plots, and 100 units (125 kg/ha) to a second plot. No additions were made to the third plot. The plots were netted to eliminate the risk of grain loss through bird attack.

Just before anthesis 80% of the spikelets in each of 50 ears, and 20% of the spikelets in each of another 50 ears, in all three plots were inoculated with strain 330 of C.purpurea (section 2.6). A further group of 50 similarly sized ears in each plot were labelled to provide controls. The ears in each of the nine treatment groups (3 levels of nitrogen x 3 levels of inoculation) were numbered 1-50 at the time of inoculation so that random number tables (Fisher & Yates, 1963) could be used to determine the order in which they were sampled.

Five ears were collected from each treatment group at weekly intervals from anthesis to harvest, and stored deep frozen ($-20^{o}C$). Within two weeks of collection the developing seeds and ergots were excised from the five ears in each group. The number of inoculated and uninoculated spikelets in each group, as well as the number and fresh weight of the seeds and ergots was recorded. The seeds and ergots were rinsed to remove any adhering honeydew and conidia and then macerated in a small volume of distilled water, using a vortex homogeniser. After lyophilisation the dry weight of each sample was determined.

In order to ascertain whether respiratory oxidation of lipids could account for the observed reduction in mean ergot weight during the later stages of ergot development (section 12.2), the lipid content of selected groups of ergots was determined (section 2.12). The groups of ergots used for this analysis were those collected 31, 38, 44 and 51 days after inoculation in the 20% inoculation + 50N treatment group.

10.2 Results and discussion

Emergence and early development was uniform in all three plots and the wheat was notably free from extraneous disease. There were no visible differences between the three plots until one week after anthesis (5th

July) when an increased chlorophyll content was apparent in the two plots to which additional nitrogen had been supplied. The ears in the plots to which 50 and 100 units of nitrogen had been added were larger than in the untreated plot, although no attempt was made to quantify this observation. No visual distinction, in terms of chlorophyll content or ear size, could be made between the plots to which 50 and 100 units of nitrogen had been applied.

The proportion of ergots to seeds in each group of ears coincided with the proportion of spikelets inoculated (table 9). Although most of the ears in which 80% of the spikelets were inoculated produced slightly fewer than 8 ergots for every 2 seeds, those ears inoculated at the 20% level produced slightly more than 2 ergots for every 8 seeds. This may be attributed to the greater number of uninoculated florets adjacent to inoculated ones, in the latter group of ears which could be secondarily infected by the ergot fungus. It may also represent ergot saturation of the more heavily infected ears.

The dry matter content of the seeds (figure 28) increased steadily in all 9 treatments from about 35% approximately 9 days after anthesis (10 days after inoculation) to a maximum level around 90%, 43 days after anthesis. The large decrease in water content between 37 and 43 days post anthesis is in accord with normal wheat development and was not affected either by the level of applied nitrogen or the presence of ergots in the ear.

The dry matter content of the ergots was more variable. While this may have been due to desiccation during dissection of the ergots from the ears, it is notable that the water content of ergots from the lightly (20%) infected ears was always lower than that of ergots from the heavily infected ears, irrespective of the level of added nitrogen.

In nearly all the groups of ears in which 80% of the spikelets were inoculated, the number of seeds/uninoculated spikelet was greater than in those groups which were either inoculated at the 20% level or left uninoculated (figure 29). This most probably does not indicate enhanced seed set but reflects the presence of seeds in the terminal floret(s) of some of the inoculated spikelets. There were approximately 2 ergots/inoculated spikelet in all the groups of ears so some of the inoculated spikelets should have had the capacity to produce one or two seeds as well. However, the control ears only produced about 2 seeds

Table 9

The percentage of ergots $\left[\frac{\text{Ergot no.x100}}{\text{Ergot no.+Seed no.}}\right]$ in each group of ears used in the spring wheat nutrient competition experiment

Treatment Group: time interval between inoculation & harvest	+ units of nitrogen	% spikelets inoculated: 0%	20%	80%
10 days	+ ON	0	22	77
	+ 50N	0	22	66
	+ 100N	0	21	76
17 days	+ ON	0	23	82
	+ 50N	0	23	77
	+ 100N	0	22	73
24 days	+ ON	0	24	77
	+ 50N	0	24	74
	+ 100N	0	24	77
31 days	+ ON	0	25	80
	+ 50N	0	21	76
	+ 100N	0	21	77
38 days	+ ON	0	25	77
	+ 50N	0	25	80
	+ 100N	0	22	75
44 days	+ ON	0	21	73
	+ 50N	0	22	72
	+ 100N	0	21	79
51 days	+ ON	0	20	76
	+ 50N	0	18	77
	+ 100N	0	19	77

Figure 28

Variations in the percentage dry matter of ergots and spring wheat seeds during development

a) +ON

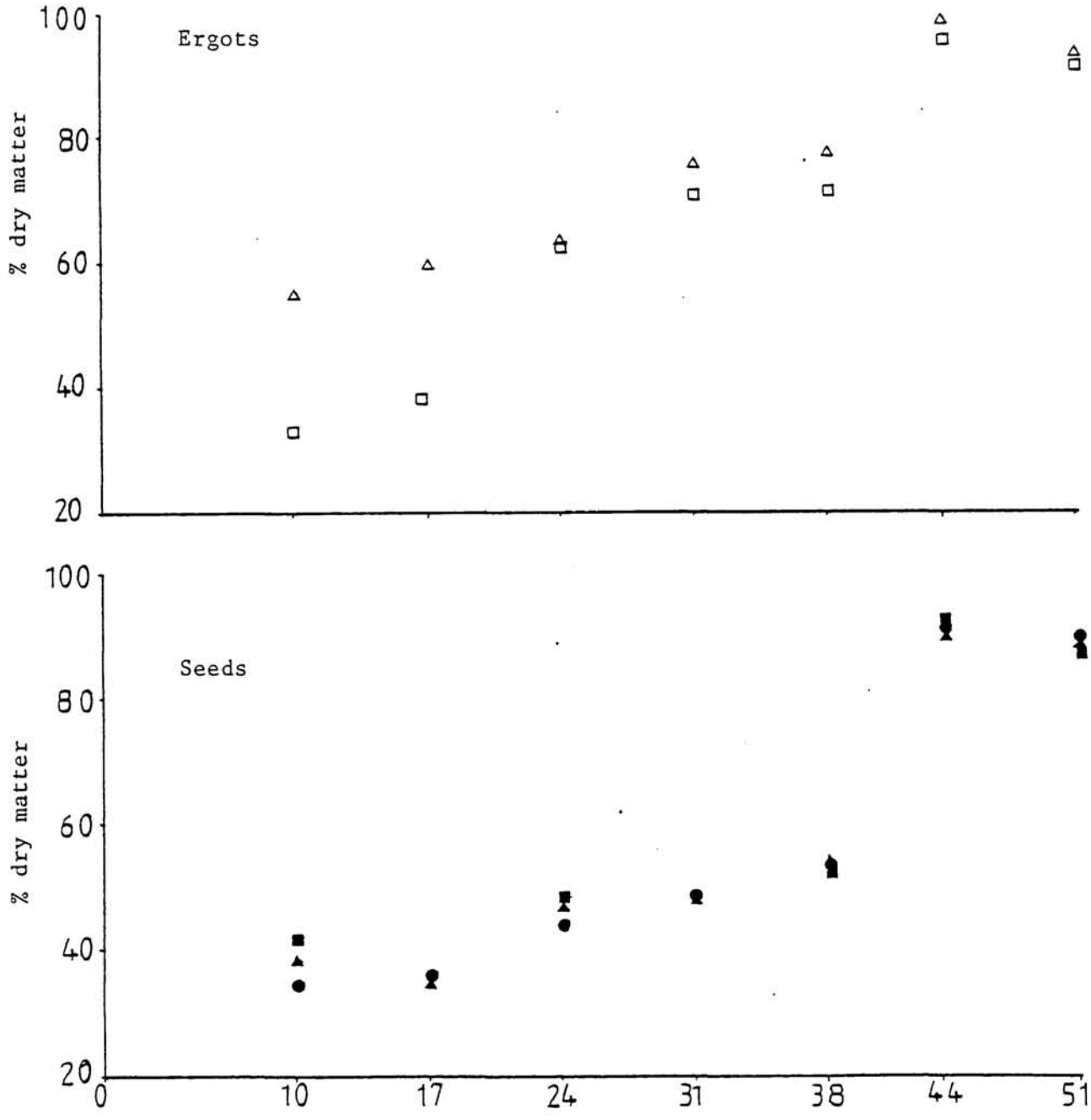
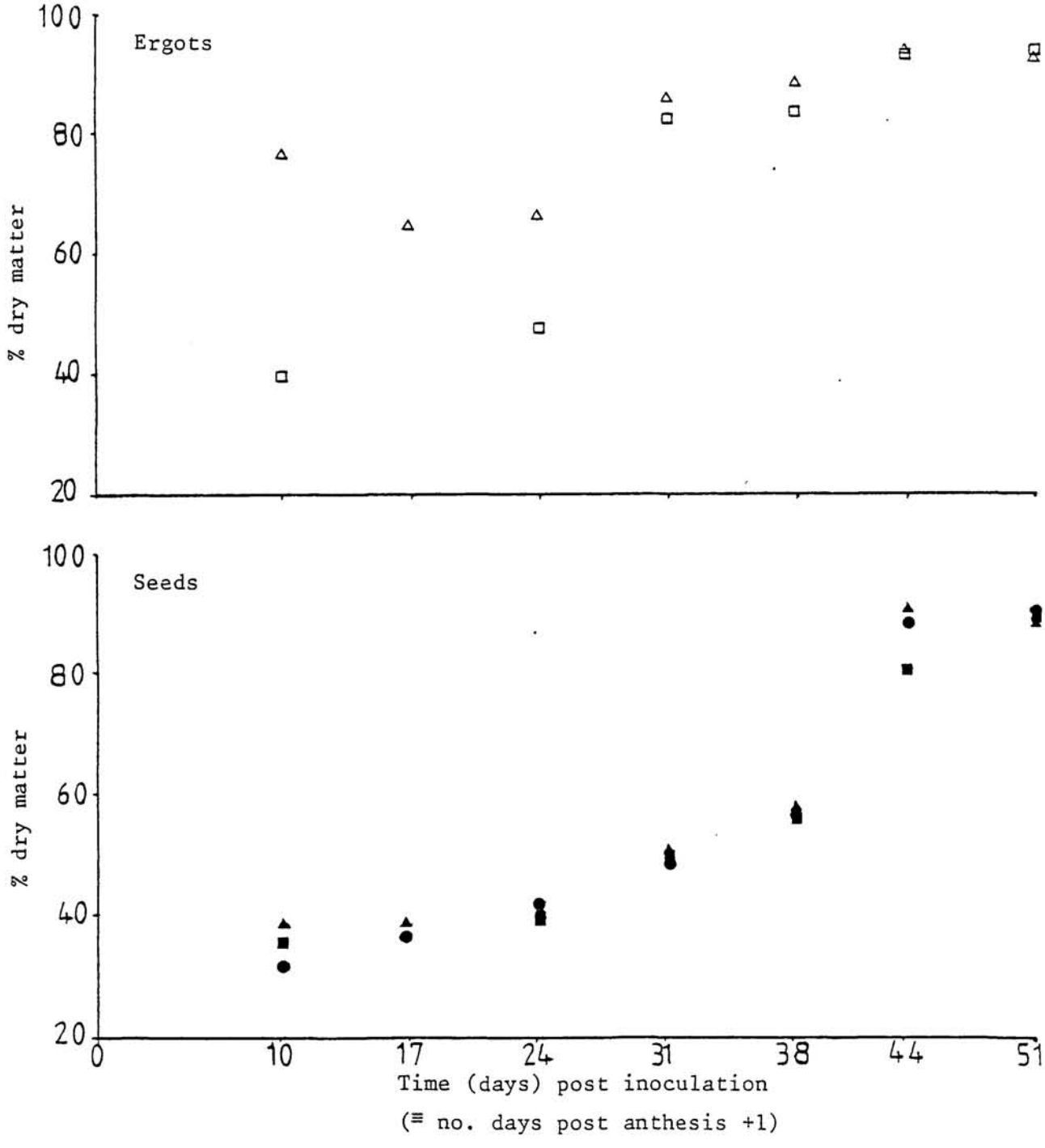


Figure 28 - cont'd

b) +50N



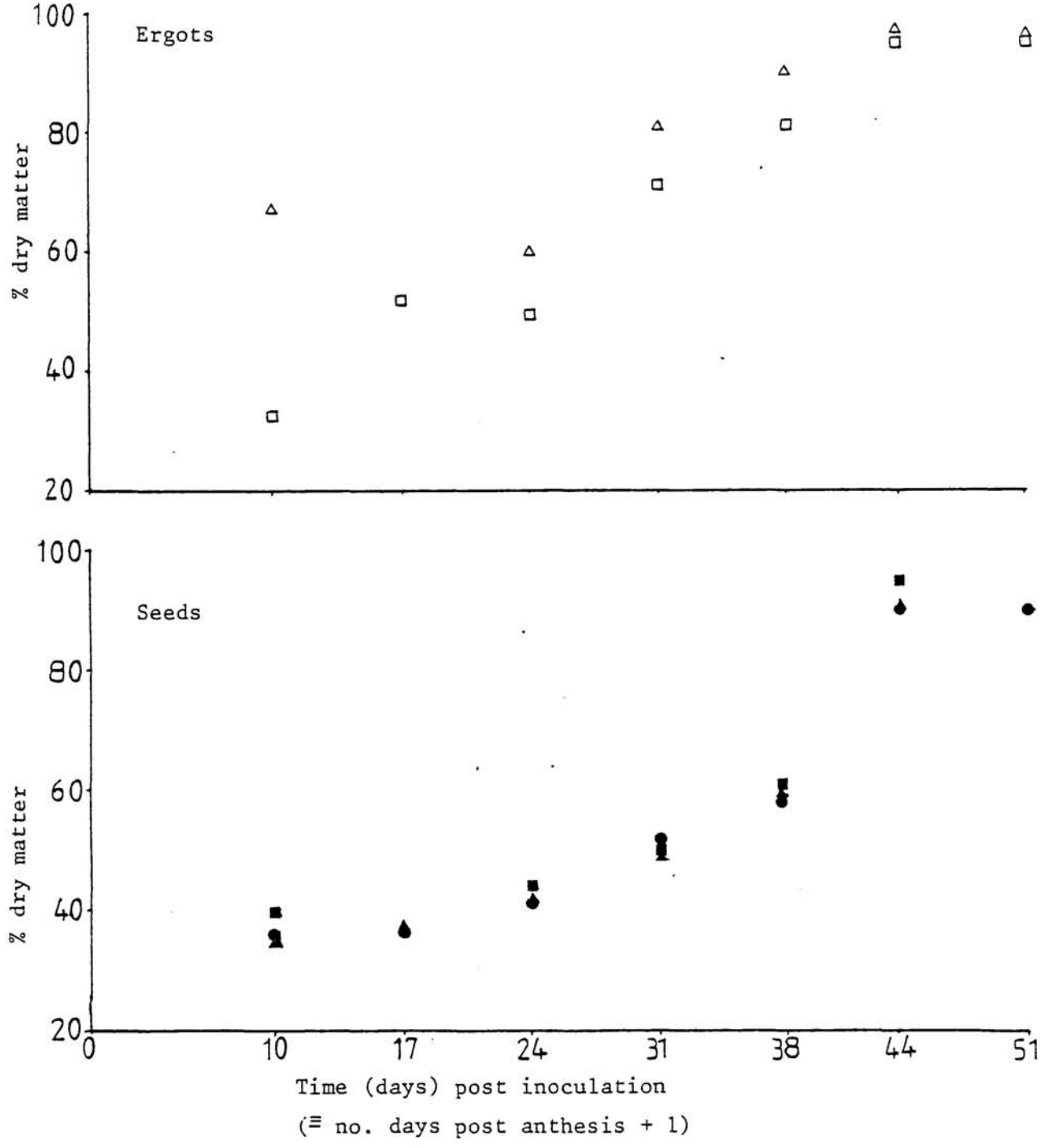
● = 0% spikelets inoculated

▲ △ = 20% spikelets inoculated

■ □ = 80% spikelets inoculated

Figure 28 - cont'd

c) +100N



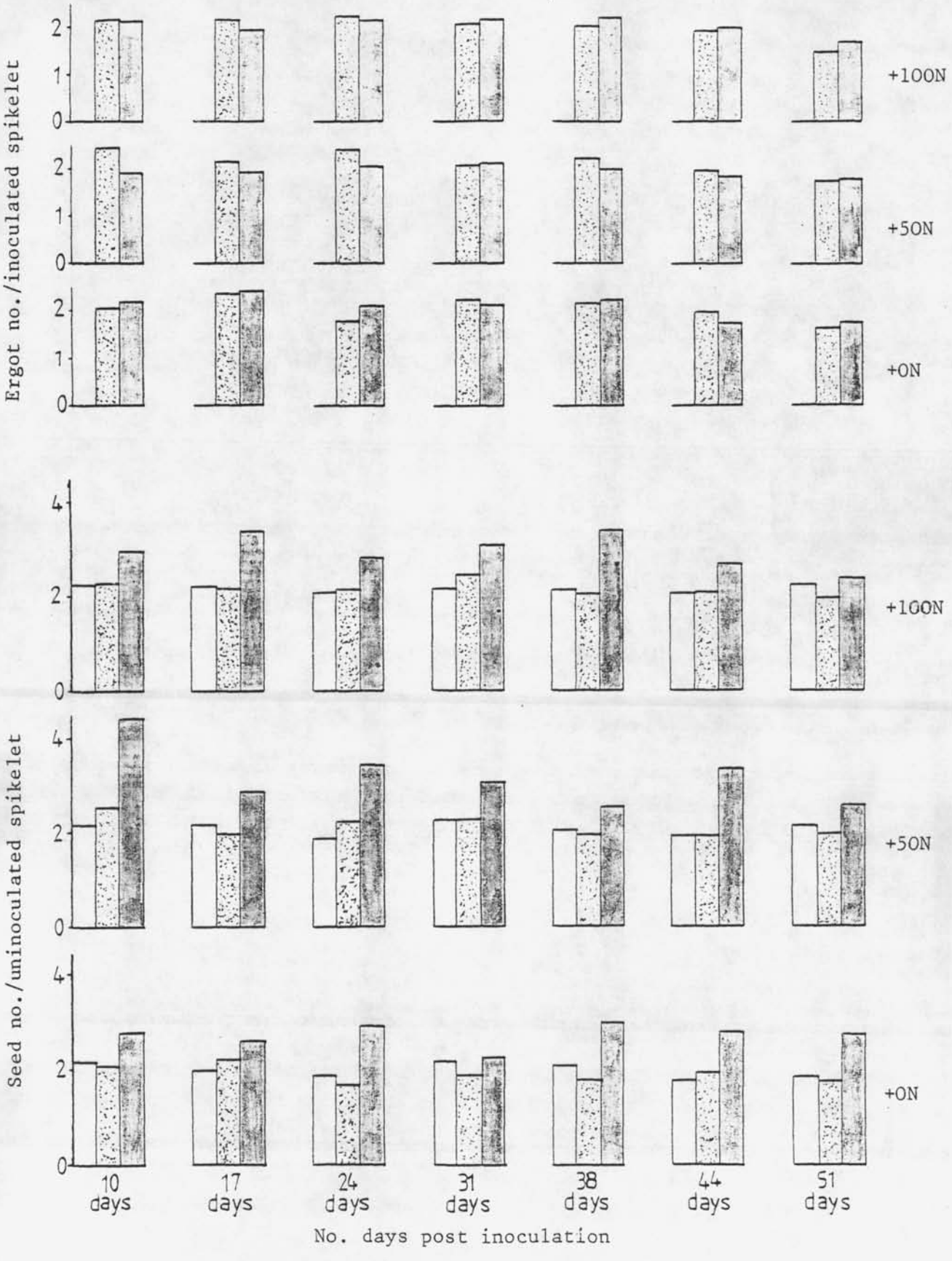
● = 0% spikelets inoculated

▲ △ = 20% spikelets inoculated

■ □ = 80% spikelets inoculated

Figure 29

Variation in seed number/control spikelet and ergot number/inoculated spikelet



per spiklet so this represents an increase in the proportion of productive florets in the heavily infected ears. The lower numbers of ergots/inoculated spikelet recorded for the ears collected 44 and 51 days after inoculation may have been caused by some of the larger ergots falling out of the ears. During dissection of the ears any empty florets were recorded, and the numbers of seeds and ergots in that ear adjusted accordingly. The internal surfaces of the lemma and palea are stained brown by close contact with an ergot, so it was possible to ascertain whether an empty floret contained an ergot or a seed. This should have minimised error from this source but probably did not eliminate it completely.

Figure 30 shows the variation in the mean dry weight of seeds with time. There are no detectable differences between any of the nitrogen treatments or inoculation levels. This contrasts the considerable variation in the mean dry weight of ergots between treatments (figure 31). At all three nitrogen levels the inoculation of 80% of the spikelets produced lighter ergots than when only 20% of the spikelets were inoculated. Infected wheat grown in the plots to which 50 or 100 units of nitrogen had been added, produced much heavier ergots in those ears which had been inoculated at the 20% level, compared to the untreated plot. No large increase in weight was recorded in the ears which had had 80% of the spikelets inoculated. These differences were not apparent during the first three weeks of ergot development but were well established 31 days after inoculation.

There was an unexpected reduction in mean ergot dry weight during the last one or two weeks of development. The lipid content of ergots from a selected sequence of groups are summarised in table 10. The maximum difference between the lipid content of these groups is 7% and the variation is apparently non-systematic. Since these ergots showed a dry weight reduction of about 30% between 38 and 51 days an even greater percentage reduction in lipid content would have been necessary to account for it. Clearly this did not occur. It therefore seems unlikely that oxidative respiration could be responsible for the weight loss. Although the loss of some ergots from ears during the later stages of development was taken into account when determining the mean ergot weights, no allowance was made for the preferential loss of the larger ergots. It seems reasonable to suppose that the larger ergots would be dislodged from the ripening ears more easily than smaller ergots, thus leading to a reduced <u>mean</u> ergot weight.

Figure 30

Variation in the mean dry weight of spring wheat seeds during development

a) +ON

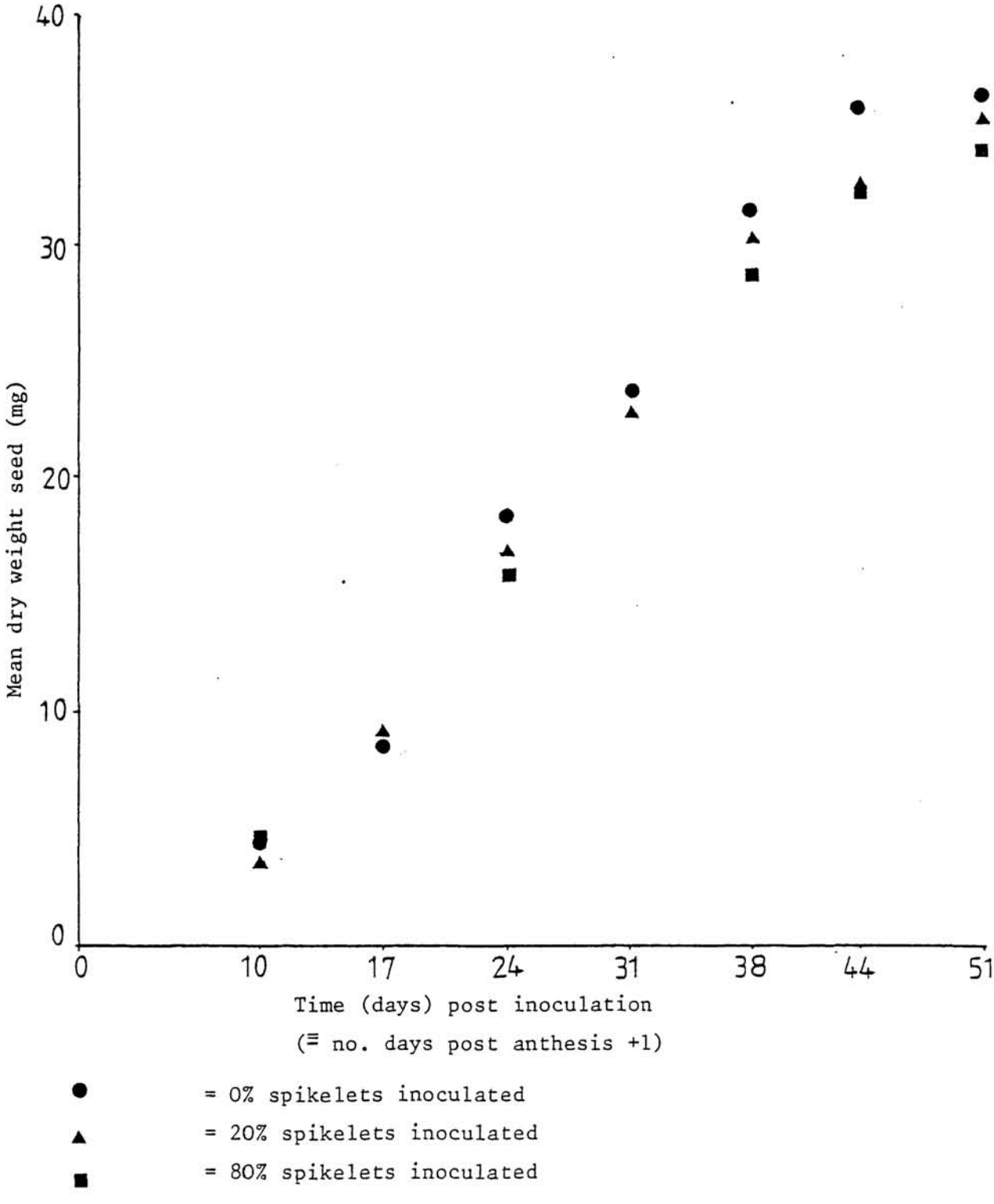
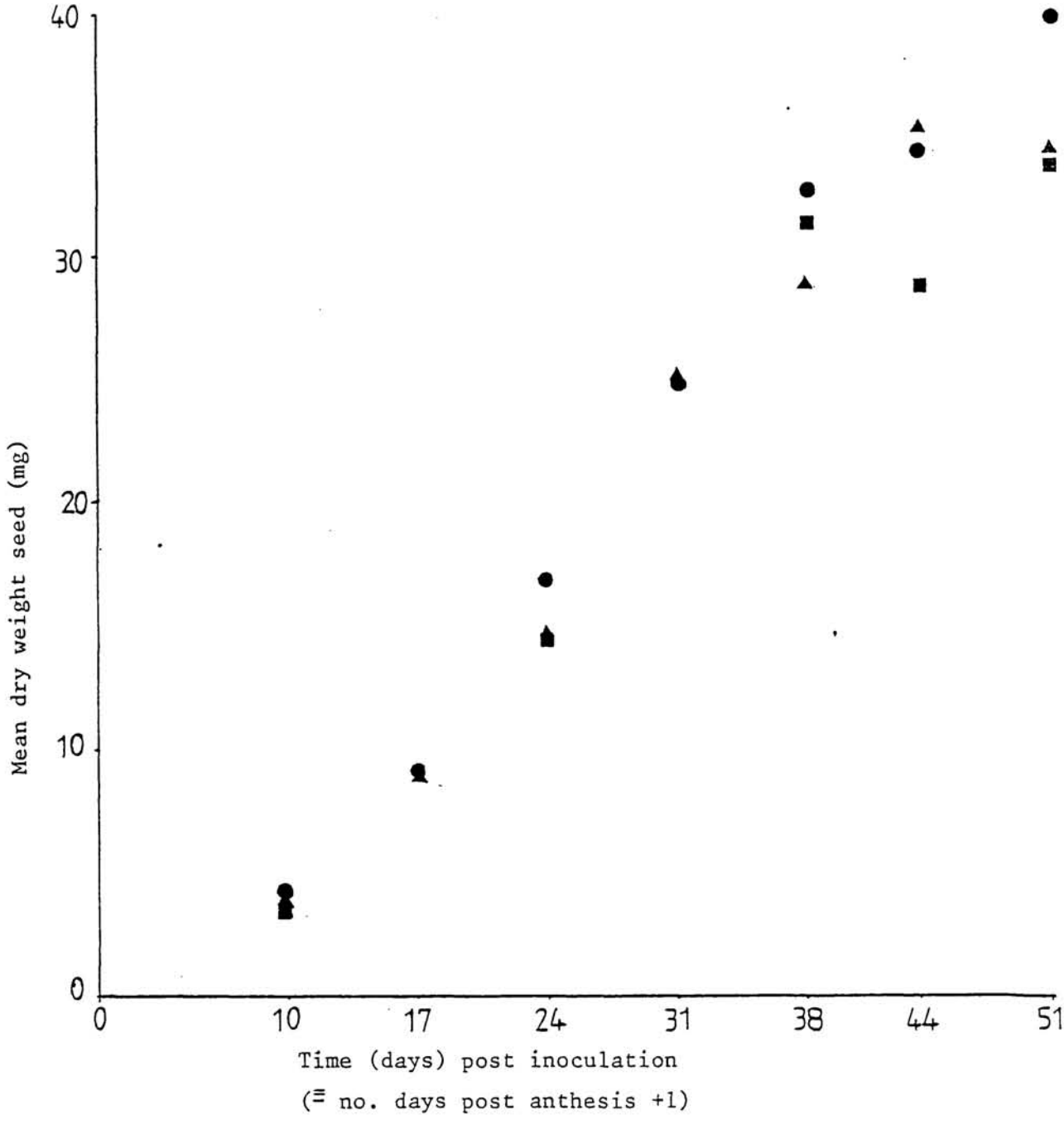


Figure 30 - cont'd

b) +50N



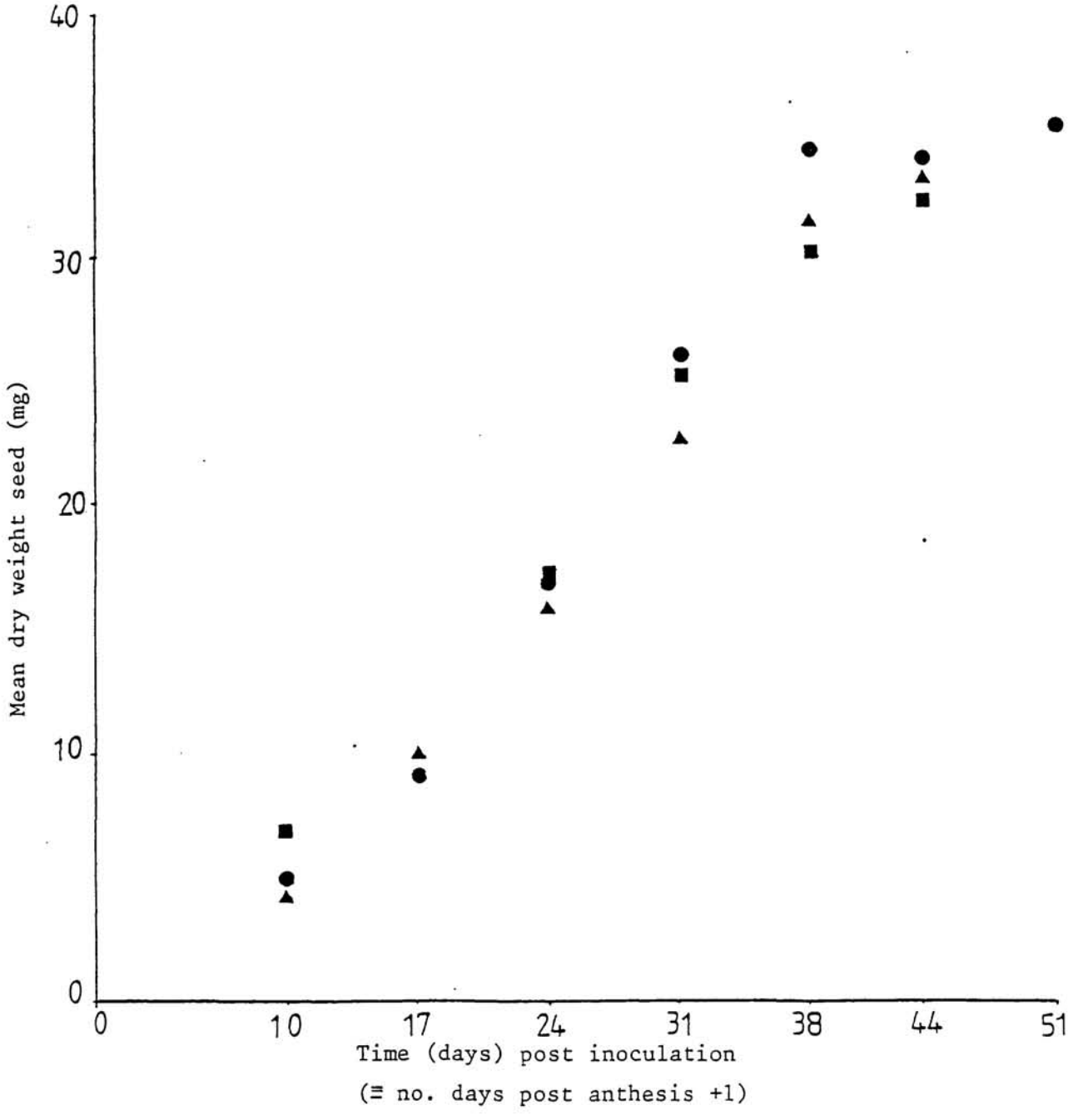
● = 0% spikelets inoculated

▲ = 20% spikelets inoculated

■ = 80% spikelets inoculated

Figure 30 - cont'd

c) +100N



● = 0% spikelets inoculated

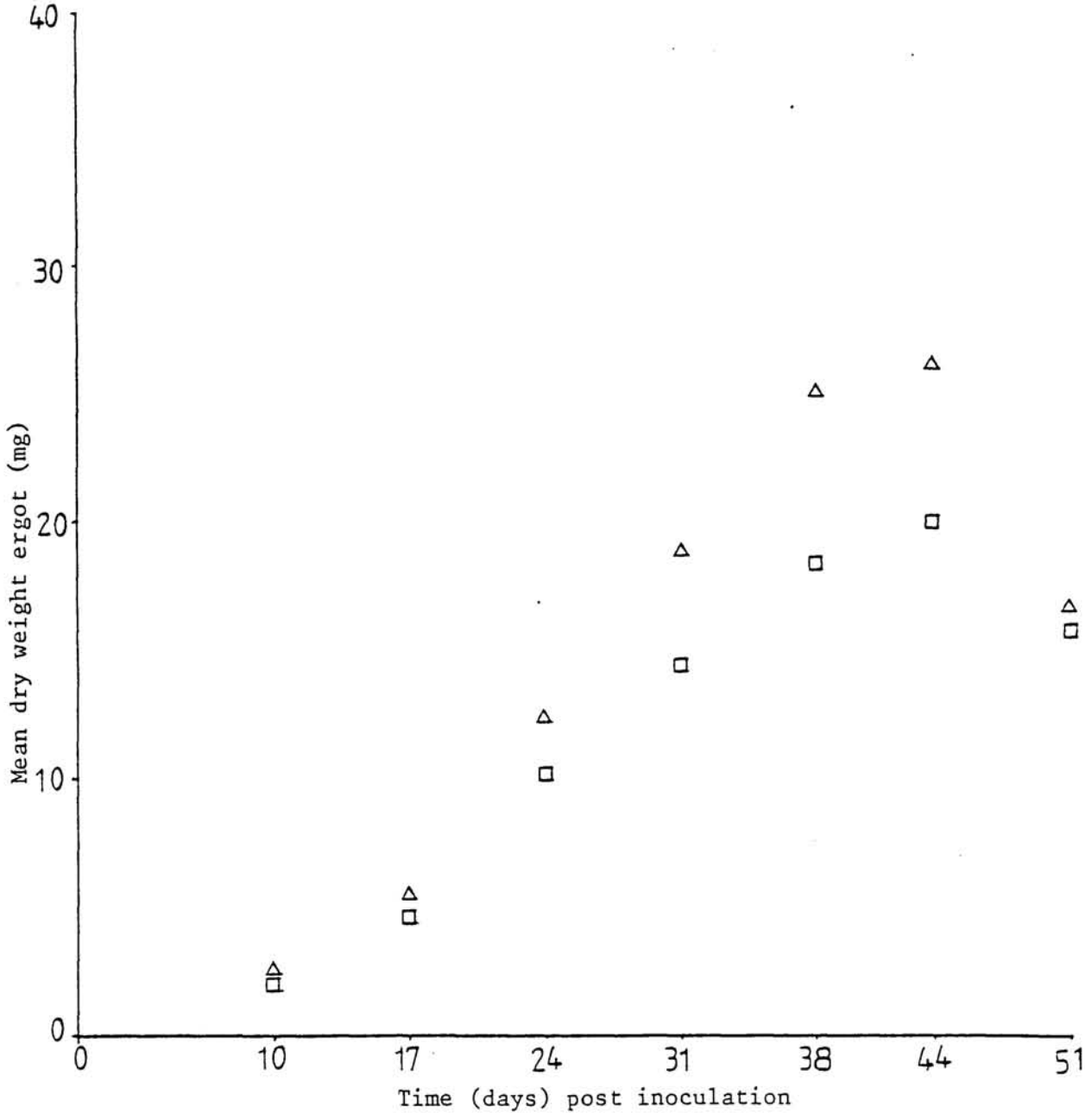
▲ = 20% spikelets inoculated

■ = 80% spikelets inoculated

Figure 31

Variation in the mean dry weight of ergots during development

a) +ON

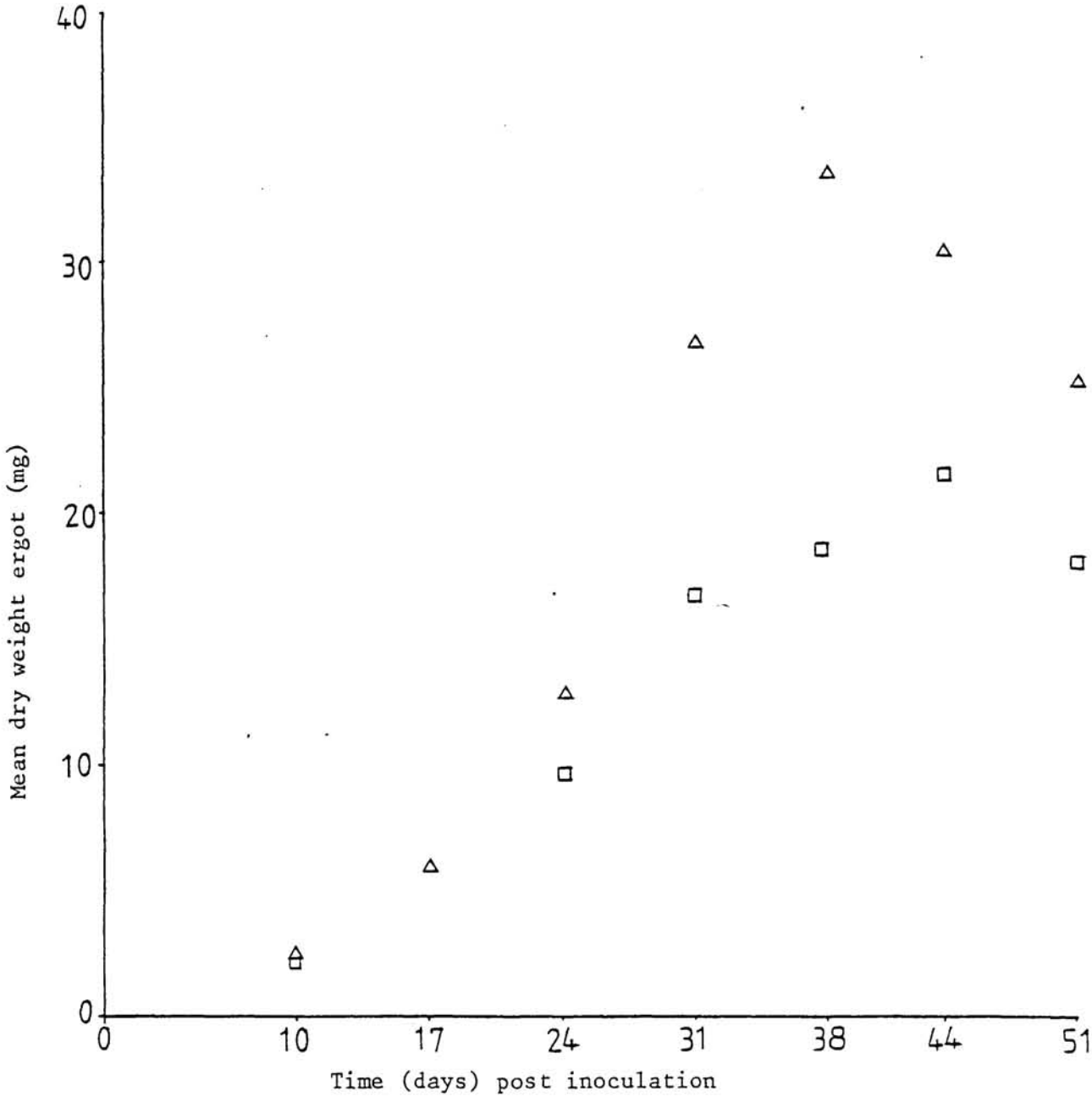


△ = 20% spikelets inoculated

□ = 80% spikelets inoculated

Figure 31 -cont'd

b) +50N

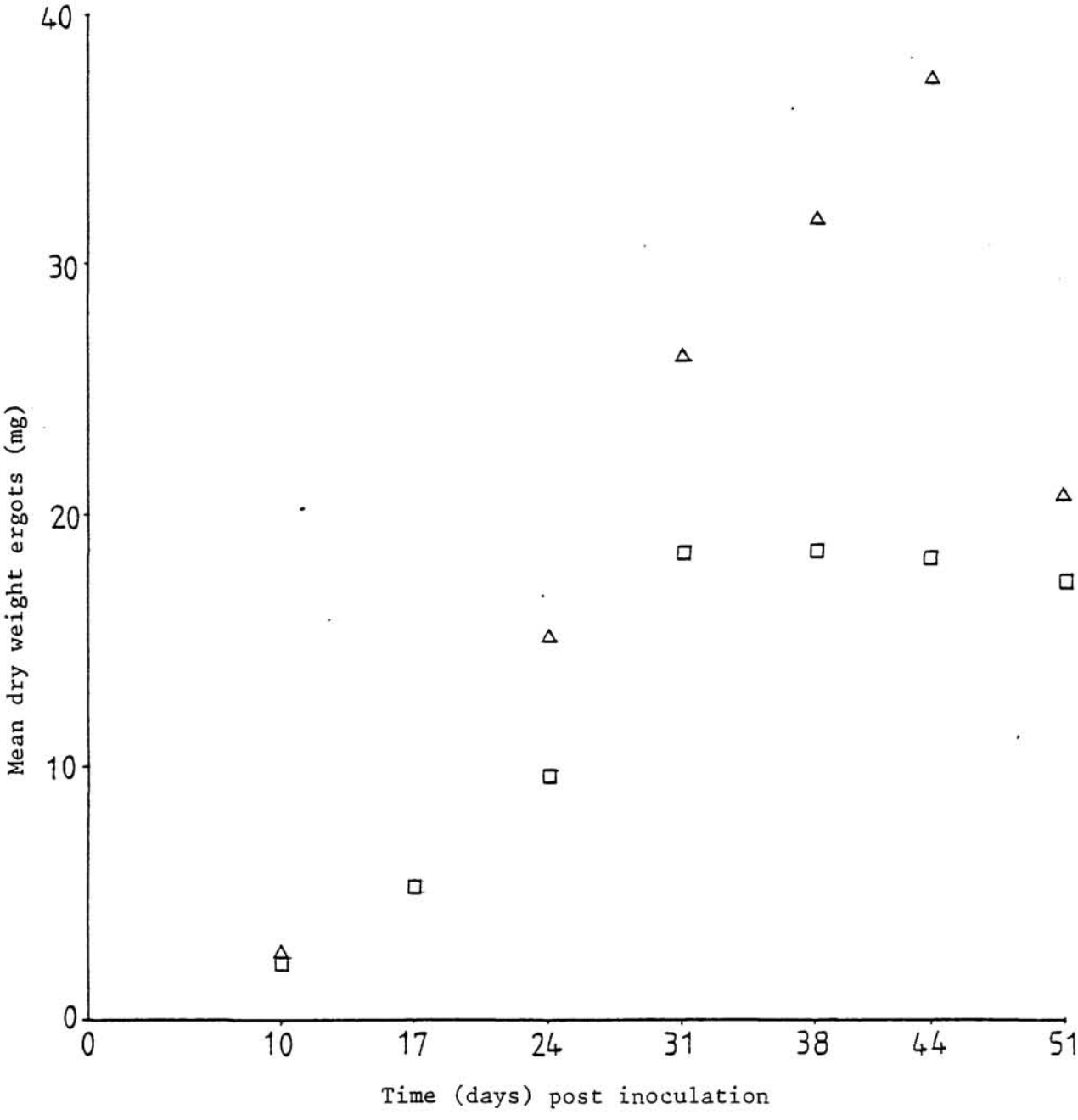


△ = 20% spikelets inoculated

□ = 80% spikelets inoculated

Figure 31 - cont'd

c) +100N



△ = 20% spikelets inoculated

□ = 80% spikelets inoculated

Table 10

The lipid content of selected groups of ergots in the spring wheat nutrient competition experiment

Treatment group	% lipid
+50N, 20% inoculation: 31 days	39
" " 38 "	34
" " 44 "	37
" " 51 "	32

10.3 Experimental - Winter wheat

During the 1976/77 growing season a second field experiment, similar in design to that described for spring wheat in section 10.1, was conducted at Model Farm.

An experimental plot was sown with winter wheat, cv. Bouquet. Half of this plot was treated 'normally' to provide a control: dressings and cultivations were carried out in accordance with RHM agronomic practise for winter wheat in that field. The other half of the plot was similarly treated except for the omission of a top dressing of nitrogen (applied as Nitram) in April.

Three groups of 50 ears in both halves of the plot were labelled, inoculated, harvested and analysed as described for the spring wheat experiment. The only difference was that strain 25B was used instead of strain 330. In order to inoculate all the ears at the same physiological age, ie. just before anthesis, the wheat in the low nitrogen area of the plot was inoculated 3 days later than the rest.

10.4 Results and discussion

Emergence and early development of the wheat was uniform throughout the plot but by early May the wheat in the low nitrogen area was much shorter, more yellow and more sparse than in the normally treated area. As in the spring wheat experiment the actual proportion of ergots to seeds in the ears differed very little from the inoculated levels of 20% and 80% (table 11). The decrease in the proportion of ergots to seeds during the last few weeks of development probably reflects the greater ease with which ergots are dislodged from more mature ears.

The increase in dry matter of seeds from all the ears grown at the lower level of nitrogen (figure 32) follows a pattern similar to that seen in the spring wheat experiment and corresponds to that commonly described for wheat. Seeds from ears grown at the higher nitrogen level showed an identical increase in dry matter during the first five weeks but the sharp loss of water in the sixth week did not occur.

In contrast to the spring wheat experiment there were only slight differences in the water content of ergots from heavily and lightly infected ears (figure 32). Nearly all the groups of ears which were only lightly infected with C.purpurea produced ergots with a slightly higher

Table 11

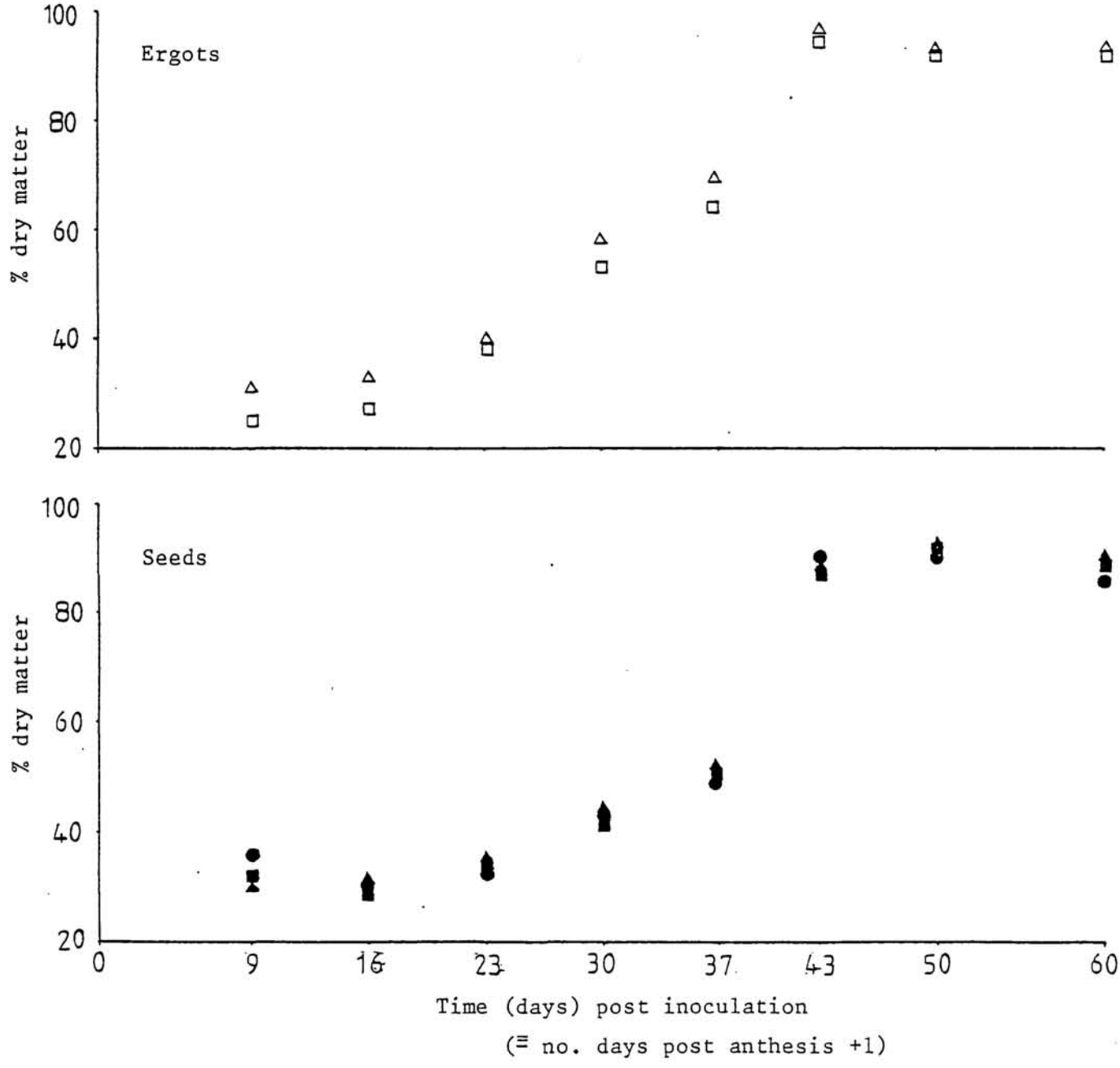
The percentage of ergots $\left[\frac{\text{Ergot no.x100}}{\text{Ergot no.+Seed No.}}\right]$ in each group of ears used in the winter wheat nutrient competition experiment

Treatment Group	% spikelets inoculated		
	0%	20%	80%
9 days, Low N	0	20	78
9 days, Normal N	0	20	79
16 days, Low N	0	22	82
16 days, Normal N	0	22	82
23 days, Low N	0	20	81
23 days, Normal N	0	22	80
30 days, Low N	0	22	82
30 days, Normal N	0	21	81
37 days, Low N	0	21	80
37 days, Normal N	0	22	78
43 days, low N	0	19	81
43 days, Normal N	0	21	80
50 days, low N	0	17	78
50 days, Normal N	0	19	78
60 days, Low N	0	17	78
58 days, Normal N	0	16	75

Figure 32

Variation in the percentage dry matter of ergots and winter wheat seeds during development

a) Low nitrogen



● = 0% spikelets inoculated

▲ △ = 20% spikelets inoculated

■ □ = 80% spikelets inoculated

Figure 32 - cont'd

b) Normal nitrogen

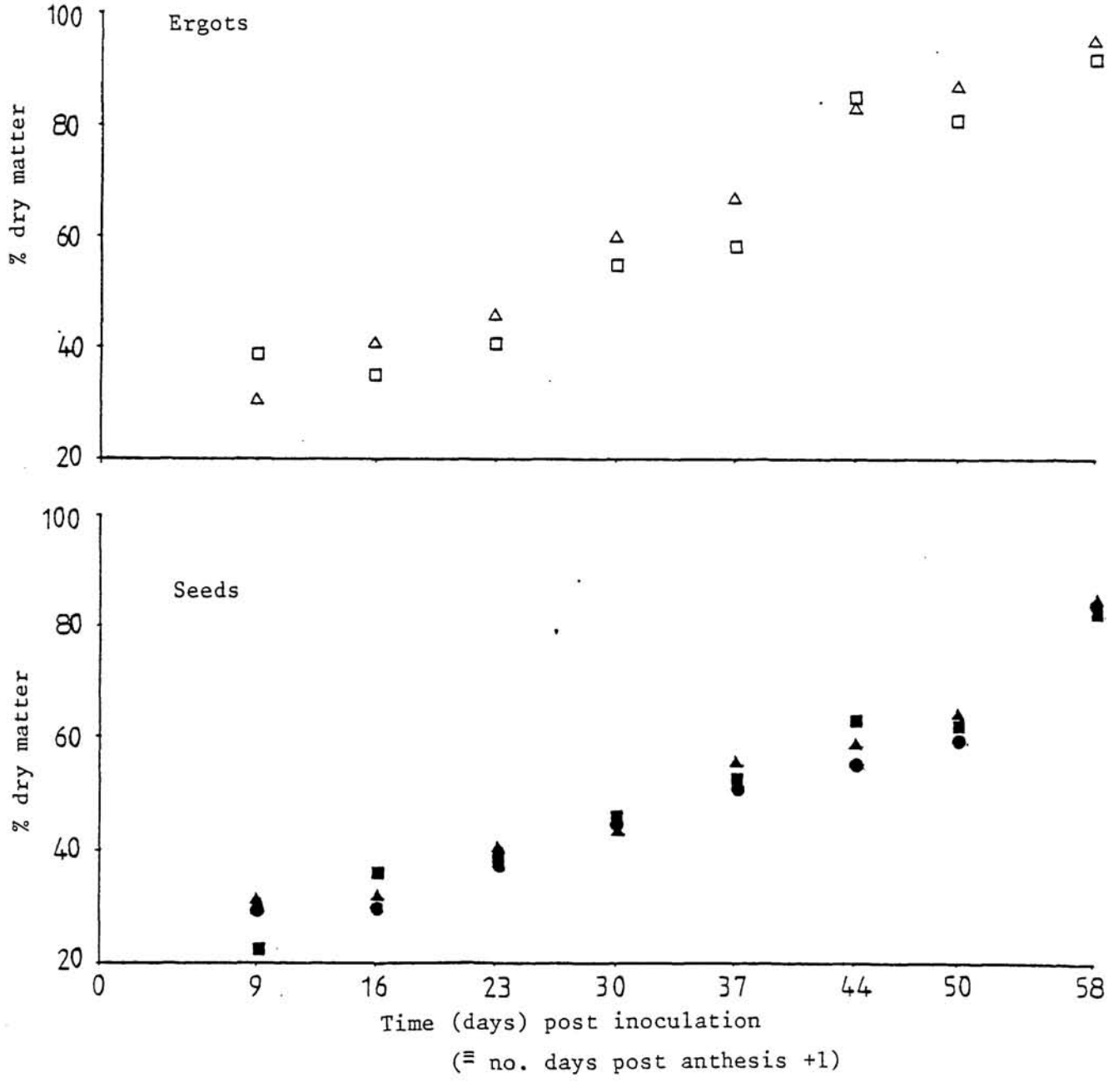


Figure 33

Variation in the mean dry weight of winter wheat seeds with time and soil nitrogen

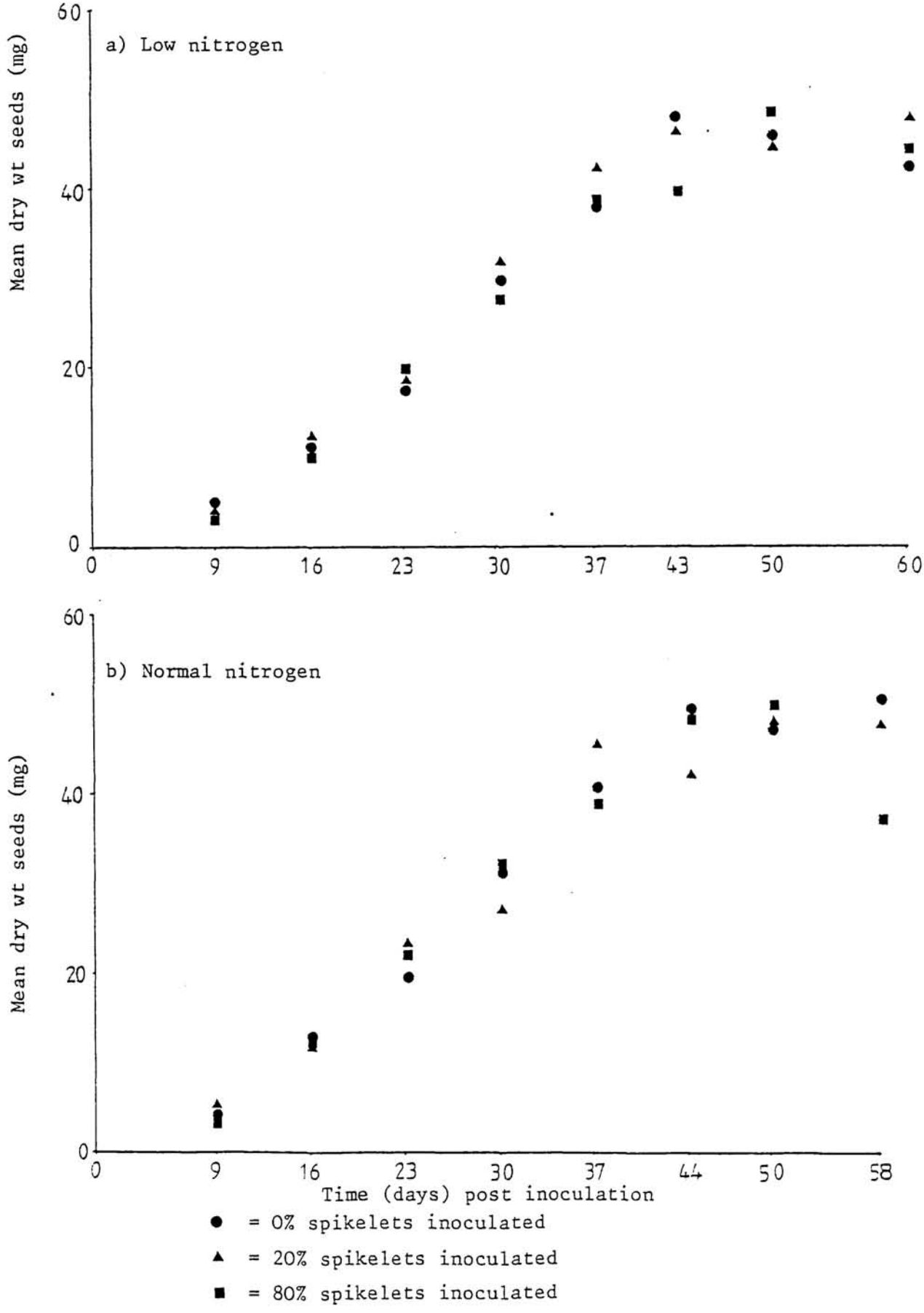
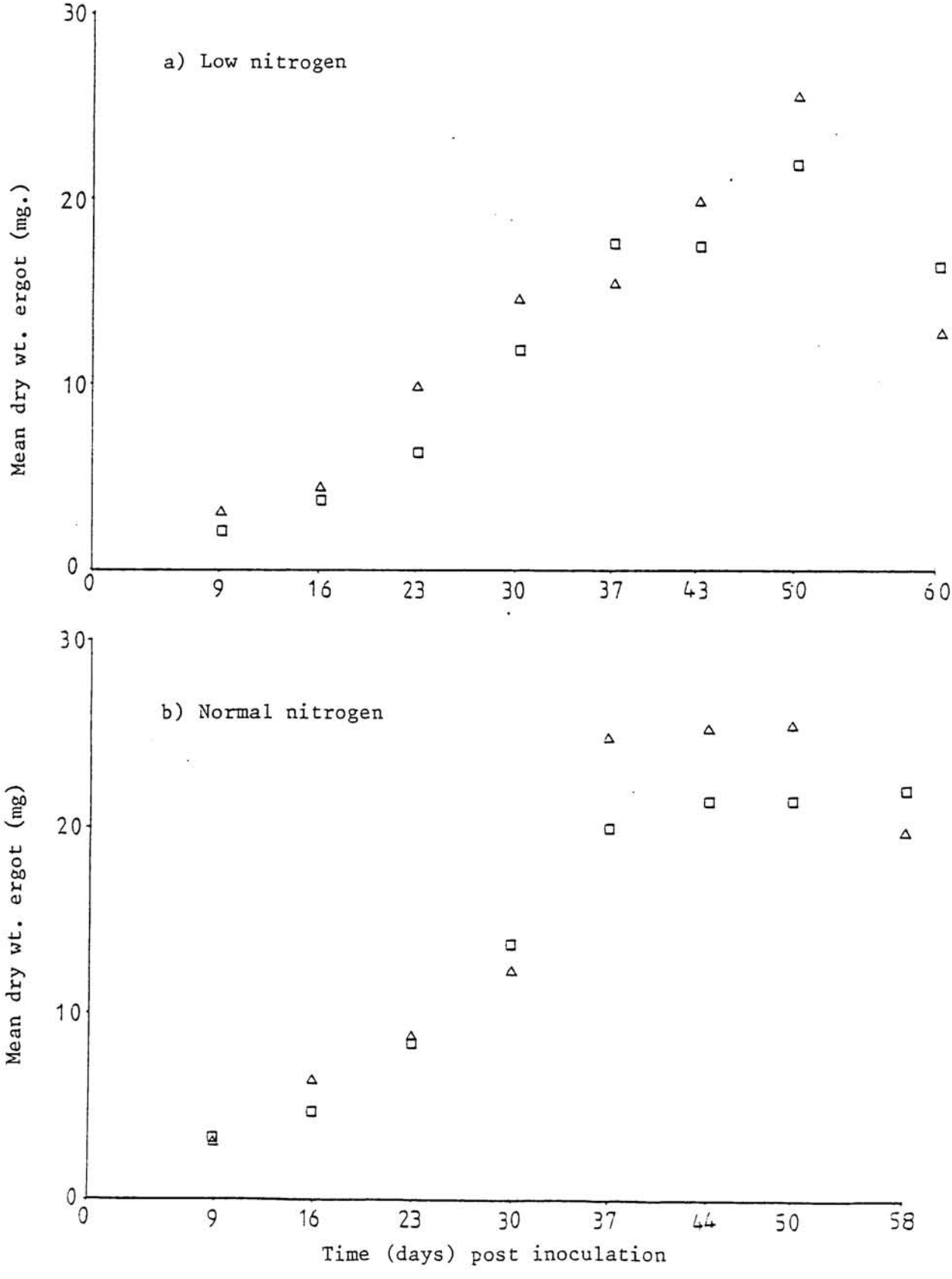


Figure 34

Variation in the mean dry weight of ergots with time and soil nitrogen



proportion of dry matter when compared with heavily infected ears. The differences were much less marked than in the spring wheat experiment: this could be due to the differences between the spring and winter varieties used, or the field conditions to which they were exposed.

As in the spring wheat experiment the mean dry weight of seeds (figure 33) was unaffected by the amount of applied nitrogen or by the presence of ergots in the ear. Considering the stunted appearance of the ears in the low nitrogen area of the plot the lack of any retardation in weight increase or in final grain weight was surprising.

In the low N area of the plot the increase in dry weight of ergots resulting from both the 20% and 80% inoculation levels was linear until a mean sclerotium weight of c.23 mg was attained approximately 50 days after inoculation (figure 34). During the final 10 days of maturation a sharp decrease in mean ergot weight was recorded. Ergots from both groups of ears grown at the normal level of nitrogen showed a similar increase in dry weight until 30 days after inoculation (which also coincided with the increase recorded for the low nitrogen groups). During the following week a sharper increase in dry weight was recorded and the ergots from the 20% inoculated ears became appreciably heavier than those from the 80% inoculated ears.

The results present some evidence for a decline in mean ergot weight shortly before harvest. This may reflect a shedding of large sclerotia as commented upon in section 10.2.

10.5 Experimental (Glasshouse)

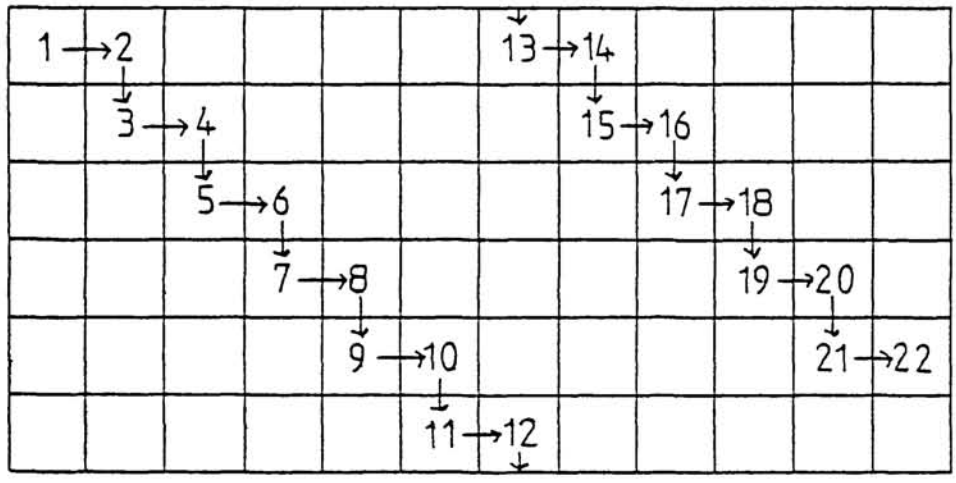
To supplement the results obtained from the spring and winter wheat field experiments on the influence of nitrogen on ergot development, a glasshouse experiment was set up at the Chelsea Physic Garden.

Potting composts containing three different levels of nutrient base were prepared as summarised below:

Treatment	Composition
Low Base	5g base per 9l compost
Half Base	30g " " " "
Full Base	55g " " " "

Figure 35

The position of a pot on the glasshouse bench, at weekly intervals, during the nutrition experiment



The 'full base' compost was similar to that normally used for growing cereals in the glasshouse at the Chelsea Physic Garden. 5" plastic plant pots were each filled with compost to within ¾" of the rim to give 24 pots containing each compost/base mixture.

Spring wheat, cv. Sappo, was germinated by incubating the seeds at room temperature on moist filter paper in petri dishes. Five freshly germinated seeds were placed in each plant pot and lightly covered with the appropriate compost/base mixture. Pre-germinated seeds were used for this experiment to ensure that five seedlings emerged in each pot. The pots were numbered 1-72 and positioned randomly on the greenhouse bench in a 12 x 6 block (random number tables - Fisher & Yates, 1963). To minimise any effects of uneven lighting or heating, the pots were moved one place forwards or sideways each week as shown in figure 35.

Just before anthesis, 75% of the spikelets in each ear, in 18 of the 24 pots of each compost/base group, were inoculated with strain 330 of C.purpurea. Ears were inoculated over a period of five days to ensure that they were all inoculated at a similar physiological age.

Two weeks after anthesis a supplementary fertiliser treatment was applied to half the pots in each compost/base group. Liquinure fertiliser (Fisons) was diluted as recommended by the manufacturer. The plant pots were transferred from the capillary bench to saucers which contained 50 ml of the Liquinure solution or 50 ml of water. Four weeks later a further 50 ml of Liquinure or water were added to each saucer. The treatments are summarised in table 12. The pots were kept moist throughout the experiment by a combination of overhead watering, to help rinse honeydew from the ears, and basal watering to ensure the movement of Liquinure into the compost.

Throughout the experiment tillers were removed once a week from as close to the soil as was possible, so that each plant only produced one main shoot.

At maturity the ears from each pot were harvested and the spikelet number, seed number, ergot number, seed weight and ergot weight were recorded for each ear. Any pots which produced two or more late anthesing ears (see section 10.6) were excluded from the analyses.

Table 12

Summary of the treatments groups in the glasshouse nutrient competition experiment

Treatment			No. pots
Low Base	No Liquinure	Control	3
" "	" "	Infected	9
" "	With Liquinure	Control	3
" "	" "	Infected	9
Half Base	No Liquinure	Control	3
" "	" "	Infected	9
" "	With Liquinure	Control	3
" "	" "	Infected	9
Full Base	No Liquinure	Control	3
" "	" "	Infected	9
" "	With Liquinure	Control	3
" "	" "	Infected	9

10.6 Results and discussion

The use of pre-germinated seeds resulted in the uniform emergence and early development of five seedlings in each pot. Development continued to be uniform for about eight weeks but although most of the ears anthesed on the same day, some smaller ears anthesed up to 5 days later. These late-anthesing ears were allowed to grow to maturity but were excluded from the analyses. There was no apparent relationship between small ear size and the amount of base in the compost, or the position of the pot on the bench. All the ears from the few pots which produced two or more late-anthesing ears were discarded.

Honeydew exudation was very copious from all the infected ears. As much as possible was removed daily to avoid any reduction in ear photosynthesis which may have otherwise occurred.

Figure 36 and appendix IIIia show that the seed/uninoculated spikelet in each ear was not affected either by the presence of ergots in the ear or the amount of nutrients available to the developing plants.

The mean seed weight increased with increasing base content of the compost but was unaffected by the addition of Liquinure (figure 37; appendix Iia). Seed from infected ears grown in the low base compost, either with or without Liquinure, had a significantly greater mean seed weight than seeds from the corresponding healthy ears (appendix Iib). Seed from infected and control ears grown in the half base compost, with or without Liquinure, showed no such difference. In the presence of the full concentration of base, the seeds from infected ears had a slightly reduced mean seed weight compared to the controls, but this difference may only be regarded as significant for the ears grown in the absence of Liquinure. It is therefore concluded from these results that for wheat grown under the conditions of this experiment, the influence of ergots on the remaining seeds in an infected ear is slightly detrimental when nutrients are abundantly available, but may be beneficial when nutrients are limited. These conclusions refer to nutrients which are available throughout the development of the wheat; the results were not affected by any increased availability of nutrients during the later stages of ear development.

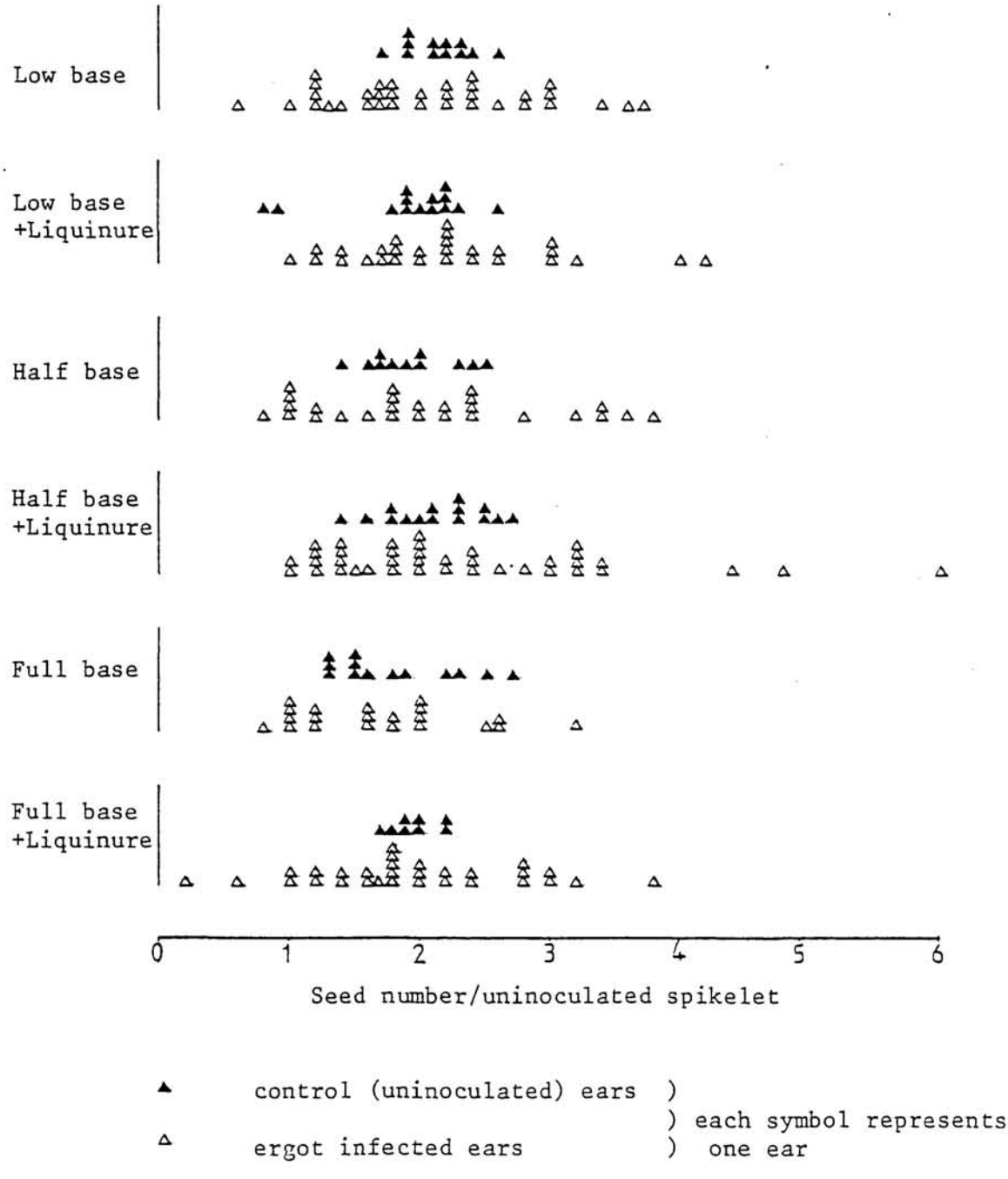
The range of mean ergot weights for each nutrient treatment is given in figure 38. The average ergot weight increased with increasing base

content of the compost, but only in one of the treatments (half base) did the addition of Liquinure have any significant effect (appendix Iic). The mean number of ergots per inoculated spikelet also increased with increasing base content (figure 39) and there were significant differences between nutrient treatments (appendix IIIid).

The inter-relationships between mean seed weight and ergot weight/ spikelet are summarised in figure 40. The influence of base concentration on both parameters can be seen but only one of the treatments (full base, no Liquinure) showed any significant correlation (appendix IIia).

Figure 36

Variation in seed number/uninoculated spikelet with nutrient treatment (glasshouse experiment)



Variation in mean seed weight with nutrient treatment (glasshouse experiment)

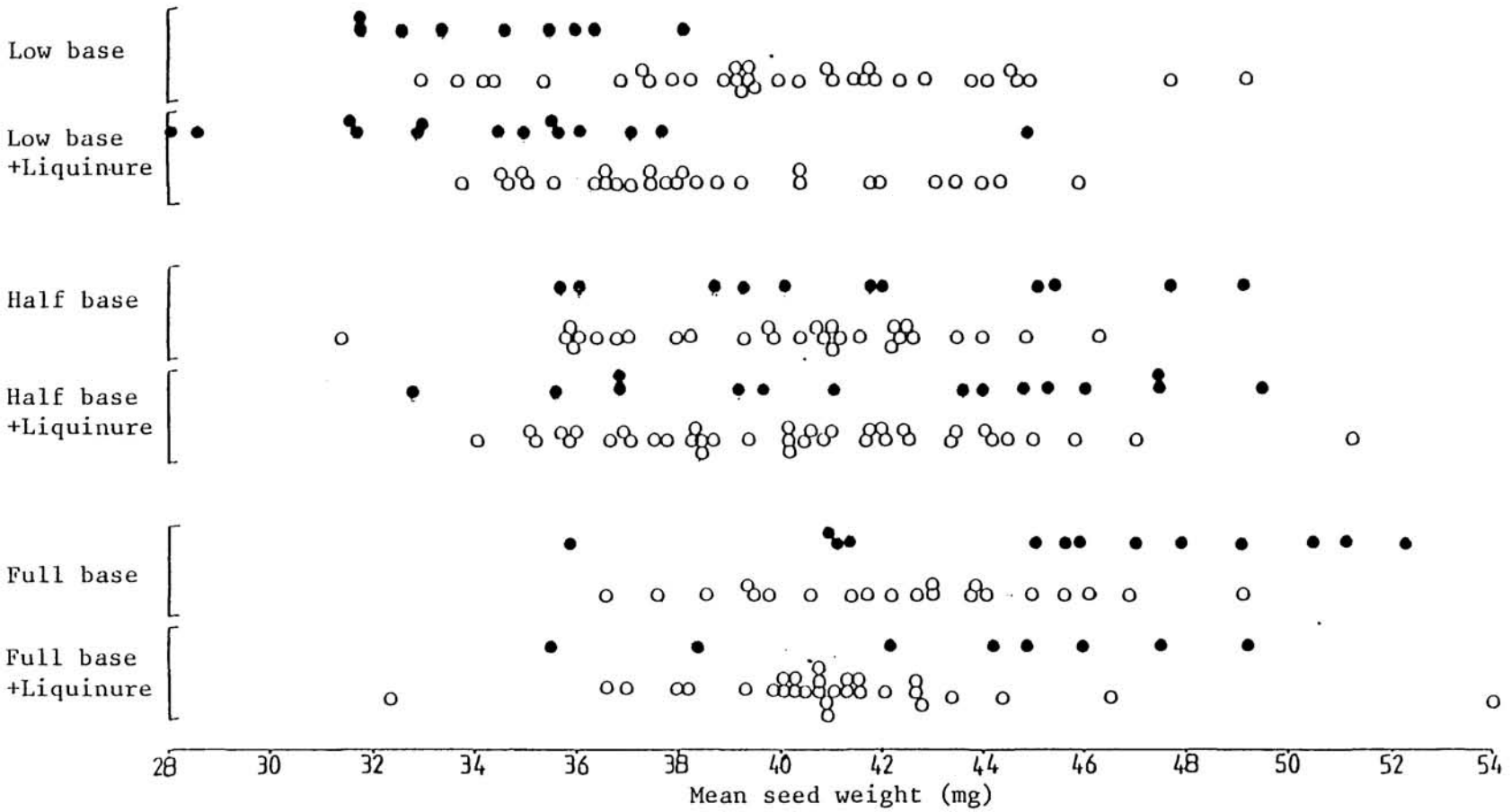
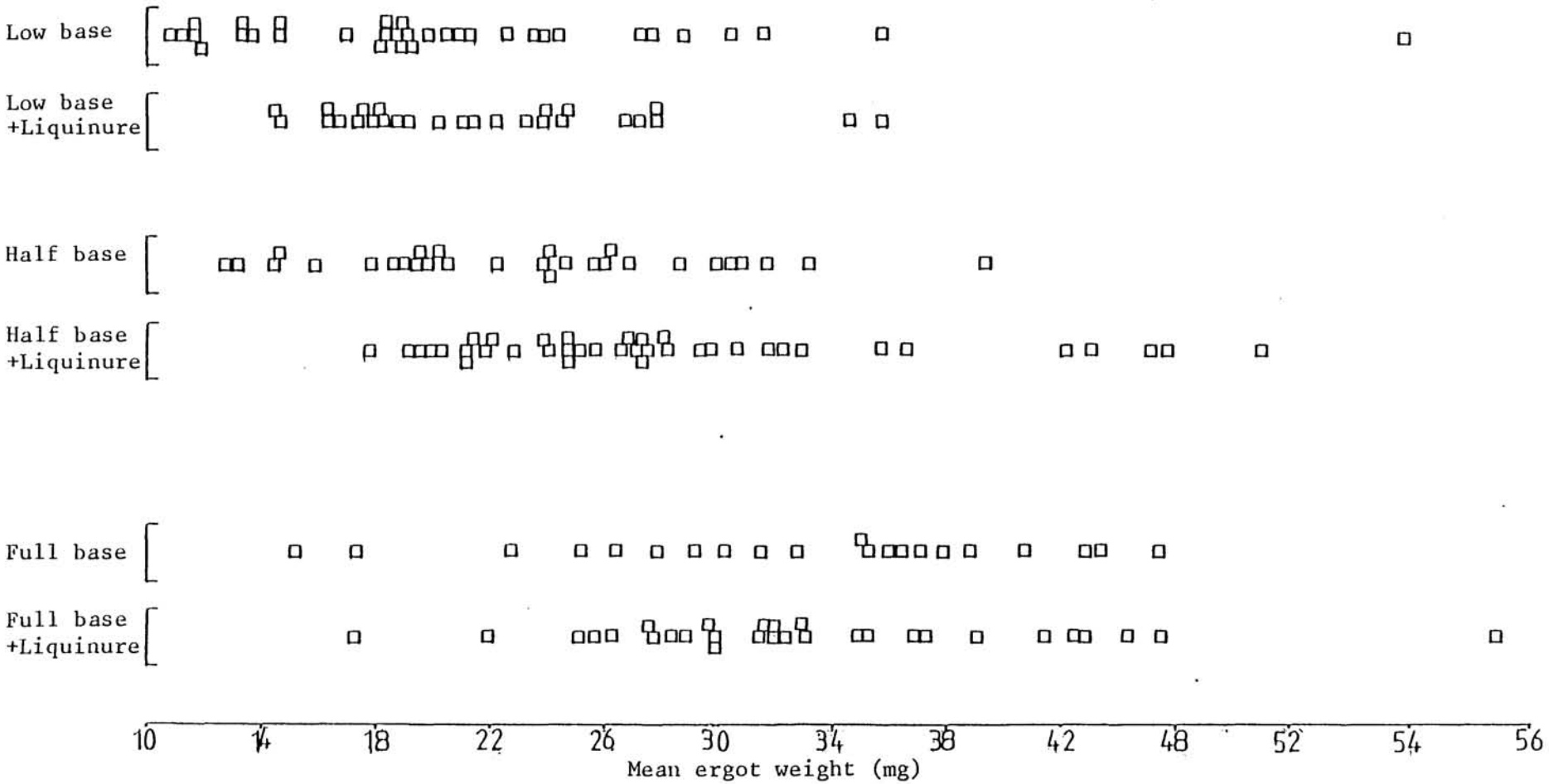


Figure 37

Variation of mean ergot weight with nutrient treatment (glasshouse experiment)

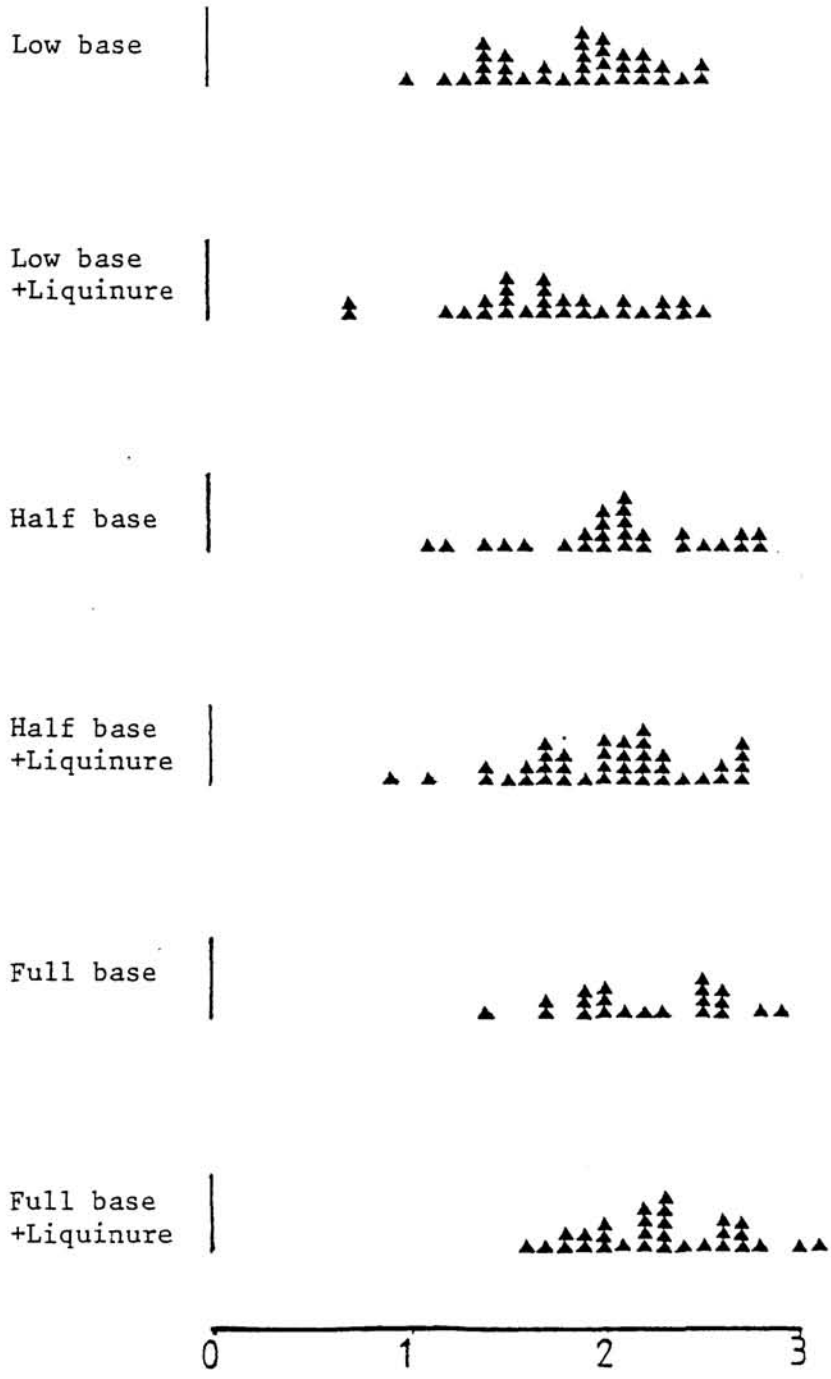


- each symbol represent one ear

Figure 38

Figure 39

Variation in ergot number/inoculated spikelet with nutrient treatment (glasshouse experiment)



- each symbol represents one ear

Inter-relationship between mean seed weight and ergot weight/spikelet (glasshouse experiment)

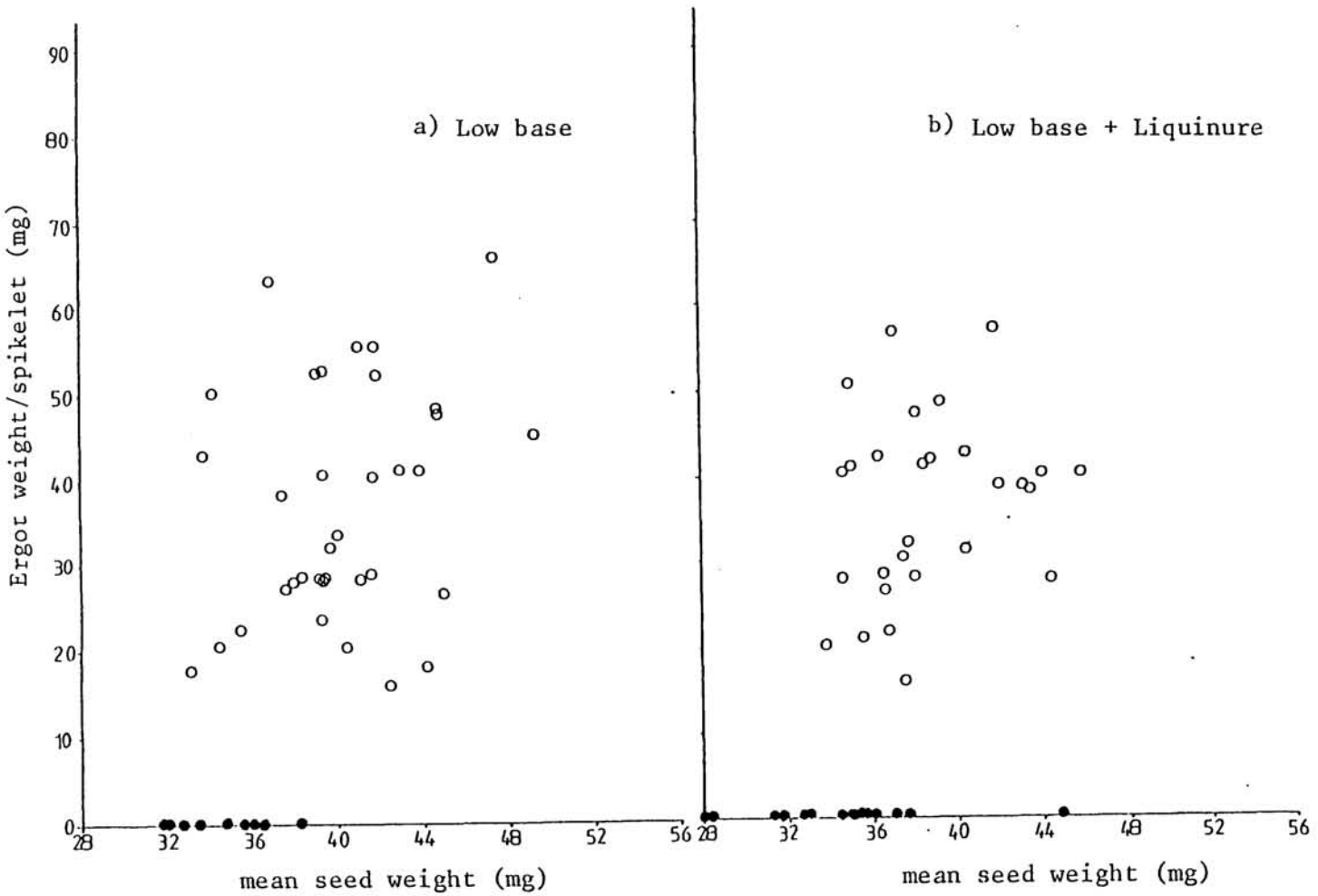


Figure 40

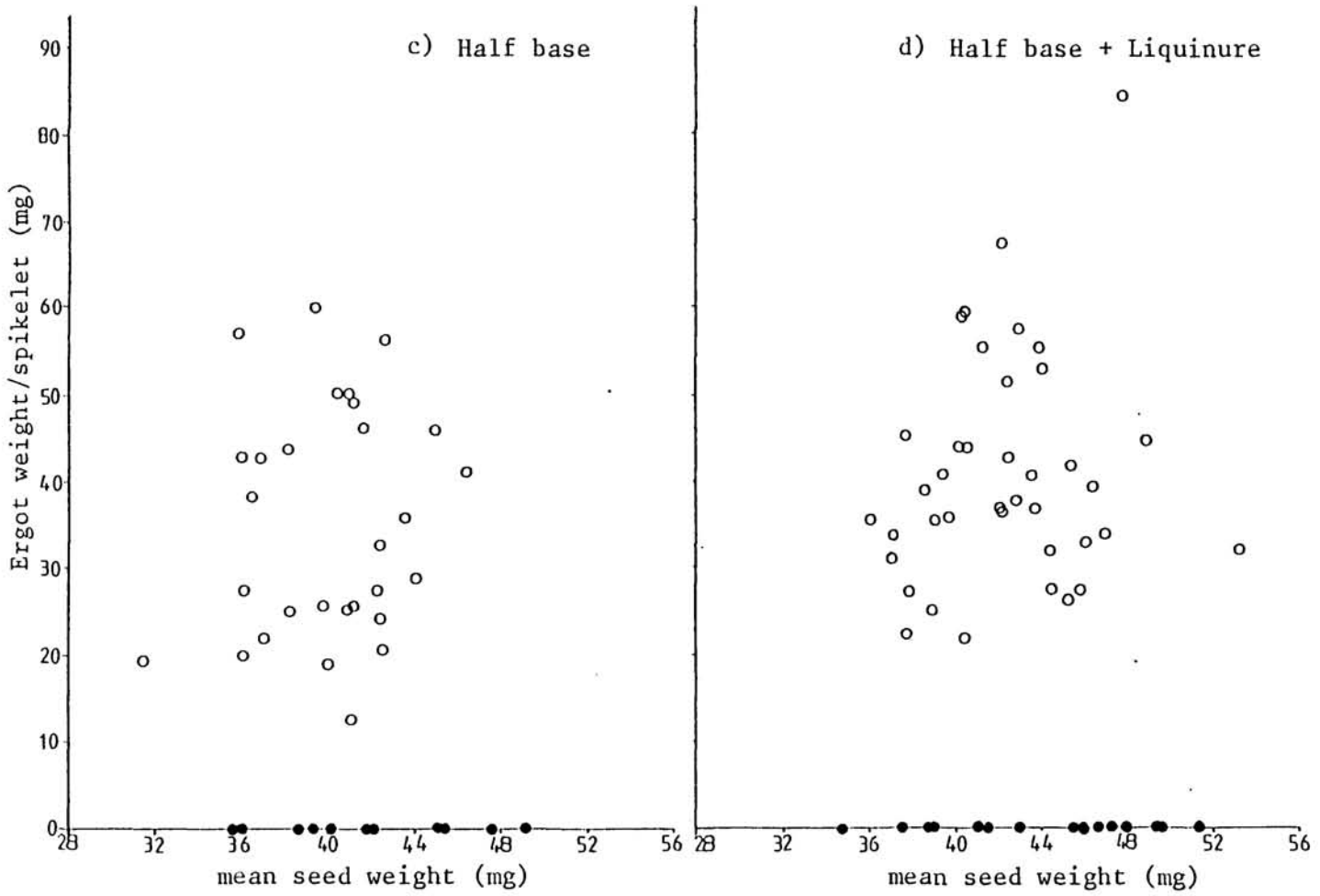
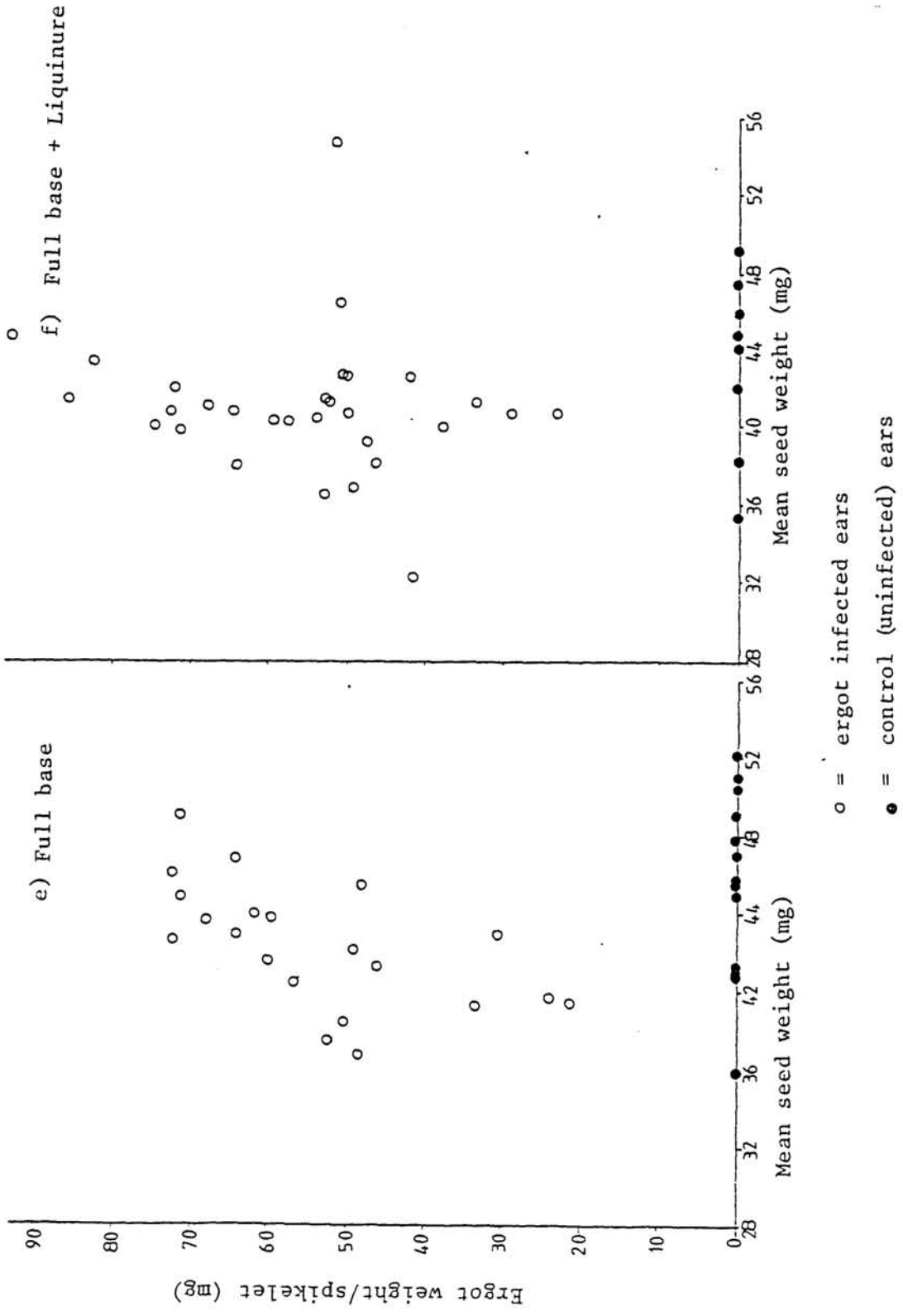


Figure 40 cont'd

Figure 40 cont'd



11. THE EFFECT OF ERGOTS ON THE QUALITY OF ADJACENT SEED

11.1 Experimental

Alternate plots of male sterile and fertile wheat varieties were sown at Hall Barn Farm on 30th October 1974, as shown in figure 41. The experiment was confined to a 10 foot wide strip running across the plots.

Fifty ears of similar size were selected in each male sterile variety. These were inoculated (section 2.6) just before pseudoanthesis, with strain 25B of C.purpurea, to give ten replicates/variety of each of the inoculation patterns (A-E) shown in figure 42. When mature, the ears were harvested by hand and stored dry, at room temperature, pending analysis.

For analysis each ear in groups A,B,C and E was divided into two or three sections as shown in figure 42. Each ear in group D was analysed separately but all the ergots were counted as section 'e' and all the seeds as section 'x'. The spikelet number was recorded for each section of every ear, as was the seed and ergot number and weight.

11.2 Results and discussion

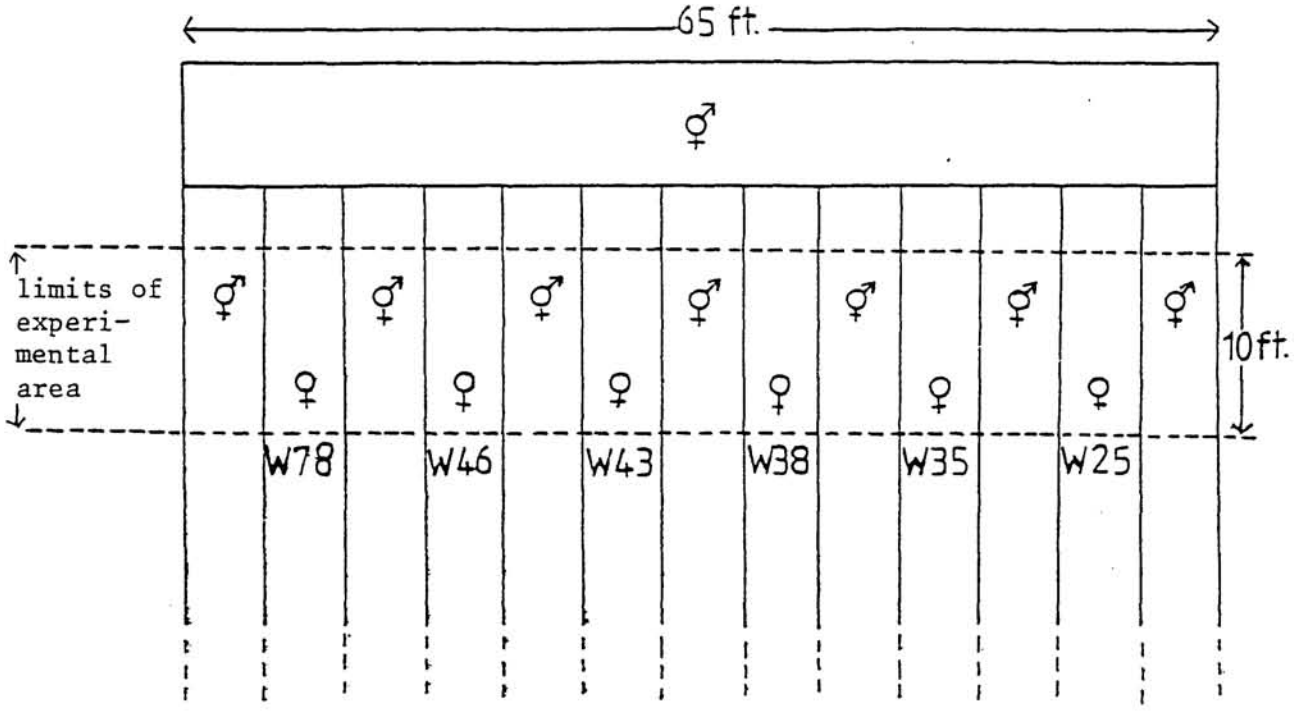
Development of infected and uninfected ears was normal and the 'nick' between the male sterile and pollinator varities was reasonable.

Using Analysis of Variance, differences in mean seed weight, mean seed number and mean ergot weight were sought between the treatments (Aa, Ab, Ac, Be, Bbc, Ca, Ce, Cc, Dx, De, Eab, Ee) within each variety, and between the varieties for each treatment. The results are expressed in table 13. Means which are connected by the same straight line are not significantly different from each other at the 95% significance level or greater. The corresponding Analysis of Variance tables are given in Appendix III.

Apart from variety 25 there were significant differences in mean seed weight between the treatments of each variety. Treatments Aa and Aab consistently appear near the top of the list of means when they are arranged in descending order (table 13), and treatments Ac and Cc are consistently near the bottom of the list. This reflects the reduced productivity of the upper spikelets as compared to the rest of the ear, and is normal for wheat.

Figure 41

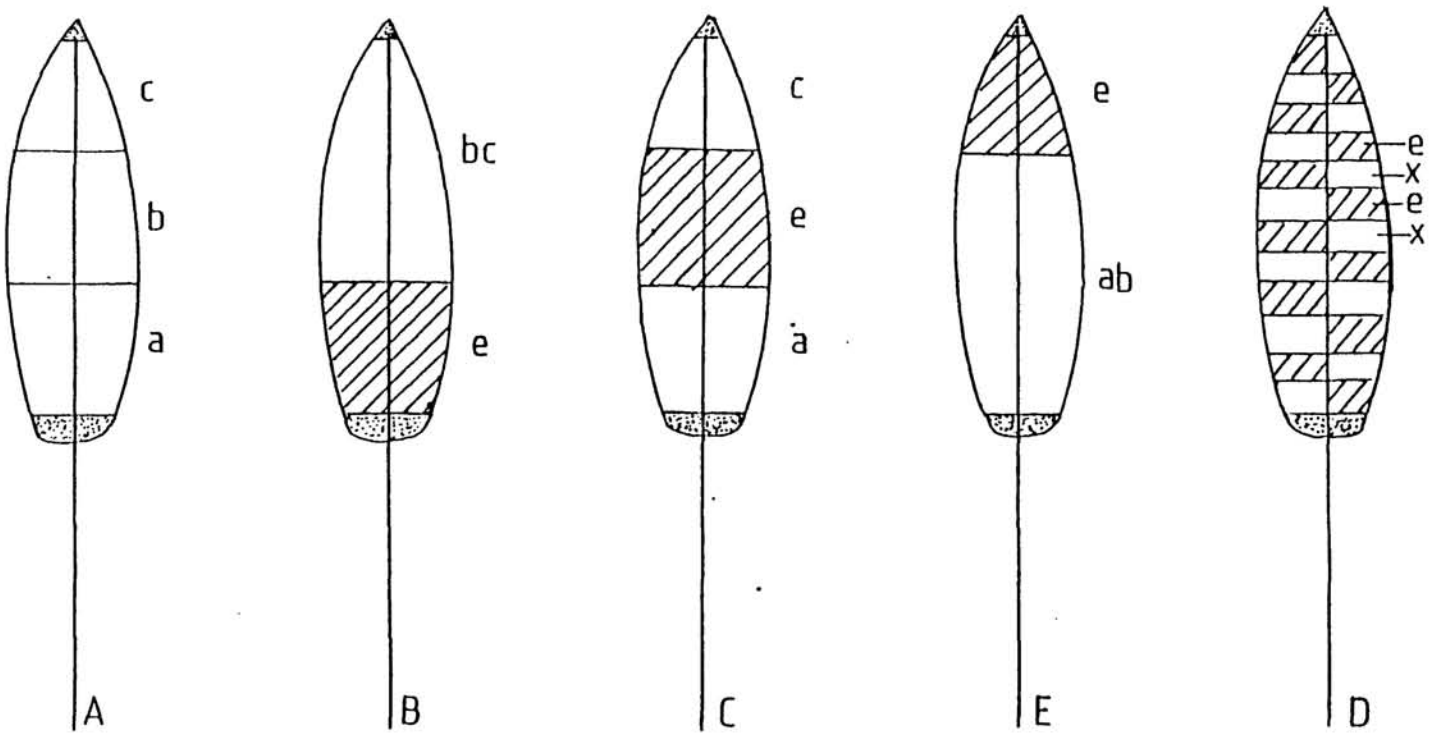
Field design of an experiment to assess the influence of ergot on adjacent seeds



♂ = var. 181-70 (pollinator)

♀ = male sterile - varieties shown under each symbol

Inoculation patterns used to assess variation in the influence of ergot on adjacent seeds according to the position of ergots in the ear



control - uninoculated

lower third of ear inoculated

middle third of ear inoculated

upper third of ear inoculated

alternate pairs of spikelets inoculated

= uninoculated

= discarded (basal and terminal spikelet of each ear)

= inoculated

Table 13

Variation in seed weight, seed number and ergot weight with position of ergots in an ear

a) Mean seed weight

Variety 25

Treatment	mean
Aab	46.303
Aa	43.739
Ca	42.508
Eab	41.293
Ac	37.689
Cc	36.689

Variety 35

Treatment	mean
Aab	49.379
Aa	49.046
Bbc	48.423
Eab	47.933
Abc	47.477
Aabc	47.400
Dx	44.955
Ca	44.640
Ac	43.376
Cc	37.821

Variety 38

Treatment	mean
Aa	48.904
Aab	48.209
Eab	47.477
Aabc	46.354
Abc	42.787
Ca	42.634
Dx	39.170
Ac	37.327
Bbc	33.004
Cc	30.894

Table 13 cont'd

Variety 43

Treatment	mean
Eab	49.801
Aa	47.845
Aab	46.799
Bbc	45.194
Aabc	44.791
Ca	43.259
Abc	43.216
Dx	40.902
Ac	40.653
Cc	36.892

Variety 46

Treatment	mean
Eab	48.961
Ca	48.744
Aab	48.044
Aa	46.735
Aabc	45.267
Bbc	45.049
Abc	44.218
Dx	44.019
Cc	43.770
Ac	39.032

Variety 78

Treatment	mean
Aa	48.001
Aab	47.843
Aabc	44.869
Eab	44.402
Abc	42.961
Ca	40.811
Dx	39.637
Cc	37.258
Ac	36.429
Bbc	36.417

Table 13 cont'd

b) Mean seed number per spikelet

Variety 25

Treatment	mean
Eab	0.82
Aab	0.73
Ac	0.63
Aa	0.63
Ca	0.60
Cc	0.55

Variety 35

Treatment	mean
Ca	1.12
Bbc	1.01
Abc	1.00
Aab	0.99
Eab	0.86
Ac	0.84
Cc	0.81
Aa	0.81

Variety 38

Treatment	mean
Aa	1.62
Aab	1.51
Ca	1.46
Bbc	1.21
Eab	1.08
Abc	1.01
Cc	0.89
Ac	0.73

Variety 43

Treatment	mean
Aab	1.04
Aa	1.03
Abc	1.01
Ac	0.95
Cc	0.91
Eab	0.87
Bbc	0.86
Ca	0.78

Table 13 cont'd

Variety 46

Treatment	mean
Aab	1.26
Abc	1.20
Aa	1.15
Ac	1.05
Eab	1.02
Bbc	0.95
Cc	0.94
Ca	0.92

Variety 78

Treatment	mean
Abc	1.21
Aab	1.16
Aa	0.99
Ac	0.98
Bbc	0.94
Eab	0.92
Ca	0.83
Cc	0.78

c) Mean ergot weight

Variety 35

Treatment	mean
De	40.896
Ce	34.650
Ee	33.858
Be	33.798

Variety 38

Treatment	mean
Ee	47.512
Ce	44.828
Be	37.851
De	33.767

Variety 43

Treatment	mean
De	49.591
Ce	49.508
Be	40.302
Ee	37.325

Table 13 cont'd

Variety 46

Treatment	mean
Be	37.387
Ee	36.418
De	34.107
Ce	31.103

Variety 78

Treatment	mean
Ce	39.050
Ee	30.668
De	27.329
Be	26.030

Individual seeds from ears in which the lower third had been inoculated (Bbc) weighed significantly less than seeds from the relevant control group (Abc) in varieties 38 and 78. However, in varieties 35, 43 and 46, seeds from these infected ears were heavier than those from control ears, although none of these differences were significant.

Seeds from the lower third of ears which had been inoculated in the middle (ie. seeds in group Ca) had a significantly lower average weight than seeds in the comparable control group (Aa) of varieties 38, 43 and 78. However in variety 46, seeds from group Ca were heavier than those from group Aa ears, but this difference was not significant. Seeds from the upper third of these centrally inoculated ears (Cc) weighed significantly less than the control seeds (Ac) in varieties 35 and 38. This contrasts with the equivalent groups of seeds from varieties 46 and 78 which were slightly heavier in infected ears, although again this difference was not significant.

There were no significant differences between seeds from ears which had the upper third of spikelets inoculated (ie seeds in group Eab) and their controls (Aab). In two varieties (43 and 46) the control seeds weighed less than those from infected ears but this was not significant at the 95% level.

Seeds from ears which had alternate spikelets inoculated (Dx) had lower average weights than the control seeds (Aabc) in all varieties but this difference was only significant for variety 38.

Mean ergot weight was not significantly affected in any of the varieties by the position of the ergots within the ear. Only variety 38 showed significant differences in mean seed number per spikelet between treatments. However none of these significant differences were between any of the treatment groups (Bbc, Ca, Cc, Dx, Eab) and their controls (Abc, Aa, Ac, Aabc, Aab).

In conclusion the results of this experiment suggest that the influence of ergot infection on the average weight of adjacent wheat seeds may depend, at least in part, on the variety. In variety 38, seeds from four of the five treatment groups compared above, weighed significantly less than the seeds from control ears. This contrasts with variety 46 in which seeds from infected ears tended to be heavier than those from healthy ears, but this difference never reached significance at the 95%

level. It is not possible to ascertain from these results whether or not the position of ergots within an ear affects the influence of the disease on developing seeds, but they do suggest that the possibility is worth pursuing.

11.3 Experimental

Male sterile winter wheat, variety W46, was grown in an experimental plot at Model Farm during 1975/6, adjacent to a plot of fertile W46. A varied number of spikelets in each of 50 ears were inoculated (section 2.6) with strain 330 of C.purpurea just before pseudoanthesis. Twenty ears of a size similar to those inoculated were labelled to provide controls. The inoculated and labelled ears were grown to maturity and harvested by hand. All ears were stored dry at room temperature until analysed.

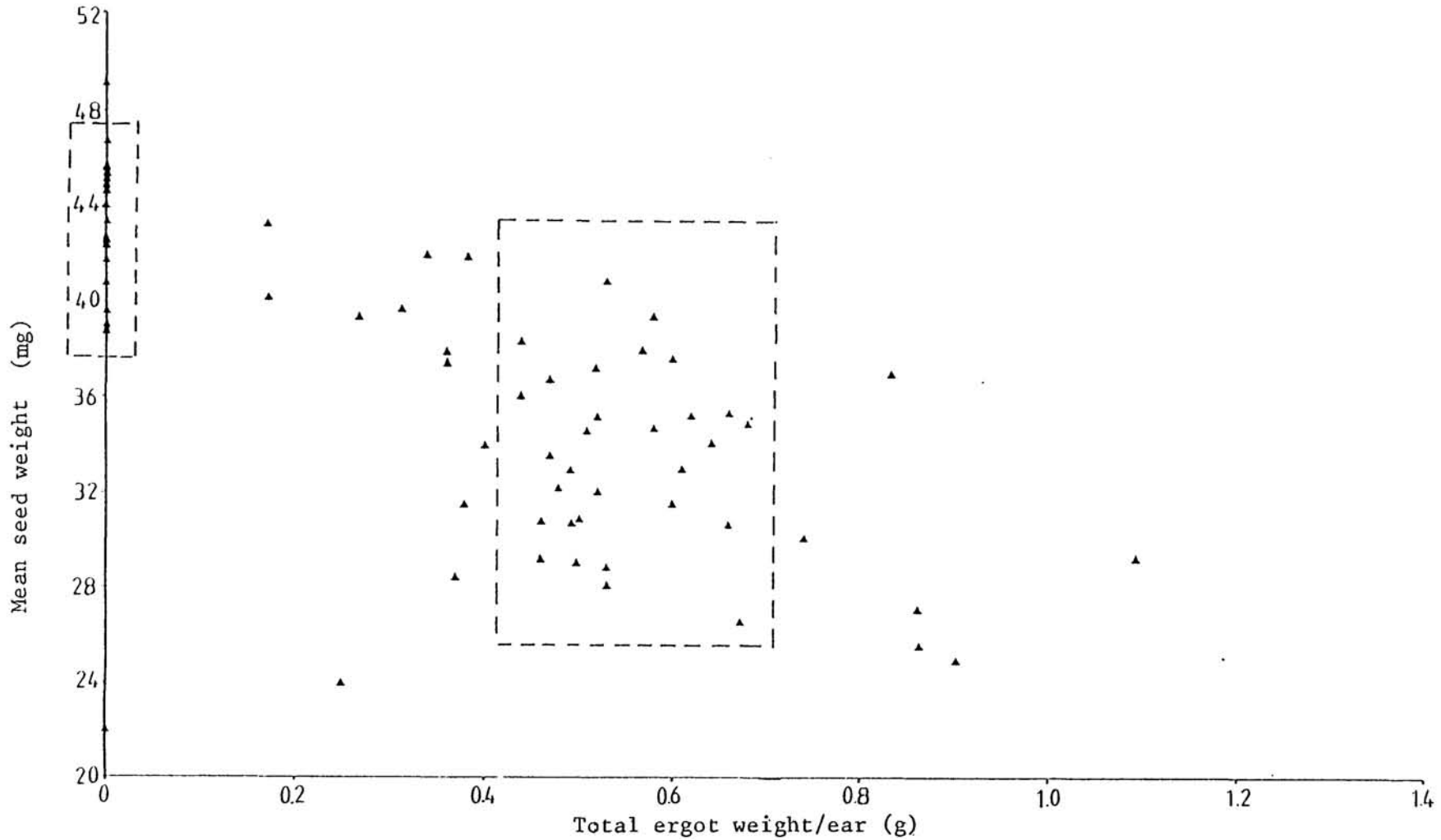
Spikelet number, seed number, ergot number, seed weight and ergot weight were recorded for each inoculated and control ear. 100 seeds from two selected groups of ears (basis of selection discussed in section 11.4) were tested by R.H.M. for germination and vigour (germination under stressful conditions) using their standard techniques.

11.4 Results and discussion

The variation of mean seed weight with total ergot weight is shown for each of the experimental ears in figure 43. Although there is considerable scatter among the values plotted, this graph shows that, in the majority of ears examined, the mean seed weight was reduced by the presence of ergots in the ear. Analysis by Student's t-test confirms that this reduction in mean seed weight is significant at the 0.1% level (appendix Iiiia). There was also a strong positive correlation between increasing mean seed weight and decreasing total ergot weight (P=0.001; appendix IIiia).

Figures 44 and 45 also show that the mean seed weight is reduced by the presence of ergot. Although there is no significant correlation between increasing mean ergot weight and decreasing mean seed weight there is a significant correlation (P=0.02) between increasing ergot number and decreasing mean seed weight (appendix IIiia).
The scatter shown in these figures may be at least partially caused by variation in the positions of ergots and seeds within each ear. The seeds were divided into two groups for vigour testing as shown in figure

Variation of mean seed weight with total ergot weight in individual ears



- seeds within the two boxes (dashed line) were used for vigour testing

Figure 44

Variation of mean seed weight with mean ergot weight in individual ears

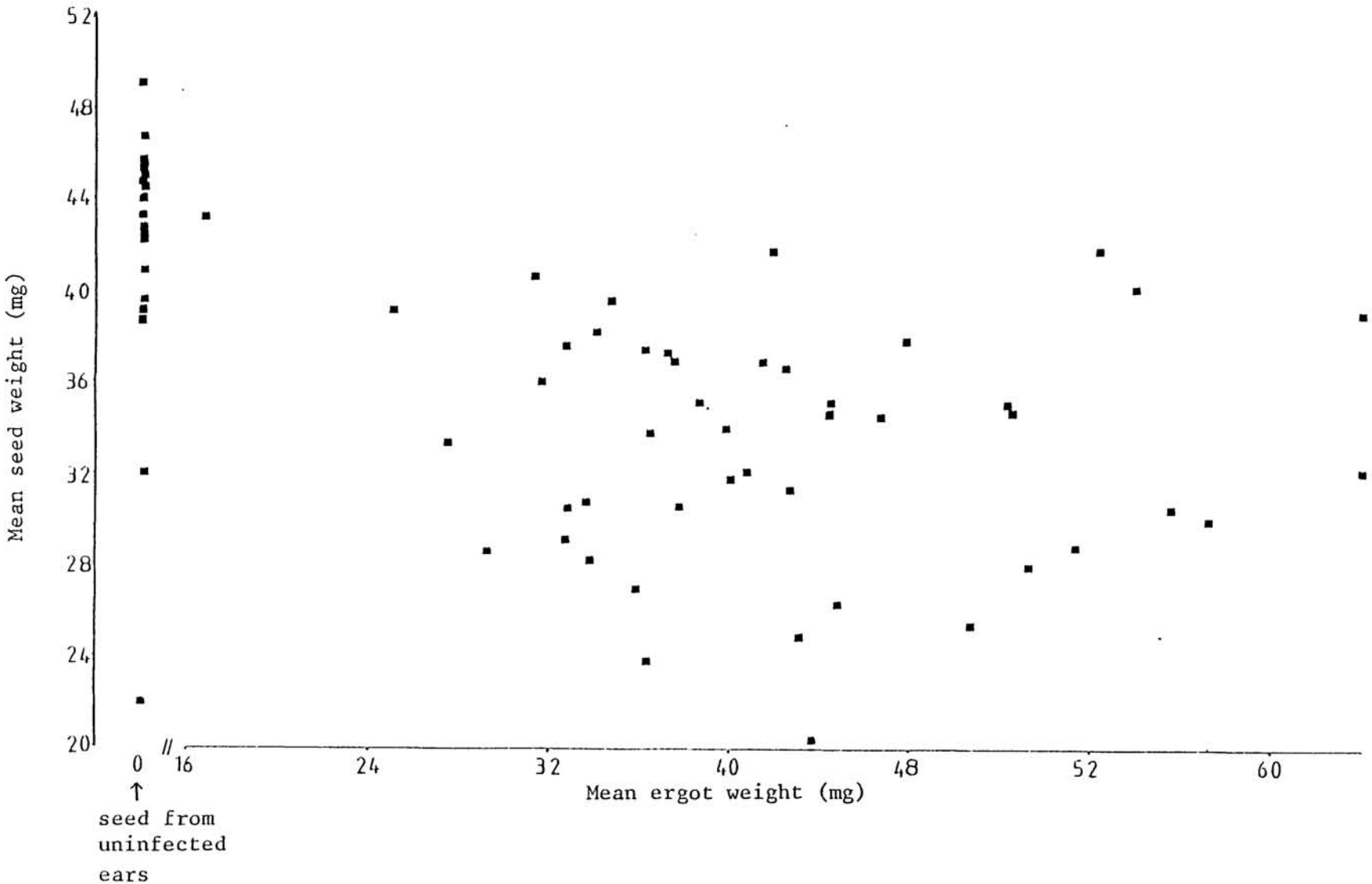
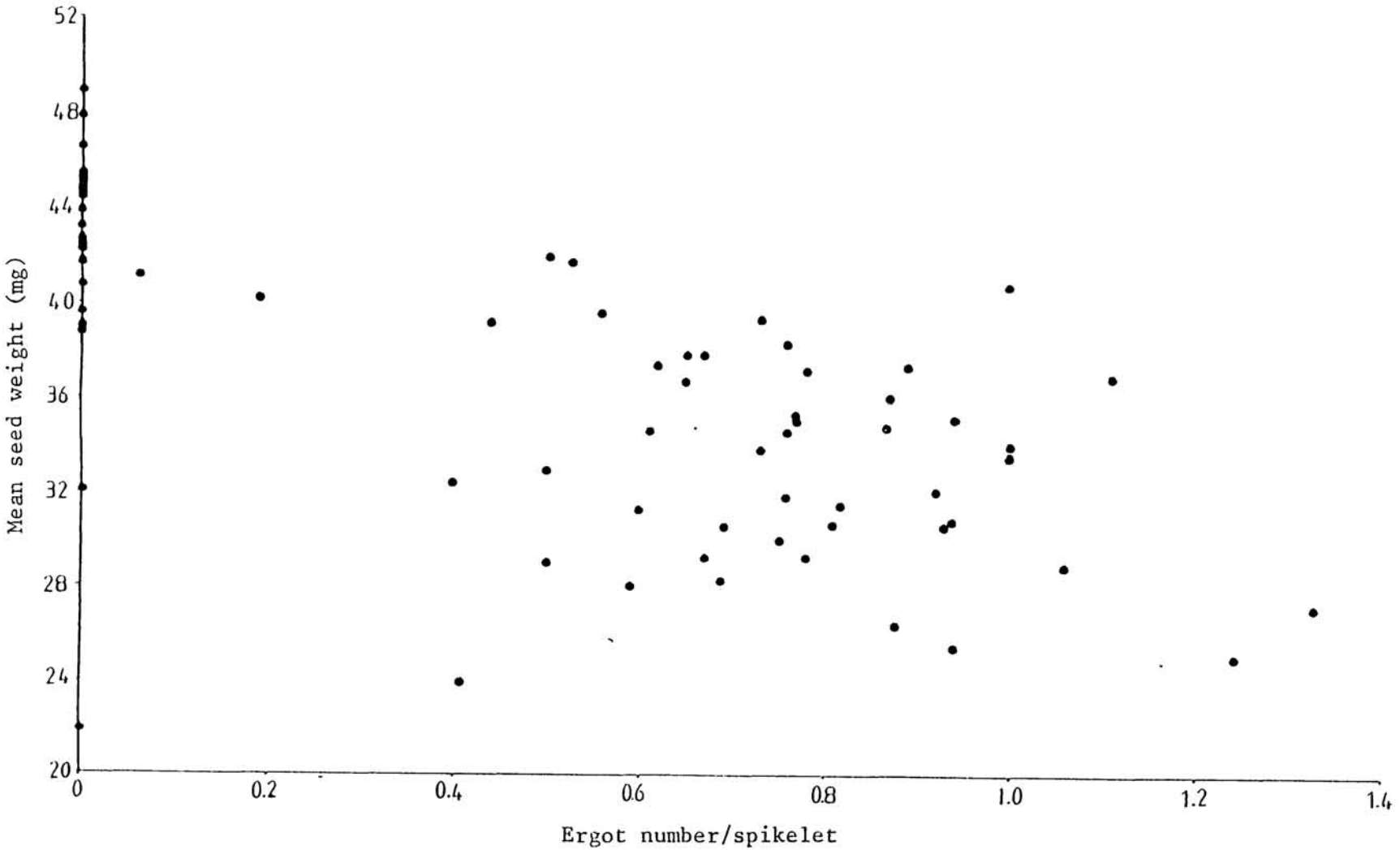


Figure 45

Variation of mean seed weight with the number of ergots/spikelet within individual ears



43. In view of the wide scatter of points it was decided to exclude the extremes of both the control and infected groups, even though such a division was rather arbitary.

In the vigour test 53% of the control seeds germinated, compared to 78% of the seeds from infected ears. All the germinated seeds from infected ears passed the vigour test (ie. 78% vigour was expressed) compared to a reduced number in the controls (45% vigour). The seeds were stored for six months (in loosely stoppered glass tubes at room temperature) prior to testing, which may at least partially explain the very low values obtained for germination and vigour.

It is possible that the presence of ergot in the infected ears changed the quantity and spectrum of plant nutrients available to developing seeds. While this might result in reduced mean seed weights it could also enhance the germination capacity and vigour of the seeds. Since there were insufficient seeds available in either group to repeat the test no confidence limits can be placed on these results, and they will not be discussed further.

It is concluded from this experiment that mean seed weight is influenced more by total ergot weight than by either mean ergot weight or number. This supports the hypothesis that sclerotia can act as a metabolic sink. Figure 44 shows that only in a few of the ears were the ergots heavier on average than the seeds, which suggests that the superior metabolic activity of the fungus was the cause of the reduction in seed weight. Honeydew exudation was extremely sparse in this group of infected ears and so did not provide a wateful drain on plant nutrients.

12. CARBON DIOXIDE FIXATION BY SPHACELIA AND SCLEROTIA OF C.PURPUREA

12.1 Experimental

Fourex rye, was grown in 5" plastic pots buried in an open plot (treatments 1-6; table 14) or the glasshouse (treatments 7-14) at the Chelsea Physic Garden, London. Prior to anthesis eighty percent of the spikelets of two thirds of the plants were inoculated (section 2.6) with parasitically produced spores of strain 25B of C.purpurea..

Each group of ears, or isolated parasitic tissues (see below), were pulse-fed with $^{14}CO_2$ for five hours after which gaseous exchange was immediately terminated. The methods were those described in sections 2.7 & 2.8. The variation in treatment of each group of plants is summarised in table 14. Treatments 1-6 and 7-14 were carried out as two discrete series of experiments.

Comparison of the relative abilities of excised and in situ (plant-attached) ears to fix carbon dioxide was achieved by duplication of four of the experimental treatments. Once it had been confirmed that excised ears were as efficient at fixing CO_2 as ears attached to the plants, all further experiments used excised ears as they were easier to manipulate. Water uptake was maintained by standing the excised ears, immediately after excision, in a flask of CO_2-free water, the neck of which was sealed from the atmosphere by foam plastic and Apiezon grease (figure 46).

In order to separate any fungal CO_2 fixation from plant CO_2-fixation the extent of $^{14}CO_2$ uptake into fungal tissues was compared in the light and the dark.

Illumination for experiments in the light was provided by a 700 watt mercury vapour lamp (Philips MBRF/U). A mirror was positioned behind the plant chamber to facilitate bilatteral illumination of the ears.

Twenty hours before administration of a $^{14}CO_2$ pulse in the dark, the plants, or excised ears, were transferred to a light-tight cupboard in a double-doored darkroom. This dark treatment ensured that no photosynthetically powered CO_2 fixation could occur during the ^{14}C feeding period. To enable safe insertion of ears into the system, and

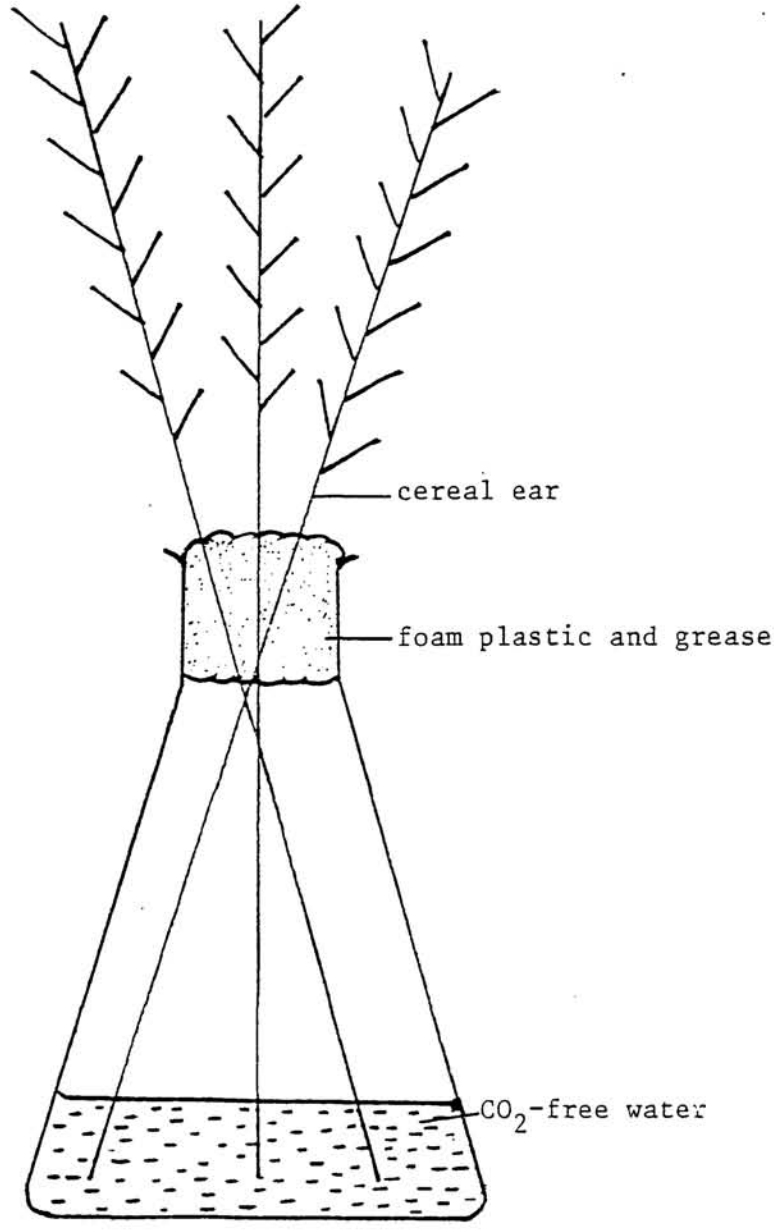
Table 14

Summary of treatments used in carbon dioxide fixation experiments

Treatment no.	Infected/ Uninfected	Light/Dark during $^{14}CO_2$ supply	Ears in situ/ ears excised/ fungus alone	No. days between inoculation and feeding	No.of ears fed
1	Uninfected	Dark	in situ ears	20	9
2	Infected	Light	in situ ears	21	8
3	Infected	Dark	in situ ears	22	8
4	Uninfected	Dark	excised ears	25	10
5	Infected (floral parts removed)	Dark	excised ears	26	17
6	Infected	Not fed	in situ ears	25	9
7	Infected	Not fed	excised ears	27 & 30	17
8	Infected	Dark	sphacelial tissue alone	12 (sphacelial)	19
9	Infected	Light	excised ears	28	13
10	Uninfected	Dark	excised ears	29	8
11	Infected	Dark	Whole ergots alone	30	14
12	Infected	Dark	macerated ergots alone	30	15
13	Infected	Dark	excised ears	27	12
14	Uninfected	Light	excised ears	28	8

Figure 46

Preparation of excised ears for feeding with $^{14}CO_2$



periodic monitoring of the experiment, a very dim primary green light was positioned in front of the apparatus. This 'photosynthetically safe' light consisted of a fluorescent tube completely enclosed within a metal box except for one slit 40 cm x 8 cm. The slit was covered with four layers of primary green celluloid film (cinemoid no. 39, λ550 nm); the intensity of the emitted light was the minimum required to be of assistance to the operator.

Once ^{14}C had been detected in ergots from infected ears which had been exposed to $^{14}CO_2$ in the dark, the possibility of uptake of gas by host tissues, and its subsequent translocation to the sclerotia, was further reduced by removing most of the actively photosynthetic tissues from a group of ears (treatment 5, table 14). As many as possible of the floral parts were excised from these ears while leaving the sclerotia attached to the host. Owing to the tenuous nature of this attachment it was not always possible to remove every vestige of floral tissue. Any sclerotia which became detached were discarded.

Final separation of any host and parasite CO_2 uptake was achieved by exposing isolated sclerotia and sphacelial tissues to radiolabelled CO_2. These tissues were kept moist after excision and during the feeding process as described in section 2.8. Macerated, as well as whole, sclerotia were fed to enable assessment of any effect of increased surface area on CO_2 uptake.

Within a few days of administration of the $^{14}CO_2$ pulse, the deep frozen ears were dissected at room temperature to separate seeds and ergots from all other tissues. After weighing, the tissues were quickly rinsed with distilled water to remove excess honeydew and spores. The tissues were homogenised in a minimal volume of distilled water using a vortex homogeniser, freeze dried, and weighed prior to storage at $4^{o}C$ in a desiccator.

1-2g of each sample, or all of the available tissue if this weighed less than 1g, was extracted using the successive procedures described in section 2.11. For samples 1-6 the ethanolic method of lipid extraction was used but for samples 7-14 the chloroform/methanol system was tried. This change in method was induced by the apparent presence of lipid in the hot T.C.A. extracts of samples 1-6 which was indicative of incomplete lipid extraction during stages b) and c) of the series. Sclerotial tissue from treatments 2,5 and 6 was extracted

in duplicate to enable assessment of the error accrued during the extraction process.

Aliquots of each seed and ergot extract were assayed for radioactivity by scintillation counting (section 2.9.3).

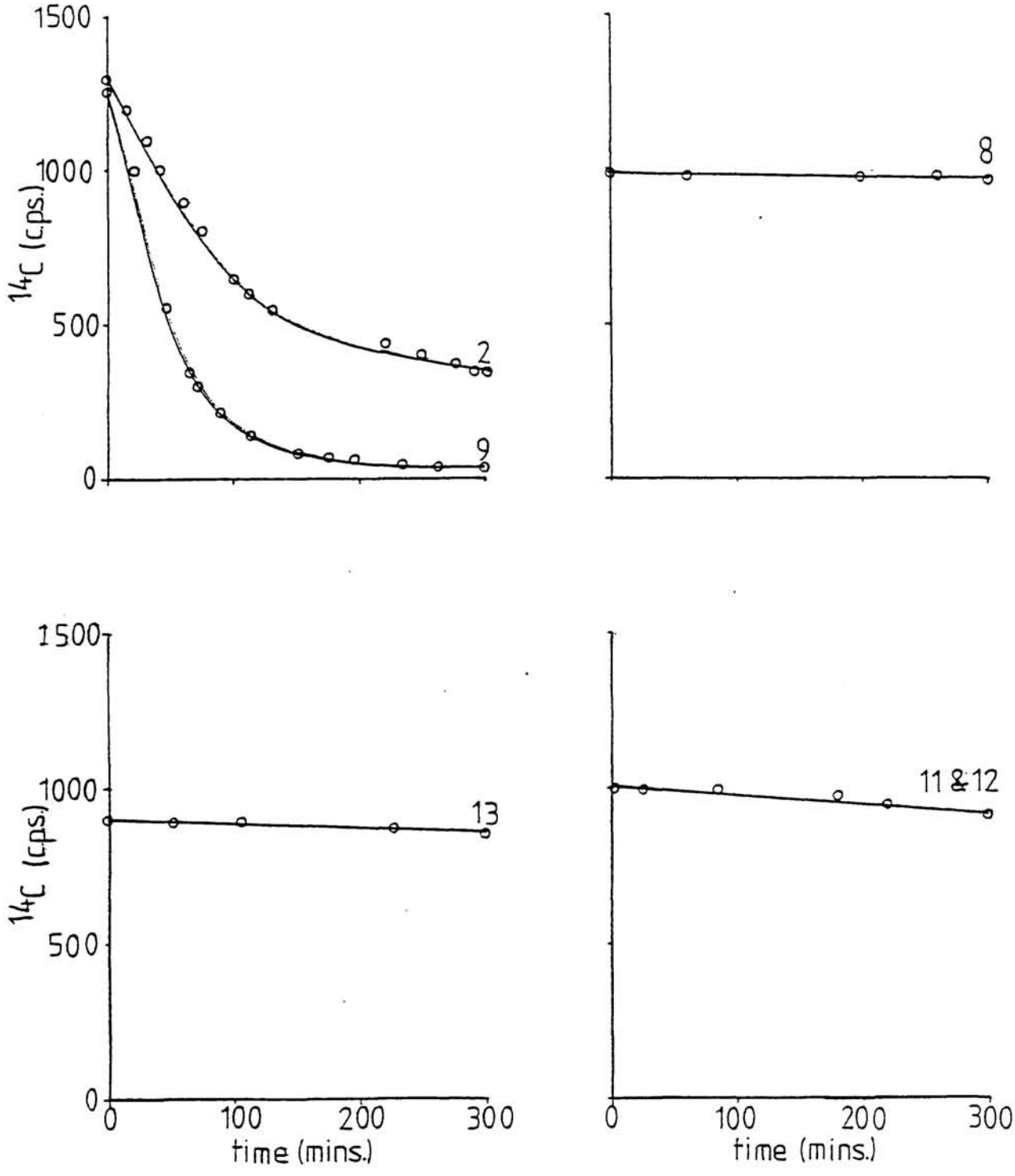
To determine the principal sugars and amino acids into which the ^{14}C had been incorporated a few of the ergot extracts (samples 2,5&6), obtained using 5% T.C.A. in the cold (4°C), were further processed. The T.C.A. was removed from these samples by shaking three times with an equal volume of diethyl ether. The carbohydrates and amino acids in the resulting aqueous solution were separated from each other by ion exchange (section 2.16), and dried under vacuum. The amino acid extracts were resolved by two dimensional paper chromatography (section 2.17) and the carbohydrates by descending paper chromatography (section 2.18). The incorporation of ^{14}C into individual amino acids and carbohydrates was detected by autoradiography of the chromatograms (section 2.9.1). Any labelled compounds were eluted from the chromatogram (section 2.9.2) and the isotopic content was quantified by scintillation counting (section 2.9.3) of a portion of the eluate. The identity of the labelled carbohydrates was confirmed by G.L.C: the remaining eluate from each chromatogram area was taken to dryness and the borate complexes were decomposed by the repeated (5-10x) addition of acidified methanol (2 ml glacial acetic acid in 100 ml methanol) which was then evaporated using an infra red lamp and fan. The free sugars were silylated and analysed by the methods described in section 2.19.

12.2 Results and discussion

Owing to the very hot, dry growing seasons of 1976, plants used for the first series of experiments showed signs of premature browning, especially of the awns, before administration of the $^{14}CO_2$ pulse. No browning of plants grown in the glasshouse during 1977 for the second series of experiments (treatments 7-14) was observed before feeding. Most sclerotia were just beginning to protrude from the florets at the time of $^{14}CO_2$ supply in each experiment. The more active metabolic state of the ears used in the second series of experiments was demonstrated by their more rapid uptake of $^{14}CO_2$ in the light, than was recorded for ears of the first series (figure 47). This was not, however, followed by an increase in the total amount of ^{14}C recovered

Figure 47

The depletion of $^{14}CO_2$ supplied to infected rye ears and isolated parasitic tissues in an enclosed system



Summary of treatments

2 = infected/in situ/light/1st series
9 = infected/excised ears/light/2nd series
8 = sphacelial tissues/dark
13 = infected/excised ears/dark
11&12 = whole and macerated sclerotia/dark

from the sclerotial tissues of ears fed in the light (table 15). In contrast, seeds from the light fed ears of the second series had a total ^{14}C content 2.5 times greater than that of the seeds from the corresponding ears in the first series of experiments (table 16). This suggests that the ergots exerted a preferential pull on fixed carbon, thus satisfying their own carbon demand at the expense of the adjacent seeds. In the first series of experiments, in which less ^{14}C was fixed by the ears, this resulted in a reduced ^{14}C content of the seeds. These conclusions are in accord with those of Corbett et al (1974) who first reported the superior metabolic activity of ergots and their ability to divert nutrients away from adjacent seeds in rye ears. The sink effect of the ergots was further demonstrated in the present studies by the lower ^{14}C content of seeds from infected ears (treatment 9) than seeds from healthy rye ears (treatment 14) which did not have to compete for their carbon.

The duplicate extractions carried out on two groups of ergots from $^{14}CO_2$ -fed ears gave values for both the total sclerotial ^{14}C content, and the ^{14}C content of each fraction, which are in close agreement (table 15). There was very little, if any, difference in the amount of ^{14}C extracted from tissues of in situ and excised ears. The only notable differences occurred in the seeds of ears fed in the light and these have already been mentioned.

The uninfected ears which were supplied with $^{14}CO_2$ in the dark incorporated a small, but measurable, amount of ^{14}C (treatments 1,4&10; table 16). Although it is possible that $^{14}CO_2$ was absorbed into the CO_2-free water, in which the excised ears were standing, (and transported via the plants' vascular system to the ears) this could not account for the ^{14}C uptake by ears fed in situ (treatment 1). It is more likely that either the twenty hour dark treatment was too short to exhaust the plants' energy reserves completely, or the green light allowed very low levels of photosynthesis to occur, thus providing the energy necessary for CO_2 fixation. All the tissues were rinsed before homogenisation thus eliminating the possibility that the isotope extracted was merely due to absorption of $^{14}CO_2$ in surface water. Furthermore, if this was the origin of the ^{14}C extracted from the seeds, all the activity would have appeared in the first extraction (a) and would not have been distributed throughout the extracts in the manner recorded (table 16).

The distribution of ^{14}C in successive extractions of ergots from ears supplied with $^{14}CO_2$ Table 15

Treatment	Treatment no.	Total ^{14}C uCi/g.d.wt	µCi/g.d.wt in extracts				% ^{14}C/extract			
			a	b+c or x	d	e	a	b+c or x	d	e
Inf./Light/in situ ears	2*	19.86/19.14	17.44/17.12	2.01/1.66	0.20/0.14	0.21/0.22	87.8/89.4	10.1/8.7	1.0/0.7	1.1/1.1
Inf./Light/exc. ears	9	21.24	19.83	0.88 x	0.49	0.04	93.4	4.1	2.3	0.2
Inf./Dark/in situ ears	3	1.17	0.51	0.27	0.10	0.29	43.6	23.1	8.5	24.8
Inf./Dark/exc. ears	13	1.03	0.92	0.07 x	0.02	0.02	89.3	6.8	1.9	1.9
Inf./Dark/floral parts removed	5	0.56	0.29/0.31	0.09/0.11	0.03/0.03	LOST/0.11	55.4	19.6	5.4	19.6
Inf./Dark/whole ergots	11	1.82	1.66	0.12 x	0.02	0.02	91.2	6.6	1.1	1.1
Inf./Dark/mac. ergots	12	0.71	0.65	0.04 x	0.01	0.01	91.5	5.6	1.4	1.4
Inf./Dark/sphac.	8	3.54	2.90	0.37 x	0.08	0.19	81.9	10.5	2.3	5.4

* = extractions carried out in duplicate

All values corrected for 30 µCi/mg Ba $^{14}CO_3$

The distribution of ^{14}C in successive extractions of seeds from ears supplied with $^{14}CO_2$ Table 16

Treatment	Treatment no.	Total ^{14}C uCi/g.d.wt	uCi/g.d.wt in extract				% ^{14}C/extract			
			a	b+c or x	d	e	a	b+c or x	d	e
Uninf./Light/exc. ears	14	65.13	63.85	0.52 x	0.17	0.59	98.0	0.8	0.3	0.9
Inf./Light/in situ ears	2	20.02	16.30	2.03	0.79	0.90	81.4	10.1	3.9	4.5
Inf./Light/exc. ears	9	49.52	48.44	0.44 x	0.43	0.21	97.8	0.9	0.9	0.4
Uninf./Dark/in situ ears	1	0.23	0.15	0.03	0.01	0.03	65.2	14.7	4.3	13.0
Uninf./Dark/exc. ears	4	0.29	0.20	0.05	0.02	0.03	69.0	17.2	6.9	10.3
Unif./Dark/exc. ears	10	0.65	0.59	0.02 x	0.03	0.01	90.8	3.1	4.6	1.5
Inf./Dark/in situ ears	3	0.30	0.18	0.06	0.01	0.05	60.0	20.0	3.3	16.7
Inf./Dark/exc. ears	13	0.49	0.38	0.06 x	0.03	0.02	77.6	12.2	6.1	4.1

All values corrected for 30 μCi/mg Ba $^{14}CO_3$

There was no difference between the total ^{14}C content of seeds from infected and uninfected ears fed in the dark, from which it is inferred that there was no translocation of fixed CO_2 from ergots to seeds during the five hour experimental period.

Preliminary evidence of CO_2 fixation by sclerotial tissue was obtained by analysis of the total ^{14}C content of dark fed, infected ears (treatments 3 and 13; table 15). Although this was not conclusive, because of background levels of CO_2 fixation by the dark treated plant tissues, active involvement of the sclerotial tissues seemed probable since the ^{14}C content of the ergots was greater than that of the seeds (table 16).

Ergots from the ears of treatment 5, in which as much photosynthetic tissue as possible was removed from the ears before feeding, had a total isotope content which was half that of ergots from intact ears, and similar to the ^{14}C content of seeds from uninfected ears fed in the dark. Although most of the floral tissues were excised, small amounts were left, when necessary, to avoid dislodging the sclerotia. These floral vestiges, and the green spike to which the sclerotia were attached, may have been able to fix CO_2, so little emphasis can be put on the isotope content of these ergots. Furthermore, some $^{14}CO_2$ may have been absorbed by the CO_2-free water and transported to the ears in the manner previously cited.

The total ^{14}C content of the ergots of treatments 11 and 12, which were completely isolated from all plant tissues before feeding, confirms that the sclerotia of C.purpurea are capable of fixing gaseous carbon dioxide.

There was insufficient space in the feeding chamber to allow a monolayer arrangement of the macerated tissues, and the adhering surface water was excessive and formed a complete film over the tissues. Both these factors would have reduced gas flow around the tissues and probably resulted in a surface area of tissue exposed to the circulating gas, which was less than that of whole ergots. This would account for the higher ^{14}C content of the whole sclerotia than that recorded for the macerated ergots.

The amount of ^{14}C extracted from sphacelial tissues, after they were fed in isolation from plant and sclerotial tissues, shows a high level of

CO_2 fixation which is almost twice that of sclerotial tissues. This is consistent with the higher metabolic activity of spacelial tissues (Corbett et al, 1974) and probably also reflects the loosely packed nature, and high surface area to weight ratio, of these tissues.

In most of the treatments 80-90% of the total amount of ^{14}C in the ergots was extracted with 5% T.C.A. at 4°C (extraction a; table 15). This was to be expected because metabolism was terminated only five hours after commencement of the feeding, thus allowing insufficient time for the ^{14}C to be incorporated into more complex molecules. The proportion of ^{14}C in the low molecular weight fractions in the ergots of treatments 3 and 5 (44 and 55% respectively) is considerably lower than in the other treatments. It seems reasonable to suppose that the poor metabolic state of the ears used in the first series of experiments, which included treatments 3 and 5, resulted in a carbon deficit in the developing ergots. Thus, when $^{14}CO_2$ was supplied to these ears the ^{14}C was more rapidly incorporated into the lipids and high molecular weight compounds, so reducing the proportion of fixed ^{14}C in low molecular weight compounds (extract a) and increasing the proportion in other fractions (b-e).

The proportion of ^{14}C in the other fractions are also in close agreement, with the exception of treatments 3 and 5. The low activity of the nucleic acid fraction (extract 'd') is not surprising since nucleic acids are only a minor component of cells, and much of the fixed ^{14}C would not have had time to be incorporated into these compounds. The greater incorporation of ^{14}C into the insoluble fraction of the sphacelial tissues (treatment 8) was not surprising since the proportion of cell wall components is greater than in sclerotial cells. The specific activities of the sclerotial lipids extracted in the chloroform/methanol system (x) were also determined and bore a direct, proportional relationship to the values quoted in table 15.

Analysis of the proportion of the total ^{14}C in each extract of the seeds (table 16) shows considerable variation, principally between years. Although in the absence of any replicated extractions, it is not possible to say whether this was due to incomplete extraction of any of the fractions, it is considered to be more probably due to physiological variation between the plants.

Table 17 shows that ergots in ears supplied with $^{14}CO_2$ in the light had a much greater proportion of the fixed ^{14}C in the carbohydrates than in the amino acids (treatment 2). This contrasts with the ergots from ears pulsed with $^{14}CO_2$ in the dark in which the ^{14}C content of the carbohydrates was less than that of the amino acids (treatments 3&5). The values given in table 17 for the total ^{14}C content of both amino acids and carbohydrates are minimum values because they were derived by summation of the activities of the individual compounds counted, and only the most heavily labelled compounds were eluted.

Analysis of the activity of individual amino acids (table 18) shows a varied distribution and the only notable feature was that glutamic acid, valine and alanine were usually labelled. The apparent inconsistencies in the labelling of individual amino acids was probably due to the limitations of the chromatographic system used, which made complete separation of every compound difficult. Furthermore, the very low isotope content of individual compounds necessitated very long periods (1-2 months) of exposure to the X-ray film and resulted in diffuse spots on the autoradiograms. In view of these technical problems it is thought that very little emphasis should be put on the activity of individual amino acids.

Analysis of the carbohydrates from the cold T.C.A. extraction (table 18) shows that the activity of the mannitol is consistently greater than that of glucose (even when expressed in terms of specific activity, table 19), and the activity of arabinitol was low. This is consistent with the findings of Dickerson et al (1976). The higher specific activity of fructose in the present studies may have resulted from a free sugar deficit in the 1976 material (see earlier in this section) which necessitated the uptake of fructose by the fungus. Sucrose and trehalose were not completely separated from each other or from oligosaccharides by the paper chromatography system used, and thus no assessment of their radioactivity was made. Since Dickerson et al (1976) showed these two sugars to be an important component of ergot carbohydrates their omission from the present analysis would have contributed to the disparity between the total ^{14}C content of fraction 'a' and the sum of the activities of the compounents measured (table 17).

Table 17

Distribution of fixed ^{14}C between amino acids and carbohydrates

Treatment no.	µC/g.d.wt in cold T.C.A. extract (a)	Total ^{14}C in amino acids (µCi/g.d.wt)	Total ^{14}C in carbohydrates µCi/g.d.wt	%activity in amino acids	%activity in carbohydrates
2	18.72	0.45	7.46	2.4	39.8
2	18.38	0.58	7.44	3.2	40.5
3	0.56	0.07	0.03	12.5	5.4
5	0.34	0.04	0.03	11.8	8.8
5	0.36	0.03	0.02	8.3	5.6

Table 18

Analysis of the labelled amino acids and carbohydrates in the low molecular weight fraction of ergots from ears pulsed with $^{14}CO_2$

Amino acid/ Carbohydrates	µCi/g.d.wt. tissue extracted				
	Treatment 2		Treatment 3	Treatment 5	
	extraction i	extraction ii		extraction i	extraction ii
Glutamic acid	0.06	0.11	0.01	0.01	0.02
Valine	0.27	0.12	-	0.01	0.00
Alanine	0.12	0.11	0.02	0.02	0.01
Lysine	-	0.12	0.01	-	-
Arginine	-	-	0.03	-	-
Phenyl alanine	-	0.12	-	-	-
Sum of amino acid activity	0.45	0.58	0.07	0.04	0.03
Glucose	0.93	1.79	<0.01	0.01	0.01
Mannitol	5.73	4.94	0.02	0.02	0.01
Fructose	0.78	0.68	-	-	-
Arabinitol	0.02	0.03	-	-	-
Sum of carbohydrate	7.46	7.44	~0.03	0.03	0.02

All values corrected for 30 µCi/mg Ba $^{14}CO_3$

- = no activity detected on chromatogram, therefore not eluted and counted.

Two separate but identical extractions were performed on ergots from treatments 2 and 5.

Two chromatograms were run for each extraction. Each was eluted and counted separately and the mean results are shown above.

Table 19

Specific activity of carbohydrates in ergots from ears supplied with $^{14}CO_2$ for five hours

Treatment no.	Carbohydrate	mg carbohydrate/ g.d.wt. ergot	specific activity of carbohydrate(1) (μCi/g)
2	Glucose	6.47	282
	Mannitol	14.62	374
	Fructose	0.93	677
	Arabinitol	1.98	15
3	Glucose	6.53	1
	Mannitol	7.14	2
	Fructose	N.D.	N.D.
	Arabinitol	N.D.	N.D.
5	Glucose	5.66	2
	Mannitol	2.95	7
	Fructose	N.D.	N.D.
	Arabinitol	N.D.	N.D.

(1) = All values are the mean of 3 chromatograms

N.D. = not detected

13. THE INFLUENCE OF SEED SET ON SECONDARY ERGOT INFECTION OF MALE STERILE WHEAT

13.1 Experimental

Work by Shaw (1975) suggested that the extent of secondary infection of male sterile wheat by C.purpurea is inversely related to seed set, but poor nick between pollinators and male steriles made the results inconclusive.

To investigate the subject further, especially at higher levels of seed set (i.e. within a few feet of the pollinator), an experiment similar to that conducted by Shaw was carried out in the 1976/7 season. To avoid having a gap between the male sterile and fertile varieties, a small proportion of rye was sown with the fertile seed to act as a marker. A mixture of four fertile varieties was used to maximise the chance of good 'nick' between the male steriles and at least one pollinator. The plots were direct drilled and orientated so that a south westerly prevailing wind blew pollen directly towards the male steriles (figure 48). The block of plots was surrounded on three sides by barley to ensure that wheat pollen could reach the male steriles from one direction only.

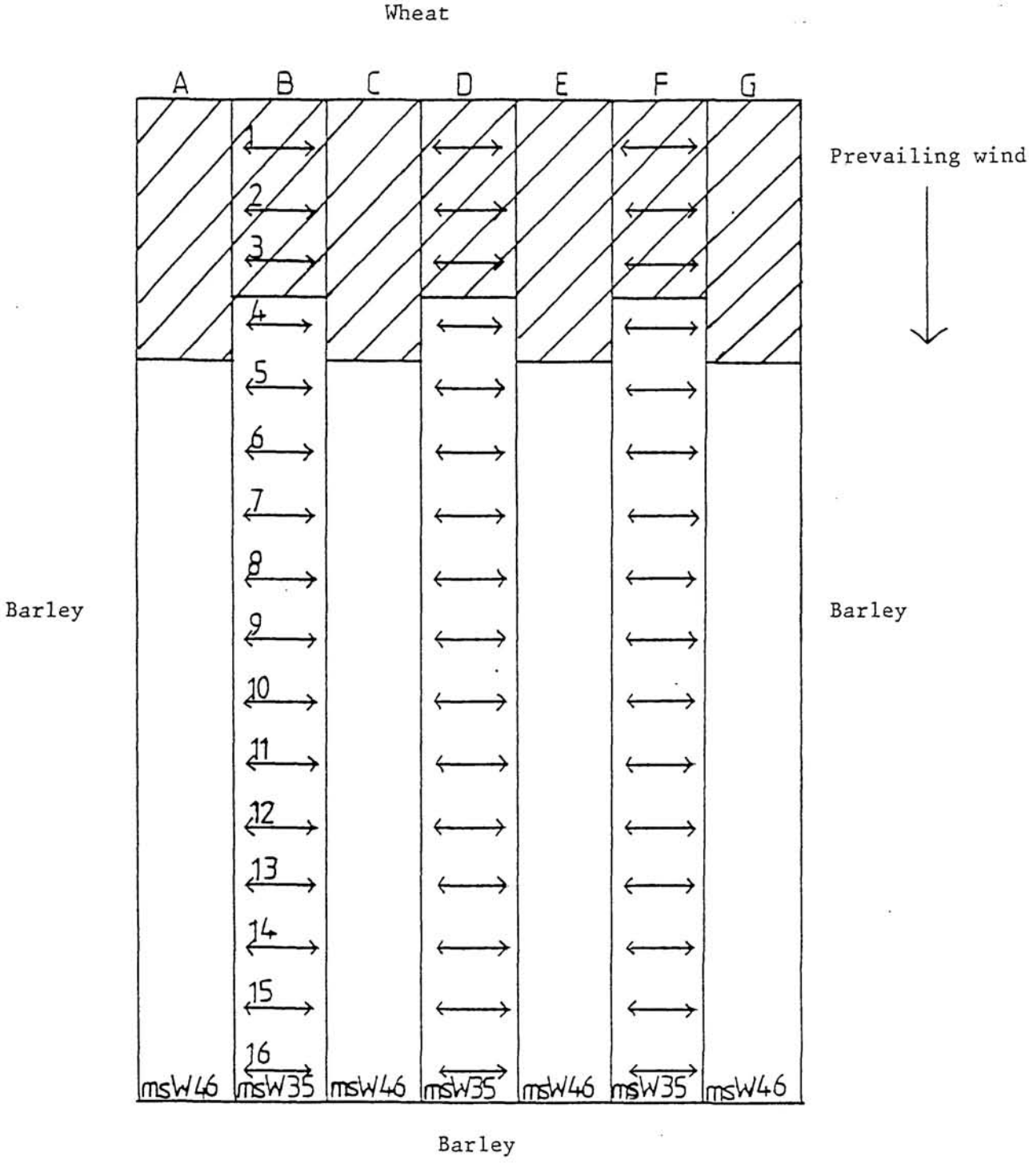
At 3 ft intervals in the three plots of msW35, ten uniformly sized ears were tagged as soon as they had emerged from boot (figure 48). The spacing of these ears across each plot was as uniform as possible. Just before anthesis/pseudoanthesis the tagged ears were inoculated at the 80% level with strain 330 of C.purpurea, as described in section 2.6. The four plots of msW46 were left uninoculated to minimise the chance of cross infection between the plots.

Fertile ears were rogued daily from the male steriles to ensure that pollen could only reach the gaping ears from one direction.

The wheat was grown to maturity and the tagged, primarily inoculated ears were discarded before harvest. The plots were divided into sections and harvested by hand: ears within a 2 ft long strip around each group of ten inoculated ears constituted one sample (figure 49). There was a very heavy storm just before harvest which dislodged some of the larger ergots from the ears. The dislodged ergots were collected from the ground, after the strips had been hand harvested, and were included in the samples.

 Figure 48

Plot layout for experiment to examine the influence of seed set on secondary ergot infection of male sterile wheat

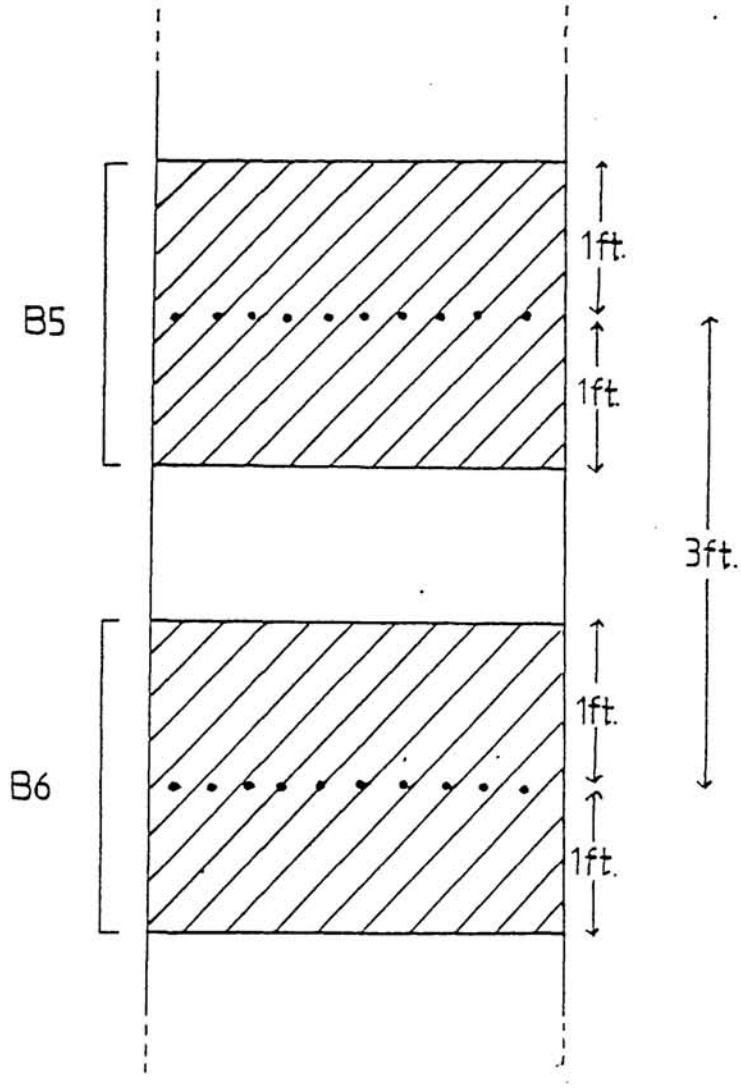


⟷ = 10 ears inoculated (primary infection)

▨ = fertile wheat varieties (W62, W42, W35, W46) and rye

Figure 49

Sample composition at harvest in experiment to examine the influence of seed set on secondary ergot infection of male sterile wheat



• • = primary infected ears

= harvested ears

The ears from each strip were counted, mechanically threshed and the seeds and ergots weighed. Very small and immature ears, which were obviously very late tillers, were excluded from the analyses as the chances of them receiving pollen were minimal, and their seed set would therefore have been unrepresentative of that sample.

Results have been expressed in terms of mean ergot weight/ear and mean seed weight/ear but it is recognised that expression on a numerical basis would have been preferable. However mechanical threshing broke many of the larger ergots and this, together with the large number and size of samples made enumeration both invalid and impractical.

13.2 Results and discussion

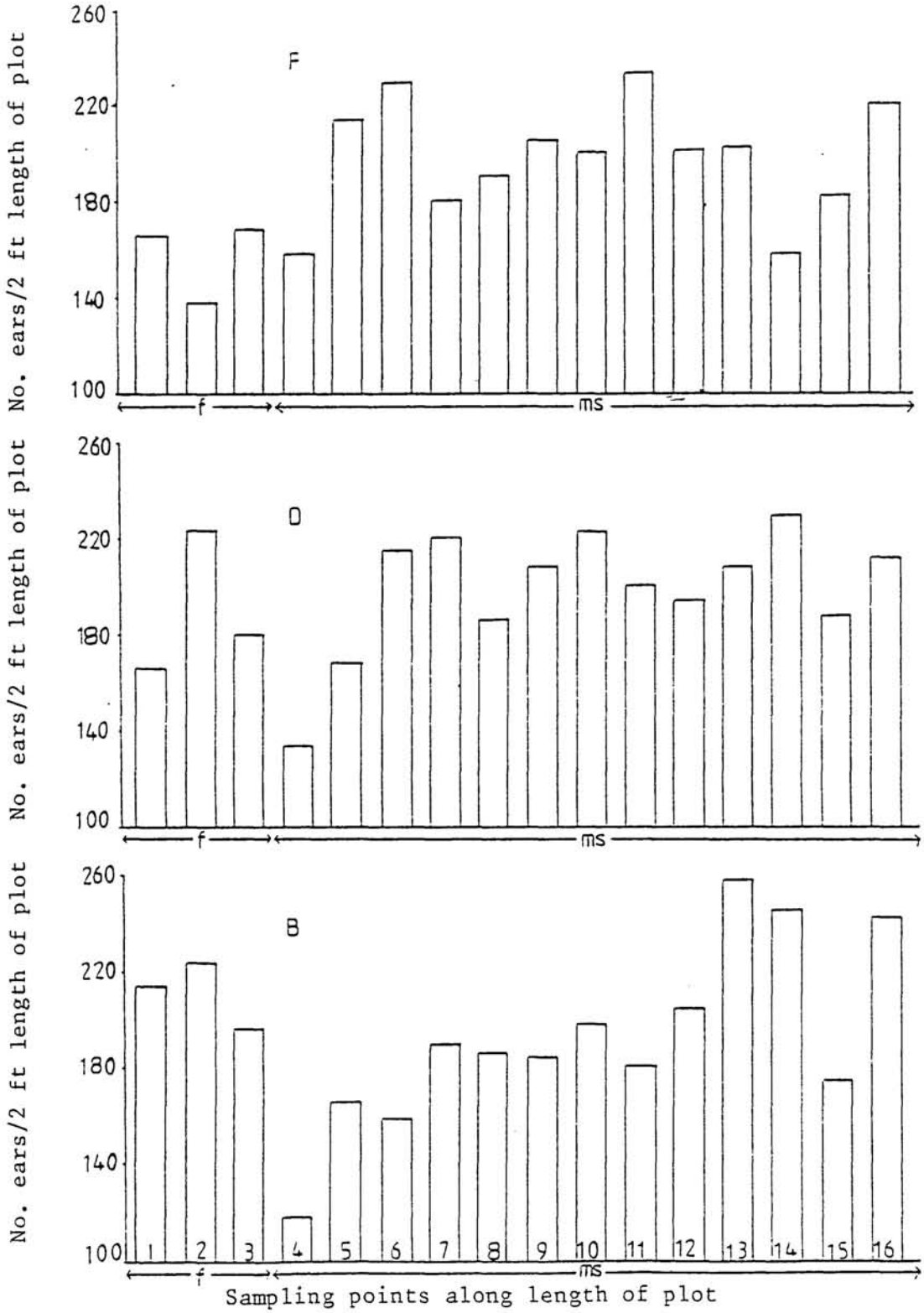
At anthesis it became apparent that the male sterile strips contained many fertile ears. These plants were rogued from the male steriles as soon as they were recognised, but inevitably this was not until at least one anther in one ear of each plant had been extruded. In consequence they may have shed some pollen, but the effect on the pollen gradient was minimised by removing the rogue plants daily.

There was considerable variation in the density of ears in the plots at harvest (figure 50) which may be attributed, in part, to uneven seedling emergence following the very wet conditions which prevailed during and immediately after drilling. The removal of rogue fertile plants also contributed to the patchy distribution of male sterile ears. This is thought to have been the main cause of the very low ear densities at sample points B4 and D4 as these samples were taken from the transition zone between pollinators and male steriles.

Samples B1-3, D1-3 and F1-3 consisted solely of fertile wheat ears (the rye was discarded prior to threshing), whereas samples B4-16, D4-16 and F4-16 contained only male sterile ears. The low values for the average weight of seed per male sterile ear in samples B6-16, D5-16 and F5-16 (figure 51), reflects the uniformly poor seed set among these male steriles. The samples B4,B5,D4 and F4, showed values intermediate between the fertiles and the previously mentioned male steriles, which may be attributed to their proximity to the pollinator. The seed weight/ear in samples B4, D4 and F4 was inversely related to the density of male sterile ears. It therefore seems likely that the rogue fertile ears in these samples were not removed until after they had shed some

Figure 50

Variation of final ear density within plots



f = fertile varieties

ms = male sterile variety

Figure 51

The Variation of seed set and secondary ergot infection with distance from the pollinator

a) Plot B

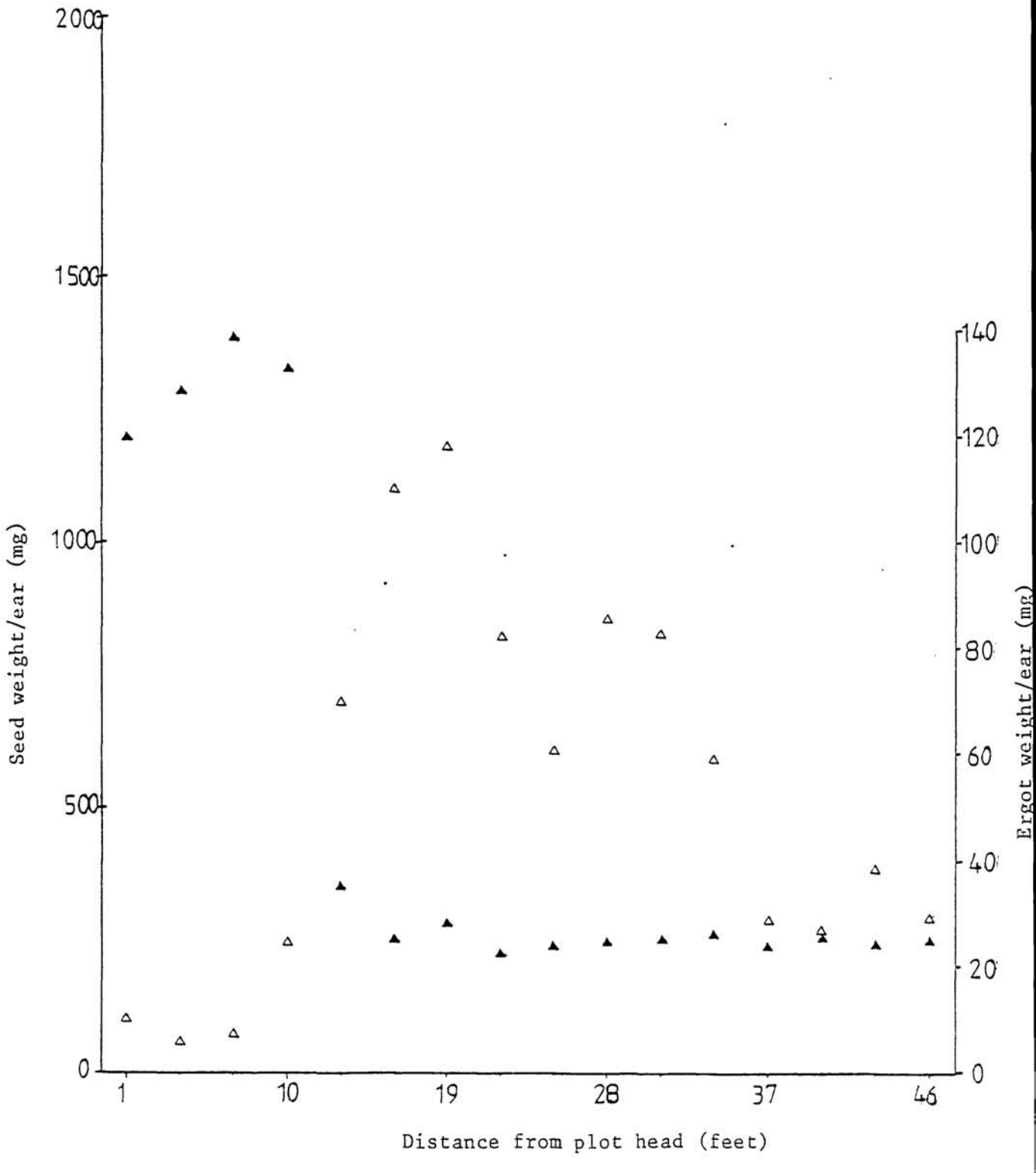
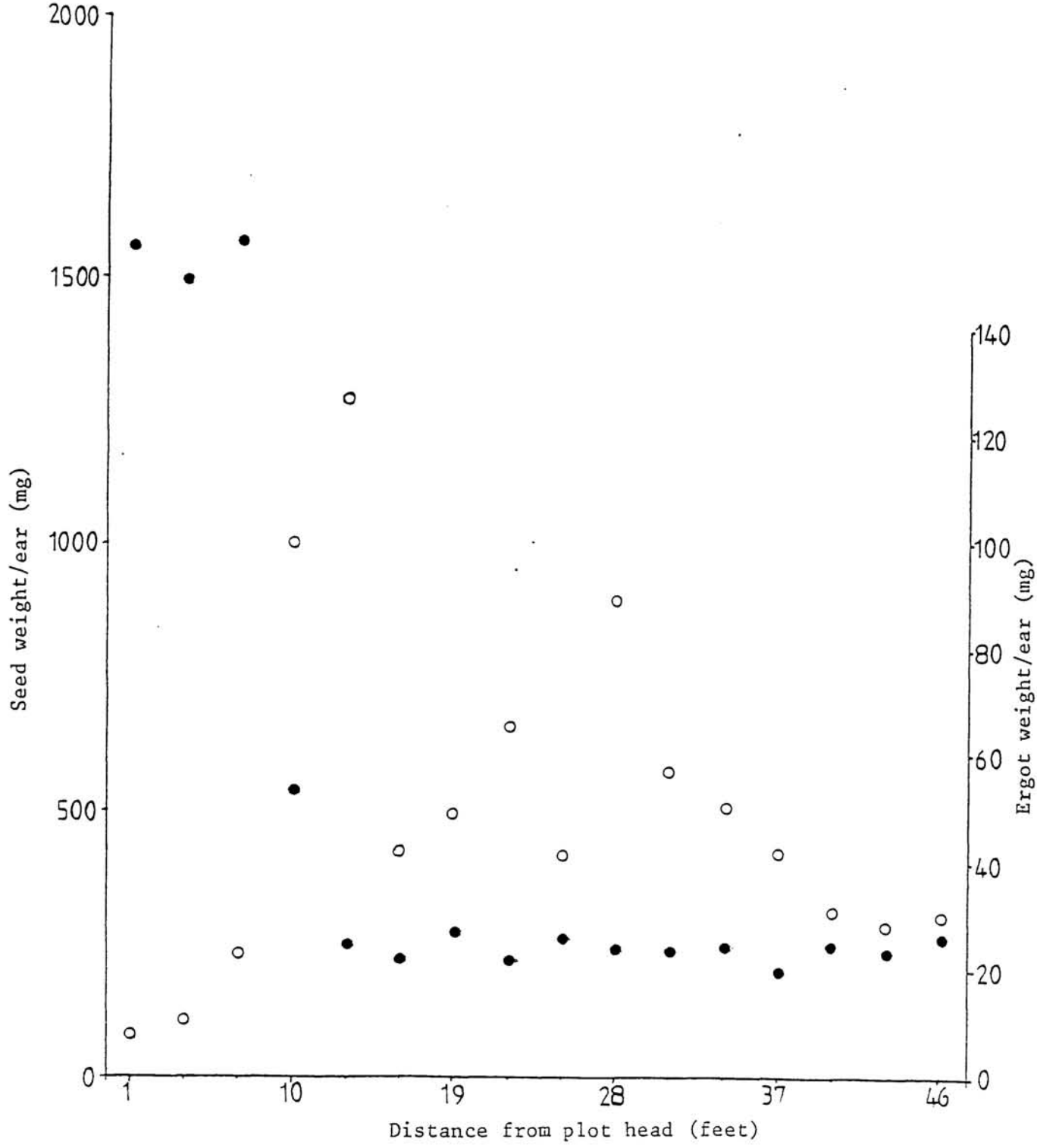


Figure 51 cont'd

b) Plot D

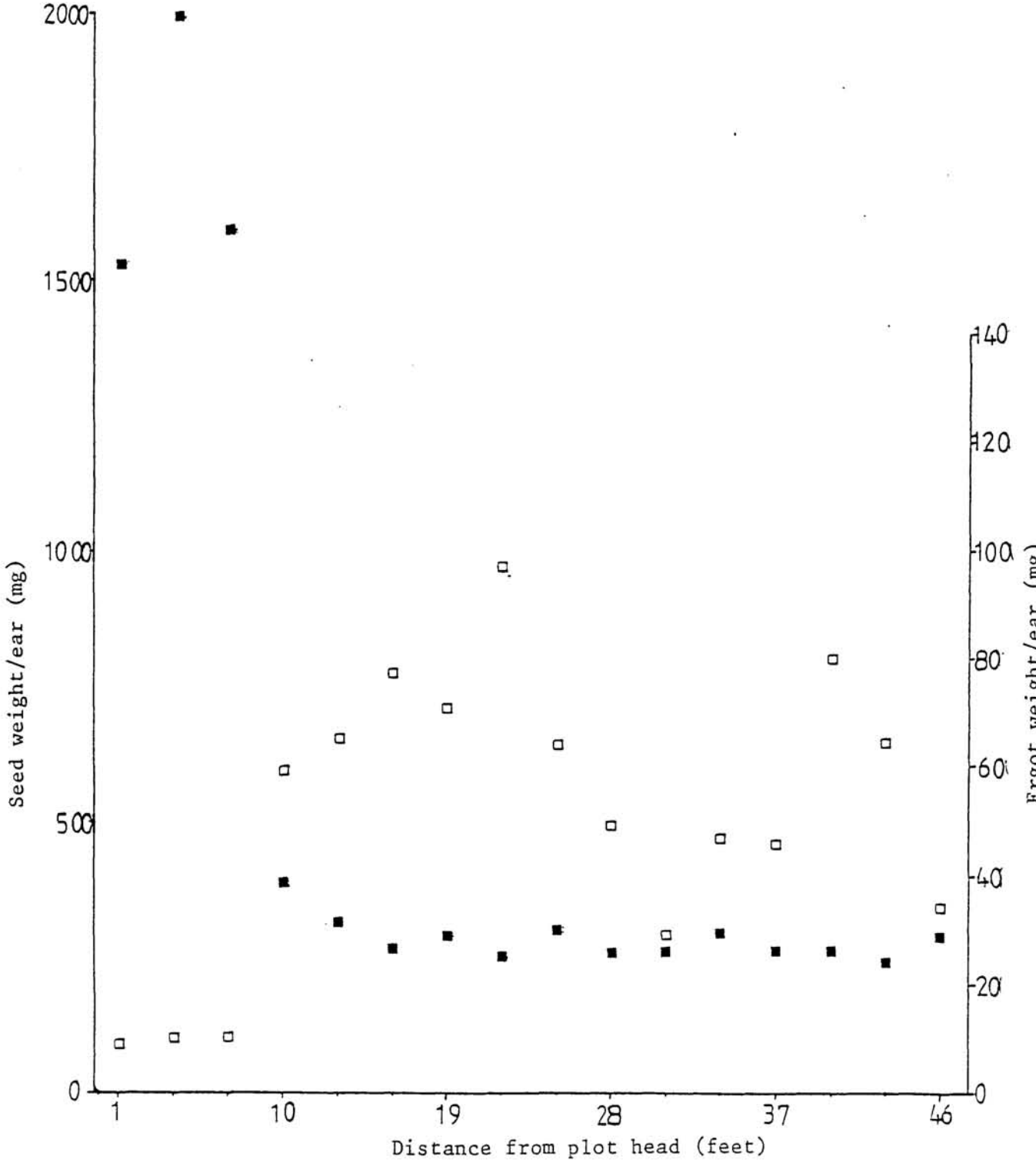


● = seed

○ = ergot

Figure 51 cont'd

c) Plot F



pollen which fertilised some of the gaping male steriles. The high seed set in sample B4 showed that the male sterile variety could achieve a value similar to conventional varieties and presumably was enabled to do so by the high proportion of rogue self-fertiles in this section of the plot.

An inverse relationship between seed and ergot weight per ear was evident through samples 1-5 in each plot. This provides additional experimental evidence in support of the field observations that secondary ergot infection is more widespread in male sterile wheat than conventional wheat and that it is favoured by low seed set. Samples 6-16 in each plot showed a consistently low level of seed set and a higher, but variable, amount of ergot compared to the fertile samples. Although the wind blew steadily from the south west (ie. from the pollinators to the male steriles) throughout the 4-5 days of the pollinating period, it was accompanied by heavy and frequent rain showers. This may account for the low seed set among the male sterile lines even when only four feet from the pollinator.

There was considerable variation in ergot weight per ear between samples with similar values for mean seed weight per ear. Heavy secondary infection of a few ears adjacent to the primary inoculated ears could account for these variations in ergot weight per ear, especially since ear distribution was not uniform in each sample area and rogue fertile ears may have caused local variations in seed set.

It is concluded from this experiment that although secondary spread of ergot is probably inversely related to changes in seed set, there are other factors, such as plant density, which must also influence secondary infection.

14. GENERAL DISCUSSION

Competitive interactions occur throughout the life cycle of C.purpurea (figure 52).

- When a sclerotium germinates, a developing stroma may have to compete for nutrients with other stromata.
- Ascospores which are subsequently released may have to compete, on the host stigma, with pollen grains and other ergot spores for entry into a receptive ovary (Shaw and Mantle 1980a).
- Throughout development each sclerotium has to compete for nutrients with seeds and any other ergots which are growing in the same ear.

Competitive interactions may therefore occur between fungus and fungus as well as between fungus and seed (figure 53).

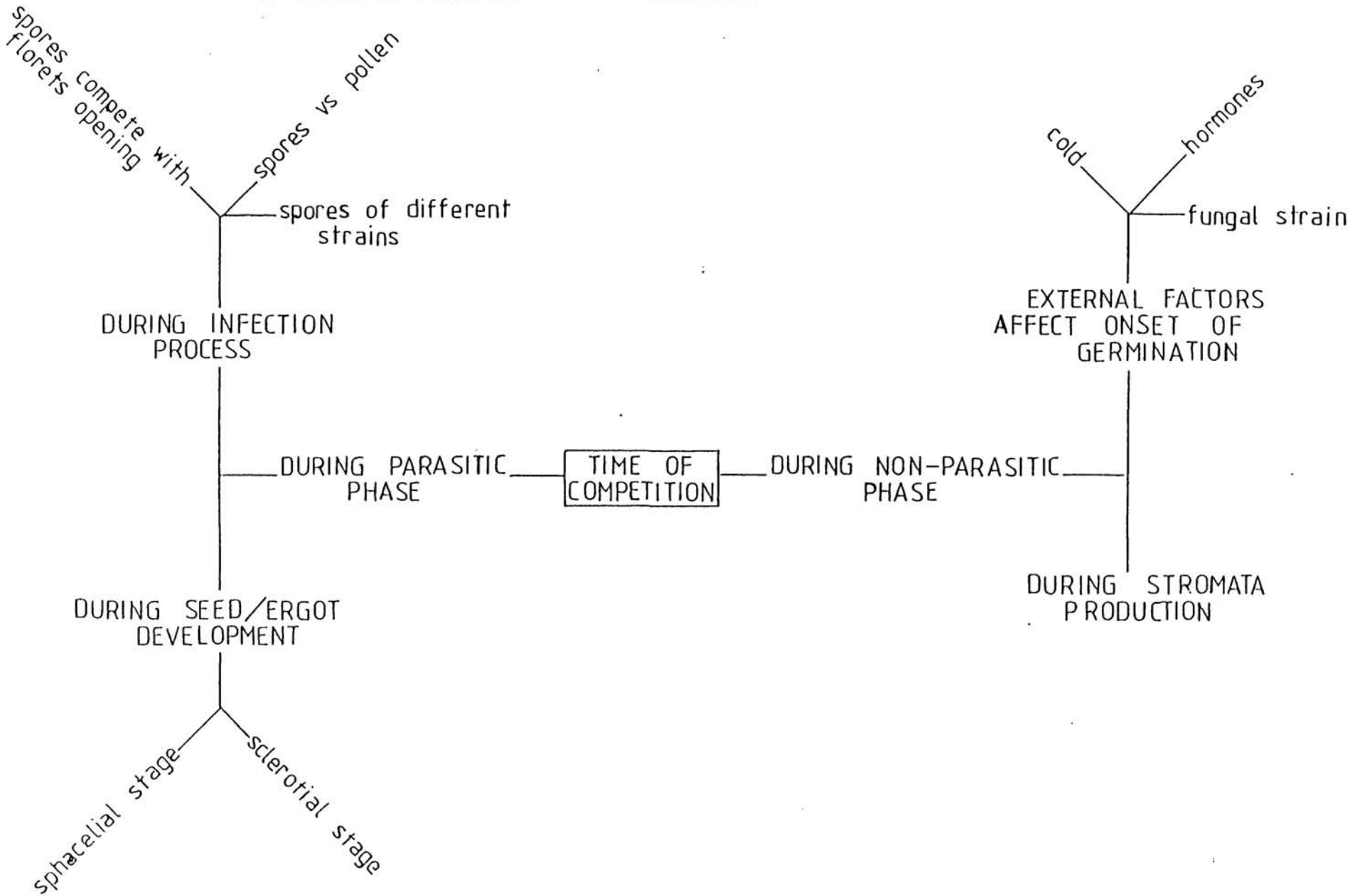
Throughout germination, developing stromata have to compete with each other for the limited nutrient reserves which are available within a single sclerotium. The germination experiments reported in section 6 showed that although substrates for stromatal growth are mainly derived locally, there is evidence that nutrients may also be obtained from adjacent regions, possibly as the local source becomes depleted. The ^{14}C bands were no more distinct in the ungerminated sclerotia than in those which had been through the germination process; this experimentally supports the hypothesis that the abundant lipid reserves were not exhausted, even locally, during stromata formation.

When a sclerotium contains two or more strains, its germinative ability is not simply a reflection of the proportion of the two single strains (section 9). Even when a strain characterised by relatively high percentage germination was in considerable excess of a poorly germinating strain, a very low proportion of the mixed strain sclerotia germinated. The form of this interaction remains unclear, but the existence in some strains of a factor(s) which is inhibitory or antagonistic to germination seems probable.

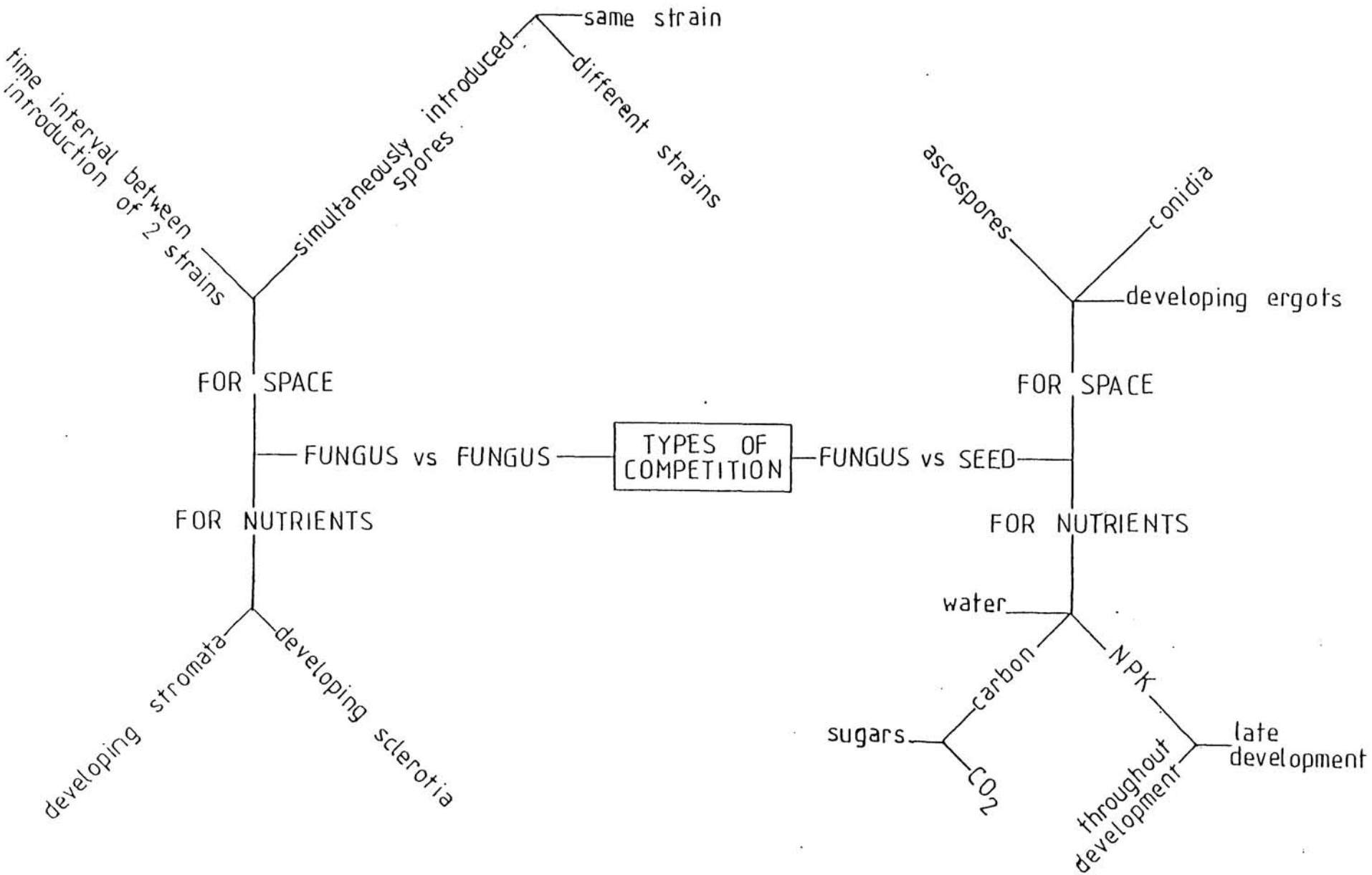
Spores of C.purpurea have to compete with pollen grains for entry into a receptive ovary. Consequently the level of ergot infection in a crop is potentially high if the seed set is low (section 13) since the latter reflects low pollen levels (Shaw 1975).

Competitive interactions during parasitic and non-parasitic stages of C.purpurea

Figure 52



Competitive interactions between fungus and fungus and between fungus and seed during the parasitism of cereals by C.purpurea



When an equal number of spores of two strains of C.purpurea are introduced simultaneously into a host floret, both strains can contribute to the resultant sclerotium (section 9). It is therefore clear that spores of more than one strain can germinate and together colonise a host ovary. There is no evidence for competition between strains during entry into nutritious ovary tissue and it may be assumed that, at this stage, spores of each strain are acting independently.

However, competition may occur if a second strain is introduced into an infected floret about one week after the ovary is initially infected. This is evident from the unexpectedly dramatic reaction in host and parasite reported in section 9. This antagonism could not have simply been caused by the second strain producing a non-specific fungitoxic compound because it did not occur when the same two strains were inoculated together.

Whether there is a critical stage at which the first infecting strain is sensitive to antagonism by another strain is not yet known. The present studies have shown an apparent paradox in that the antagonising pathogen (strain B) eliminated strain E and caused floret necrosis. The efficiency of strain B as a pathogen is without question as it regularly destroyed ovaries when inoculated alone, but its sclerotium forming capacity was much less evident than that of strain E. Whether this indicates an inferior general parasitic ability or results from a hyper-pathogenic capacity is not clear. In any event, the simplest mechanism by which ovary abortion and glume necrosis could both be produced would be by vascular insufficiency. Efficient ergot parasitism (ie. development of a sclerotium) depends on an adequate nutrient supply, which may be facilitated by β-glucanase action on vascular tissue (Dickerson, et al 1979; Shaw and Mantle 1980a). General disruption of vascular function in the rachilla would therefore appear to offer a mechanism for the antagonistic reaction.

As a sclerotium develops it has to compete for nutrients with the seeds and any other ergots which are growing in the same ear. In the present studies (section 10) sclerotia from sparsely infected ears were generally heavier than sclerotia from extensively infected ears. It seems probable that the constraint imposed on sclerotia in the heavily infected ears was the finite amount of nutrients which could be obtained from the host. Thus the developing sclerotia had to compete with each other in this respect and a reduced mean sclerotium weight resulted.

As sclerotia develop within a cereal ear they inevitably compete with the seeds for host-supplied nutrients. The amount of nutrients which a plant is able to supply is partially dependent on the nutrient status of the soil. In consequence the severity of any competition which may occur between seeds and ergots might be expected to increase with reduced availability of soil nutrients. In practice, however, at extremely low nutrient levels, higher mean seed weights were recorded in infected than in healthy ears (section 10.6). One possible explanation is that the exudation of honeydew from infected florets increased the rate of vascular flow to the ear, and the seeds as well as the ergots were able to utilise the soil derived nutrients which it contained. In contrast, when NPK nutrients were abundantly available throughout the development of the wheat plant, ergot infection was detremental to seed growth (section 10.6). However, seed weight was no different in infected and healthy ears when nutrients were supplied shortly before or after anthesis. It can therefore be concluded that the competition seen in rye between seeds and ergots (Corbett, et al 1974) cannot necessarily be extended to other cereals. The superior efficiency of wheat as an agricultural crop indicates that it has sufficient spare capacity to accommodate the competitive demands of the ergot parasite. Furthermore, the ability of C.purpurea sclerotia to fix carbon dioxide (section 12) suggests that the fungus may be at least partially self-supporting in terms of its carbon requirement.

15. REFERENCES

AMOS A.J. (1973) Ergot - recent work reduces risks. Journal of Flour & Animal Feed Milling 155, 26-27.

BARGER G. (1931) Ergot and ergotism. Gurney & Jackson, London.

BERNIER C.C. (1978) Evaluation of resistance to Claviceps purpurea in the genus Triticum. 3rd International Congress of Plant Pathology, Munich, August 16-23.

BOVE F.J. (1970) The story of ergot. J. Karger, Basel, Switzerland.

BRENTZEL W.E. (1927) Studies on ergot of grains and grasses. North Dakota Agricultural Experiment Station. Bulletin 348.

CAMPBELL W.P. (1958) Infection of barley by Claviceps purpurea. Canadian Journal of Botany 36, 615-619.

CAMPBELL W.P. (1959) Inhibition of starch formation by Claviceps purpurea. Phytopathology 49, 451-452.

CAMPBELL W.P. & FRIESEN H.A. (1959) The control of ergot in cereal crops. Plant Disease Reporter 43, 1266-1267.

COOKE R.C. & MITCHELL D.T. (1967) Germination pattern and capacity for repeated stroma formation by Claviceps purpurea. Transactions of the British Mycological Society 50, 257-283.

COOKE R.C. & MITCHELL D.T. (1970) Carbohydrate physiology of sclerotia of Claviceps purpurea during dormancy and germination. Transactions of the British Mycological Society 54, 93-99.

CORBETT K., DICKERSON A.G. & MANTLE P.G. (1974) Metabolic studies on Claviceps purpurea during parasitic development on rye. Journal of General Microbiology 84, 39-58.

CORBETT K., DICKERSON A.G. & MANTLE P.G. (1975) Metabolism of the germinating sclerotium of Claviceps purpurea. Journal of General Microbiology 90, 55-68.

CUNFER B.M. (1976a) Water potential of ergot honeydew and its influence upon colonization by micro-organisms. Phytopathology 66, 449-452.

CUNFER B.M. (1976b) Inhibition of conidial germination by Claviceps purpurea honeydew fluid. Canadian Journal of Botany 54, 2541-2545.

CUNFER B.M., MATHRE D.E. & HOCKETT E.A. (1975) Factors influencing the susceptibility of male-sterile barley to ergot. Crop Science 15, 194-196.

CUNFER B.M. & SECKINGER A. (1977) Survival of Claviceps purpurea and Claviceps paspali sclerotia. Mycologia 69, 1142-1148.

DARLINGTON L.C., CARROLL T.W. & MATHRE D.E. (1976) Enhanced susceptibility of barley to ergot as a result of barley stripe mosaic infection. Plant Disease Reporter 60, 584-587.

DICKERSON A.G. (1972) A β-D-fructofuranosidase from Claviceps purpurea. Biochemical Journal 129, 263-272.

DICKERSON, A.G., MANTLE P.G. & NISBET L.J. (1976) Carbon assimilation by Claviceps purpurea growing as a parasite. Journal of General Microbiology 97, 267-276.

DICKERSON A.G., MANTLE P.G. & SZCZYRBAK C.A. (1970) Autolysis of extracellular glucans produced in vitro by a strain of Claviceps fusiformis. Journal of General Microbiology 60, 403-415.

FISHER R.A. & YATES F. (1963) Statistical tables for biological, agricultural and medical research. 6th edition. Oliver & Boyd, Edinburgh.

FOSTER J.W., CARSONS S.F., RUBENS S. & KAMEN M.D. (1941) Radioactive carbon as an indicator of carbon dioxide utilization. VII. The assimilation of carbon dioxide by molds. Proceedings of the National Academy of Science, USA 27, 590-597.

FUTTRELL M.C. & WEBSTER O.J. (1966) Host range and epidemiology of the sorghum ergot organism. Plant Disease Reporter 50, 828-831.

GRIFFIN D.M. (1972) Ecology of soil fungi. Chapman & Hall, London.

HARDISON J.R. (1972) Prevention of ascocarp formation in Claviceps purpurea by fungicides applied over sclerotia at the soil surface. Phytopathology 62, 609-611.

HARDISON J.R. (1975) Chemical prevention of ascocarp formation in Claviceps purpurea. Phytopathology 65, 502-503.

HARTMAN R.E., KEEN N.T. & LONG M. (1972) Carbon dioxide fixation by Vertocillium albo-atrum. Journal of General Microbiology 73, 29-32.

HULSE J.H. (1974) Triticale in developing nations. In: Triticale, first man-made cereal. Ed. Tsen C.C. pp2-7. American Association of Ceral Chemists, Minnesota.

JENKINSON J.G. (1958) Ergot infection of grasses in south-west England. Plant Pathology 7, 81-85.

KIRCHHOFF H. VON (1929) Contribution to the biology and physiology of the ergot fungus. Zentralblatt für Bakteriologie, Parasitenkunde, u. Infectionskrankeheiten und Hygiene. Abt. II 77, 310-369.

KISS A. (1974) Cultivation problems of Triticale in Hungary. In: Triticale, first man-made cereal. Ed. Tsen C.C. pp15-20. American Association of Cereal Chemists, Minnesota.

KOBEL H. & SANGLIER J.J. (1978) Formation of ergotoxine alkaloids by fermentation and attempts to control their biosynthesis. In: Antibiotics and other second metabolites; biosynthesis and production. FEMS symposium no. 5. Ed. Hütter R., Leisinger T., Nüesch J. & Wehrli W. Academic Press. pp233-242.

KUHN J. (1863) Untersuchungen uber die Entstehung das Kuhstliche Hervorrufen und die Verhutung des Mutterkorns. Landwitsch. Instituts der Universität Halle. pp1-36.

KYBAL J. (1963) Response of ergot fungus to the nutrition available from the host plant. Phytopathology 53, 363.

LARGE E.C. (1954) Growth stages in cereals: illustration of Feekes scale. Plant Pathology 3, 128-129.

LARMOUR R. & MARCHANT R. (1977) Carbon dioxide fixation and conidiation in Fusarium culmorum grown in continuous culture. Journal of General Microbiology 99, 59-68.

LOVELESS A.R. (1971) Conidial evidence for host restriction in Claviceps purpurea. Transactions of the British Mycological Society 56, 419-434.

LUTTRELL E.S. (1977) The disease cycle and fungus-host relationships in Dallisgrass ergot. Phytopathology 67, 1461-1468.

LUTTRELL E.S. (1980) Host-parasite relationships and development of the ergot sclerotium in Claviceps purpurea. Canadian Journal of Botany 58, 942-958.

LYNCH V.H. & CALVIN M. (1952) Carbon dioxide fixation by micro-organisms. Journal of Bacteriology 63, 525-531.

MacCAULEY B.J. & GRIFFIN D.M. (1969a) Effect of carbon dioxide and oxygen on the activity of some soil fungi. Transactions of the British Mycological Society 53, 53-62.

MacCAULEY B.J. & GRIFFIN D.M. (1969b) Effect of carbon dioxide and the bicarbonate ion on the growth of some soil fungi. Transactions of the British Mycological Society 53, 223-238.

MANTLE P.G. (1967) Emergence and phytopathological properties of a new strain of Claviceps purpurea on rye. Annals of Applied Biology 60, 353-356.

MANTLE P.G. (1969a) The role of alkaloids in the poisoning of mammals by sclerotia of Claviceps species. Journal of Stored Products Research 5, 237-244.

MANTLE P.G. (1969b) Studies on Claviceps purpurea (Fr.) Tul. parasitic on Phragmites communis Trin.. Annals of Applied Biology 63, 425-434.

MANTLE P.G. (1972) An unusual parasitic association between Claviceps purpurea and rye. Transactions of the British Mycological Society 59, 327-330.

MANTLE P.G. & SHAW S. (1977) A case study of the aetiology of ergot disease of cereals and grasses. Plant Pathology 26, 121-126.

MANTLE P.G., SHAW S. & DOLING D.A. (1977) Role of weed grasses in the aetiology of ergot disease in wheat. Annals of Applied Biology 86, 339-351.

MANTLE P.G. & TONOLO A. (1968) Relationship between the morphology of Claviceps purpurea and the production of alkaloids. Transactions of the British Mycological Society 51, 499-505.

MATHRE D.E. (1978) In: Plant disease: an advanced treatise. Ed. Horsefall J. & Cowling, E.C.

McLAUGHLIN J.L., GOYAN J.E. & PAUL A.G. (1964) Thin layer chromatography of ergot alkaloids. Journal of Pharmaceutical Sciences 53, 306-310.

MIROCHA C.J. & DEVAY J.E. (1971) Growth of fungi on an inorganic medium. Canadian Journal of Microbiology 17, 1373-1378.

MITCHELL D.T. & COOKE R.C. (1968) Some effects of temperature on germination and longevity of sclerotia in Claviceps purpurea. Transactions of the British Mycological Society 51, 721-729.

MORRIS L.J., HALL S.W. & JAMES A.T. (1966) Biosynthesis of ricinoleic acid by Claviceps purpurea. Biochemical Journal 100, 29C-30C.

MOWER R.L. & HANCOCK J.G. (1975a) Sugar composition of ergot honeydews. Canadian Journal of Botany 53, 2813-2825.

MOWER R.L. & HANCOCK J.G. (1975b) Mechanism of honeydew formation by Claviceps species. Canadian Journal of Botany 53, 2826-2834.

MOWER R.L., SNYDER W.C. & HANCOCK J.G. (1975) Biological control of ergot by Fusarium. Phytopathology 65, 5-10.

NISBET L.J. (1975) Differentiation of Claviceps purpurea during parasitic and exenic culture. Ph.D. Thesis, University of London.

NISBET L.J., DICKERSON A.G. & MANTLE P.G. (1977) Changes in respiration patterns of Claviceps purpurea during parasitic development. Transactions of the British Mycological Society 69, 11-14.

PLATFORD R.G. & BERNIER C.C. (1970) Resistance to Claviceps purpurea in spring and durum wheat. Nature 226, 770.

POMERANZ Y., ROBBINS G.S. & BRIGGLE L.W. (1975) Amino acids in sound and ergot infected grasses and cereals. Cereal Chemistry 52, 108-114.

PURANIK S.B. & MATHRE D.E. (1971) Biology and control of ergot on male sterile wheat and barley. Phytopathology 61, 1075-1080.

RAMSTAD E. & GJERSTAD G. (1955) The parasitic growth of Claviceps purpurea (Fr.) Tul. on rye and its relation to alkaloid formation. Journal of the American Pharmaceutical Association, Scientific Edition, 44, 741-743.

RATANOPAS S. & BERNIER C.C. (1972) Evaluation of the resistance to Claviceps purpurea in two wheat cultivars. Proceedings of the 38th session of the Canadian Phytopathology Society, 39-40.

SHAW B.I. (1979) Infection of cereals by C.purpurea and the role of some enzymes in parasitism. Ph.D. Thesis, University of London.

SHAW B.I. & MANTLE P.G. (1980a) Host infection by Claviceps purpurea. Transactions of the British Mycological Society 75, 77-90.

SHAW B.I. & MANTLE P.G. (1980b) Parasitic differentiation of Claviceps purpurea. Transactions of the British Mycological Society 75, 117-121.

SHAW S. (1975) Aetiology of ergot disease of male sterile wheat. Ph.D. Thesis, University of London.

SIMPSON C.F. & WEST E. (1952) Ergot poisoning in cattle. Florida Agriculture Experimental Station, circular S-43, 6pp.

SMITH D., MUSCATINE L. & LEWIS D. (1969) Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. Biological Reviews 44, 17-90.

SIEGEL S. (1956) Nonparametric statistics for the behavioural sciences. McGraw Hill.

SOSULSKI F. & BERNIER C.C. (1975) Ergot tolerance in spring rye. Canadian Plant Disease Survey 55, 155-157.

STALLKNECHT G.F. & MIROCHA C.J. (1970) Carbon dioxide fixation by uredospores of Uromyces phaseoli and its incorporation into cellular metabolites. Phytopathology 60, 1338-1342.

STALLKNECHT G.F. & MIROCHA C.J. (1971) Fixation and incorporation of carbon dioxide into ribonucleic acid by germinating uredospores of Uromyces phaseoli. Phytopathology 61, 400-405.

STAPLES R.C. & WEINSTEIN L.H. (1959) Dark carbon dioxide fixation by uredospores of rust fungi. Contributions from the Boyce Thompson Institute 20, 71-82.

SWEELEY C.C., BENTLEY R., MAKITA M. & WELLS W.W. (1963) Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. Journal of the American Chemical Society 85, 2497-2506.

TABAK H.H. & COOKE W.B. (1968) The effects of gaseous environments on the growth and metabolism of fungi. Botanical Review 34, 126-252.

TREVELYAN W.E., PROCTER D.P. & HARRISON J.S. (1950) Detection of sugars on paper chromatograms. Nature 166, 444-445.

YANAGITA T. (1963) Carbon dioxide fixation in germinating conidiospores of Aspergillus niger. Journal of General and Applied Microbiology 3, 343-351.

YARWOOD C.E. & JACOBSEN L. (1955) Accumulation of chemicals in diseased areas of leaves. Phytopathology 45, 43-48.

ZAKI A.I. & MIROCHA C.J. (1965) Carbon dioxide fixation by rust infected bean plants in the dark. Phytopathology 55, 1303-1308.

16. APPENDICES - STATISTICS

I. STUDENT'S T-TESTS

i. Competition for nutrients between seeds and ergots: glasshouse experiment (section 10.6)

a) Comparison of mean seed weights in ears grown with an without Liquinure

Treatment	No. ears	Mean seed weight (mg)	d.f.	t	P
Low base, controls: with Liquinure	14	34.421	21	0.029	n.s.
without Liquinure	9	34.467			
Low base, infected: with Liquinure	28	38.686	60	1.671	0.1 (n.s)
without Liquinure	34	40.221			
Half base, controls: with Liquinure	15	42.027	24	0.062	n.s.
without Liquinure	11	41.909			
Half base, infected: with Liquinure	39	40.390	66	0.512	n.s.
without Liquinure	29	39.945			
Full base, controls: with Liquinure	8	43.487	19	1.035	n.s.
without Liquinure	13	45.677			
Full base, infected: with Liquinure	30	41.100	49	1.334	n.s.
without Liquinure	21	42.409			

b) Comparison of mean seed weights in control (healthy) and infected ears, after different regimes

Treatment	No. ears	Mean seed weight (mg)	d.f.	t	P
Low base, no Liquinure: controls	9	34.467	41	4.318	0.001
infected	34	40.221			
Low base, with Liquinure: controls	14	34.421	40	3.565	0.001
infected	28	38.686			
Half base, no Liquinure: controls	11	41.909	38	1.516	n.s.
infected	29	39.945			
Half base, with Liquinure: controls	15	42.027	52	1.317	n.s.
infected	39	40.390			
Full base, no Liquinure: controls	13	45.677	32	2.401	0.025
infected	21	42.409			
Full base, with Liquinure: controls	8	43.487	36	1.610	n.s.
infected	30	41.100			

c) Comparison of mean ergot weights in ears grown with and without Liquinure

Treatment	No. ears	Mean ergot weight (mg)	d.f.	t	P
Low base: with Liquinure	28	21.457	60	0.330	n.s.
without Liquinure	34	20.812			
Half base: with Liquinure	39	28.256	66	2.810	0.01
without Liquinure	29	23.193			
Full base: with Liquinure	30	33.527	49	0.352	n.s.
without Liquinure	21	32.662			

ii. Competition between strains of C.purpurea in vivo (section 9.4)

a) Comparison of percentage seedling emergence in seeds from infected and healthy ears

Treatment	no. samples	mean % emergence	d.f.	t	P
Controls	16	88.812	30	2.986	0.01
E	16	94.500			
Controls	16	88.812	30	2.461	0.02
E-B	16	82.937			

iii. The effect of ergots on the quality of adjacent seed (section 11.4)

a) Comparison of mean seed weights in control (healthy) and infected ears

Treatment	no. ears	mean seed weight (g)	d.f.	t	P
Control ears	20	41.645	66	5.862	0.001
Infected ears	48	33.627			

II. CORRELATION COEFFICIENTS

i. Competition for nutrients between seeds and ergots: glasshouse experiment (section 10.6)

a. Evaluation of the extent of correlation between mean seed weight and ergot weight per spikelet in infected ears grown in different nutrient regimes

Treatment	No. ears	Mean seed weight (mg)	Mean ergot weight/ spikelet (mg)	r	P
Low base, no Liquinure	34	40.221	26.621	0.252	n.s.
Low base, with Liquinure	28	38.686	26.696	0.236	n.s.
Half base, no Liquinure	29	39.945	35.286	0.123	n.s.
Half base, with Liquinure	39	40.390	40.990	0.148	n.s.
Full base, no Liquinure	21	42.409	53.509	0.582	0.01
Full base, with Liquinure	30	41.100	56.035	0.150	n.s.

ii. The effect of ergots on the quality of adjacent seed (section 11.4)

a. Evaluation of the extent of correlation between mean seed weight and the presence of ergots in the ear

Treatment	No. ears	Mean seed weight (mg)	Mean ergot weight (mg)	r	P
Random inoculations	48	33.627	43.848	-.1282	n.s.

Treatment	No. ears	Mean seed weight (mg)	Mean total ergot weight (mg)	r	P
Random inoculations	48	33.627	529.081	-.4651	0.001

Treatment	No. ears	Mean seed weight (mg)	Mean ergot no./ spikelet (mg)	r	P
Random inoculations	48	33.627	0.743	-.367	0.02

III. ANALYSIS OF VARIANCE

i. Competition for nutrients between seeds and ergots: glasshouse experiment (section 10.6)

a. Mean seed number per uninoculated spikelet

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	11	7.981	0.725	-0.185	n.s.
Error	240	-938.244	-3.909		
Total		-930.263			

b. Mean seed weight

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	2	884.790	442.395	10.920	0.001
Error	246	9965.808	40.511		
Total		10850.599			

c. Mean ergot weight

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	5	2725.35	545.07	8.887	0.001
Error	175	10733.58	61.335		
Total		13458.94			

d. Mean ergot number per inoculated spikelet

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	5	6.513	1.302	7.310	0.001
Error	172	30.653	0.178		
Total		37.167			

ii. The effect of ergots on the quality of adjacent seed

a) Mean seed weight

Variety 25

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	5	653.354	130.671	1.89	n.s.
Error	52	3594.558	69.126		
Total		4247.913			

Variety 35

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	9	1107.399	123.044	3.645	0.001
Error	88	2970.889	33.760		
Total		4078.287			

Variety 38

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	9	3089.103	343.234	8.283	0.001
Error	77	3190.679	41.437		
Total		6279.782			

Variety 43

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	9	1296.738	114.082	2.849	0.01
Error	88	4450.118	50.569		
Total		5746.856			

Variety 46

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	9	790.801	87.867	2.001	n.s./ 0.05
Error	88	3863.541	43.904		
Total		4654.341			

Variety 78

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	9	1606.983	178.554	5.188	0.001
Error	79	2718.841	34.416		
Total		4325.824			

b) Mean seed number per spikelet

Variety 25

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	5	0.468	0.093	0.917	n.s.
Error	52	5.307	0.102		
Total		5.775			

Variety 35

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	7	0.833	0.119	0.570	n.s.
Error	70	14.611	0.209		
Total		15.444			

Variety 38

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	7	6.891	0.984	3.308	0.01
Error	61	18.150	0.297		
Total		25.042			

Variety 43

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	7	0.603	0.086	0.627	n.s.
Error	71	9.751	0.137		
Total		10.354			

Variety 46

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	7	1.126	0.161	1.473	n.s.
Error	71	7.749	0.109		
Total		8.875			

Variety 78

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	7	1.315	0.188	1.732	n.s.
Error	62	6.724	0.108		
Total		8.039			

c) Mean ergot weight

Variety 35

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	3	350.751	116.917	0.533	n.s.
Error	36	7900.076	219.446		
Total		8250.827			

Variety 38

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	3	818.476	272.825	2.504	n.s.
Error	24	2614.818	108.951		
Total		3433.295			

Variety 43

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	3	1152.512	384.171	0.816	n.s.
Error	34	15998.090	470.532		
Total		17150.602			

Variety 46

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	3	213.742	71.247	0.506	n.s.
Error	33	4649.406	140.891		
Total		4863.148			

Variety 78

Source of variation	d.f.	Sum of squares	Mean square	F	P
Between treatments	3	915.258	305.086	2.186	n.s.
Error	31	4325.345	139.527		
Total		5240.604			

IV. KOLMOGOROV-SMIRNOV TWO SAMPLE TEST

The Kolmogorov-Smirnov two sample test is a test of whether two independent samples have been drawn from populations with the same distribution. The two-tailed test is sensitive to any kind of difference in the distribution such as dispersion and skewness. If the two samples have been drawn from the same population distribution then the cumulative distributions of both samples should show no more than random deviations from the population distribution. If the random deviation is exceeded at one or more point, this suggests that the samples came from populations with different distributions.

$S_{n_1}(X)$ = Cumulative step function of sample 1

$S_{n_1}(X) = \frac{K}{n_1}$ where K = no. of scores $\leqslant$ X

$S_{n_2}(X)$ = Cumulative step function of sample 2

$S_{n_2}(X) = \frac{K}{n_2}$ where K = no. of scores $\leqslant$ X

$D = \text{maximum } \left[S_{n_1}(X) - S_{n_2}(X)\right]$

The critical value for D may be read off a table (Siegel 1956); if the calculated value of D is equal to or larger than the critical value it is probable, at the chosen level of significance, that the two samples came from populations with different distributions.

i. Germination studies on ^{14}C-labelled sclerotia (section 6.4)

Observed frequencies of stromata

Treatment	% distance from base of sclerotium										
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Total
14 days	1	3	5	11	15	8	2	0	0	0	45
21 days	3	3	4	2	20	14	8	6	2	4	66
38 days	1	0	1	2	10	23	15	15	20	12	99
Control	7	7	14	27	24	12	27	10	8	1	137

Cumulative frequencies of stromata

Treatment	% distance from base of sclerotium									
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
14 days	1	4	9	20	35	43	45	45	45	45
21 days	3	6	10	12	32	46	54	60	62	66
38 days	1	1	2	4	14	37	52	67	87	99
Control	7	14	28	55	79	91	118	128	136	137

Calculation of D when comparing each treatment to the control

From tables (Siegel 1956):

$$\text{critical value of } D = 1.36 \sqrt{\frac{n_1 + n_2}{n_1 n_2}} \quad \text{at 5\% level of significance}$$

$$\text{or } D = 1.63 \sqrt{\frac{n_n + n_2}{n_1 n_2}} \quad \text{at 1\% level of significance}$$

14 days

	% distance from base of sclerotium									
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
$S_{45}(X)$	.022	.089	.200	.444	.778	.956	1.0	1.0	1.0	1.0
$S_{137}(X)$	.051	.102	.204	.401	.576	.664	.861	.934	.993	1.0
$S_{137}(X)-S_{45}(X)$	.029	.013	.004	-.042	-.201	-.292	-.139	-.066	-.007	0

∴ observed D = .292

critical D = .277 if P = 0.01

∴ the distribution of stromata is significantly different (P=0.01) in the sclerotia pulsed with ^{14}C at 14 days and the controls.

21 days

	% distance from base of sclerotium									
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
$S_{66}(X)$	.045	.091	.151	.182	.485	.697	.818	.909	.939	1.0
$S_{137}(X)$	.051	.102	.204	.401	.576	.664	.861	.934	.993	1.0
$S_{137}(X)-S_{66}(X)$	.006	.011	.053	.219	.091	-.033	.043	.025	.054	0

∴ observed D = .219

critical D = .204 if P = 0.05

(critical D = .244 if P = 0.01)

∴ the distribution of stromata is significantly different (P=0.05) in the sclerotia pulsed with ^{14}C at 21 days and the controls.

38 days

	% distance from base									
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
$S_{99}(X)$	.010	.010	.020	.040	.141	.374	.525	.677	.879	1.0
$S_{137}(X)$	.051	.102	.204	.401	.576	.664	.861	.934	.993	1.0
$S_{137}(X)-S_{99}(X)$	.041	.092	.184	.361	.435	.290	.336	.257	.114	0

∴ observed D = .435

critical D = .257 if P = 0.001

∴ the distribution of stromata is significantly different (P=0.001) in the sclerotia pulsed with ^{14}C at 38 days and the controls.

17. ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. P.G. Mantle, most sincerely for all the patient help and encouragement he has given me throughout the studies described in this thesis. I am also grateful to Dr. A.G. Dickerson for his helpful supervision during Dr. Mantle's sabbatical year.

Grateful thanks are also due to the staff of the Crop Science Department of the Lord Rank Research Centre for their assistance with the field experiments, and in particular to Dr. D.A. Doling and Dr. R.J. Ellis for many invaluable discussions.

I am indebted to Mr. R. Freer for technical advice, and to the other members of staff of the Biochemistry Department of Imperial College, who provided technical assistance. I also appreciate the advice of many people on statistical analysis and the co-operation of the staff of the Chelsea Physic garden with the greenhouse experiments.

Thank you also to Mrs. D.J. Morris for typing this manuscript so carefully, and to my colleagues at the British Gas Corporation who have endured my divided interests for so long.

This work was carried out during the tenure of a Science Research Council C.A.S.E. Award. I am grateful to the Science Research Council and to R.H.M. (Research) Ltd. for their financial support.