THE UNIVERSITY OF CALGARY

AN ECOPHYSIOLOGICAL STUDY OF THE INTERACTION OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI AND SULPHUR DIOXIDE ON THE GROWTH OF *Phleum pratense* L.

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "An Ecophysiological Study of the Interaction of Vesicular-Arbuscular Mycorrhizal Fungi and Sulphur Dioxide on the Growth of *Phleum pratense* L.", submitted by M. Jill Clapperton in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

This study examined the effects of colonisation by vesicular-arbuscular (VA) mycorrhizal fungi on *Phleum pratense* L., a co-dominant grass species in a sub-montane mixed grass prairie. Field soil was collected at this site and was used in experiments based on the 'most probable number' technique to study the effects of VA mycorrhizal inoculum density on the growth of *Phleum pratense*. I found that plant growth measured as the number of tillers, shoot and root dry weights, and root:shoot ratio, was inversely related to increasing proportions of field soil in which the plants were grown, and also to VA mycorrhizal colonisation. It was hypothesised that the negative effects of VA mycorrhizal colonisation on *Phleum pratense* were associated with the known carbon demand on plants by the VA mycorrhizal fungi. In order to study the effects of additional stress on the symbiosis, I used the gaseous air pollutant sulphur dioxide (SO_2), which is known to affect plant carbon assimilation, partitioning and translocation processes, as a tool to modify plant growth and assimilate partitioning, and determined the consequent effects on colonisation by VA mycorrhizal fungi. Fumigation with SO_2 decreased the amount of assimilate translocated to the root, and this was associated with an inhibition of the ability of VA mycorrhizal fungi to colonise and proliferate within the roots. Colonisation by VA mycorrhizal fungi in turn appeared to increase the sensitivity of *Phleum pratense* to SO_2 fumigation. Since tolerant grass populations were found to occur at a field site exposed to chronic low

concentrations of SO_2, the possibility that SO_2 tolerance affected assimilate translocation and possibly VA mycorrhizal fungi was explored. SO_2-tolerant and non-tolerant genotypes of *Phleum pratense* were identified. It was then determined that assimilate partitioning to the roots was less affected by SO_2 exposure in the SO_2-tolerant genotype than in the non-tolerant genotype. The results of a two-year field study using the SO_2-tolerant and non-tolerant genotypes and plants from commercial seed of *Phleum pratense* showed that the SO_2-tolerant genotype was more colonised by VA mycorrhizal fungi than the other genotypes. However, in the field study the most significant effect on plant productivity was the compounding effect of grazing and SO_2 exposure.

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Engineering loaned me an SO_2 analyser when mine was being repaired.

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INTRODUCTION

Vesicular-arbuscular (VA) mycorrhizal fungi generally form mutualistic symbioses, with the primary benefit to the plant being improved nutrient uptake. The plant can also benefit from increased drought tolerance (c.f. Nelson, 1987) and disease resistance (c.f. Bagyaraj, 1984) conferred by VA mycorrhizal infection. The net result is usually increased plant growth. However, growth depressions in response to VA mycorrhizal infections have been reported (Buwalda & Goh, 1982; Koide, 1985; Fitter & Nichols, 1988). This lack of positive response to VA mycorrhizal colonisation has been attributed to root morphology (Baylis, 1970), high availability of inorganic nutrients (Buwalda & Goh, 1982) in particular phosphorus (Anderson & Liberta, 1989), photoassimilate carbon drain (Buwalda & Goh, 1982), and interferring soil microorganisms (Hetrick *et al.*, 1988). Fitter & Nichols (1988) suggested that there is a need to study the effects of VA mycorrhizal fungi on plant growth in 'natural soil media' as interactions between soil microorganisms and VA mycorrhizal fungi are often responsible for the lack of positive plant response to VA mycorrhizal fungi in field experiments. The main objective of this study was to examine the relationship between plant growth and VA mycorrhizal fungi in a way which would reflect the the association between the plant and fungus in a natural ecosystem.

To examine the effects on plant growth of colonisation by VA mycorrhizal fungi from field soil, and of field soil

organisms, I used the 'most probable number' (MPN) method for quantifying microbial populations (Alexander, 1982). The objective of this part of my study was to define the relationship between VA mycorrhizal inoculum density and plant growth; and to examine the possibility that the plant growth response to VA mycorrhizal fungi was directly related to the amount of colonisation by VA mycorrhizal fungi.

In this study (Chapter 1) I examined the effects of field soil dilution (and therefore decreasing VA mycorrhizal inoculum density) and the application of increased fertilizer on the growth of *Phleum pratense* L.. *Phleum pratense* was chosen because it was a co-dominant grass species at the field site where the field soil used as inoculum was collected. The diluent soil was the same soil which was used as a source of VA mycorrhizal inoculum but which had been partially sterilized using microwave irradiation. Thus, the host, inoculum, and diluent soil all had similar origins. This is important because Adelman & Morton (1986) have shown that percent VA mycorrhizal colonisation, sporulation, and the extent of extra-matrical hyphae were greatest for a host/ VA mycorrhizal/ diluent soil combination with the same origin. The soil was irradiated with microwaves for a number of reasons. Microwave irradiation of soils releases less amino acids and carbohydrates than other methods, with little or no effect on the concentration of mineral nutrients (Ferriss, 1984). Microwave irradiation acts effectively to destroy the infectivity of VA mycorrhizal fungi and some other soil fungi

(Ferriss, 1984; Gibson, Fox & Deacon, 1988) while leaving many soil bacteria alive. Some populations of soil fungi can be re-established if microwave-irradiated soils are left uncovered for several days (Clapperton & Parkinson, unpublished). The use of such partially sterilized soil as diluent soil permits comparisons between the growth of mycorrhizal and non-mycorrhizal plants while subject to the influence of other soil microorganisms. There is good evidence that VA mycorrhizal fungi interact with other soil organisms in the mycorrhizosphere (Meyer & Linderman, 1986 a, b; Hetrick *et al.* 1988; Paulitz & Linderman, 1990) and that these organisms influence the plant response to colonisation by VA mycorrhizal fungi.

Most studies have concentrated on the rewards and penalties afforded plants by VA mycorrhizal fungi. However, there are also examples of studies of effects of plants on colonisation by VA mycorrhizal fungi. Nelson (1987) suggested that a positive correlation between plant biomass and fungal spore numbers in response to moisture availability was plant-mediated by changes in plant size and the availability of photosynthates. Colonisation appears to be affected indirectly by other factors which alter plant productivity. For example low light intensity (Daft & El-Giahmi, 1978), decreased photoperiod (Johnson *et al.*, 1982), defoliation (Daft & El-Giahmi, 1978; Wallace, 1981; Bethlenfalvay & Dakessian, 1984), and ozone (McCool & Menge, 1983) and sulphur dioxide (SO_2) exposure (Rice *et al.*,

1979) have all been shown to affect colonisation by VA mycorrhizal fungi. It has been suggested that the effects of ozone and SO_2 on plant assimilate partitioning affect the ability of VA mycorrhizal fungi to colonise and proliferate within plant roots (McCool, 1988). Indeed, estimates of the carbon cost to the plant for maintaining VA mycorrhizal symbiosis range between 4 and 20 percent of the total photoassimilate (Pang & Paul, 1980; Bethlenfalvay *et al.*, 1982; Jakobsen & Rosendahl, 1990).

In the second part of my study (Chapters 2 & 3) I used the gaseous air pollutant SO_2, which is known to affect assimilate partitioning and translocation to roots, as a tool to modify plant growth and assimilate partitioning, and determined consequent effects on colonisation by VA mycorrhizal fungi.

The effects of SO_2 on plant physiological processes can vary depending on the plant species, concentration and duration of SO_2 exposure, and the environmental conditions during exposure. The stomatal pore is thought to be the major pathway for the uptake of gaseous SO_2 (Spedding, 1969). The stomata may be induced to either open or close depending on the SO_2 exposure concentration and plant species (Majernik & Mansfield, 1970; Unsworth, Biscoe & Pinckney, 1972; Black & Unsworth, 1980; Taylor, Reid & Pharis, 1981). Inhibition of photosynthesis is one of the first effects of SO_2 on plant physiology. Photosynthesis is inhibited initially by the accumulation of sulphate, sulphite, and their hydrated forms within the leaf stomatal cavity (Ziegler, 1972; Anderson,

Muschinek & Marques, 1988). This leads to structural damage within the plastids and particularly in the thylakoids (Wellburn *et al.*, 1972; Wellburn, 1985; Shimazaki, 1988). Long-term SO_2 exposure then ultimately leads to reduced dry matter production (Bell & Clough, 1978; Ashendon, 1979), primarily associated with the roots (Jones & Mansfield, 1982 a,b; Mansfield & Jones, 1985). These losses in dry weight are likely a consequence of disrupted photosynthesis coupled with changes in the assimilate translocation processes (Noyes, 1980; Teh & Swanson, 1982; Minchin & Gould, 1986; Gould, Minchin & Young, 1988).

In light of the effects of SO_2 fumigation on plant photosynthesis, and on assimilate translocation and partitioning, and of the carbon requirements of VA mycorrhizal fungi, it is likely that there is a compounding adverse effect of SO_2 on such symbiotic associations. This hypothesis was tested in the experiments reported in chapters 2 & 3. Objectives of this part of my study were to examine the effects of low-concentration SO_2 exposure of *Phleum pratense* on ^{14}C-labelled photoassimilate partitioning and the ability of VA mycorrhizal fungi to colonise, and proliferate in, the roots.

An important feature of my study was the use of SO_2 fumigation chambers designed and constructed to fumigate only the shoots of plants, thus avoiding any direct effects of SO_2 on soil microorganisms and VA mycorrhizal fungi. I used ^{14}C-radioactive labelling to determine the assimilate

partitioning patterns and the proportion of assimilate as amino acids, soluble carbohydrates and organic acids in SO_2-fumigated and 'control', mycorrhizal and non-mycorrhizal plants. Once again I used a host and inoculum (field soil) combination which were naturally associated. The non-mycorrhizal soil was the same field soil but which had been microwave-irradiated. In all cases I endeavoured to maintain experimental conditions which were as close as possible to those of the sub-montane mixed grass prairie where the grass species and soil in my experiments originated. The concentrations of SO_2 used in this study were similar to those experienced at field sites in southern Alberta which are exposed to SO_2 from sour gas processing plants.

There has been a great emphasis placed on the need for field studies in order to increase our understanding of how organisms function in 'natural' ecosystems. Clapperton & Parkinson (1990) have described three field sites located in a sub-montane mixed grass prairie in southern Alberta which had similar vegetation and soils. The two field sites used in the present study and referred to as sites 1 and 2, were described field sites 1 and 3 (respectively) in Clapperton & Parkinson (1990). The sour gas processing plant which is the SO_2 point source in the vicinity of the field sites has operated for approximately 28 years. Populations of grasses which have been exposed to low-concentrations of SO_2 for at least 20 years are known to have developed tolerance to SO_2 exposure (Ayazloo & Bell, 1981).

Grasses from areas subject to chronic low-concentration SO_2 exposure often have decreased rates of senescence, lower shoot and root weights and lower root:shoot ratios than grasses from unpolluted areas (Bell & Clough, 1973; Ayazloo & Bell, 1981; Ayazloo, Garsed & Bell, 1982). These results suggest that assimilate translocating and partitioning mechanisms may be less affected by chronic SO_2 exposure in tolerant than in non-tolerant plants. Therefore, it is possible that soil- and root- inhabiting microorganisms may also be affected by plant tolerance, and in turn influence the process of tolerance. My objectives for examining tolerance to SO_2 were to identify SO_2-tolerant and non-tolerant genotypes of *Phleum pratense* collected from the field sites, and then to determine the effects of low-concentration SO_2 fumigation on growth and assimilate partitioning in the deemed SO_2-tolerant and non-tolerant genotypes.

The screenings for SO_2 tolerance were conducted in the SO_2 fumigation chambers on tillers from genotypes of *Phleum pratense* which had been collected at random from both the field sites. The criteria used to screen plants for tolerance were based on plant dry matter production, and were similar to those described by Ayazloo & Bell (1981). The deemed SO_2 tolerant and non-tolerant genotypes were then used in a two-year field study (Chapter 5) in which the growth and VA mycorrhizal colonisation of *Phleum pratense* genotypes were compared for polluted and unpolluted field sites over two growing seasons.

The use of field plots along pollution concentration gradients from gaseous air pollution point sources has the advantage over laboratory and field studies using open-top chambers in that there are no modifications to environmental conditions and, abiotic and biotic factors (Colvill *et al.*, 1985). Abiotic and biotic factors which act with SO_2 to modify the plant response in the field are; drought (Lucas, 1990), grazing (Lauenroth *et al.*, 1984), light intensity and photoperiod (Davies, 1980; Jones & Mansfield, 1982a), associations with microorganisms (Heagle, 1973; McLeod *et al.*, 1988), nitrogen nutrition (c.f. Winner *et al.*, 1985), and temperature (Jones & Mansfield, 1982a).

Studies of plant growth around known SO_2 sources could be valuable for comparing plant sensitivity to SO_2 exposure (Karnosky, 1985). There is also the need for more information on the interaction of SO_2 effects and grazing, trampling and climatic factors on plant growth under field conditions (Bell, 1985). In keeping with these suggestions I designed a 2-year field study with the objective of comparing the growth and colonisation by VA mycorrhizal fungi in SO_2-tolerant and non-tolerant genotypes and in seedlings from commercial seed of *Phleum pratense* at two field sites. The one field site was exposed to SO_2 and the other was a reference site with no higher than expected ambient concentrations of SO_2.

It has become increasingly apparent that there is a need for more interdisciplinary studies in the biological sciences. The contributions of soil microorganisms, especially organisms

in the rhizosphere and/or mycorrhizosphere, to plant growth are only now being fully appreciated.

CHAPTER 1: A Study of the Relationship Between Plant Growth and Colonisation by VA Mycorrhizal Fungi.

INTRODUCTION

VA mycorrhizal symbiosis is most often associated with improved plant growth. Plants associated with vesicular-arbuscular (VA) mycorrhizas are known to have improved inorganic nutrition (Cooper, 1984), greater rates of photosynthesis (Allen *et al.*, 1981), and improved resistance to drought (c.f. Nelson, 1987) and pathogens (Graham & Menge, 1982). The host plant maintains the fungi by providing assimilated carbon compounds (Ho & Trappe, 1973).

However, in some cases plant growth depression has been attributed to VA mycorrhizal symbiosis (Buwalda & Goh, 1982; Koide, 1985). This lack of positive response to VA mycorrhizal colonisation has been attributed to root system architecture (Baylis, 1970), and high concentrations of available inorganic nutrients (Buwalda & Goh, 1982; Anderson & Liberta, 1989). In both these cases infection is inhibited, and in the latter, the symbiosis can also become non-functional and an energy drain on the host. Koide (1985) has reported a negative correlation between the degree of VA mycorrhizal colonisation and leaf area.

In preliminary studies using the most probable number method (MPN) (Alexander, 1982; Wilson & Trinick, 1982) to

examine the VA mycorrhizal inoculum density of two prairie soils, I noticed a negative correlation between plant growth and inoculum density. This led me to conduct the study I present in this chapter. My objective was to examine the relationship between VA mycorrhizal inoculum density and plant growth.

In this chapter I have examined the effects of field soil dilution (and hence decreasing VA mycorrhizal inoculum density) and the application of increased fertilizer, on the growth of *Phleum pratense* L.. Since it is possible that any observed plant growth effects could be host species specific, included in my study was a second host grass species, *Agropyron trachycaulum* (Linke) Malte.. Unlike *P. pratense*, *A. trachycaulum* was not native to the field sites where the VA mycorrhizal inoculum for this study was collected. Therefore, differences in VA mycorrhizal colonisation patterns and percentage of root colonised by VA mycorrhizal fungi between the two different host grasses would be expected. It has been shown that percent VA mycorrhizal infection, intensity of infection, sporulation, and the extent of extra-matrical hyphae are greatest for a host/ VA mycorrhizal inoculum/ diluent soil combination which have the same origins (Adelman & Morton, 1986). Abbott & Robson (1982) concluded that in general there was a relationship between the number of spores and the development of infection for individual VA mycorrhizal-forming fungi. Indeed, if there is a relationship between the degree of VA mycorrhizal

colonisation and the degree of plant growth stimulation or depression then this relationship will likely be effected by changes in VA mycorrhizal inoculum density.

MATERIALS AND METHODS

VA mycorrhizal inoculum

The VA mycorrhizal inoculum used in these experiments was soil from a sub-montane mixed grass prairie in southern Alberta, Canada (Clapperton & Parkinson, 1990). The soil was black chernozemic sandy-silty loam. It was collected from the top 21 cm, sieved (2mm mesh), and stored for two weeks at 4°C in sealed, air-filled polyethylene bags prior to use. Soil nutrient analysis (Alberta Environment, Earth Sciences Division, Lethbridge, Alberta) showed the soil contained 2.3 mg kg^{-1} phosphorus, 18.1 mg kg^{-1} available nitrogen, 28.0 mequiv $100g^{-1}$ calcium, 5.3 mequiv $100g^{-1}$ magnesium, and 8.9 percent organic matter. The pH of the soil was 5.91 as determined by saturated paste.

The VA mycorrhizal fungi present in the soil were *Glomus fasiculatum* Thaxter sensu Gerd., *Glomus macrocarpum* Tul. and Tul., var. macrocarpum, *Glomus microcarpum* Tul. and Tul., *Glomus mosseae*, and a species of *Gigaspora*. There were 13.7 spores g^{-1} soil, and the most probable number of infective propagules (Fisher & Yates, 1963) as estimated from unfertilized *Phleum pratense* was 25.7 g^{-1} soil.

The soil used for control (non-mycorrhizal) plants was a subsample of the soil used as inoculum. This soil had been microwave-irradiated to a final temperature of 80°C. Next the soil was covered and allowed to cool to room temperature. Soil was then remoistened and allowed to rest uncovered for two weeks before being used in the experiments. Microwave irradiation is a method for partial soil sterilization which has been shown to release less amino acids and carbohydrates than other methods, with little or no effect on the concentration of mineral nutrients (Ferris, 1984). The most significant effect of microwave irradiation on soil is in reducing the population of microorganisms (Ferris 1984; Gibson, Fox, & Deacon, 1988).

Preparation of the dilution series

A series of five two-fold dilutions (w/w) of field soil were made with microwave-irradiated soil used as the diluent to give final concentrations of 0.5, 0.25, 0.125, 0.063, 0.031 g field soil g^{-1} total. Included with the dilution series were controls (only microwave-irradiated soil), and undiluted field soil. The soils for each dilution and control series were packed into 25ml syringe barrels. There were a total of thirty-five tubes per experiment, five replicates per dilution or control.

Effect of fertilizer and inoculum density of VA mycorrhizal fungi on Phleum pratense

Seeds of *Phleum pratense* were germinated on moistened filter paper for 7 d before planting. A 7 d-old seedling was planted into each of ten tubes for each dilution and controls, for a total of 70 tubes. Then five tubes from each dilution or control were selected at random to receive increased fertilizing. These plants were fertilized twice-weekly with Peter's Professional 20-10-20 fertilizer (Peter's Fertilizer Products, Pennsylvania) at a concentration of 0.25 g l^{-1} P, and were watered once per week with distilled water. The remaining five plants per dilution and control were watered three-times weekly with distilled water, and fertilized once every second week; these plants are referred to hereafter as unfertilized. In both treatments all tubes were watered or fertilized to saturation. Plants were grown in a chamber at temperatures held between 20°C and 25°C, at 300 µmol m^{-2} s^{-1} PAR, with a 16 h photoperiod. Plants were harvested after four weeks. At harvest, plants were removed from the tubes and the roots washed free of soil. The number of tillers and fully expanded leaves were counted before the shoots and roots were separated, weighed and dried at 80°C for 48h. The roots were then stained (Phillips & Hayman, 1970) and assessed for VA mycorrhizal colonisation using a modification (Zak & Parkinson, 1982) of the line-intercept method (Newman, 1966).

Effect of Field Soil Dilution on Agropyron trachycaulum (Malte) Link.

Seeds of *Agropyron trachycaulum* were germinated on moist filter papers for 9 d before planting. One seedling of *A. trachycaulum* was planted into each of five tubes per dilution or control. Plants were grown, watered, and fertilized as described for unfertilized *P. pratense*.

Statistics

All data were examined for normality using probability plots (Ryan, Joiner, & Ryan, 1976), before two way or one way analyses of variance were performed. All means were separated using Tukey's test for multiple comparisons (Zar, 1984). The data for length of root with VA mycorrhizal infection and percent VA mycorrhizal infection were transformed using the square root. Unless stated otherwise $\alpha=0.01$. Quadratic regression analysis was performed using field soil proportion and VA mycorrhizal colonisation, and VA mycorrhizal colonisation and shoot weight or root: shoots ratios as variables.

RESULTS

There appeared to be a positive correlation between the proportion of field soil and the percentage of colonisation by VA mycorrhizal fungi in all the grasses used in this study (Fig. 1). Indeed, it was determined using regression analysis (quadratics) that the percentage of colonisation by VA mycorrhizal fungi could be predicted (r^2 = 0.95 for fertilized and 1.0 unfertilized *P. pratense*, and 0.93 for *A. trachycaulum*) from the proportion of field soil present.

Effects of fertilizer and VA mycorrhizal inoculum density on Phleum pratense

As anticipated there was a significant effect of both fertilizer and dilution on VA mycorrhizal colonisation (Fig.1 & Table 1). In the unfertilized plants the percentage of the root colonised by VA mycorrhizal fungi increased with increasing proportions of field soil. The ability of VA mycorrhizal fungi to colonise the roots of *P. pratense* was severely inhibited by the fertilizer. There were also effects on the form of the colonisation; the percentages of hyphae and arbuscules in the roots changed with the application of fertilizer (Table 1). There were consistently fewer arbuscules formed in the roots of fertilized than in unfertilized *P. pratense*.

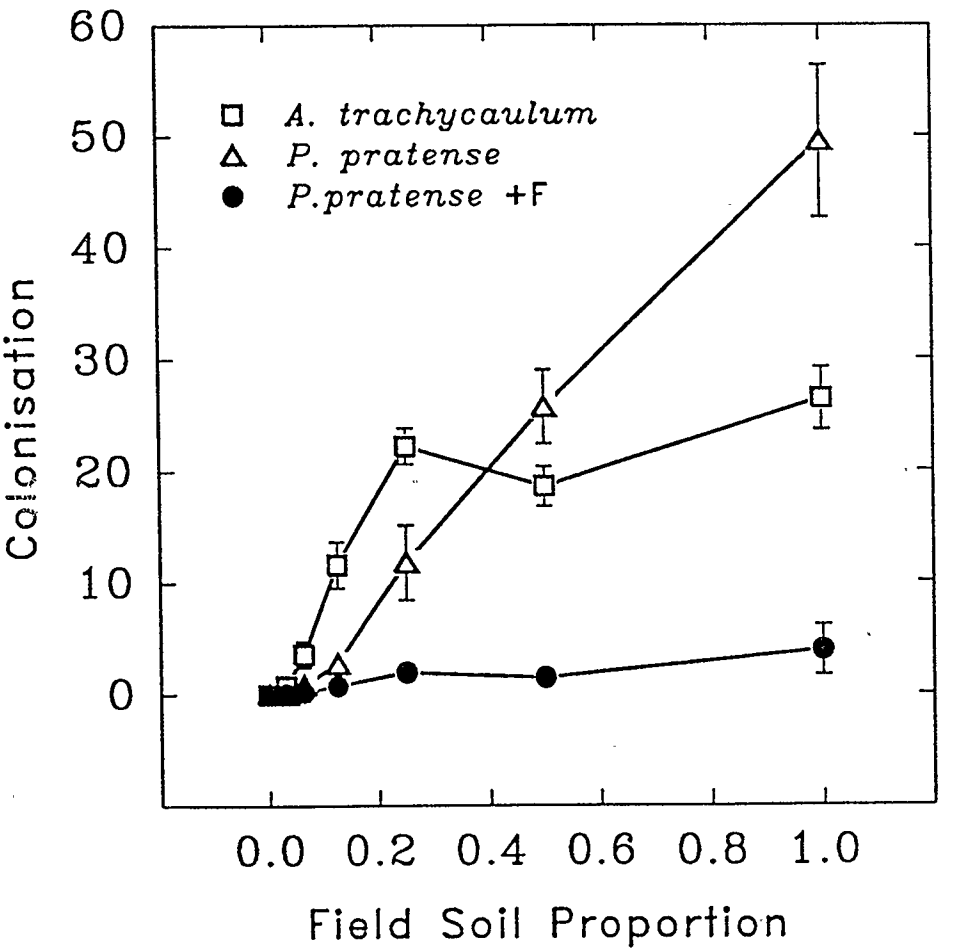

Figure 1. Mean percentage of root colonised by VA mycorrhizal fungi (± S.E.) for *Agropyron trachycaulum* and fertilized (F) and unfertilized *Phleum pratense* grown in increasing proportions of field soil.

Table 1. The percent of total VA mycorrhizal colonisation comprised of hyphae or arbuscules, and length of root colonised (cm) by VA mycorrhizal fungi in fertilized and unfertilized *Phleum pratense* at each dilution of field soil.

Treatment	Hyphae	Arbuscules	Colonised Root Length
No Dilution (1.0)			
Fertilized	71	29	9.3 E
Not Fertilized	24.3	75.7	113.2 B
0.5			
Fertilized	82.5	17.5	6.3 F
Not Fertilized	28.1	71.9	175.3 A
0.25			
Fertilized	70	30	7.7 EF
Not Fertilized	40.0	58.5	76.7 C
0.125			
Fertilized	67.6	51.4	3.7 G
Not Fertilized	43.7	56.3	14.2 D
0.063			
Fertilized	100	0.0	1.5 H
Not Fertilized	50.0	50.0	2.1 G
0.031			
Fertilized	0.0	0.0	0.0 H
Not Fertilized	0.0	0.0	0.0 H
Control (0.0)			
Fertilized	0.0	0.0	0.0 H
Not Fertilized	0.0	0.0	0.0 H

Differences between means in columns are not significant when followed by the same letter, $\alpha = 0.01$, 5 replicates.

There was a significant interaction (α=0.05) between fertilizer and the effect of soil dilution on the number of tillers produced per plant (Fig. 2). Fertilizing the plants appeared to improve the ability of the plants to produce tillers most notably at the 0.5 soil dilution. The number of tillers produced by unfertilized *P. pratense* seedlings showed a marked response to dilution. The number of tillers produced per plant was highest at the 0.125 dilution and in the control (microwave-irradiated) soil (Fig. 2).

The number of leaves per plant was significantly affected by both the fertilizer and soil dilutions (Fig. 2). Fertilized plants consistently had greater numbers of leaves per plant; this was particularly marked in plants grown in undiluted field soil.

The dry weight of the shoots responded significantly to both fertilizer and soil dilution. The shoot weights of both fertilized and unfertilized plants increased with increasing proportions of field soil up to the 0.5 soil dilution for fertilized and the 0.125 soil dilution for unfertilized *P. pratense* and then declined significantly (Fig. 3). Quadratic regression analysis showed that the percentage of colonisation by VA mycorrhizal fungi can be used to predict shoot dry weight in fertilized (r^2 = 0.89) and unfertilized *P. pratense* (r^2 = 0.99). Plants given fertilizer had consistently greater shoot dry weights than did unfertilized plants. The plants grown in undiluted field soil (1.0 g field soil g^{-1}) in both treatments had the lowest shoot and root dry weights (Fig. 3). However, the

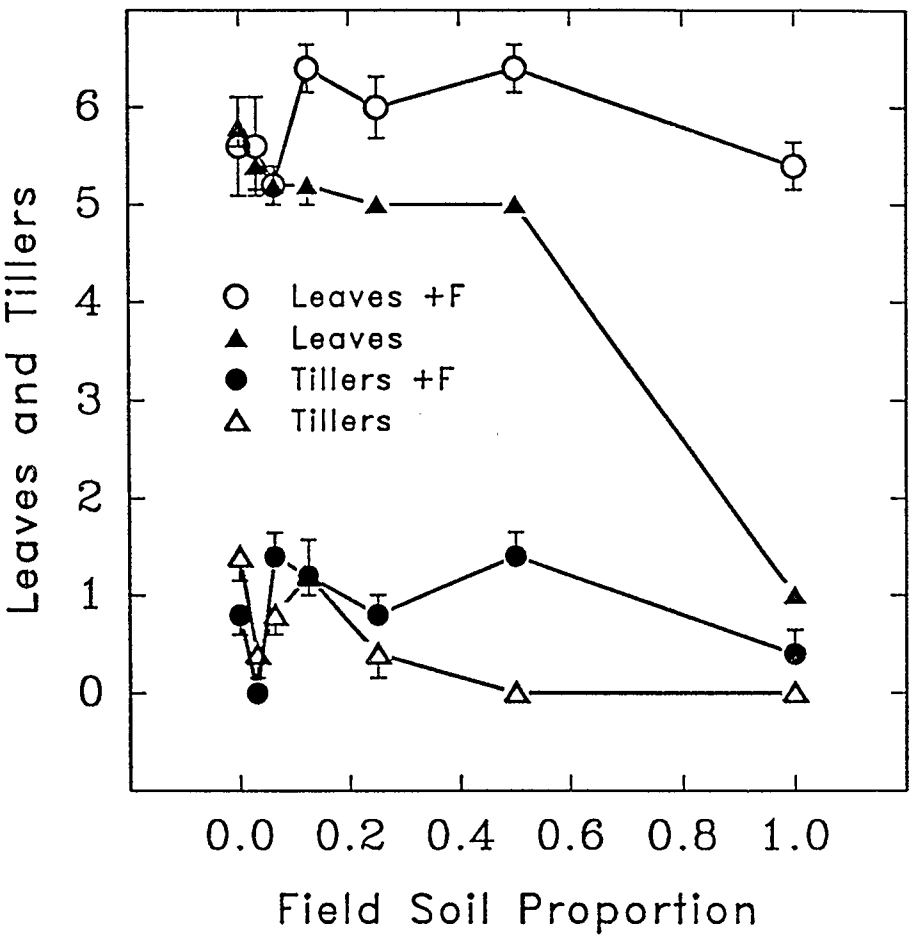

Figure 2. The effect of increasing field soil proportions on the mean number of leaves and tillers produced by fertilized (F) and unfertilized *Phleum pratense*.

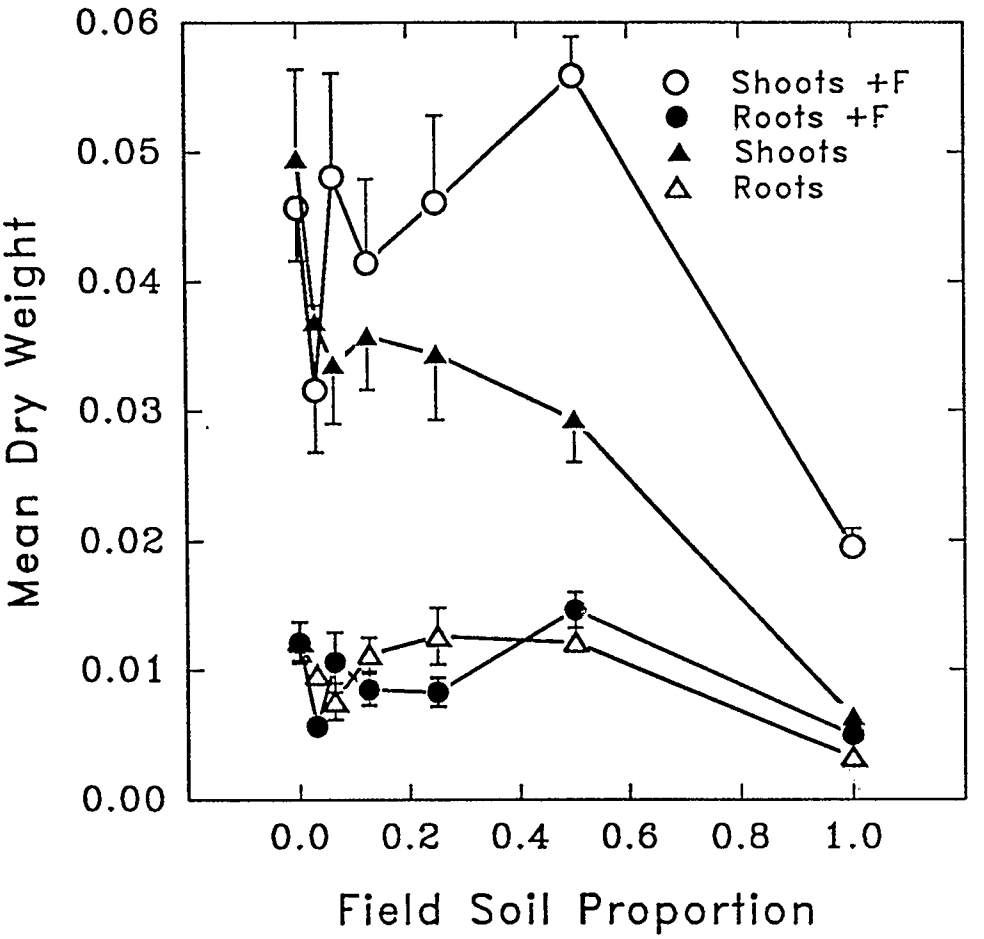

Figure 3. The effect of increasing proportions of field soil on the mean shoot and root dry weights (g) (± S.E.) of fertilized (F) and unfertilized *Phleum pratense*.

roots showed no significant effect of fertilizer. Root dry weight was greatest in the 0.5 soil dilution for fertilized plants and in the 0.250 soil dilution for unfertilized plants (Fig. 3).

The effect of VA mycorrhizal inoculum density on Agropyron trachycaulum

There was a significant effect of soil dilution on the percent (Fig. 1) and length of root (Table 2) colonised by VA mycorrhizal fungi. The percent colonisation as arbuscules was greater than that as hyphae at all dilutions (Table 2). Indeed, the percent colonisation as arbuscules tended to increase with increasing dilution of field soil. This represented a different pattern of VA mycorrhizal colonisation from that seen in unfertilized *P. pratense* (Table 1).

Growth of *A. trachycaulum* was significantly affected by increased proportions of field soil but not to the same extent as unfertilized *P. pratense*. The number of tillers and leaves produced per plant declined initially with increasing proportions of field soil, increased at the 0.125 dilution, and declined again thereafter (Fig. 4). Shoot dry weight and root dry weight (Fig. 5) followed the same pattern. It was determined using quadratic regression analysis that shoot dry weight in *A. trachycaulum* could be predicted ($r^2 = 0.92$) from the percentage of VA mycorrhizal colonisation.

Table 2. The percent of total VA mycorrhizal colonisation comprised of hyphae or arbuscules, and length of root (cm) colonised by VA mycorrhizal fungi in *Agropyron trachycaulum* at each dilution.

Dilution	Hyphae	Arbuscules	Colonised Root Length
No Dilution (1.0)	40.3	59.8	111.2 A
0.5	31.9	68.1	98.0 A
0.25	36.1	63.9	140.1 A
0.125	19.4	80.6	85.0 A
0.063	19.6	80.4	23.0 B
0.031	24.1	75.9	5.4 C
Control (0.0)	0.0	0.0	0.0 C

Differences between means in columns are not significant when followed by the same letter, $\alpha = 0.01$, 5 replicates.

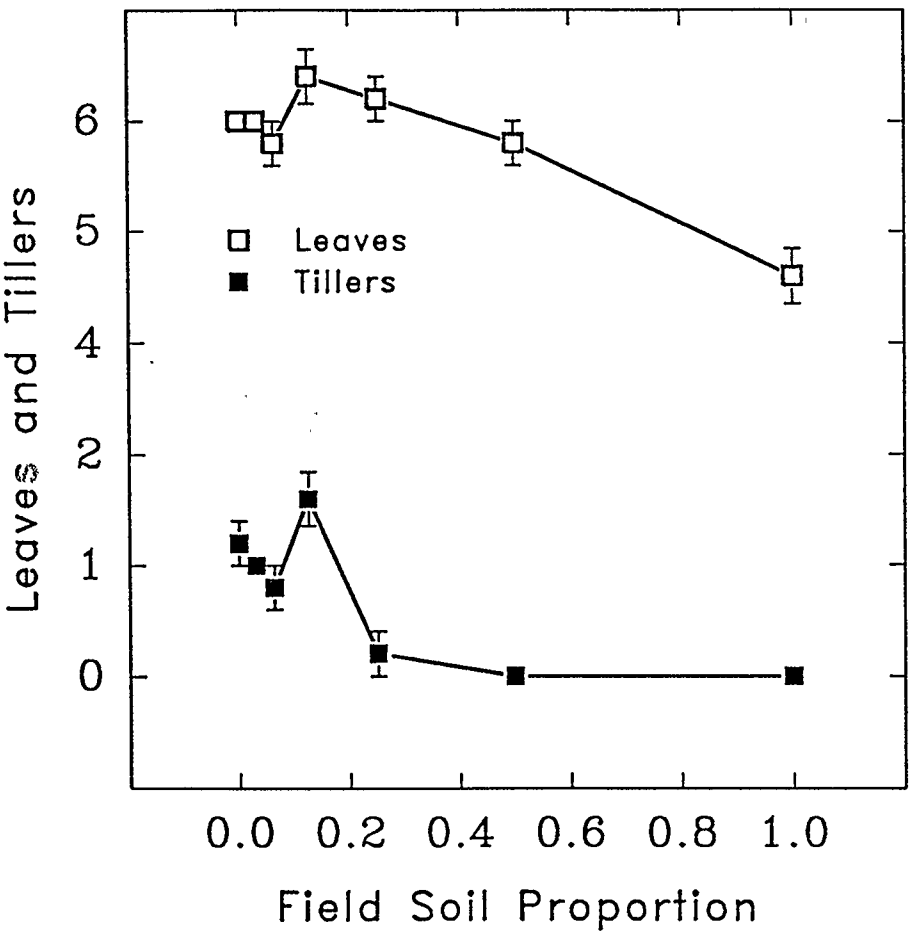

Figure 4. The effects of increasing proportions of field soil on the mean number of leaves and tillers (±S.E.) of *Agropyron trachycaulum*.

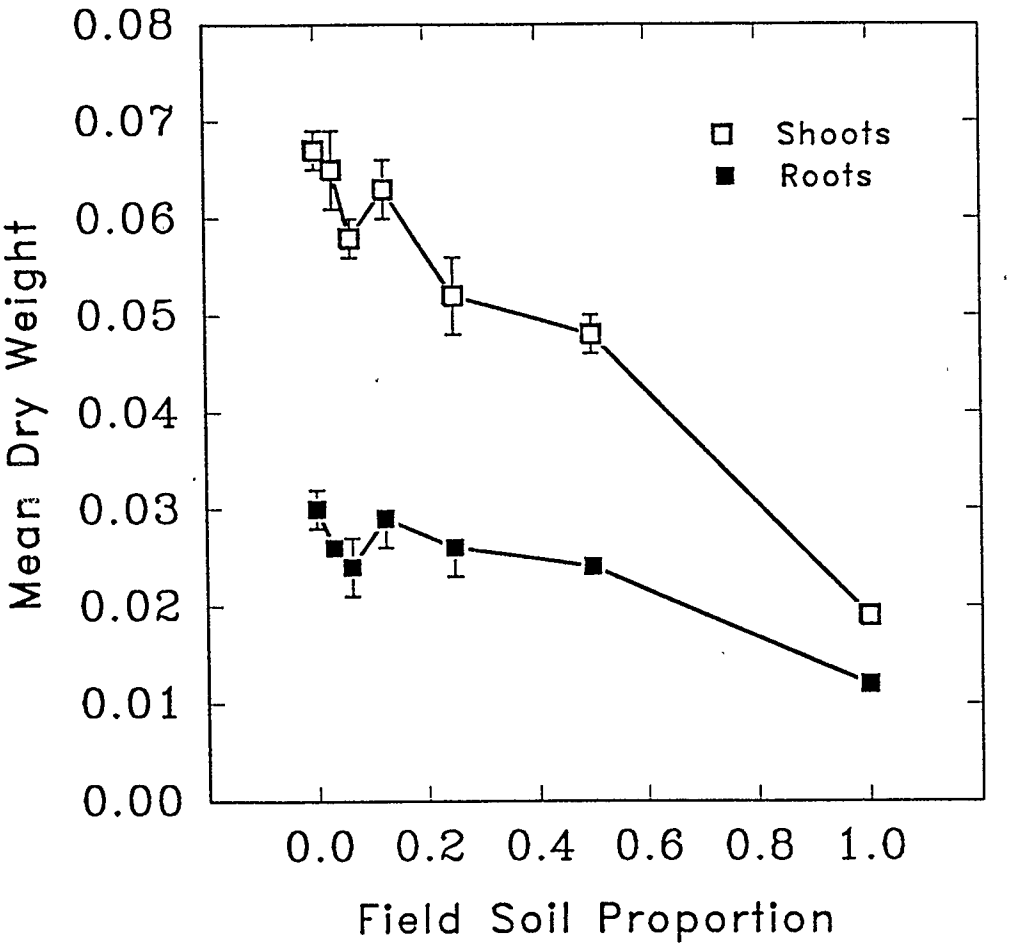

Figure 5. The effects of increasing proportions of field soil on the mean shoot and root dry weights (g) (± S.E.) of *Agropyron trachycaulum* .

Effect of field soil proportion and VA mycorrhizal fungi on root:shoot ratios

The root:shoot ratios for all grasses increased with increasing proportions of field soil (Fig. 6). Regression analysis using quadratic regression equations showed that there was a significant relationship between root:shoot ratios and VA mycorrhizal colonisation in fertilized (r^2 = 0.65) and unfertilized (r^2 = 0.99) *P. pratense* and *A. trachycaulum* (r^2 = 0.92).

Other experiments using microwave-irradiated soil

In these experiments plant growth was promoted in microwave-irradiated soil (control) (Figs. 3 & 5). The initial addition of non-sterile soil decreased the ability of the microwave-irradiated soil to promote plant growth. In other studies, from which the present was derived, I saw similar patterns of plant growth in response to increasing proportions of field soil (Fig. 7). The diluent soil in these studies was microwave-irradiated field soil from the same field site as described previously, and field soil from a similar field site. The diluent soils had been microwave-irradiated to a final temperature of 91°C for 40 min. The final temperature of the soil was higher and the duration of microwave-irradiation was longer than in the present study. In both these dilution series the growth of the control plants (0.0 g field soil g^{-1} diluent soil) was lower compared with plants grown in the initial proportions of field soil. Plant growth increased from

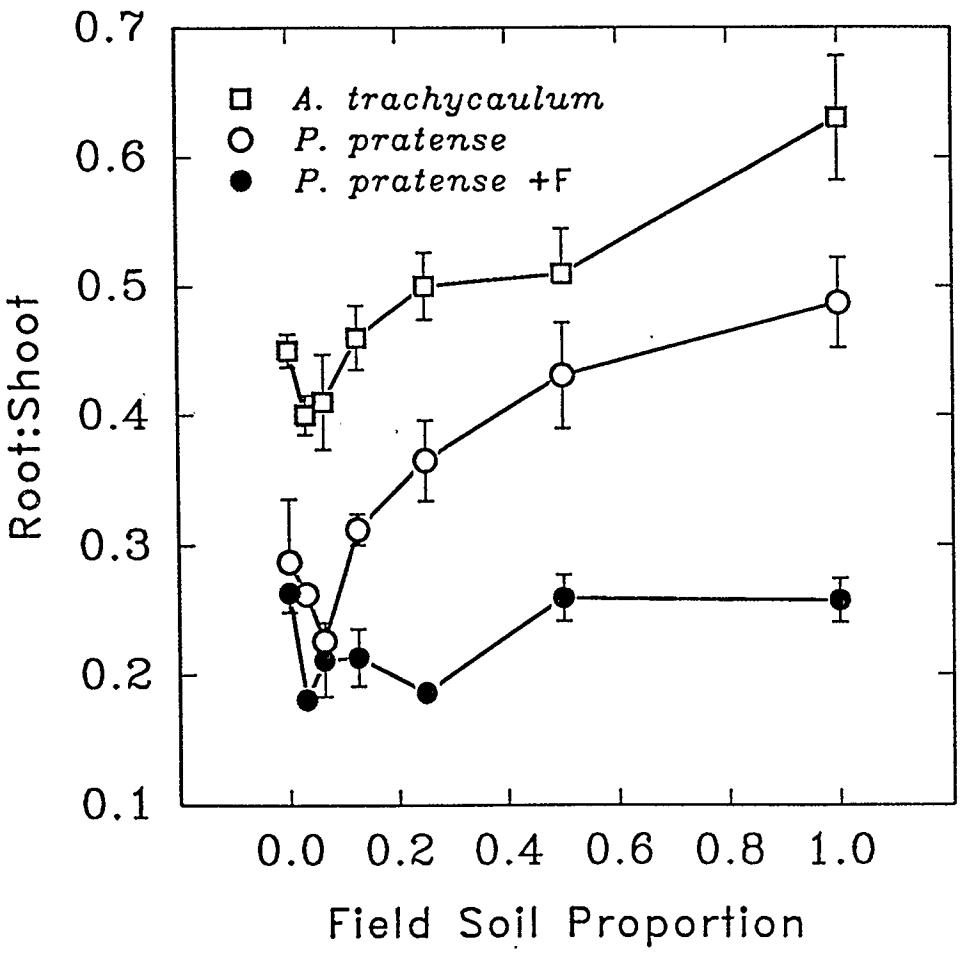

Figure 6. The effects of increasing proportions of field soil on the mean root:shoot ratio (± S.E.) of *Agropyron trachycaulum* and fertilized (F) and unfertilized *Phleum pratense.*

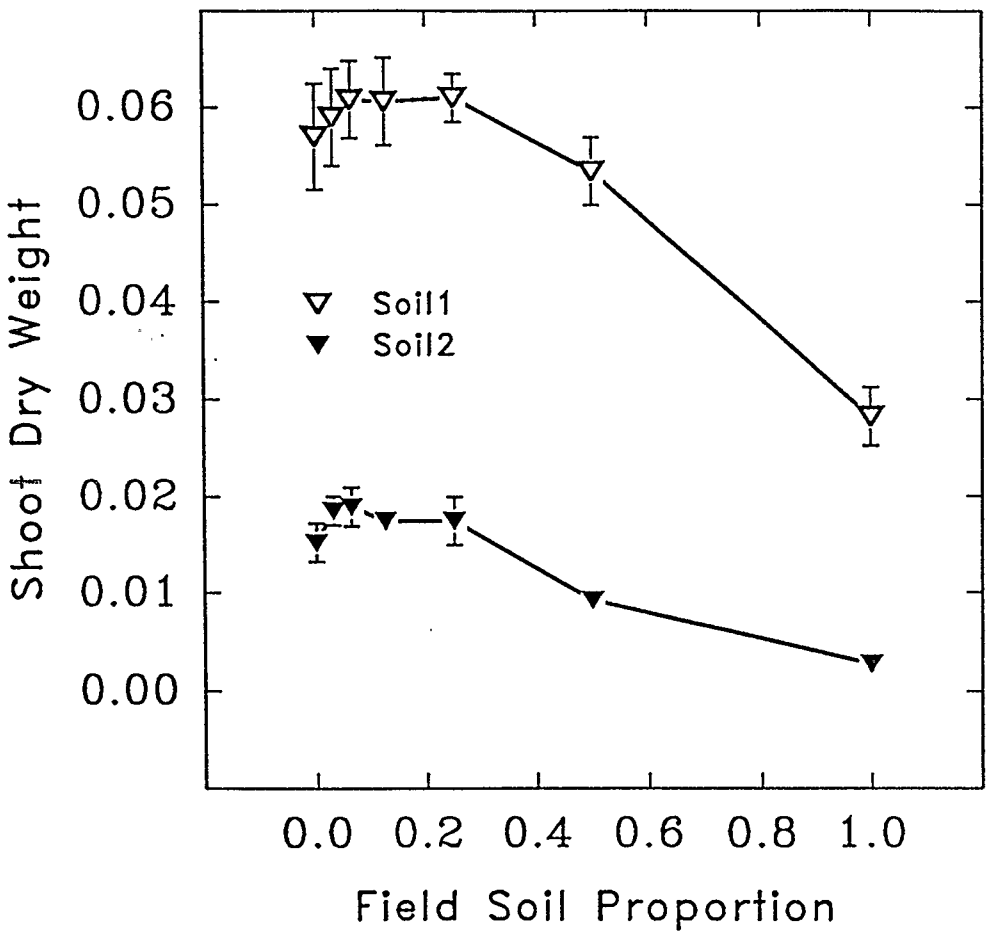

Figure 7. The effects of increasing proportions of field soil, diluted with microwave-irradiated field soil on the mean dry shoot weight (g) of *Phleum pratense*. Soil 1, microwave-irradiated soil supplemented with a field soil filtrate; Soil 2, soil from field site 1 diluted with microwave-irradiated site 1 soil and no added filtrate.

controls to a maximum at the 0.125 soil dilution and then significantly declined to the 1.0 g field soil g^{-1} soil. The addition of a soil filtrate (Paulitz & Linderman, 1989) to the diluent soil did not affect the pattern of plant growth in the controls or in increasing proportions of field soil.

DISCUSSION

The results show that there was a positive correlation between the proportion of field soil and the degree of VA mycorrhizal colonisation. However, the pattern of colonisation and the length of root colonised differed between treatments and grass species (Tables 1&2). The increased application of fertilizer inhibited the ability of VA mycorrhizal fungi to colonise the roots of *Phleum pratense* . It also apparently inhibited the ability of the fungi to form functional symbioses as there was a smaller proportion of arbuscules formed in the roots of fertilized *P. pratense* compared with unfertilized *P. pratense* (Table 1). The arbuscule is considered to be the primary site of nutrient exchange between the fungi and the plant (Bonfante-Fasolo, 1984). Hence, the presence of arbuscules can be considered an important measure of the amount of exchange and functioning of a host/ VA mycorrhizal symbiosis.

Hetrick, Kitt & Wilson (1986) suggested that higher available phosphorous (P) concentrations in soil reduce the

need for colonisation by VA mycorrhizal fungi. Anderson & Liberta (1989) showed that there was a close link between soil inorganic nutrients, especially P, and the ability of VA mycorrhizal fungi to form mutually beneficial associations. Certainly, the results of my study are consistent with the results of these studies. However, in my study plant growth was negatively correlated with increased VA mycorrhizal colonisation.

Plant dry weight decreased initially with increasing field soil proportion and then increased slightly at the 0.125 soil dilution for unfertilized *P. pratense* and increased up to the 0.5 soil dilution for fertilized *P. pratense* (Fig. 3) and significantly declined thereafter. The numbers of tillers and leaves showed the same trend (Fig. 2). This transient increase in plant growth coincided with the onset of the development of significant mycorrhizal colonisation (Fig 1; Table 1) and thus might have resulted from a promotive effect of VA mycorrhizal fungi. However, any promotive effect was apparently masked by a negative influence of the increasing proportions of field soil. Evidence for this negative influence is seen in the experiment using *Agropyron trachycaulum*, where plant growth steadily declined (Fig. 5) even after there was no further increase in VA mycorrhizal colonisation. Thus, it appeared that some constituent (other than VA mycorrhizal fungi) in the field soil was inhibiting plant growth.

Since there was little effect of fertilizer ($\alpha = 0.01$) on the growth of *P. pratense* in the microwave-irradiated soil (0.0 g field soil g^{-1} soil) and in lower proportions of field soil, it appeared that the positive effect of fertilizer on plant growth in the higher proportions of field soil was not due directly to the provision of mineral nutrients to the plant, but indirectly through the mitigation of the negative influence of the field soil on plant growth. Other studies have shown plant growth inhibition when plants are grown in non-sterile soil compared with plants grown in sterile soil (Hetrick, Kitt & Wilson, 1986; Anderson & Liberta, 1989). Some of these studies have shown that fertilizer application and particularly the addition of phosphorus can increase the growth of plants in non-sterile soil (Hetrick *et al.*, 1988; Baas, 1990), prompting the suggestion that microbial competition for, and immobilisation of, nutrients was involved in the plant response to non-sterile soil (Hetrick *et al.*, 1988). Baas, Van Dijk, & Troelstra (1989) suggested that VA mycorrhizal fungi may compensate for phosphorus immobilisation by other rhizosphere organisms and increase phosphorus uptake, although enhanced plant growth was not always obvious. However, other rhizosphere organisms are also known to increase phosphorus mobilisation in the presence of VA mycorrhizal fungi (Kucey, 1987). Indeed, Fyson & Oaks (1990) suggested that although VA mycorrhizal fungi were associated with increased plant growth in the presence of rhizosphere soil, it was not possible to rule-out involvement

by other organisms, particularly other fungi. In another study, Hetrick *et al.* (1988) suggested that fungi, more than bacteria, were responsible for non-mycorrhizal plant growth stimulation in non-sterile soil.

It has been shown that root colonisation by VA mycorrhizal fungi can affect the populations of rhizobacteria in the mycorrhizosphere (Ames, Reid, & Ingham, 1984; Meyer & Linderman, 1986a; Paulitz & Linderman, 1989). So, I suggest that there is a point or a field soil dilution at which the plant, VA mycorrhizal fungi, and other rhizosphere organisms reach an equilibrium which results in improved plant growth. The point at which this equilibrium occurs appears to be affected by the amount of available nutrients. In other studies it has been shown that mixtures of organisms often promote plant growth better than plants inoculated with only one of the organisms. VA mycorrhizal/*Rhizobium*/ or plant growth promoting rhizobacteria (PGPR) symbioses significantly improve plant growth compared with plant growth after inoculation with either VA mycorrhizal fungi, *Rhizobium*, or PGPR alone (Bagyaraj, 1984; Meyer & Linderman, 1986b). Analogous studies with PGPR have shown that species specific populations in the rhizosphere (Fallik, Okon, & Fischer, 1988; Holl *et al.*, 1988) and low to moderate nitrogen fertilizer regimes are often required to promote plant growth.

It was not the intent of this study to examine the effect of partially sterilized soil on plant growth. However, the plants

grown in microwave-irradiated soil (controls) in my study were of comparable size with the larger plants in the higher series of dilutions. Promotion of plant growth in microwave-irradiated soil has previously been reported (Ferriss, 1984; Gibson, Fox, & Deacon, 1988). Indeed, Gibson, Fox, & Deacon (1988) suggested that many plant soil-borne parasites are heat-sensitive, and as a consequence of microwave irradiation can be reduced to less competitive soil populations, thus promoting plant growth. Partial soil sterilization with microwave irradiation tends to have a devastating effect on fungal populations (Ferriss, 1984; Speir *et al.*, 1986; Gibson, Fox, & Deacon, 1988), leaving the more heat-tolerant bacteria alive. In my study I left the partially sterilized soil saturated to field capacity and uncovered on a bench at room temperature for two weeks to increase fungal populations in the soil (Clapperton & Parkinson, unpublished). Fungal populations in non-sterile soils have been linked to plant growth promotion in field soils (Hetrick *et al.*, 1988; Koide & Li, 1989).

Partial soil sterilization using microwave irradiation is perhaps the method which best maintains the integrity of soil inorganic and organic nutrient status but disrupts the soil micro-flora and -fauna components (Ferriss, 1984). Thus, it should be recognised that comparisons of plant growth in partially sterilized and field soils were made with the assumption that there was no contribution to plant growth by soil organisms other than the VA mycorrhizal fungi. In light

of the field soil dilution experiments, it appears that this was an incorrect assumption. In my opinion the use of microwave-irradiated soils represented the least expensive and best compromise for the field situation for examining the contribution of VA mycorrhizal fungi to plant growth. Statements as to the effects of VA mycorrhizal fungi on plant growth in my study have been made in light of the potential contribution of other soil organisms to plant growth. However, I believe that I have used a more 'natural' non-mycorrhizal soil in using microwave-irradiated field soil for comparison of the effects of VA mycorrhizal fungi on plant growth.

The results of this study indicate that there might be little host species specificity with regard to plant growth in response to increasing proportions of field soil. The growth response pattern of the host to increasing proportions of field soil appeared to be similar for both *A. trachycaulum* (Fig. 5) and unfertilized *P. pratense* (Fig. 3); however, the pattern of VA mycorrhizal infection was different (Fig. 1; Tables 1&2). At the 0.125 soil dilution both the percent and length of root infected with VA mycorrhizal fungi was similar for both *A. trachycaulum* and unfertilized *P. pratense*. However, the percentage of infection as arbuscules or hyphae differed significantly between the two species. This suggested that different species of VA mycorrhizal fungi might have predominated in the colonisation of *A. trachycaulum* and *P. pratense*, or that host genetic differences allowed for a different pattern of VA mycorrhizal colonisation.

Nevertheless, the pattern of plant growth promotion over the series of field soil dilutions and in the control soil was similar for both host species. Clapperton, Pearce, & Pharis (unpublished) have seen similar effects on plant growth in response to VA mycorrhizal inoculum dilution in *Zea mays* using inoculum from pot cultures of *Glomus aggregatum* and field soil.

The mechanisms by which increasing proportions of field soil inoculum affect plant growth are not inherently obvious. However, evidence suggests that there is a relationship between assimilate partitioning to the roots and shoots and the concentration of field soil inoculum, and that root colonising organisms were most likely to have effected the changes in assimilate partitioning. Indeed, as the proportion of field soil increased so did the colonisation by VA mycorrhizal fungi, and so did the root:shoot ratios. Studies have shown that VA mycorrhizal plants translocate more carbon to the roots than non-mycorrhizal plants (Kucey & Paul, 1982; Douds, Johnson & Koch, 1988; Wang *et al.*, 1989). The fertilizer appeared to inhibit colonisation and the formation of arbuscules by VA mycorrhizal fungi. Consequently, the root:shoot ratios of fertilized *P. pratense* remained lower and unaffected by increasing proportions of field soil. This supports the suggestion that colonisation by VA mycorrhizal fungi was affecting the partitioning of assimilate to the roots (see Chapter 3). This is further supported by the lack of significant differences in the

number of tillers produced by fertilized *P. pratense* and the decreasing number of tillers produced by unfertilized *P. pratense* with increasing proportions of field soil (Fig. 2). Tiller production is directly linked to photoassimilate availability (Aspinall, 1961).

CONCLUSIONS

The increased availability of nutrients from the added fertilizer inhibited the ability of VA mycorrhizal fungi to colonise and form a functional symbiosis in the roots. However it was likely that the overall increased plant growth of fertilized *P. pratense* was a result of the increased availability of nutrients to the plant due to decreased microbial competition for nutrients in all proportions of field soil.

It is likely that VA mycorrhizal colonisation can be used as a measureable indicator of factors which affect the mycorrhizosphere, as well as those factors which affect plant carbon partitioning and assimilation.

Experiments using anti-fungal and bacterial agents as treatments over a field soil dilution series would examine the relative contributions of fungi and bacteria to plant growth at increasing proportions field soil. It would also be necessary to identify organisms present in the diluent soil before conducting further experiments.

CHAPTER 2: Effects of Sulphur Dioxide Fumigation on Colonisation by VA Mycorrhizal Fungi and Growth of *Phleum pratense* .

INTRODUCTION

Sulphur dioxide (SO_2) is a well known aerial pollutant. It is a highly reactive gas which by itself or combined with other elements acts to damage terrestrial ecosystems. In most terrestrial ecosystems vegetation effectively removes large quantities of sulphur dioxide (SO_2) by dissolution in water on the leaves and other plant surfaces (Fowler & Unsworth, 1974) or by absorption through stomata (Majernik & Mansfield, 1970). Therefore, in undisturbed ecosystems it is quite possible that any effects of SO_2 exposure on soil microorganisms and especially microorganisms associated with the mycorrhizosphere (Linderman, 1988) would presumably be indirect, or plant-mediated.

Continuous exposure of plants to low concentrations of SO_2 is known to ultimately reduce dry matter production (Bell & Clough, 1973), with initial losses in dry weight primarily associated with roots of SO_2 fumigated plants (Jones & Mansfield, 1982a). These losses in dry weight are likely as a consequence of disrupted photosynthesis (Black, 1982) coupled with changes in assimilate translocation processes (Noyes, 1980).

There is a correlation between the amount of vesicular-arbuscular (VA) mycorrhizal colonisation and the amount of

carbohydrate in and exuded from host roots (Menge *et al.*, 1978; Ratnayake, Leonard & Menge, 1978; Graham, Leonard & Menge, 1981). Host plants are known to translocate carbohydrate to VA mycorrhizal symbionts (Ho & Trappe, 1973), prompting the suggestion that VA mycorrhizal colonisation may be affected indirectly by factors which alter host photosynthesis (Cooper, 1984). Therefore, it is possible that VA mycorrhizas are affected by the changes in host plants which have been exposed to SO_2. Indeed, Rice *et al.* (1978) using an open-air fumigation system, reported reduced VA mycorrhizal colonisation in *Agropyron smithii* with increasing SO_2 concentration. In a preliminary study Clapperton and Parkinson (1990) determined that there was a lower VA mycorrhiza fungal inoculum potential at two prairie field sites which had been continuously exposed to low concentrations of SO_2 compared than at a control field site.

I have examined the effect of continuous low-concentration SO_2 exposure of *Phleum pratense* shoots on the ability of VA mycorrhizal fungi to colonise and proliferate in roots. An important feature of this study was the use of SO_2 fumigation chambers which allowed only the shoots of plants inside the chamber to be fumigated. Therefore, only the effects of SO_2 on the plants and its subsequent effect on VA mycorrhizal fungi were studied. Because I compared the effect of SO_2 on both VA mycorrhizal and non-mycorrhizal plants I was also able to more directly examine the effects of SO_2 on VA mycorrhizal infection and plant growth. In

Experiment 1 I examined the effect of SO_2 fumigation of *Phleum pratense* shoots on the ability of VA mycorrhizas to infect the roots. In Experiment 2 I examined the effect of SO_2 fumigation of *Phleum pratense* shoots on the proliferation of VA mycorrhizal fungi within the roots.

MATERIALS AND METHODS

Fumigation Chambers

Both of the experiments in this study were conducted in specially designed, controlled-environment fumigation chambers (Fig. 8 & 9). The chambers were constructed from perspex which was lined with fluorinated ethylene propylene (FEP) teflon. Air was circulated from the upper air flow plenum, creating a laminar-type flow over the plants to the lower air flow plenum which funneled the air around each individual plant to the shoot/soil interface and then back through the air temperature control module.

The environment inside the chambers was controlled and monitored by a microprocessor-based controller (D. Savage, unpublished). The desired concentration of SO_2 was obtained by combining a known flow rate of SO_2 with a known flow rate of SO_2-free air. The SO_2 concentration was measured at the inlet, outlet, and root chamber outlet of the chamber using a Monitor Labs 8850 Influorescent SO_2 Analyser (Monitor Labs Inc., San Diego, California). The pneumatics of the

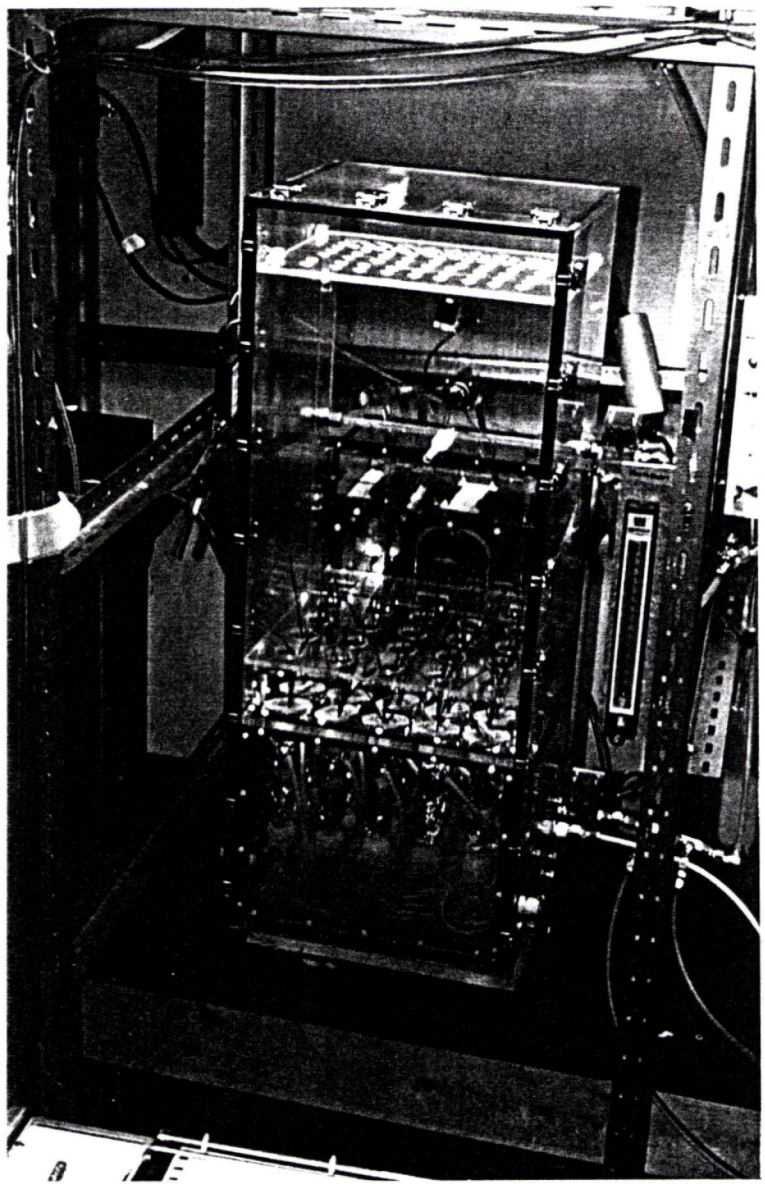

Figure 8. A photograph of one of the fumigation chambers.

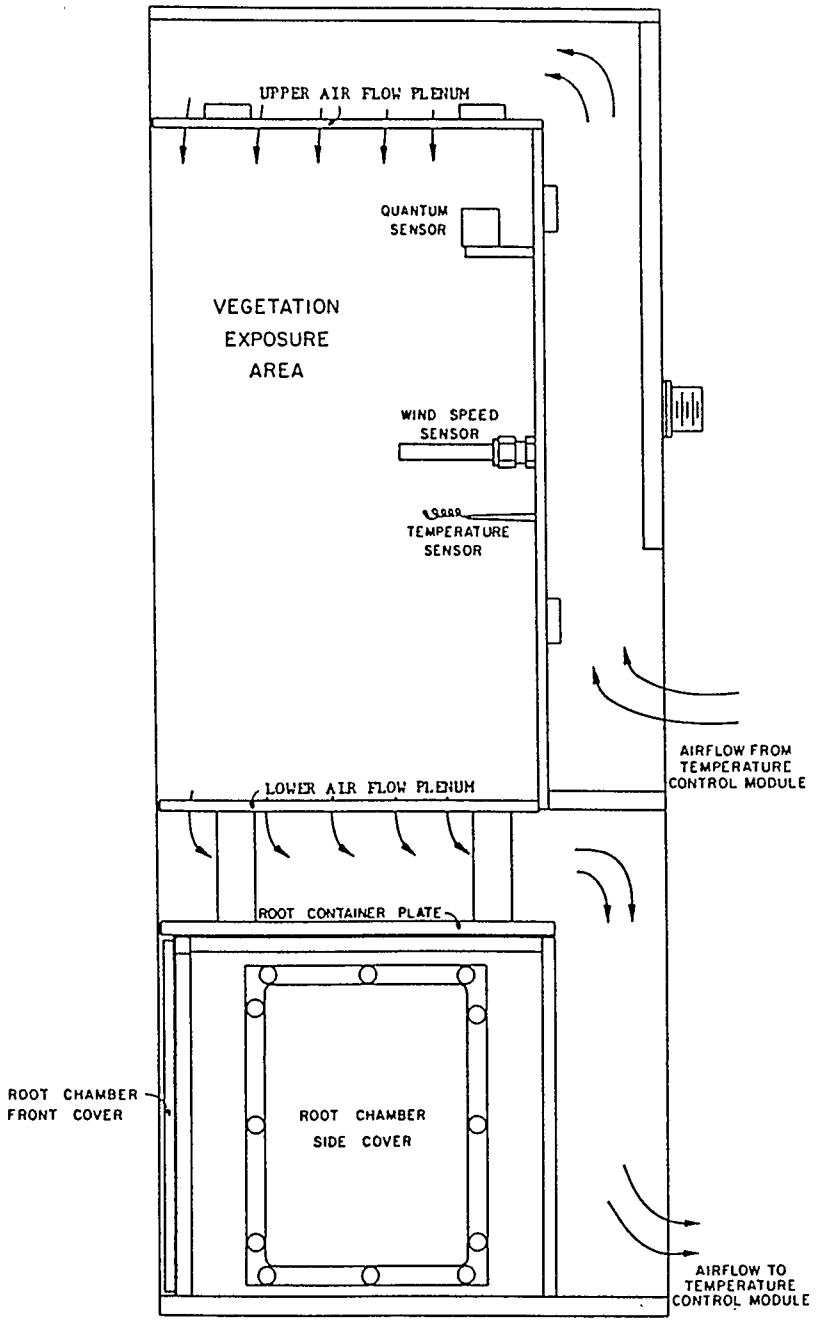

Figure 9. A side-view of the fumigation chambers.

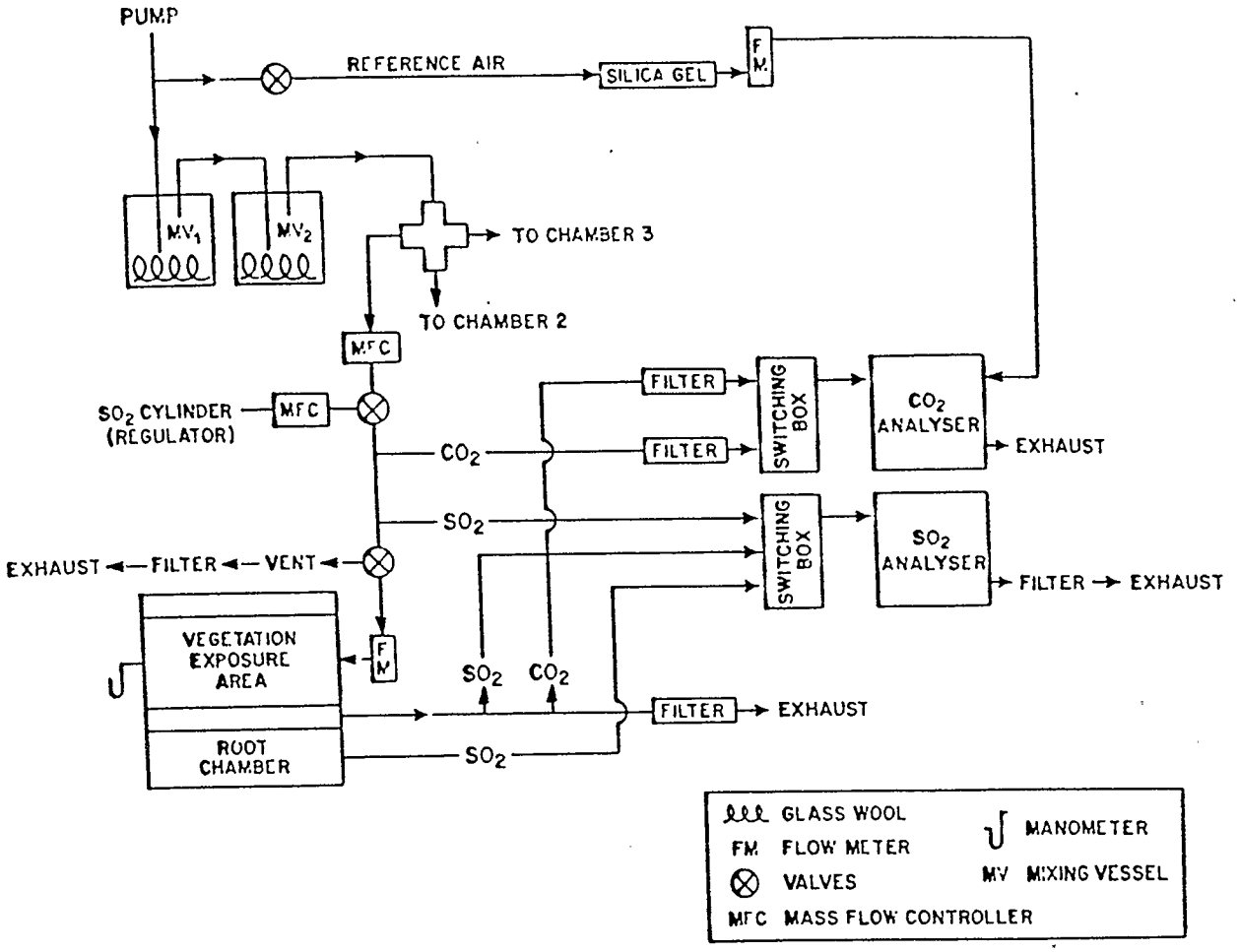

Figure 10. The schematic diagram of the fumigation system.

fumigation system are shown in Figure 10. More specific details are given in Clapperton (1986).

VA mycorrhizal inoculum

The source of VA mycorrhizal inoculum used in these studies was field soil containing spores, hyphae, and infected root fragments. The VA mycorrhizal fungi present in the soil were: *Glomus fasiculatum* Thaxter sensu Gerd., *Glomus macrocarpum* Tul. and Tul., var macrocarpum, *Glomus microcarpum* Tul. and Tul., *Glomus mosseae*, and a species of *Gigaspora* (Clapperton & Parkinson, 1990).

The soil for the study was collected from a reference sub-montane mixed grass prairie field site in southern Alberta, Canada (Clapperton & Parkinson, 1990). The soil was black chernozemic containing 2.3mg kg^{-1} phosphorus, 18.1 mg kg^{-1} available nitrogen, and 28.0 mEq $100g^{-1}$ calcium. Soil was sieved (2mm mesh), and stored for less than one month at 4°C in a sealed air filled polyethylene bags prior to use.

The soil used as a growing medium for non-mycorrhizal plants was the same soil which had been microwave irradiated (see Materials and Methods, Chapter 1) to a final temperature of 80°C. Therefore, soil was irradiated for less than twenty minutes at a medium power setting. The soil was then covered and allowed to cool to room temperature. Then remoistened and allowed to rest uncovered for one week. Soil was then placed in an air-filled sealed polyethylene bag and stored at 4°C until use (less than one month).

Fumigation Experiments

Experiment 1

Seeds of *Phleum pratense* L. were germinated on moistened filter paper for six days prior to planting. *Phleum pratense* was chosen as a host, as it was a co-dominant grass species at the reference field site where the VA mycorrhizal inoculum was collected (Clapperton & Parkinson, 1990). Seedlings were selected for planting based on uniformity of size. A homogeneous mixture of field soil (mycorrhizal treatment) or microwave-irradiated soil (non-mycorrhizal treatment) was packed into each of 20 (total 40), 65 ml syringe barrels, and a six day-old seedling planted into each. A field soil filtrate was prepared by mixing 500 g of field soil with 1.5 l of distilled water, stirring for ten minutes, and filtering through a 38μm filter. Fifteen millilitres of distilled water was dispensed into each of the non-mycorrhizal treatment containers. The shoot/soil interface of each plant was covered with a 3mm layer of fine charcoal to absorb SO_2 which might otherwise have been absorbed by the soil. The charcoal was then covered with a small amount of polyester batting to prevent the charcoal from blowing off during fumigation. Plants were watered three times weekly, including once with Peter's professional 20-10-20 fertilizer (Peter's Fertilizer Products, Pennsylvania) at a concentration which gave 25μg P. Plants were watered through a port located one-third of the distance from the top of the plant

container. Thus, any problems with the dissolution of SO_2 in the surface water were avoided. Plants were grown under 0.433 mol m^{-2} s^{-1}PAR. The photoperiod was 16 h. The temperature was 21 and 12°C, day- and night- time, respectively. The wind speed in the chamber was maintained at 0.5 m s^{-1} and the airflow through the chambers was 20 l min^{-1}, providing one air change every two minutes. The humidity inside the chamber varied between 52 and 64% RH. Plants were exposed to between 0.04 - 0.07 µl l^{-1} SO_2 for the duration of the experiment. Plants were harvested after six weeks. At harvest plants were removed from the container and roots washed free of soil. Leaf area was measured using an Ikegami leaf area meter (Ikegami Tsushinki Co., Ltd., Japan). Shoots and roots were separated, weighed, and dried at 80°C for 48 hr. Prior to drying a 0.11 g fresh weight subsample was taken from the roots. This subsample was stained (Phillips & Hayman, 1970) and assessed for mycorrhizal colonisation using a modification (Zak & Parkinson, 1982) of the line-intercept method (Newman, 1966). The dry weight of the subsample was estimated from a ratio of fresh weight to dry weight from the remaining roots. Estimates of total root length, and length of root colonised by VA mycorrhizal fungi were calculated by dividing the measured length of root values by the dry weight and then multiplying by the total dry weight of the roots (Cook, Jastrow, & Miller, 1988). There were seven replicate plants per treatment.

Experiment 2

Experiment 2 followed the same procedures as outlined under Experiment 1 with some exceptions. The plants were watered three times weekly, and fertilized once every second week. Plants were grown first in SO_2-free air for three weeks, after which they were exposed to between 0.05 and 0.07 $\mu l\ l^{-1}$ SO_2 for another three weeks, and harvested. There were eight replicate plants per treatment.

Statistics

Data for VA mycorrhizal infection were analysed using a one-way ANOVA (Zar, 1984). All other data were analysed using a two-way ANOVA (Zar, 1984). The shoot and root dry weights were transformed using Log 10 in Experiment 2. Means were separated using Tukey's test for multiple comparisons (Zar, 1984).

RESULTS

Plants in both experiments showed similar amounts of VA mycorrhizal colonisation (Table 3) but the total root lengths were shorter in Experiment 2. There was only a small effect of SO_2 fumigation on the percent of total root length colonised

Table 3. Lengths of root with VA mycorrhizal infection (including arbuscules, hyphae and vesicles), lengths of root with arbuscules, and total root length (cm), compared in plants exposed to SO_2 and controls in Experiments 1 and 2.

Treatment	Replicates	Infection	Arbuscules	Root Length
Experiment 1				
Control	7	356±125(16%)*A	286±100(12%) A	2303±413A
SO_2	7	187±84(10%)B	151±25(8%)B	1965±378A
Experiment 2				
Control	6	320±83(36%)A	276±67(31%)A	884±151A
SO_2	6	281±62(30%)A	230±50(24%)A	974±198A

Differences between means in columns are not significant when followed by the same letter $\alpha = 0.2$, ± S.E.

*Percent of root length

by VA mycorrhizal fungi, and with arbuscules alone, in Experiment 1 (α= 0.2), indicating that SO_2 fumigation affected the ability of the mycorrhizal fungi to establish themselves in the roots of *P. pratense*, but in Experiment 2, measures of colonisation and the number of arbuscules decreased even less in response to SO_2.

SO_2 exposure reduced the dry matter production of shoots and roots in non-mycorrhizal plants in both experiments (Tables 4 & 5). The plants infected with VA mycorrhizal fungi showed a tendency to reduced dry matter production in shoots and roots in response to SO_2 exposure in Experiment 1 but not in Experiment 2. The effect of VA mycorrhizal fungi was most marked in Experiment 2, and masked any effects of SO_2 on plant productivity. The other parameters measured the number of tillers produced per plant and the total leaf area tended to be lower in non-mycorrhizal plants exposed to SO_2 (Tables 4 & 5). The overriding effect on all the parameters measured in both experiments was VA mycorrhizal colonisation. Plants with VA mycorrhizal colonisation had fewer tillers, smaller leaf area, and lower shoot and root dry weights (Tables 4 & 5) than non-mycorrhizal plants.

DISCUSSION

The decrease in measures of VA mycorrhizal colonisation, and root length colonised with arbuscules in response to SO_2

Table 4. The mean number of tillers, leaf area (cm^2), shoot and root dry weights (g) of mycorrhizal (M) and non-mycorrhizal (NM) *Phleum pratense* in Experiment 1.

Treatment	Tillers	Leaf Area	Shoot Wt.	Root Wt.
NM	5.7A (±1.0)	100.0A (±5.5)	0.54A (±0.05)	0.23A (±0.02)
NM + SO_2	5.1A (±0.6)	85.6A (±3.9)	0.44A (±0.05)	0.11B (±0.02)
M	2.9B (±0.3)	60.6B (±3.23)	0.28B (±0.02)	0.11B (±0.01)
M + SO_2	3.3B (±0.2)	51.4B (±0.26)	0.22B (±0.01)	0.08B (±0.01)

Differences between means in columns are not significant when followed by the same letter. $\alpha = 0.05$, (± S.E.).

Table 5. The mean number of tillers, leaf area (cm^2), shoot and root dry weights (g) of mycorrhizal (M) and non-mycorrhizal (NM) *Phleum pratense* in Experiment 2.

Treatment	Tillers	Leaf Area	Shoot Wt.	Root Wt.
NM	5.9A (±0.9)	116.0A (±8.5)	0.68A (±0.07)	0.19A (±0.02)
NM+SO_2	5.3A (±0.7)	95.4B (±8.5)	0.52A (±0.07)	0.10B (±0.02)
M	0.13B (±0.14)	38.5C (±1.4)	0.08B (±0.01)	0.03C (±0.004)
M+SO_2	0.13B (±0.14)	36.7C (±1.7)	0.06B (±0.01)	0.03C (±0.004)

Differences between means in columns are not significant when followed by the same letter $\alpha = 0.05$, (± S.E.).

exposure were 47 percent each in Experiment 1 (Table 3). The results for Experiment 2 show a 12 and 17 percent decrease in VA mycorrhizal colonisation and root length containing arbuscules (respectively) in response to SO_2 exposure. Thus, it appeared that SO_2 exposure of *Phleum pratense* shoots limits the ability of VA mycorrhizal fungi to colonise and form a functional symbiosis. However, arbuscule formation seemed to be somewhat more sensitive to SO_2 exposure. These results suggest that the ability of VA mycorrhizal fungi to colonise the host, and to a lesser extent, proliferate within the host, was limited by the host as a consequence of SO_2 exposure. The productivity of VA mycorrhizal fungi has often been linked to host productivity, and SO_2 is known to reduce plant productivity due to its effect on plant photosynthesis. Therefore, my results are likely explained by changes in the quantity of carbon in and exuded by the roots as a consequence of the affects of SO_2 exposure on assimilate translocation (Teh & Swanson, 1982; Minchin & Gould, 1986), to the roots (Jones & Mansfield, 1982a). The effects of SO_2 on root dry weight are clearly seen in the non-mycorrhizal plants which had been exposed to SO_2 (Tables 4 & 5).

Growth of external hyphae, or fungal productivity may also be limited by changes in plant assimilate partitioning. Indeed, Brewer and Heagle (1983) have shown that exposure of soybeans to acid rain reduced the number of chlamydospores produced by *Glomus geosporum*. In a continuing field study there were fewer spores and a lower inoculum potential at

two native prairie field sites which had been continuously exposed to SO_2 for 23 years than at a reference site (Clapperton & Parkinson, 1990), further indicating that there is likely a host-mediated effect of SO_2 fumigation on VA mycorrhizal fungal productivity.

Interestingly, the VA mycorrhizal plants in Experiment 2 produced less root dry matter than in Experiment 1. Plants in Experiment 2 were fertilized less often than in Experiment 1 in order to encourage more VA mycorrhizal infection. However, the non-mycorrhizal plants in both Experiments 1 and 2 were similar in size and other measured parameters. This indicated that the change in the fertilizer regime had affected the VA mycorrhizal fungi. The increased VA mycorrhizal colonisation in plants in Experiment 2 probably explains the significantly (α=0.05) decreased number of tillers, smaller leaf area and shoot and root dry weights in VA mycorrhizal plants from Experiment 2 compared with VA mycorrhizal plants from Experiment 1. Tiller production and survival are linked to assimilate production by plants (Aspinall, 1961). It appeared that the increased colonisation by VA mycorrhizal fungi in Experiment 2 might have increased the carbon drain on the plants. Experiments using radioactive carbon will be used to examine the relationship between the host, VA mycorrhizal fungi, and host stress (Chapter 3).

The superior growth and biomass production of the non-mycorrhizal plants compared with the mycorrhizal plants

could be partially attributed to the microwave-irradiated soil (Ferriss, 1984; Gibson, Fox & Deacon, 1984) used for non-mycorrhizal plants. Preliminary studies produced similar results when non-mycorrhizal plants were grown in an autoclaved peat:vermiculite mixture (2:1), and mycorrhizal plants were grown in peat:vermiculite:field soil mixture (3:1:2) (Clapperton & Parkinson, unpublished). I suggest that improved plant growth in microwave-irradiated soil could have been due to the changes in the number and species composition of soil microbes as a result of microwave irradiation (see Chapter 1).

CONCLUSIONS

The ability of VA mycorrhizal fungi to infect roots of *Phleum pratense* was affected by SO_2 exposure. Proliferation of VA mycorrhizal fungi within the root does not appear to be particularly affected by SO_2 exposure of host shoots. Evidence from other sources suggests that growth of VA mycorrhizal fungi external to the root, and VA mycorrhizal productivity may however be affected. Plants colonised by VA mycorrhizal fungi showed a reduction in tillering, leaf area, and shoot and root dry weights regardless of SO_2 exposure. This was attributed to VA mycorrhizal fungi being an overriding sink for plant assimilate. The effect of SO_2 on non-mycorrhizal plants was the same as predicted from other published studies on grasses.

CHAPTER 3: Effects of Sulphur Dioxide Fumigation and VA Mycorrhizal Fungi on ^{14}C - Partitioning in *Phleum pratense*.

INTRODUCTION

Long-term fumigation with sulphur dioxide (SO_2) inhibits photosynthesis (Black, 1982), the net rate of assimilation (Bell, Rutter & Relton, 1979; Jones & Mansfield, 1982a), and the distribution of assimilates (Ashendon & Mansfield, 1977; Ashendon, 1978; Jones & Mansfield, 1982b; Murray, 1985). These changes eventually lead to reduced dry matter production (Bell & Clough, 1973) particularly in the roots (Jones & Mansfield, 1982b; Whitmore & Mansfield, 1983).

Changes in patterns of assimilate partitioning in response to long-term low-concentration SO_2 fumigation have been measured by changes in root:shoot dry weight ratios (Ashendon & Mansfield, 1977; Ashendon, 1978; Jones & Mansfield, 1982a) and reduced carbon movement to the roots, and from leaves (Koziol & Cowling, 1978; Jones & Mansfield, 1982b; McLaughlin & McConathy, 1983). Some studies have suggested that changes in the pattern of assimilate partitioning are caused by changes to the carbon translocating mechanism (Noyes, 1980; Teh & Swanson, 1982; Minchin & Gould, 1986). Recently, Gould, Minchin & Young (1988) have shown that SO_2 fumigation can inhibit phloem loading and reduce the speed at which assimilate is translocated from the leaves.

Vesicular-arbuscular (VA) mycorrhizas form symbiotic relationships with most herbaceous plants. In grasslands it has been shown that members of the Gramineae are particularly well colonised compared with other grassland plants (Sparling & Tinker, 1975; Read, Koucheki, & Hodgson, 1976). VA mycorrhizas are known to promote plant growth, primarily by improving plant nutrition. However, in some cases plant growth depressions have also been attributed to VA mycorrhizal symbiosis (Buwalda & Goh, 1982; Koide, 1985). Estimates of the carbon cost to the plant for maintaining VA mycorrhizal symbioses range between 4 and 20 percent of the total photoassimilate (Pang & Paul, 1980; Bethlenfalvay *et al.*, 1982; Kucey & Paul, 1982; Snellgrove *et al.*, 1982; Harris, Pacovsky & Paul, 1985; Jakobsen & Rosendahl, 1990). Koch & Johnson (1984) have shown that mycorrhizal root systems acquire a greater percentage of the photoassimilate than do the root systems of non-mycorrhizal plants.

In light of the effects of SO_2 fumigation on plant physiology and in particular carbon partitioning, and the carbon requirement of VA mycorrhizal fungi, there is a "conflict of interest". Indeed, results in Chapter 2 showed that low-concentrations of SO_2 affected the ability of VA mycorrhizal fungi to colonise and proliferate within roots.

The objective of this study was to examine the effect of long-term low-concentration SO_2 fumigation and VA mycorrhizal colonisation on the partitioning of ^{14}C-labelled

photoassimilate in *Phleum pratense* L. subsequently fed $^{14}CO_2$.

MATERIALS AND METHODS

VA mycorrhizal inoculum

The source and preparation of inoculum is the same as given in Chapter 2.

Fumigation chambers

The fumigation chambers are as described in Chapter 2.

Fumigation Experiment

Seeds of *Phleum pratense* L. were germinated on moistened filter paper for 10 d prior to planting. *Phleum pratense* was chosen as a host because it was a co-dominant grass species at the site where the inoculum was collected (Clapperton & Parkinson, 1990). Seedlings of uniform size were selected for planting. A homogeneous mixture of field soil or microwave-irradiated soil was packed into 65 ml syringe barrels (20 per soil), and a 10 d-old seedling planted into each. The soil was covered with a 3mm layer of charcoal to absorb SO_2. The charcoal was then covered with a small amount of polyester batting to prevent it from blowing off

during fumigation. Plants were watered three times weekly, including once with Peter's Professional 20-10-20 fertilizer (Peter's Fertilizer Products, Pennsylvania) at a concentration which gave 25 μg P per plant. Plants were watered through a port located one-third of the way from the top of the syringe barrel. Plants were grown under a photon flux density which ranged between 422 and 303 μmol m^{-2} s^{-1} PAR. The photoperiod was 15 h and the temperature climbed to 21°C during the day and decreased gradually to 12°C at night. The windspeed in the chamber was maintained between 0.3 and 0.5 m^{-2} s^{-1} and the air flow through the chambers was 20 l min^{-1}, providing one air change every 2 min. The relative humidity varied between 43 and 61%. Plants were exposed to between 0.04 and 0.065 μl l^{-1} SO_2 for the duration of the experiment. Plants were harvested after six weeks. At harvest five pairs of plants from each treatment were selected at random for ^{14}C-labelling. The remaining plants in each chamber were removed from the syringe barrel and the roots washed free of adhering soil. Leaf area was measured using an Ikegami leaf area meter (Ikegami Tsushinki Co., Ltd., Japan). Shoots and roots were separated and dried at 80°C for 48h, and then weighed. Roots were subsequently stained (Phillips & Hayman, 1970) and assessed for VA mycorrhizal infection using a modification (Zak & Parkinson, 1982) of the line-intercept method (Newman, 1966). There were five replicates per treatment for VA mycorrhizal assessment.

^{14}C - labelling

The five plants from each treatment from each chamber were placed in a fumehood under a bank of Gro-lux lights (Sylvania). Each plant was labelled with $^{14}CO_2$ released from 74 kBq (20μCi) of liquid ^{14}C-sodium bicarbonate source. Plants were labelled individually in 3 l polyethylene bags which were sealed around the syringe barrel near the top with an elastic band. The soil surface was sealed with Parafilm (American National Can, Connecticut) before labelling. The ^{14}C-sodium bicarbonate was contained in a sealed 10 ml glass vial, inside the bag with the opening of the vial level with the rim of the syringe barrel, and attached to the syringe barrel with an elastic band. The $^{14}CO_2$ was released by opening the vial, puncturing the bag with a syringe, and introducing 500 μl of 85% lactic acid into the vial. The hole left by the syringe was then sealed with tape. After 15 min the reaction was stopped using 500 μl of 2N NaOH, the vials were resealed, and the bags were slashed with a razor blade and then removed. The plants were then left under the lights for 12h.

The roots of the plants were then quickly washed free of soil, and the roots and shoots separated, frozen, and freeze-dried for 48h.

^{14}C extraction procedure

In all cases the roots and shoots of individual plants in each treatment were extracted separately; replicates were not pooled.

The roots and shoots were weighed and then ground in liquid nitrogen in a mortar. The ground sample was extracted in two equal volumes of warmed 80% methanol and then filtered through Whatman 2 filter paper in a Büchner funnel. The filter paper with the extracted residue was dried in a convection oven at 70°C and stored for oxidation. The filtrate or extract was then subsampled to count radioactivity; the scintillation fluid used was BioSafe II (Research Products International, Illinois). All vials were counted for 2 min. in a Packard - Bell 2200CA Liquid Scintillation Analyser (Canberra - Packard Canada Ltd.). The filtrate was then diluted to 50% (v/v) with double distilled water, and the methanol was removed *in vacuo*. The remaining aqueous extract was frozen at -20°C and then freeze-dried.

The freeze-dried extract was dissolved in 5 ml of warm double distilled, demineralized water, and passed through a C_{18} - Sep-Pak column (Waters Associates, Massachusetts) to remove pigments and the eluate was sampled for counting. The C_{18} column was then rinsed with 5 ml of 100% methanol. The methanol (methanol wash) was collected separately from the aqueous filtrate, and an aliquot was taken for counting.

A 1 ml aliquot of the extract was put onto the first of two ion exchange columns that were connected in series. The first ion exchange column contained AG50W-X8 (100-200 mesh) and the second contained AG1-X8 (200-400 mesh). The sample was eluted first with 20 ml of distilled water. The eluate was collected and an aliquot taken for counting. This eluate represented the neutral soluble carbohydrate and sugar fractions of the extract. The two columns were then detached and eluted separately. The first column was eluted with 30 ml of 4N ammonium hydroxide, to remove amino acids from the column. The second column was eluted with 4N formic acid, to remove organic acids. Aliquots of eluates from both columns were taken for counting.

Oxidation procedure

The sample residues remaining on the filter papers from the Büchner funnel were weighed. The ^{14}C in labelled compounds remaining in these residuals (i.e., not extracted by 80% MeOH) determined by oxidation followed by scintillation counting. The residues were oxidised using a Harvey Biological Oxidiser OX500 (RJ Harvey Instrument Corporation, New Jersey). Gaseous products of combustion were trapped in an absorbing agent and scintillant (RJ Harvey Instrument Corp. Carbon 14 Cocktail) in glass vials. The vials were counted for ^{14}C-radioactivity for 2 min in a Packard 2200CA

Tri-carb Liquid Scintillation Analyser (Canberra-Packard Canada, Ltd.).

Statistics

Data were examined for normality using probability plots (Ryan, Joiner, & Ryan, 1976) before two way or one way analyses of variance were performed ($\alpha = 0.01$). Data from the methanol wash were transformed using the Log_{10} transformation to overcome heteroscedacity of variance. Means were separated using Tukey's test for multiple comparisons, $\alpha = 0.01$ (Zar, 1984).

RESULTS

The mycorrhizal plants in both treatments had fewer tillers, less leaf area, reduced shoot and root dry weights, and greater root:shoot ratios than non-mycorrhizal *P. pratense* (Table 6). However, mycorrhizal plants incorporated more ^{14}C-radioactivity per g dry weight than non-mycorrhizal plants in both treatments (Table 7). Mycorrhizal plants had significantly more ^{14}C-label per g dry weight in shoot and root extracts, and in the shoot methanol wash fraction (which contained pigments and other non-polar compounds). In terms of photosynthetic activity, mycorrhizal plants in both treatments incorporated significantly more ^{14}C-radioactivity

Table 6. The mean number of tillers, leaf area (cm^2), shoot and root dry weights (g), and the root:shoot ratio of non-mycorrhizal (NM) and mycorrhizal (M) *Phleum pratense* fumigated (SO_2) or not fumigated with sulphur dioxide.

Treatment	Tillers	Leaf Area	Shoot wt.	Root wt.	Root:Shoot
NM	5.6A (±0.5)	114.4A (±4.3)	0.671A (±0.033)	0.212A (±0.013)	0.32B (±0.02)
NM+SO_2	6.0A (±0.6)	122.4A (±6.9)	0.734A (±0.046)	0.200A (±0.023)	0.28B (±0.04)
M	0.6B (±0.2)	36.3B (±0.5)	0.059B (±0.004)	0.036B (±0.002)	0.63A (±0.05)
M+SO_2	0.0B (±0.0)	34.8B (±0.6)	0.047B (±0.005)	0.026B (±0.003)	0.53AB (±0.06)

Differences between means in columns are not significant when followed by the same letter, $\alpha = 0.01$, ±S.E.

Table 7. The effect of fumigation with SO_2 on mycorrhizal (M) and non-mycorrhizal (NM) *Phleum pratense*. The mean amount of ^{14}C-radioactivity (kBq) per g dry weight in the aqueous extract, methanol wash (shoots only), residue in the shoots and roots, and the mean total ^{14}C-radioactivity per g shoot dry weight.

Treatment	Shoots				Roots			^{14}C/Shoot
	Extract	Wash	Residue	Total	Extract	Residue	Total	
NM	32.3 B	0.83 BC	16.5 A	49.6 B	18.9 B	4.3 A	23.3 B	59.6 B
NM+SO_2	40.3 B	0.24 C	26.4 A	67.0 B	10.8 B	6.1 A	16.9 B	68.3 B
M	117.2 A	1.6 B	31.0 A	149.8 A	46.4 A	6.3 A	52.6 A	299 A
M+SO_2	131.2 A	3.1 A	22.8 A	157.0 A	27.0 B	3.4 A	30.4 AB	260 A

Differences between means in columns are not significantly different when followed by the same letter, $\alpha = 0.05$.

per g dry shoot weight than did non-mycorrhizal plants (Table 7).

The non-mycorrhizal plants in both treatments contained a greater percentage of the total ^{14}C-radioactivity in recovered shoot extracts, and a lower percentage shoot and root residues than mycorrhizal plants (Table 8). In some cases exposure to SO_2 increased the percentage of ^{14}C-label extracted from shoots and reduced the percentage from roots, and appeared to have a differential effect on the percentage of ^{14}C-label in shoot and root residues of mycorrhizal and non-mycorrhizal plants (Table 8). However, the overall effect of SO_2 was less marked than that of mycorrhizal colonisation.

In terms of changes in the distribution of the ^{14}C-label, mycorrhizal plants tended to have less of the label in the shoots and more in the roots (Table 8). The mycorrhizal plants had a lesser proportion of ^{14}C-labelled carbohydrates in the shoots compared with non-mycorrhizal plants (Table 9). In the roots, mycorrhizal plants had a significantly greater proportion of ^{14}C-labelled amino acids and organic acids (Table 10). Fumigation with SO_2 also had a significant effect on the distribution of ^{14}C-labelled compounds.

Mycorrhizal and non-mycorrhizal plants which had been fumigated with SO_2 had a greater percentage of the ^{14}C-label in the shoots, and a lower percentage in the roots compared with non-fumigated plants (Table 8). This translated into an increased percentage of amino acids and soluble carbohydrates and a decreased percentage of organic acids in

Table 8. The effect of fumigation with SO_2 on mycorrhizal (M) and non-mycorrhizal (NM) *Phleum pratense*. The mean percentage of total ^{14}C radioactivity (kBq) incorporated into the aqueous extract and residue, and the total percent ^{14}C-radioactivity in the shoots and roots.

Treatment	Shoots			Roots		
	Extract	Residue	Total	Extract	Residue	Total
NM	55±1 AB	32±1 B	87±2 AB	9±2 A	4±1 A	11±2 AB
NM+SO_2	58±2 A	35±2 B	92±2 A	5±2 B	3±0.5 A	7±2 B
M	41±3 C	42±2 A	83±4 B	9±2 A	8±3 A	17±4 A
M+SO_2	50±3 B	37±2 AB	88±2 AB	6±1 AB	6±1 A	12±2 AB

Differences between means in columns are not significant when followed by the same letter $\alpha = 0.05$, ±S.E..

Table 9. The percentage of recovered radioactivity incorporated into amino acids, organic acids, and carbohydrates in the shoots of non-mycorrhizal (NM) and mycorrhizal (M) *Phleum pratense* fumigated (SO_2) or not fumigated with sulphur dioxide.

Treatment	Amino Acids	Organic Acids	Carbohydrates
NM	1.0±0.2 C	12.0±0.9 A	67.2±2.2 B
NM+SO_2	2.3±0.3 B	9.2±1.3 B	80.0±0.8 A
M	2.1±0.1 BC	15.5±0.3 A	59.5±1.9 C
M+SO_2	4.3±0.3 A	12.8±0.8 A	71.5±1.2 B

Differences between means in coloumns are not significant when followed by the same letter, $\alpha = 0.01$, ±S.E.

Table 10. The percentage of recovered radioactivity incorporated into amino acids, organic acids, and carbohydrates in the roots of non-mycorrhizal (NM) and mycorrhizal (M) *Phleum pratense* fumigated (SO_2) or not fumigated with sulphur dioxide.

Treatment	Amino Acids	Organic Acids	Carbohydrates
NM	0.18±0.04 B	0.73±0.08 B	18.8±2.1 A
NM+SO_2	0.23±0.02 B	0.73±0.21 B	7.5±1.5 B
M	0.48±0.04 A	3.3±0.9 A	19.2±0.8 A
M+SO_2	0.53±0.08 A	1.8±0.3 AB	9.1±1.1 B

Differences between means in columns are not significant when followed by the same letter, $\alpha = 0.01$, ±S.E.

the shoots, and a reduced percentage of soluble carbohydrates in the roots of fumigated plants (Tables 9 & 10). In general, these changes were reflected in the tendency towards increased shoot and reduced root dry matter in fumigated plants (Table 6).

Mycorrhizal plants which were fumigated with SO_2 had shorter roots, less root length colonised by VA mycorrhizal fungi, and a smaller proportion of the root length infected with arbuscules than non-fumigated mycorrhizal plants (Table 11).

DISCUSSION

Plants colonised by VA mycorrhizal fungi regardless of fumigation had fewer tillers, less leaf area, lower shoot and root dry weights, and higher root:shoot ratios than non-mycorrhizal plants. These results agree well with the findings discussed in Chapter 2 and are likely related to the effects of field soil on plant growth (Chapter 1). Nevertheless, mycorrhizal plants in both treatments incorporated a greater amount of radioactivity per g dry shoot weight indicating that these plants had increased rates of photosynthesis. Plants colonised by VA mycorrhizal fungi are known to have increased rates of photosynthesis (Allen *et al.*, 1981; Bildusas *et al.*, 1986; Brown & Bethlenfalvay, 1987) and more chlorophyll (Allen *et al.*, 1981) than non-mycorrhizal plants.

Table 11. Length of root with VA mycorrhizal infection (cm) (including hyphae, arbuscules, and vesicles), length of root with arbuscules (cm), and total root length (cm) of mycorrhizal (M), non-mycorrhizal (NM), and *Phleum pratense* fumigated (SO_2) or not fumigated with sulphur dioxide.

Treatment	Replicates	Infection	Arbuscules	Root length
M	5	484±23(32%)*A	310±10(20%) A	1536±67 A
M+SO_2	5	158±47(20%) B	108±35(13%) B	698±122 B

Differences between means in columns are not significant when followed by the same letter, $\alpha = 0.05$, ±S.E.

*Percent of root length

Winner & Mooney (1980b) have suggested that plants with high photosynthetic capacities are particularly sensitive to photosynthetic damage from exposure to air pollutants. Therefore, photosynthesis in plants colonised by VA mycorrhizal fungi may be more sensitive to SO_2 fumigation. Indeed, the mycorrhizal plants which were exposed to SO_2 had chlorotic lesions and some dead leaves not seen in the non-fumigated, and non-mycorrhizal fumigated plants.

The tendency to increased shoot dry weight in fumigated non-mycorrhizal plants, compared with the tendency to decreased plant dry weight in fumigated mycorrhizal plants further illustrated the possibility that mycorrhizal plants may be more sensitive to SO_2 fumigation. In contrast, SO_2 fumigation appeared to stimulate photosynthesis in non-mycorrhizal plants. Non-mycorrhizal plants exposed to SO_2 incorporated more ^{14}C-label per g shoot weight than non-fumigated plants (Table 2). Low-concentration SO_2 fumigation has been shown to stimulate photosynthesis (Black, 1982).

The results of my study agree well with the results of other studies which have examined the effects of long-term low-concentration SO_2 on the partitioning of assimilates (Jones & Mansfield, 1982 a&b; Whitmore & Mansfield, 1983; Gould & Mansfield, 1988). The SO_2 fumigated mycorrhizal and non-mycorrhizal plants showed a greater tendency to retain labelled assimilates in the shoots (Table 8) and had lower root:shoot ratios (Table 6) than non-fumigated plants.

Plants that have been exposed to low-concentrations of SO_2 are known to translocate less assimilate to the roots (Jones & Mansfield, 1982b; McLaughlin & McConathy, 1983; Gould & Mansfield, 1988). Indeed, it has been shown that SO_2 fumigation inhibits both phloem loading and the speed at which assimilates are translocated from the leaves (Gould, Minchin & Young, 1988). This will likely affect the availability of photoassimilate for fungal consumption.

It has been shown that the carbon energy cost to the plant for maintenance of VA mycorrhizal symbiosis is between 4 and 20 percent of the total photoassimilate (c.f. Harris & Paul, 1987; Douds, Johnson, & Koch, 1988; Jakobsen & Rosendahl, 1990). It is also known that root systems colonised by VA mycorrhizal fungi acquire a greater percentage of photoassimilate than non-mycorrhizal roots (Koch & Johnson, 1984; Douds, Johnson & Koch, 1988; Wang *et al.*, 1989).

There was a tendency for fumigated mycorrhizal plants to have a greater proportion of ^{14}C - radioactivity in the roots compared with fumigated non-mycorrhizal plants (Table 8). This suggests that VA mycorrhizal fungi might have acted to increase the amount of labelled assimilate translocated to the roots. Nevertheless, the length of root colonised by VA mycorrhizal fungi in the fumigated plants was severely reduced compared with non-fumigated mycorrhizal plants (Table 11). It has been shown that factors which cause a decrease in the amount of soluble carbohydrate in the roots

can affect the ability of VA mycorrhizal fungi to infect and proliferate in host roots (Thomson, Robson & Abbott, 1990). Indeed, SO_2 fumigation significantly reduced the amount of labelled soluble carbohydrates in the roots of both mycorrhizal and non-mycorrhizal plants (Table 10). The length of root infected with arbuscules was also significantly reduced in the SO_2 fumigated plants. Since the arbuscule is thought to be the primary site of exchange between the plant and fungus (Bonfante-Fasolo, 1984), a decrease in the formation of arbuscules by the fungus could indicate a functional decrease in the symbiosis.

Fumigation with SO_2 also changed the relative proportions of labelled amino acids and organic acids in the roots and shoots of mycorrhizal and non-mycorrhizal plants. The amino acid content of shoots has been reported to increase in response to SO_2 exposure (c.f. Rowland, Borland & Lea, 1988). Karolewski (1984 & 1985; in Rowland, Borland & Lea, 1988) showed that protein-forming amino acids increased in the pool of free amino acids in trees that were exposed to SO_2. Increased concentrations of the sulphur containing amino acids cysteine, methionine, and glutathione have also been found in response to SO_2 fumigation (Garsed & Read, 1977; Grill, Esterbauer & Klosch, 1979; Malhotra & Sarkar, 1979). It is also possible that the increased concentrations of amino acids in response to SO_2 exposure resulted from increased rates of protein degradation associated with SO_2 fumigation (Rowland, Borland & Lea,

1988). Unfortunately, I was unable to perform amino acid analysis in my study.

While the content of labelled amino acids increased in plants exposed to SO_2 the ^{14}C-labelled organic acid content decreased. It is possible that the two are linked. The enzyme isocitrate dehydrogenase which catalyses the reaction of isocitrate to α-ketoglutaric acid in the TCA cycle is reportedly stimulated by SO_2 fumigation (c.f. Rowland, Borland, & Lea, 1988). The product of this reaction, α-ketoglutaric acid, can be used to produce chlorophyll, amino acids and lipids. However, this scenario should have resulted in an increase in amino acids and an increase in organic acids, not seen in my experiments. Amino acid and organic acid analysis would be required to examine this hypothesis.

Malate dehydrogenase, another enzyme in the TCA cycle, which catalyses the reaction of malate to oxaloacetic acid, is inhibited by SO_2 fumigation (Sarkar & Malhotra, 1979). The inhibition of this enzyme in conjunction with the stimulation of isocitrate dehydrogenase would thus lead to a temporary increase in organic acids, again not seen in my results. The interpretation of the results of the changes in ^{14}C-labelled organic compounds in response to SO_2 fumigation is limited in that these experiments were harvested at only one point in time. A time-course experiment with analysis of individual amino acids, organic acids, and carbohydrates would have revealed more about the fate of ^{14}C-labelled assimilate in response to SO_2 fumigation.

The decreased content of labelled organic acids in the roots and shoots of SO_2 fumigated plants suggests that respiration might have been reduced in response to SO_2 exposure (Tables 4 & 5). However, there is no consensus on the effects of SO_2 on plant respiration. It does appear that any response of respiration to SO_2 fumigation is concentration dependent (Black, 1982; Dizengremel & Citerne, 1988; Darrall, 1989). Cowling & Koziol (1978), using low-concentrations of SO_2 in a long-term fumigation experiment, saw no change in respiration.

Mycorrhizal plants generally had a significantly greater proportion of amino acids and organic acids in both shoots and, particularly, in roots than non-mycorrhizal plants (Tables 9 &10). This would suggest that the VA mycorrhizal plants, both fumigated and non-fumigated, were respiring at a faster rate than non-mycorrhizal plants. Baas, van der Werf & Lambers (1989) have shown that plants colonised by VA mycorrhizal fungi have increased root respiration.

CONCLUSIONS

Colonisation by VA mycorrhizal fungi has an overriding effect on carbon assimilation, use, and translocation in plants. The effect of SO_2 fumigation on these same physiological processes puts an increased strain on the plant which eventually takes its toll on the VA mycorrhizal fungi.

In this study there were no significant effects of low-concentration SO_2 fumigation on some of the the gross parameters of plant growth such as plant dry weight and tiller production. However, there were significant effects of SO_2 exposure at the physiological and biochemical levels, providing further evidence for 'invisible injury' due to SO_2 exposure. It is likely that with time these physiological effects of SO_2 fumigation on the plant would have affected plant dry weight. I observed that SO_2-fumigated plants had more dead leaves and yellowing leaf tips compared with non-fumigated plants.

This study also suggests that factors such as SO_2 exposure which affect the partitioning of assimilates and availability of soluble carbohydrates in roots will affect the VA mycorrhizal symbiosis.

CHAPTER 4: A Possible Relationship Between ^{14}C-Assimilate Partitioning and Tolerance of *Phleum pratense* to Sulphur Dioxide Fumigation

INTRODUCTION

Little is known about the ecological and genetic effects of sulphur dioxide (SO_2) on plant populations and communities. However, there is a demonstrated genetic variability in response to air pollutants. Differences in response to SO_2 have been reported between species (Winner & Mooney, 1980a,b; Dodd, Laurenroth & Heitschmidt, 1982; Whitmore & Mansfield, 1983; Gould, Minchin & Young, 1988) and for populations within species (Miller, Howell & Caldwell, 1974; Taylor & Murdy, 1975; Ayazloo & Bell, 1981). Bradshaw (1977) suggested that environmental factors that adversely affect plant growth can exert selection pressures on plant populations by acting on their inherent genetic variability.

Bell and Clough (1973) showed that *Lolium perenne* from an area with a history of SO_2 pollution had a tolerance to SO_2 exposure. Evidence since then suggests that plant tolerance to SO_2 is widespread (Ayazloo & Bell, 1981; Karnosky, 1985). Tolerance to acute SO_2 exposure is known to develop in 3 to 4 years in *P. pratense* (Wilson & Bell, 1985). However, it has been shown that tolerance to acute SO_2 exposure is independent of tolerance to chronic or long-term low-concentration SO_2 exposure (Ayazloo, Garsed & Bell, 1982).

Chronic SO_2 injury includes increased leaf senescence (Kropff, 1990), decreased photosynthesis (Black, 1982) and dry matter production (Bell & Clough, 1973), and changes to the translocation (Minchin & Gould, 1986) and partitioning of assimilates (Teh & Swanson, 1982). Ayazloo, Garsed & Bell (1982) suggested that chronic effects of SO_2 involve a gradual physiological change and that the ability to lessen the effect of SO_2 was a potential mechanism of tolerance.

Plants from areas subject to long-term low-concentration SO_2 exposure have been shown to have decreased rates of senescence, lower shoot and root weights, and lower root:shoot ratios in response to SO_2 than plants from unpolluted areas (Ayazloo & Bell, 1981; Ayazloo, Garsed & Bell, 1982). These results suggest that assimilate translocating and partitioning mechanisms may be less affected by chronic SO_2 exposure in tolerant than in non-tolerant plants.

The objectives of this study were to :

1. Identify SO_2-tolerant and non-tolerant genotypes of *P. pratense* collected from the field sites.

2. Examine the effects of continuous SO_2 fumigation at field-like low concentrations on growth and assimilate partitioning in the SO_2-tolerant and non-tolerant genotypes.

MATERIALS AND METHODS

Field sites and experimental plants

The two field sites were located in the foothills along the eastern slopes of the Rocky Mountains in a sub-montane mixed grass prairie approximately 25 km southwest of Pincher Creek, Alberta, Canada. The SO_2 point source is a sour gas processing plant which has been operating for the past 28 years. The gas plant emitted approximately 24.8 tonnes SO_2 per day during 1989 and 1990 (Shell Canada Resources Ltd., 1991). The first site (site 1) was located approximately 5 km from the gas plant. The second site (site 2) was approximately 20 km from the gas plant.

Five plants of *P. pratense* were collected at random from sites 1 and 2.

Screening of plants for tolerance to SO_2

The plants collected in the field were propagated by individual cuttings, and then by individual tillers. These stock plants were then continually propagated in the greenhouse until there were enough tillers of similar age for screening experiments. Ten tillers at the three fully expanded leaf stage were removed from clones of each of two randomly selected stock plants from each site.

Plants were screened in the fumigation chambers described in Chapter 2. The exposure concentrations of SO_2 used used in the initial screening varied between 0.1 and 0.5 $\mu l\ l^{-1}$. Photoperiod was 16 h at a photon flux density which

varied between 374 and 421 μmol m^{-2} s^{-1} PAR. Day- and night-time temperatures were 21 and 12°C, respectively. Humidity varied between 50 and 64% RH. Plants were watered three times weekly including once with 20-10-20 fertilizer (Peter's Professional Fertilizer, Pennsylvania, U.S.A.). After three weeks of fumigation, some plants had chlorotic lesions and dead leaves so the experiment was terminated. At harvest, plant roots were washed free of soil, and roots and shoots were separated and dried for 48 h at 80°C, and then weighed.

The criteria used to select genotypes which were considered to be SO_2-tolerant or non-tolerant were as follows. SO_2-tolerant genotypes were considered to be plants that showed little or no visible leaf damage in response to SO_2. There were also to be no significant differences in root or shoot dry weights between SO_2-fumigated and control plants. Conversely, non-tolerant genotypes were those plants that showed a significant difference in root and shoot dry weights between SO_2-fumigated and control plants, and had considerable leaf damage and numerous dead leaves in response to SO_2 fumigation.

Assimilate partitioning and SO_2-tolerance

SO_2 fumigation

Twenty tillers of equivalent age and size (i.e. at the three fully expanded leaf stage) from each of a deemed SO_2-tolerant stock plant and a non-tolerant stockplant of *P. pratense* were planted in 65 ml containers with potting mix (3:1:1 v/v peat:sand:vermiculite). Ten tillers from each stock plant were fumigated with SO_2, while the other ten were not fumigated, i.e., were grown in 'control' conditions. The position of the containers inside the chambers were selected at random. However, clones from the same stock plant or genotype were placed in the same position inside each chamber to minimize variation between treatments from chamber effects. The exposure concentrations of SO_2 during the experiments varied between 0.05 and 0.07 $\mu l\ l^{-1}$. The photoperiod was 15 h and the photon flux density varied between 303 and 427 $\mu mol\ m^{-2}\ s^{-1}$. The humidity ranged between 57 and 66% RH. The windspeed varied between 0.3 and 0.5 $m\ s^{-1}$. The day- and night-time temperatures inside the chambers were 21 and 12°C, respectively. Plants were watered three times weekly including once with 20-10-20 fertilizer (Peter's Professional Fertilizer, Pennsylvania, U.S.A.). The experiment was terminated after two weeks. This was when some plants started to show the first signs of leaf damage.

^{14}C - labelling

At harvest, 7 plants of each stock plant from each treatment were selected for radioactive labelling with $^{14}CO_2$. Plants were each exposed for 30 min (as described in Chapter 3) to $^{14}CO_2$ released from 60 kBq of ^{14}C sodium bicarbonate. The label was chased for 12h after which time the roots were washed free of soil, shoots and roots were separated, freeze-dried, and then weighed.

^{14}C oxidation

The proportion of ^{14}C in the shoots and roots of individual plants was determined by sample oxidation using a Harvey Biological Oxidiser OX500 (RJ Harvey Instrument Corporation, New Jersey) as described in Chapter 3.

RESULTS

Identification of SO_2 tolerance

The response of the different stock plants to SO_2 fumigation varied. Plants fumigated with SO_2 generally had some dead leaves, more tillers, and reduced shoot and root dry weights (Table 12). Stock plant 3 had the fewest dead leaves per plant and thus the least visible injury due to SO_2. Fumigation with SO_2 significantly reduced the root:shoot ratio

Table 12. The effect of SO_2 fumigation on the mean number of dead leaves and tillers, and shoot and root dry weights (g) of four different stock plants of *Phleum pratense*.

	Stock plant 1		Stock plant 2		Stock plant 3		Stock plant 4	
	Control	SO_2	Control	SO_2	Control	SO_2	Control	SO_2
Dead Leaves	0.0	5.8	0.0	2.0	0.0	0.8	0.0	1.4
Tillers	2.0AB	2.8AB	1.0B	3.5A	1.5B	2.3AB	3.0A	3.0A
Shoot wt.	0.337CD	0.272D	0.439AB	0.440AB	0.507A	0.415BC	0.379BC	0.348CD
Root wt.	0.224A	0.076D	0.178BC	0.189AB	0.212AB	0.204AB	0.179BC	0.144C
Root:Shoot	0.681A	0.283C	0.411BC	0.435BC	0.424BC	0.498B	0.481B	0.435BC

Differences between means in rows are not significant when followed by the same letter, $\alpha = 0.05$.

of stock plant 1. In contrast, the root:shoot ratio tended to increase in stock plants 2 &3 and especially in stock plant 3 (Table 12).

Generally, stock plant 1 appeared to be the plant most affected by SO_2 fumigation. In contrast stock plants 2 and 3 appeared to be the least affected, and stock plant 4 had and intermediate response to SO_2 fumigation.

It was determined using a squashed root tip stained with 2% acetorcein that each of the genotypes or stock plants had 42 chromosomes.

Assimilate partitioning and SO_2 tolerance

Stock plants 1 & 3 were used as the non-tolerant and SO_2-tolerant plants, respectively.

Despite the significant increase in the number of dead leaves per plant in the non-tolerant clones there was no significant effect of SO_2 exposure on shoot dry matter in the two genotypes (Table 13). However, the non-tolerant plants had significantly less dry root weight and root:shoot ratios than SO_2-tolerant plants in response to SO_2 exposure.

Both the non-tolerant and SO_2-tolerant clones tended to incorporate more ^{14}C-label in response to SO_2 fumigation (Table 13). There was a significantly higher percentage of label retained in the shoots of fumigated non-tolerant plants and as a consequence a significantly lower percentage of label in the roots than in non-tolerant controls and, SO_2-tolerant clones in

Table 13. The effect of SO_2 fumigation on the shoot and root dry weights (g), root:shoot ratio, and the amount of ^{14}C-label incorporated (kBq) per g dry weight of shoots and percentage of ^{14}C-label incorporated into the shoots and roots of non-tolerant (stock plant 1) and tolerant (stock plant 3) *Phleum pratense*.

Treatment	Shoot(g)	Root(g)	Root:Shoot	^{14}C/Shoot	% Shoots	% Roots
Non-Tolerant						
Control	0.127A (±0.008)	0.086A (±0.007)	0.67A (±0.1)	862AB (±71)	84.7B (±0.9)	15.3A (±0.9)
SO_2	0.121A (±0.01)	0.051B (±0.008)	0.42B (±0.13)	974A (±81)	91.8A (±1.1)	8.2B (±1.1)
Tolerant						
Control	0.126A (±0.006)	0.070AB (±0.006)	0.56AB (±0.12)	647B (±92)	83.4B (±1.5)	16.6A (±1.5)
SO_2	0.109A (±0.008)	0.043B (±0.007)	0.38B (±0.11)	912AB (±112)	86.9B (±0.8)	13.1AB (±0.8)

Differences between means in columns are not significant when followed by the same letter $\alpha = 0.01$, ± S.E.

both treatments (Table 13). In contrast, there was no significant difference in the percentage of ^{14}C-label retained in the shoots and contained in roots of SO_2-tolerant plants in both treatments.

DISCUSSION

Stock plants 1 and 2 were collected at site 2 and stock plants 3 and 4 were collected at site 1. It was hypothesised that the populations of *P. pratense* at site 2 would lack tolerance to SO_2 whereas, the populations at site 1 would have acquired tolerance because of their exposure to SO_2 emitted from the gas plant during the past 28 years. Indeed, there appeared to be no reason to reject the hypothesis (Table 12). The least tolerant plant was collected at site 2 and the most SO_2-tolerant plant was collected at site 1 (Table 12). However, stock plant 2 showed some tolerance to SO_2 exposure using my criteria as described in the Results. This suggests the possibility for tolerant and sensitive plants to exist at both sites. Wilson & Bell (1985) have suggested that there is no need for the genetic influx of tolerance to have tolerant plants. It is also interesting that both the stock plants tested from site 1 were predisposed towards tolerance. Horsman, Roberts & Bradshaw (1978) reasoned that this apparent lack of variability was probably due to overall selection of more tolerant genotypes. It must be noted at this point that it cannot be determined from this study to what extent the response of these four stock plants

or genotypes (two from each site) represented the overall response to SO_2 exposure in populations at the field sites. Obviously, I would have to collect and screen a greater number of populations of *P. pratense* at each site to be able to make any conclusions on the effects of SO_2 exposure on tolerance in populations at the field sites.

In measuring or screening for plant tolerance to SO_2 exposure I used visible damage (number of dead leaves), and changes in dry matter production and partitioning as markers of SO_2 injury (Bell & Mudd, 1976; Horsman, Roberts & Bradshaw, 1979; Ayazloo & Bell, 1981). The two plants chosen for the ^{14}C-radioactive labelling experiment and for use in the field experiments were stock plant 1 (Table 12) which appeared to be the most sensitive of the plants, and stock plant 3 (Table 12) which was the most tolerant. Stock plant 3 showed no significant change in root dry weight and had the fewest dead leaves per plant in response to SO_2 exposure.

Studies have shown that exposure to SO_2 affects plant productivity (Ashendon, 1979) and in particular root dry weight (Ayazloo & Bell, 1981; Ayazloo, Garsed & Bell, 1982; Jones & Mansfield, 1982a,b). Thus plants tolerant of SO_2 exposure would be expected to show no significant effect of SO_2 on root weight and have increased partitioning of assimilates to the roots in response to SO_2 exposure compared with non-tolerant plants. Indeed, the SO_2-tolerant clones had no dead leaves, no significant difference in shoot

dry weight, and a tendency to decreased root dry weight, and reduced root:shoot ratios in response to SO_2 exposure. In contrast, the non-tolerant clones had a significant number of dead leaves, and significantly decreased root dry weight and reduced root:shoot ratios in response to SO_2. The SO_2-tolerant clones showed a general trend toward lower root:shoot ratios compared with the non-tolerant clones. Ayazloo & Bell (1981) suggested that reduced leaf growth was the primary factor involved in the selection for tolerance to SO_2. It was suggested that increased shoot weight in response to SO_2 exposure led to increased tolerance, unless accompanied by a competitive disadvantage in some other respect (Ayazloo, Garsed & Bell, 1982).

The non-tolerant clones generally had greater incorporation of the ^{14}C label than SO_2-tolerant clones (Table 13). This indicated that the non-tolerant clones had higher rates of photosynthesis than SO_2-tolerant clones. Winner & Mooney (1980b) suggested that plants with higher intrinsic photosynthetic capacity were more sensitive to SO_2 than those with lower capacities. The data also suggest that the low concentrations of SO_2 used in my study possibly increased stomatal conductance as both genotypes had increased incorporation of ^{14}C-radioactivity in response to SO_2 fumigation. Low-concentrations of SO_2 have been known to increase stomatal conductance (Black, 1982). Higher stomatal conductance leads to increased SO_2 absorption so all other factors being equal, plants that have lower stomatal

conductance are probably more tolerant of SO_2 fumigation (Winner & Mooney, 1980b). The SO_2-tolerant clones did incorporate less ^{14}C-radioactivity than non-tolerant clones in response to SO_2 fumigation (Table 13). It is also likely that the photosynthetic efficiency (expressed as the amount of radioactivity incorporated per g dry weight) of the non-tolerant clones in my study was underestimated in SO_2 fumigated plants because of the number of dead or non-photosynthesising leaves on non-tolerant clones.

The results indicate that the SO_2-tolerant plants are possibly avoiding SO_2 injury by reduced stomatal conductance and are also compensating physiologically. SO_2-fumigated non-tolerant clones retained an increased percentage of the ^{14}C-label in the shoots and had less in roots than non-fumigated non-tolerant clones (Table 13). This suggests that assimilate translocating mechanisms in SO_2-tolerant clones were less affected by SO_2 fumigation than in the non-tolerant clones. Considering the possible and demonstrated effects of changes in assimilate partitioning on VA mycorrhizal fungi it is likely that soil and mycorrhizosphere organisms are also affected by plant tolerance to SO_2.

CONCLUSIONS

In my study, unlike other studies (Ayazloo & Bell, 1981; Ayazloo, Garsed & Bell, 1982), low-concentration SO_2

fumigation produced visible injury to the non-tolerant clones. However, it is possible that the non-tolerant clones of *Phleum pratense* in my study were more sensitive to SO_2 exposure than non-tolerant clones of other grass species used in other studies. Indeed it would have been interesting to examine the tolerance to SO_2 exposure in field populations of other grasses at the field sites. Certainly if my study had been expanded to examine SO_2 tolerance in more field populations of *Phleum pratense* including other grasses which were present at the field sites, I would have been better able to examine any within-species and between-species differences in tolerance mechanisms, and the distribution of tolerance among populations at the two field sites. Since few studies have been reported on the effects of SO_2 or other gaseous air pollutants on the distribution of plant tolerance to gaseous air pollution in North American grasslands, expanded studies such as I have suggested would certainly be beneficial and should be pursued.

CHAPTER 5: A Two-Year Field Study Examining the Effects of Field Concentrations of SO_2 on *Phleum pratense*.

INTRODUCTION

Plant response to SO_2 has been tested using closed system fumigation chambers, open-top chambers in the field, and open-air field fumigation systems. Closed system chambers can mimic field concentrations of SO_2 to some extent while allowing for the control of climatic and edaphic factors (Unsworth, 1982; Bell, 1985). Closed chambers are more appropriate for the physiological study of the effects of air pollutants, and the interaction of other air pollutants and climatic factors on plants. Open-top chambers were designed to provide climatic conditions that were as close as possible to ambient (Roberts *et al.*, 1983). However, light intensity in those chambers could be reduced by up to 25 percent (Roberts *et al.*, 1983). Greenwood *et al.* (1982) suggested that all enclosed environments are somewhat 'un-natural', since it is difficult to simulate natural variation in temperature, precipitation, light intensity, humidity, and air turbulence.

The open-air fumigation system is now probably the preferred technique for field experiments (McLeod *et al.*, 1988). This system allows experimental plants to be grown under typical agricultural or native conditions while the micro-environment of the plants is only marginally altered

by the fumigation system (Miller *et al.*, 1980). Field plots share many of the same advantages as the open-air fumigation systems. The use of field plots along pollution concentration gradients from a gaseous air pollution point source have the advantage of there being no modification of microclimate, no changes in the interactions of air pollutants, and no modifications of abiotic and biotic stresses (Colvill *et al.*, 1985).

It is known that there are substantial but invisible effects of SO_2 exposure on plants at field sites where the annual SO_2 concentration is less than 0.06 $\mu l\ l^{-1}$ SO_2 (Bell, 1985). There are also a number of factors both abiotic and biotic that can interact with SO_2 to modify the plant response in the field. Factors which are known to affect plant response to SO_2 include drought (Lucas, 1990), grazing (Lauenroth *et al.*, 1985), light intensity and photoperiod (Davies, 1980; Jones & Mansfield, 1982a), associations with microorganisms (Heagle, 1973; McLeod *et al.*, 1988), nitrogen nutrition (c.f. Winner *et al.*, 1985), and temperature (Jones & Mansfield, 1982a). It is considered likely that the factors which affect plant response to SO_2 will also affect plant tolerance to SO_2 (Ayazloo & Bell, 1981; Wilson & Bell, 1985).

Results of long-term field studies using low-concentrations of SO_2 in open-top and open-air fumigation systems have shown that plants respond as predicted from greenhouse and laboratory fumigation studies. Plants fumigated with SO_2 in the field had decreased root:shoot ratios (Ayazloo & Bell,

1981; Murray, 1985), fewer tillers (Baker, Fullwood & Colls, 1987; Wilson & Murray, 1990), fewer flowering heads (Wilson & Murray, 1990), and reduced weight of seed heads or pods (Wilson & Murray, 1990; Kropff *et al.*, 1989). Depending on the concentration of SO_2 used, plants had increased above-ground biomass (Lauenroth, Milchunas & Dodd, 1984; McLeod *et al.*, 1988) at concentrations less than 0.038 $\mu l\ l^{-1}$, or decreased above- and below-ground biomass at concentrations greater than 0.041 $\mu l\ l^{-1}$ (McLeod *et al.*, 1988; Wilson & Murray, 1990).

Karnosky (1985) suggested that comparative studies using the same genotype exposed to SO_2 in fumigation chambers, field chambers and field plots were ideal for determining the relative predictability of plant response in 'natural' conditions. He also suggested that studies of plant growth around known SO_2 sources would be valuable for comparing plant sensitivity to SO_2 exposure. Furthermore, Bell (1985) suggested that there is a need for more information on the interaction of SO_2 effects and grazing, trampling and climatic factors on plant growth under field conditions. With these suggestions in mind I designed a two year field experiment based on the following objectives:

1. To examine the effects of field concentrations of SO_2 on plant growth; specifically to compare the growth of SO_2-tolerant plants, non-tolerant plants, and seedlings from commercial seed at two field plots, one exposed to increased

concentrations of SO_2 and one with no increased exposure to SO_2.

2. To compare the rates of VA mycorrhizal colonisation between the *Phleum pratense* genotypes at the two field sites.

MATERIALS AND METHODS

Field sites

The field sites were located in a sub-montane mixed grass prairie in south western Alberta. The mean monthly temperatures at the study areas in 1989 ranged from -9.5°C in January to 16.5°C in July. The mean monthly temperature during the main growing season, May through August was 13.4°C. The total annual precipitation was 543.4 mm. The total precipitation during the growing season was 251.7 mm. In 1990, the mean monthly temperatures ranged from -5.5°C in February to 16.9°C in August. The mean monthly temperature during the growing season was 13.3°C. The total precipitation up to and including September 1990 was 389.6mm. The total precipitation during the growing season was 263.8 mm.

The soils at the field sites are classified as black chernozemic, sandy to silty loam with a lime layer between 46 and 76 cm below the surface. The results of soil analysis are given in Clapperton & Parkinson (1990). There were

significant differences between the soil chemical analysis between the two sites (sites 1 and 3 in Clapperton & Parkinson, 1990) with respect to most analyses. It was suggested that the soils at site 1 showed symptoms of soil acidification.

Analysis of vegetation (Clapperton & Parkinson, 1990) showed that *Festuca* spp (*Festuca rubra* L. and *Festuca scabrella* Torr.) and *Phleum* spp (*Phleum commutatum* Gaudin and *Phleum pratense* L.) were co-dominant at the field sites. The percent species composition for *Festuca* spp was 16.4% at site 1 and 32.7% at site 2, and *Phleum* spp represented 24.2% at site 1 and 24.1% at site 2 as determined using a modified point transect (Coupland, 1950; Becker & Crockett, 1973).

The species composition of VA mycorrhizal fungi (Clapperton & Parkinson, 1990) was similar at both field sites and included *Glomus fasiculatum* Thaxter sensu Gerd., *Glomus macrocapum* Tul & Tul. var *macrocarpum*, *Glomus microcarpum* Tul & Tul., *Glomus mosseae*, and a species of *Gigaspora*. It was determined from rates of VA mycorrhizal colonisation, spore counts, and MPN (Clapperton & Parkinson, 1990) that the soil at site 2 had a greater inoculum potential than the soil at site 1.

The SO_2 point source was a sour gas processing plant which emitted approximately 24.8 tonnes SO_2 per day in 1989 and 1990 (Shell Canada Resources Ltd.). Site 1 was located downwind approximately 5 km from the gas plant.

Site 2 was located upwind approximately 20 km from the gas plant. The annual average sulphur deposition in 1989 and 1990 as estimated from total sulphation data (Shell Canada Resources Ltd.) was approximately 19 kg ha^{-1} at site 1 and less than 1.5 kg ha^{-1} at site 3.

Field plots and experimental plants

An experimental plot was established at each field site. The 1.5 x 3.0 m rectangular plots were cultivated and the exposed soil was covered with black plastic for one year, then cultivated and covered for another winter. The plastic covering reduced the germinating and growth potential of seeds and rhizomes present in the cultivated soil.

In the spring of 1989 the plastic was removed and each plot was planted with 50 clones (at the three fully expanded leaf stage) from each of the SO_2-tolerant and non-tolerant genotypes of *Phleum pratense*. The SO_2-tolerant and non-tolerant genotypes used were those identified in the previous chapter. In addition, the plots were planted with 100 seedlings grown from commercial seed of *Phleum pratense*. The seedlings were also at the three fully expanded leaf stage of growth. The clones and seedlings were planted in individual sections with borders between the sections overlapping (Fig. 11). The clones and seedlings were identified by differently coloured strings, yellow, green and red, that were loosely tied around the base of each clone or

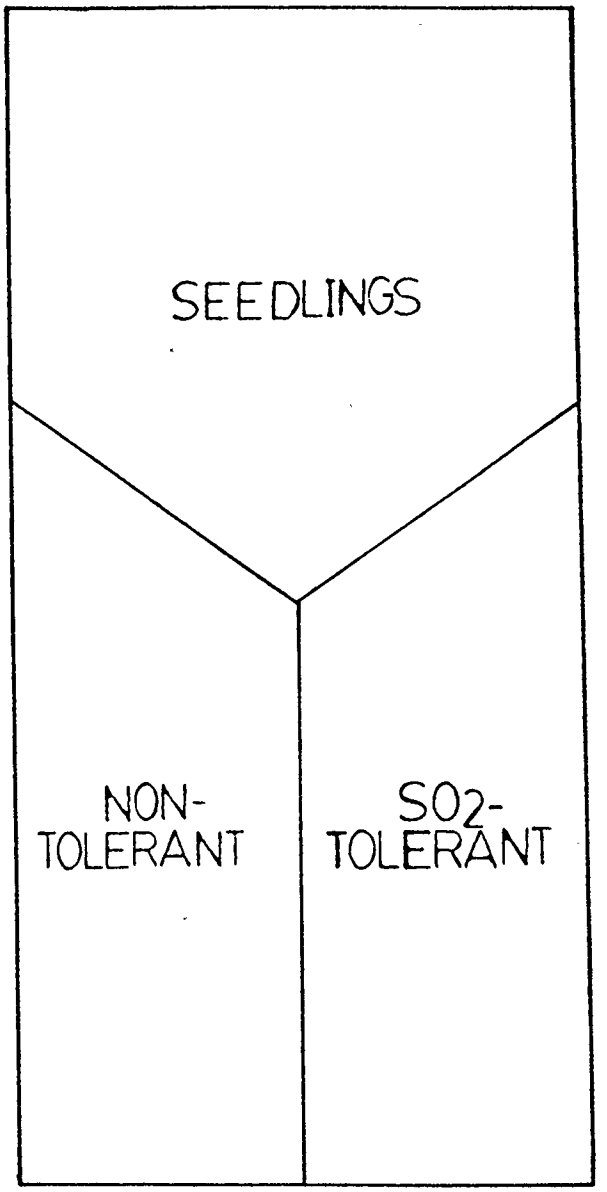

Figure 11. A diagram showing the design of the field plots. Scale: 1 cm = 0.2 m.

seedling at planting. Small coloured stakes were used to delineate the sections within each plot. The clones and seedlings were planted to give a final density of 44 plants m^{-2}. The plants were watered with 20 l per site immediately after planting, and then again in mid-July 1989.

Experimental procedure

In September 1989, twenty-five clones of each of the SO_2-tolerant and non-tolerant genotypes and twenty-five seedlings were randomly sampled from each field site. The tillers and inflorescences were counted, and the shoots were clipped 2 cm above the shoot/soil interface and dried at 70°C for 3 days and weighed. The roots from ten of each of the twenty-five clones and seedlings from each field site were sub-sampled randomly. The root samples were removed using a 7 cm diameter and 11 cm long corer giving a volume of 423 cm^3. The roots contained within the core were washed free of soil, separated from the crown, dried at 80°C for 48 h, and weighed.

In September 1990, ten of the remaining fifteen clipped plants from each of the clones of each genotype and seedlings at each of the field sites were randomly selected for harvest. Of these ten plants, five had roots harvested also. At each field site shoots were also harvested from ten plants from each of the clones and seedlings which had not been harvested the previous year. Again, five of these plants had

roots harvested also. In 1990 roots were harvested with a 10 cm diameter and 11 cm long corer, giving a volume of 864cm^3.

At harvest the inflorescences were counted, the shoots were clipped 2 cm above the shoot/soil interface, dried at 70°C for 6 days, and weighed. Roots were processed in the same way as described for the 1989 harvest.

Plants which had been clipped in 1989 will be referred to as clipped hereafter.

VA mycorrhizal colonisation

A 0.03 g subsample of dried roots was taken from root samples from each of the clones and seedlings from each site in 1989 and 1990 harvests. The root samples were cleared in boiling 10% KOH on a hot plate, and stained using Chlorazol Black E (Brundrett, Piché & Peterson, 1984). Roots were then mounted on microscope slides and assessed for colonisation by VA mycorrhizal fungi using a modification (Zak & Parkinson, 1982) of the line-intercept method (Newman, 1966). Estimates of total root length, and length of root colonised by VA mycorrhizal fungi were calculated by dividing the measured length of root values by the sub-sample dry weight and then multiplying by the total dry weight of the roots.

Statistics

Data were examined for normality using probability plots (Ryan, Joiner & Ryan, 1976). A two way analysis of variance (ANOVA) was performed on data from 1989, and a three way ANOVA on data from 1990. VA mycorrhizal colonisation data were transformed using the square root. Means were separated using Tukey's test for multiple comparisons (Zar, 1984).

RESULTS

In 1989, after the first growing season the plants at site 1 (the site closest to the gas plant) generally produced fewer tillers, and less shoot and root dry weight than plants at site 2 (the farthest site from the gas plant) regardless of genotype (Table 14). It also appears that there were more inflorescences per g shoot dry weight produced on plants at site 2 than at site 1 (Table 14). Unfortunately some of the seedlings at site 2 which were near the edge of the plot appeared to have been grazed soon after planting. This probably accounted for the significantly lower mean number of inflorescences produced per g shoot dry weight of seedlings at site 2.

Table 14. The mean number of tillers, inflorescences per (g) dry weight, shoot and root weights (g), and the estimated root dry weight (g) per 796 ml soil core volume of non-tolerant, SO_2-tolerant and plants from commercial seed seedlings (Seedlings) of *Phleum pratense* harvested in 1989.

	Tillers	Inflorescences	Shoot wt	Root wt	Estimated Root wt
Site 1					
Non-tolerant	13.6C	0.90A	6.47C	0.375B	0.75
SO_2-tolerant	25.5B	0.71B	8.89BC	0.523A	1.05
Seedings	28.9B	0.62B	11.6B	0.453AB	0.91
Site 2					
Non-tolerant	21.4BC	1.0A	9.09BC	0.403B	0.81
SO_2-tolerant	45.5A	0.86AB	15.7A	0.463AB	0.93
Seedlings	45.4A	0.39C	16.4A	0.473AB	0.95

Differences between means in columns are not significant when followed by the same letter, $\alpha = 0.05$.

The total monthly sulphur deposition at site 1 as estimated (Leahey & Schroder, 1985) from total monthly sulphation (Shell Canada Resources Ltd., 1989) during the 1989 growing season was 725 g ha^{-1} in May, 258 g ha^{-1} in June, 183 g ha^{-1} in July, and 42 g ha^{-1} in August. At site 2 the total monthly sulphur deposition during the months of May, June, July, and August was 108, 167, 75, 42 g ha^{-1}, respectively. Thus, the total sulphur deposition at site 1 during the 1989 growing season was 1.2 kg ha^{-1} and 0.4 kg ha^{-1} at site 2. In 1990, after one year of growth and after the second growing season there was no significant interaction between plant growth and sites except for the number of inflorescences produced per g shoot dry weight (Table 15). There were fewer inflorescences per g shoot dry weight produced on plants grown at site 1 than at site 2 (Table 15). There also were fewer inflorescences produced per g shoot dry weight on plants that were clipped than on unclipped plants regardless of genotype (Table 15). Plants that were clipped also produced less shoot and root dry weight than unclipped plants (Table 15).

In 1990 the total monthly sulphur deposition (Leahey & Schroeder, 1985) as estimated from total monthly sulphation (Shell Canada Resources Ltd, 1990) at site 1 during May, June, July, and August was: 167, 183, 58, 175 g ha^{-1} respectively. At site 2 total monthly sulphur deposition was: 42, 75, 58, 67 g ha^{-1} for May, June, July and August respectively. Thus, the total sulphur deposition during the

Table 15. The mean shoot and root dry weights (g) and the number of inflorescences produced per (g) dry weight of clipped (C) and unclipped (NC), non-tolerant, SO_2-tolerant plants and plants from commercial seed (Seedlings) of *Phleum pratense* harvested at the end of the 1990 growing season.

	Shoot wt		Root wt		Inflorescences	
	C	NC	C	NC	C	NC
Site 1						
Non-tolerant	14.1C	22.8B	1.48AB	1.97A	0.76C	1.36B
SO_2-tolerant	14.2C	27.0B	1.61AB	1.82A	1.38B	1.51AB
Seedlings	21.4C	45.3AB	1.85AB	2.32A	1.22B	1.45B
Site 2						
Non-tolerant	14.7C	19.9B	1.26B	1.93A	2.20A	2.01A
SO_2-tolerant	26.4BC	32.6AB	2.36A	2.05A	2.06A	1.86A
Seedlings	29.7BC	46.8A	1.52AB	2.05A	2.86A	1.91A

Differences between means in columns (and in rows for comparison between clipped and unclipped) are not significant when followed by the same letter, $\alpha = 0.05$.

Table 16. The mean percentage of root colonised by VA mycorrhizal fungi (including hyphae and arbuscules), percentage with hyphae and arbuscules, the length of root colonised by VA mycorrhizal fungi (cm), and the estimated length all roots (m) in non-tolerant, SO_2-tolerant, and plants from commercial seed (Seedlings) of *Phleum pratense*.

	Colonised(%)	Hyphae	Arbuscules	Colonised(cm)	Length
Site 1					
Non-tolerant	0.6 DE	0.2 D	0.4C	42 E	65 A
SO_2-tolerant	0.8D	0.3 D	0.5 C	89 D	93 A
Seedlings	0.2 E	0.1 D	0.1 C	19 F	82 A
Site 2					
Non-tolerant	6.2 B	3.9 B	2.3 B	381 B	61 A
SO_2-tolerant	12.0 A	5.0 A	7.0 A	593 A	51 A
Seedlings	1.7 C	1.0 C	0.6 C	135 C	87 A

Differences between means in columns are not significant when followed by the same letter, $\alpha = 0.05$.

1990 growing season was 583 g ha^{-1} at site 1 and 242 g ha^{-1} at site 2.

Colonisation by VA mycorrhizal fungi

All genotypes at site 2 were more colonised by VA mycorrhizal fungi than at site 1 (Table 16). There was also a lower VA mycorrhizal inoculum density at site 1 than at site 2 (Clapperton & Parkinson, 1990). At site 2 SO_2-tolerant clones had the greatest amount of VA mycorrhizal colonisation than non-tolerant clones and seedlings. More importantly at both field sites the SO_2-tolerant genotypes had significantly more root length colonised by VA mycorrhizal fungi (Table 16). However, there was no significant difference in the total estimated root length of plants within and between field sites (Table 16).

DISCUSSION

After the first growing season (1989) plants of all genotypes at site 1 had fewer tillers and inflorescences, and lower shoot and root dry weights than plants at site 2 (Table 14). These results agree well with the results of other field studies which have shown that grasses produce fewer tillers (Baker, Fullwood & Colls, 1987; Wilson & Murray, 1990) and

inflorescences (Wilson & Murray, 1990), and have reduced shoot and root dry weights in response to SO_2 exposure (Bell & Clough, 1973; Ashendon & Williams, 1980). After the second growing season there was a less obvious effect of SO_2 exposure on plant growth (Table 15). The total sulphur deposition at both sites in 1990 was half of that in 1989, in 1990. Nevertheless, there were significantly more inflorescences produced in plants grown at site 2 regardless of the effects of clipping. It has been reported that plants are more sensitive to SO_2 exposure immediately before and during the onset of flowering than at other growth stages (Filner *et al.*, 1984; Kropff *et al.*, 1989). Indeed, Bonte (1982) suggested that SO_2 exposure might directly alter the process of fruiting. Therefore, it seems likely that the processes involved in flowering are more sensitive to low concentrations of SO_2 than are other plant processes.

During the growing season in both 1989 and 1990 the highest exposure to SO_2 occurred in the months of May and June. *Phleum pratense* is a C_3 'cool-season' grass; the development of inflorescences and increased growth is initiated in May and June, with anthesis occurring in July. Therefore, the evidence suggests that it is likely that increased exposure to SO_2 during the period of increased growth and inflorescence development would affect plant productivity. Certainly, the results of the field study (Tables 14 & 15) support this hypothesis. I suggest that the increased effects of SO_2 exposure seen in the plants at site 1

after the first growing season (1989) were due to the compounded stress of transplanting and exposure to SO_2 during the stage of rapid vegetative growth and inflorescence initiation.

The plants grown from commercial seed were used in my study to represent the greater genetic variability present in field populations. Thus, it was hypothesized that the net growth response of these plants to SO_2 exposure was more likely to represent the net growth response of field populations. It was assumed that SO_2-tolerant and non-tolerant genotypes would be represented in the seedlings grown from commercial seed, and that no influx of tolerant genes was required to obtain SO_2-tolerant genotypes (Wilson & Bell, 1985). However, the net growth response of the seedlings to SO_2 exposure was similar to the net growth response of the SO_2-tolerant clones, regardless of SO_2 exposure (Tables 14 & 15). This result was unexpected since it was hypothesized that the net growth response of the seedlings to SO_2 exposure would be intermediate, between the growth response of the SO_2-tolerant and non-tolerant clones. Thus, it appeared that the SO_2-tolerant clones although more tolerant to SO_2 than the non-tolerant clones, were generally of intermediate tolerance.

The non-tolerant clones produced fewer tillers, more inflorescences per tiller, and had lower shoot and root dry weights than the SO_2-tolerant clones or seedlings regardless of the site at which they were grown (Tables 14 &15). It

appeared that the response of the non-tolerant clones and SO_2-tolerant clones and seedlings to SO_2 exposure (and clipping) was probably effected by differences in growth morphology between genotypes. The non-tolerant clones flowered approximately one week to two weeks earlier than the SO_2-tolerant clones and the majority of the seedlings. In the field, the non-tolerant clones at both sites appeared to be more blue-green as opposed to the more yellow-green colour of the SO_2-tolerant clones. It is possible that pigment differences could affect rates of photosynthesis and affect tillering (Salisbury & Ross, 1985). Plants with greater rates of photosynthesis are thought to be more sensitive to SO_2 injury (Winner & Mooney, 1980b).

In my study the most likely scenario is that the non-tolerant genotype has a different innate reproductive growth strategy, and puts less energy into vegetative growth and tiller production than the SO_2-tolerant genotype and most of the seedlings (Tables 14 & 15). It is even more likely that the growth morphology and reproductive strategy of the non-tolerant genotype made it particularly sensitive to grazing stress, which was compounded by SO_2 stress at site 1, compared with the SO_2-tolerant genotype and the seedlings.

Above-ground clipping of the plants at the end of the 1989 growing season resulted in reduced shoot and root dry weights and the production of fewer inflorescences in clipped plants at the end of the 1990 growing season (Table 15). Grazing generally results in reduced yields which occur first

in the roots and later in the shoots (Singh *et al.*, 1980). Clipping of the shoots has been shown to initially reduce assimilate partitioning to the roots as the new leaves of the regrowing shoots become intense carbon sinks (White, 1973 c.f. Singh *et al.*, 1980). It is likely that such changes in carbon partitioning also affect flowering processes. Grazing has been shown to affect both the intensity of flowering and seed production, depending on the growth stage when grazing occurred (c.f. Harper, 1977). The sensitivity of plants to grazing is affected by the stage of ontogeny and availability of carbohydrate reserves at the time of grazing (Singh *et al.*, 1980). Late season clipping as in my study has been shown to retard the regrowth and affect the cold tolerance of grasses (c.f. Singh *et al.*, 1980). This suggests a stress interaction in plants subjected to defoliation.

In my study, clipping appeared to increase the sensitivity of flowering to SO_2 injury, particularly in the non-tolerant genotype. There was a 65 percent decrease in the number of inflorescences per g dry weight produced by clipped non-tolerant clones at site 1 compared with those grown at site 2 (Table 15). There was a 33 and 57 percent decline in the number of inflorescences produced by the clipped SO_2-tolerant clones and seedlings (respectively) at site 1 compared with those at site 2, thus indicating there was a significantly greater effect of SO_2 on clipped non-tolerant clones than on clipped SO_2-tolerant clones and seedlings. There was also a 44 percent decline in the number of

inflorescences produced by clipped versus unclipped non-tolerant clones at site 1 (Table 15). In contrast there was a 9 and 16 percent decline in the number of inflorescences produced by SO_2-tolerant clones and seedlings (respectively). This indicated that the flowering processes in non-tolerant clones of *P. pratense* were more sensitive to clipping stress under the influence of SO_2 exposure. Generally, it appears that flowering as opposed to vegetative plant growth is more sensitive to plant stress and that SO_2 injury can be increased by compounding stresses. Furthermore, there was no significant effect of clipping on flowering in plants grown at site 2 again suggesting that clipping had increased the sensitivity of the plants to SO_2 stress.

The effects of grazing on root growth include decreased nutrient uptake from soil within 24 hours of grazing (c.f. Harper, 1977). It seems possible given the decrease in nutrient uptake as a result of grazing, that water uptake might also be affected by grazing, thus increasing plant sensitivity to drought. There is evidence that grazing can also increase plant sensitivity to cold (Singh *et al.*, 1980). This suggests that grazing, although affecting primary production, might be acting to intensify the effects of other plant stresses because of its effects on plant assimilation and carbon reserves.

Perhaps the greatest effect of the interaction between clipping and SO_2 exposure at site 1 was on the persistence or survival of non-tolerant clones. Of the 15 clipped plants

available for harvest at site 1 in 1990 only 10 had survived (personal observation). In contrast at site 2 in 1990 there were 1 or 2 clipped seedling deaths and no noted clipped SO_2-tolerant clone deaths. In 1989 it was noted that there had been two plant deaths in the non-tolerant clones and seedlings at site 1 before clipping. However, there were no plant deaths reported in 1989 or 1990 at site 2 for any of the genotypes regardless of clipping. Indeed, the sward at site 2 appeared to be much more dense than the sward at site 1.

It was unlikely that during the 1989 growing season there was any effect of VA mycorrhizal fungi on plant growth. The percentage of roots colonised by VA mycorrhizal fungi was very low and particularly low in plants grown at site 1. Clapperton & Parkinson (1990) showed that there was a lower VA mycorrhizal inoculum potential at site 1 compared with site 2. However, the field plots at both sites had initially been cultivated. There have been reports that soil disturbance from soil tillage disrupts the external hyphal network of VA mycorrhizal fungi severely affecting their infectivity (Anderson, Millner & Kunishi, 1987; Jasper, Abbott & Robson, 1989; Evans & Miller, 1990). It seems likely that the effects of soil tillage on the infectivity of VA mycorrhizal fungi explains the lack of VA mycorrhizal infection at both field sites in 1989.

Despite the small amounts of VA mycorrhizal colonisation in plants at sites 1 and 2, there were significant differences in the amounts of colonisation between genotypes at site 2.

There was a tendency for the SO_2-tolerant genotype to partition more ^{14}C-labelled compounds to the roots (Table 13, Chapter 4) than the non-tolerant genotype. It might be that this difference in carbon partitioning is reflected in the increased percentage of colonised roots in the SO_2-tolerant clones at both sites 1 and 2.

Interestingly, seedlings at both sites were the least colonised by VA mycorrhizal fungi compared with the SO_2-tolerant and non-tolerant plants. This might be related in some way to the differences between tillers and seedlings. It is possible that the tillers had still not recovered from being separated from parent plants and might have had damaged roots which might have increased the amount of carbon leaking into the rhizosphere. Infection by VA mycorrhizal has been linked to the amount of carbon in and exuded from roots (Ratnayake, Leonard & Menge, 1979). However, it is more likely that the tillers were partitioning more carbon to the roots than seedlings due to the apparent increased leaf area of the tillers.

Tillers for the most part also appeared to have slightly more root length than seedlings at transplanting. Since there is a seasonal variation in rates of VA mycorrhizal infection (Rabatin, 1979; Brundrett & Kendricks, 1988) it is possible that the apparent increased root length of tillers at transplanting early in the growing season resulted in increased amounts of colonisation by VA mycorrhizal fungi in tillers. However, I harvested plants at the end of the

growing season so if plants had been colonised early in the season infections should have proliferated more throughout the root system. Clearly this was not the case.

CONCLUSION

In terms of grassland ecology my results suggest that grazing could increase the effects of SO_2 exposure on plant productivity increasing the selection pressure on non-tolerant plants. The lack of persistence of the SO_2 non-tolerant genotype at site 1 suggests that there is likely less genetic variability between grass populations at site 1 compared with populations at site 2. It is also possible that increased grazing pressure in areas subjected to chronic SO_2 exposure affects the competition within and between grass populations at these sites, therefore affecting the genetic and species diversity at polluted sites.

It is important to note that during this two-year field study the compressor stations near the gas plant were being upgraded which meant that the gas producing capacity of the plant was cut in half (Shell Canada Resources Ltd., 1990). Nevertheless, this study showed that there were still effects of the low sulphur deposition on *Phleum pratense* at site 1 compared with site 2. It would be useful to monitor the field sites for at least another growing season to see if the increased sulphur deposition, due to the return of the gas

plant to full capacity in November 1990, has an effect on plant productivity at site 1.

DISCUSSION: Ecological Implications

Natural grasslands occur in areas too dry for forest development but not arid enough to prevent the development of a closed perennial herbaceous layer (Coupland, 1979). In these areas climate acts to control the biotic components both directly, and indirectly through its influence on soil development. Mean annual precipitation in temperate grasslands ranges between 250 and 750 mm (Coupland, 1979). Precipitation is known to determine the nature and extent of grasslands while high winds can increase the tendency for water loss through evapotranspiration which in turn can be accentuated by high summer temperatures.

The soils of temperate grasslands are characterised by an abundance of organic matter at all depths, restricted soil leaching because of insufficient water percolation, and the low solubility of minerals in basic soil solution which are slowly released from humus (Coupland, 1979).

Grassland vegetation in general is dominated by graminoids (members of the Graminaceae and Cyperaceae) and forbs. Graminoids usually represent less than 20 percent of the species richness but 90 percent and more of the canopy biomass (Coupland, 1979). Growth morphology is important in relation to environmental adaptation. Thus, the perennial habit predominates in natural grasslands

with two or more grass species providing greater than 60 percent of the total shoot biomass (Coupland, 1979).

Herbivores both large and small can be as important as climate and soil type in effecting the floristic composition of grasslands (Andrzejewska & Gyllenberg, 1980; Van Dyne *et al.*, 1980; Grime *et al.*, 1987). The effects of these animals and insects are often more obvious in man-modified ecosystems. Overgrazing by domestic livestock and ungulates in restricted habits can severely reduce the productivity of grasslands, drastically changing the plant species composition.

Floristic diversity in grasslands is also affected by VA mycorrhizal fungi (Grime *et al.*, 1987). Grime *et al.* (1987), using a mixture of pasture grasses and forbs, showed that VA mycorrhizal fungi shifted the floristic balance in favour of forbs. Buwalda (1980) showed that when clover (*Trifolium repens*) was grown with perennial ryegrass (*Lolium perenne*) in non-mycorrhizal soil the grass grew better than the clover, and the addition of VA mycorrhizal fungi improved the growth of the clover compared with that of the grass. In another experiment Fitter (1977) found that the size ratio of *Holcus lanatus* to *Lolium perenne* increased with increasing colonisation by VA mycorrhizal fungi and suggested that colonisation by VA mycorrhizal fungi reduced the growth of *Lolium perenne*. Allen & Allen (1990) concluded that VA mycorrhizal fungi might influence the regulation of plant competition when

species with different responses to colonisation are neighbours, and that the importance of VA mycorrhizal fungi to competition varies between plant species and environments.

Miller (1987) stated that the growth response of plants to VA mycorrhizal infection varies with climate, soil type, and the habitat from which the host and endophyte originate. There are reports of reduced growth of grasses in response to colonisation by VA mycorrhizal fungi (Sparling & Tinker, 1978; Chapters 1, 2, & 3 in this thesis). Evidence suggests that temperate and alpine grasses do not respond as well as tropical and warm-season grasses in terms of biomass production to VA mycorrhizal fungi (Miller, 1987; Hetrick, Wilson & Todd, 1990). It has been suggested that the lack of response of cool-season C_3 grasses to VA mycorrhizal fungi is related to plant strategies for nutrient uptake (Hetrick, Kitt & Wilson, 1988; Hetrick, Wilson & Todd, 1990). However, the majority of grasses in grasslands are colonised by VA mycorrhizal fungi (Sparling & Tinker, 1975; Read, Koucheki & Hodgson 1976; Molina, Trappe & Strickler, 1978).

Coupland (1979) stressed the need for multidisciplinary studies to obtain more information on the mechanisms and processes that control various components of grassland ecosystems. It is apparent that there is a need to consider the symbiotic relationship between VA mycorrhizal fungi

and the host plant in any interpretation of the structure and function of a community.

A plant community is conceptually composed of plant populations and their interactions. These interactions can be influenced by abiotic and biotic factors as I have previously pointed-out. Ecological systems in most cases fit a hierarchical model or organisation. In terms of response to plant stress, high-level responses (i.e. responses of plant communities) do not always consist of simple linear combinations of low-level responses (i.e. photosynthesis, assimilate partitioning, translocation) (Lauenroth & Milchunas, 1985). Indeed, it is likely that low-level responses act synergistically in many cases.

In the following discussion I will re-examine the low-level responses of *Phleum pratense* to colonisation by VA mycorrhizal fungi, and to SO_2 fumigation and grazing (the field study in chapter 5) in context with the hierarchial model, with respect to temperate and more specifically sub-montane grasslands.

The results of my study (chapter 1) show that there is a an effect of colonisation by VA mycorrhizal fungi on the growth of *Phleum pratense* and that this effect was directly proportional to the percent of VA mycorrhizal colonisation from field soil. These results agree with those of Hetrick, Wilson & Todd (1990) showing that plant dry weight and colonisation by VA mycorrhizal fungi are inversely related in cool-season C_3 grasses. In turn, the

percent of VA mycorrhizal colonisation was directly proportional to the amount of field soil used as inoculum. Adding mineral nutrients increased plant growth and inhibited the ability of VA mycorrhizal fungi to form functional symbioses. It appeared that the increased plant growth was a result of relief from mineral nutrient immobilisation by soil microbes rather than from decreased colonisation by VA mycorrhizal fungi. It has been shown that the growth of some cool-season C_3 grasses is not affected by phosphorus fertilizing, or by soil microorganisms not including VA mycorrhizal fungi (Hetrick, Wilson & Todd, 1990). Clearly the results of my study differ from theirs. However, individual plant species will respond differently to VA mycorrhizal fungi. For example, the dry weight of *Agropyron trachycaulum* plants was not as highly correlated with colonisation by VA mycorrhizal fungi as that of *Phleum pratense* (Chapter 1). This is also consistant with the suggestion (Adelman & Morton, 1986) that percent VA mycorrhizal colonisation is greatest for a host/VA mycorrhizal inoculum/diluent soil combination with the same origins. It seems likely that just as plant ecosystems are unique to certain soils and climates, organisms which comprise the mycorrhizosphere are uniquely adapted to the plant community. Therefore, it is equally as likely that factors which determine and effect plant communities will also effect mycorrhizosphere communities.

Environmental factors which are known to affect root exudation either directly or indirectly include anoxia (Hiatt & Lowe, 1967), drought (Shone, Whipps & Flood, 1983), temperature (Rovira, 1959), grazing (Singh *et al.*, 1980), andlight intensity (Rovira, 1959). All factors which affect root exudation necessarily affect mycorrhizosphere organisms. The gaseous air pollutant SO_2 is known to affect plant carbon assimilation, and translocation and partitioning to the roots. The results of my study (chapters 2 & 3) show that the direct effects of SO_2 on plant physiology indirectly affect colonisation by VA mycorrhizal fungi. Because other soil microorganisms are also associated with plant roots and affected by VA mycorrhizal colonisation it is likely that they are also affected. More importantly, the results showed that the negative effect of colonisation by VA mycorrhizal fungi on the growth of *Phleum pratense* was associated with the physiological effects of VA mycorrhizal fungi on plant carbon assimilation and partitioning (Chapter 3). It appeared that as a consequence of these effects plants had increased sensitivity to SO_2 exposure.

Interestingly, SO_2 exposure had no obvious gross morphological effect on plants grown at the field sites, while there were obvious physiological and biochemical effects on plants grown in the laboratory (Chapter 3). Dodd, Lauenroth & Heitschmidt (1982) reported no significant effects of low-concentration SO_2 exposure on

above-and below-ground biomass production in a northern mixed-grass prairie. However, they did show that the rates of increase in rhizome biomass were reduced during a 4-year study and suggested that long-term exposure of grasslands to low concentrations of SO_2 would reduce the vigour of rhizomes to such an extent that net primary production was reduced, above- and below- ground biomass dynamics altered, and floristic diversity affected.

However, many plants growing at polluted sites have increased tolerance to SO_2 exposure (Horsman *et al.*, 1978; Taylor, 1978; Ayazloo & Bell, 1981). Evidence suggests that the evolution of plant tolerance to SO_2 is widespread (Taylor & Murdy, 1975; Horsman, Roberts & Bradshaw, 1979; Ayazloo & Bell, 1981; Wilson & Bell, 1986). Ayazloo & Bell (1981) demonstrated that chronic low-concentration SO_2 exposure can cause significant changes in the characteristics of grass populations, mediated by altered intraspecific competition, rather than by eliminating sensitive genotypes.

Certainly the results of my study indicate there are intraspecific differences in tolerance to SO_2 exposure in *Phleum pratense* populations (Chapter 4) and that there is probably less genetic variability between grass populations at the field site subjected to chronic SO_2 exposure (Chapter 5). Horsman, Roberts & Bradshaw 1978 have suggested that the lack of intraspecific genetic variability at sites subjected to long-term exposure to air pollutants was

probably due to the overall selection of more tolerant genotypes. Since colonisation by VA mycorrhizal fungi influences the sensitivity of grasses to SO_2 exposure (Chapter 3) it is possible that they also influence the tolerance selection process.

Studies have shown that colonisation with VA mycorrhizal fungi increases plant photosynthesis (Allen *et al*, 1981; Bildusas *et al.*, 1986; Brown & Bethlenfalvay, 1987). Colonisation by VA mycorrhizal fungi is known to increase stomatal conductance (Allen, 1982; Allen & Allen, 1986) which is thought to be important during drought stress. Winner and Mooney (1980b) suggested that drought tolerance mechanisms were important in determining the effects of gaseous air pollutants. They showed that stomatal conductance was critical to plant SO_2 tolerance. Plants with higher stomatal conductance absorb more SO_2 and all factors being equal such plants are thought to be less tolerant (Winner & Mooney, 1980b).

In the field study SO_2-tolerant plants were significantly more colonised by VA mycorrhizal fungi than non-tolerant plants and seedlings at both field sites (Chapter 5). In the laboratory the SO_2-tolerant genotype was shown to have lower rates of photosynthesis (indicating lower stomatal conductance) and to partition more assimilate to the roots than the non-tolerant genotype (Chapter 4). It would have been interesting to determine if colonisation by VA mycorrhizal fungi increased the rate of photosynthesis

and/or stomatal conductance in SO_2-tolerant plants. If the effect of VA mycorrhizal fungi on photosynthesis is source-sink (Herold, 1980) related (Dosskey, Linderman & Boersma, 1990) then it is likely that there would be little effect of VA mycorrhizal fungi on photosynthesis in SO_2-tolerant plants due to the innately high partitioning of assimilate to the roots of SO_2-tolerant plants. However, it is possible that VA mycorrhizal fungi affect stomatal conductance directly through abscisic acid (ABA) production (Allen, Moore & Christensen, 1982). Abscisic acid affects stomata (Raschke, 1979) and has been implicated in the effects of SO_2 on stomata (Kondo & Sugahara, 1978; Taylor, Reid & Pharis, 1981). ABA is synthesised in the roots (Robertson *et al.*, 1985) and is readily transported throughout the plant (Hubick & Reid, 1988). If this is the case then the rates of photosynthesis in SO_2-tolerant plants would likely increase in response to colonisation. More importantly since such physiological effects ultimately affect biomass production, it is possible that VA mycorrhizal fungi might affect intraspecific competition between SO_2-tolerant and non-tolerant individuals in the field.

In the first year of the field study the amount of colonisation by VA mycorrhizal fungi in all *Phleum pratense* genotypes was low (Chapter 5). It is likely this was as a consequence of soil perturbation (Jasper *et al.*, 1989: Evans & Miller, 1990). However, in grasslands VA

mycorrhizal infection usually arises from contact between uninfected roots and infected roots (Sparling & Tinker, 1975; Read *et al.*, 1976) and there is evidence that most VA mycorrhizal plants are interconnected within a plant community (Whittingham & Read, 1982; c.f. Newman, 1988). It is likely that there was indeed an effect of soil perturbation on VA mycorrhizal fungi. However I suggest that the low amount of colonisation by VA mycorrhizal fungi at the field sites was related in part to differences between the ability of the fungi to colonise tillers and seedlings. It is likely that the ability of VA mycorrhizal fungi to colonise seedlings is reduced compared with tillers connected to parent plants. There is no doubt significantly more carbon is available to fungi from tillers than from seedlings.

In undisturbed grasslands the perennial habit predominates, which might explain the lack of apparent detrimental effects of VA mycorrhizal fungi on plant growth in grasslands. Most experiments which study the effects of VA mycorrhizal fungi on plant growth use seedlings. It may be that VA mycorrhizal fungi in combination with other soil organisms have a parasitic relationship with seedlings, and a symbiotic relationship with tillers because of the greater leaf area of tillers at the same age as seedlings and the ability of the parent plant or perennating organ to offset the carbon cost of the fungus. It is also likely that the ability of VA mycorrhizal fungi to

colonise tillers is related to the type of perennating organ, as plant species differ in the amounts of carbon partitioned to and reserved in perennating organs.

VA mycorrhizal fungi are thought to be important to plant growth in soils of low phosphorus content. Yet reports have shown that VA mycorrhizal infection is not a prerequisite for phosphorus uptake or nutrition in temperate C_3 grasses (Atkinson, 1983; Hetrick, Wilson & Todd, 1990). However, these studies were conducted with seedlings, under greenhouse conditions, in potting mixture (Atkinson, 1983) and field soil, non-mycorrhizal soil, and sterile soil (Hetrick, Wilson & Todd, 1990). In the greenhouse and in growth chambers root temperatures are higher than in the field. Hetrick, Kitt & Wilson (1988) suggested that cool-season C_3 grasses have evolved nutrient uptake strategies, such as extremely fiberous root systems, which limit their dependence on VA mycorrhizal fungi, because VA mycorrhizal fungi are not effective in nutrient uptake at lower temperatures (Hetrick, Wilson & Kitt, 1988; Hetrick, Wilson, & Todd, 1990). Despite the lack of apparent requirement for VA mycorrhizal fungi in temperate and alpine grasslands their presence is ubiquitous, so natural selection in favour of symbiosis must still occur.

Laurenroth & Milchunas (1985) suggested that the more connected a plant community hierarchy, the more likely the effects of SO_2 exposure and for that matter the effects

of any other stress will be found at the highest hierarchical level: the plant community.

In my study (chapter 5) there were effects of SO_2 exposure at field site 1 on dry matter partitioning in the first year, and on flowering in the second year. It was unlikely however that VA mycorrhizal fungi were involved in any of the first year effects because the amount of colonisation was extremely low. The involvement of VA mycorrhizal fungi in the response of *Phleum pratense* to SO_2 in the fields is unlikely for reasons suggested previously. More importantly, this study showed that there is a compounding effect of foliar stresses (SO_2 exposure and clipping) on plant productivity (Chapter 5) (Heasley, Lauenroth & Yorks,1984). Given continued industrial development and increased use of grasslands for livestock production, the effect of compounding stresses is worth considering.

Changes in floristic diversity are clearly the ultimate effect of the multiplicity of low-level plant responses to abiotic and biotic factors. Although the plant species composition at the two field sites was similar (Clapperton & Parkinson, 1990) it appeared that there had been some changes in the population diversity of *P. pratense* at site 1 (chapter 4). There was also a lower VA mycorrhizal inoculum density at site 1 pointing to the indirect effect of SO_2 on VA mycorrhizal fungi. Indeed, it is likely that measured amount of colonisation may be useful in

interpreting the effects of indirect and direct stress on the mycorrhizosphere.

This study illustrates the need to continue to integrate the mycorrhizosphere into models which examine plant growth and the effects of abiotic and biotic factors on plant growth. It is also obvious that long-term field studies are necessary to test the predictability of laboratory and greenhouse results to natural ecosystems.

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