V-A MYCORRHIZAS OF WINTER CEREALS IN S.E. ENGLAND: ECOLOGY, TAXONOMY AND EFFECTS OF AGROCHEMICALS

by

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ABSTRACT

The association between vesicular-arbuscular mycorrhizal fungi and winter wheat was investigated in the field, the greenhouse and the laboratory.

Extensive early autumn fractional infection of commercially grown winter cereals was observed in two fields in Sussex over a three-year period. Fluctuations in seasonal infection levels were monitored over this period and two trends were noted in different seasons, an overwinter decline and continued infection spread through the winter months. The overwinter decline in infection may be correlated with low thermal time accumulation during the autumn period and therefore early sowing may be important. This effect, however, appeared to be influenced by other factors such as soil moisture status and consequently root growth dynamics.

Three *Glomus* species were obtained in pure pot culture and their taxonomy investigated by means of morphology (light microscopy and electron microscopy) and polyacrylamide gel electrophoresis of spore esterases. Differences were noted between spores isolated from the field and their subsequent appearance in pure pot cultures.

The effect of soil pesticides on the VAM symbiosis was investigated. In general, herbicide applications at equivalent field rates had negligible effects on VAM infection development in winter wheat grown in pots in the greenhouse, and on *in vitro* spore germination. Fungicide applications, also at equivalent field rates, covered the range of response from inhibitory to stimulatory on infection levels and spore germination depending on the nature of the active moiety of the agrochemical used.

The indigenous VAM population from one of the Sussex field sites appeared less efficient at stimulating phosphorus uptake in winter wheat grown in partially sterile field soil compared with three introduced *Glomus* endophytes.

Root and rhizosphere acid phosphatase activities differed between three VAM fungal infections of wheat grown in sterile sand culture. High activity did not correlate with increased shoot P uptake.

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ABBREVIATIONS

a.i.	active ingredient
approx	approximately
aq	aqueous
BF	brightfield light micrograph
°C	degrees Centigrade
cm	centimetres
cv	cultivar
D.W.	dry weight
e.g.	for example
Fig	Figure
F.W.	fresh weight
h	hour
kg	kilogram
Klx	kilolux
kv	kilovolts
L	litre
LSD	least significant difference
M	molar
µm	micrometres
mg	milligrams
mm	millimetres
mins	minutes
NB	nutrient broth
NIC	interference contrast light micrograph
PAGE	polyacrylamide gel electrophoresis
pers.comm.	personal communication
Pi	inorganic phosphorus
rpm	revolutions per minute
secs	seconds
SEM	scanning electron micrograph
SW	sterile water
TEM	transmission electron micrograph
VAM	vesicular-arbuscular mycorrhiza(s)
var	variety
v/v	volume for volume
WA	water agar
w/v	weight by volume

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

It is now generally accepted that vesicular-arbuscular mycorrhizas (VAM) are mutualistic symbioses involved in the interchange of carbon and phosphate between plants and fungal endophytes. The passage of carbon from plant to fungus and phosphorus from fungus to plant has been clearly shown (Cox *et al.*, 1975).

VA mycorrhizas are formed in most angiosperms, some gymnosperms and in pteridophytes and bryophytes under tropical, temperate and even arctic conditions (Hayman, 1982). They form associations with most of the economically important crops including the cereals, wheat and barley. VAM fungi have not yet been successfully cultured axenically and hence controlled studies on the development of VAM infection are difficult. Substantial hyphal growth can be obtained *in vitro* from germinated spores but growth ceases when the hyphae are excised from the parent spore. It has been shown that spores of *Glomus* spp. can synthesise new m-RNA for hyphal growth (Hepper, 1979) and that they possess an Emden-Meyerhof-Parnas system, a tricarboxylic acid cycle and a hexose monophosphate shunt (MacDonald and Lewis, 1978). These results suggest that the fungi resemble saprophytic fungi more closely than most obligate biotrophs. A limited independent spread of VAM fungi in soil has also been reported, suggesting some saprophytic ability in soils (Warner and Mosse, 1980).

1.1. VAM infection development

Studies on the development of VAM infection have shown that neither spore germination nor initial direction of hyphal growth was influenced by the presence of host roots. Hyphae from spores were not attracted to roots until they approached them closely (Powell, 1976a; Mosse and Hepper, 1975). Once a fungal hypha has contacted the root surface, penetration may occur via an appressorium either directly or following ectotropic growth. It is believed that there is some stimulation of hyphal growth in the rhizosphere, and a positive correlation between root exudation and VAM infection via increased permeability of root cell membranes has been found (Ratnayake *et al.*, 1978; Azcon and Ocampo, 1981). Recent work by Azcon and Ocampo (1984) has indicated, however, that sugar concentrations inside the root and in root exudates may not be directly related to plant susceptibility to VAM infection as earlier believed. Recent evidence has implicated a volatile substance, released by suspension-cultured plant cells, in greatly improving hyphal growth of *G. caledonium* (Nicol. & Gerd.) Gerd. & Trappe in axenic culture (Carr *et al.*, 1985). Another important rhizosphere factor is the presence of active populations of microorganisms. Suggestions that they play a role in the development of VAM infection is supported by studies on the influence of free-living rhizosphere microorganisms on VA fungi under axenic conditions. It has commonly been observed that fungal and bacterial contaminants around or near VAM spores on agar media have increased hyphal growth (Dodd and Krikun, 1984) but the reasons behind this phenomenon are unknown.

Once an appressorium is produced hyphal penetration occurs into or more usually between the cortical cells. Once the first entry point is established, root colonisation extends in each direction along the root and hyphae multiply inter- and intracellularly, without invading the endodermis, stele or root meristems

(Amijee *et al.*, 1986). Recent work has indicated that the older a root becomes, the less chance there is of infection occurring successfully from appressoria (Hepper, 1985).

Shortly after infection, an invasive hypha enters a single inner cortical cell by penetrating the cell wall and invaginating the plasma membrane. This hypha shows repeated dichotomous branching and an *arbuscule* is formed. The function suggested for the arbuscule is the bidirectional transfer of nutrients, the mechanism requiring the presence of the living fungal endophyte (Cox and Tinker, 1976). Cytoplasm content increases in the infected host cell, starch disappears and nuclei become enlarged and divide. Cell organelles such as mitochondria and ribosomes also increase in number (Rhodes and Gerdemann, 1980). Arbuscules are usually functional for 4-13 days after which degeneration occurs and the host cell and organelles return to their normal state, and occasionally another arbuscule may subsequently be formed in the same cell (Hayman, 1982). Later in infection development the endophyte may form inter- or intracellular *vesicles*, which are oval to obovoid to spherical lipid-containing structures. They are thin-walled at first and are believed to have a temporary storage function, after which they may become thick-walled and chlamydospore-like.

When the internal infection has become established, external hyphae ramify, either growing along the root surface forming more penetration points or extending into the surrounding soil beyond the rhizosphere producing an extensive fine mycelial network. External hyphae are typically dimorphic with coarse, thick-walled hyphae channelling into the entry points and bearing resting spores and fine thin-walled hyphae which are more ephemeral and have an absorptive function. The density, geometry and size of the external mycelium and frequency of entry points per unit root length are of great relevance in the

functioning of VAM associations. Spore production usually signifies the maturation of the association where large resting spores and/or sporocarps are produced on the external mycelium. Some species, however, can form prolific numbers of spores within the root cortex.

1.2. Physiology of the VAM symbiosis

Current mycorrhizal research is progressing towards a better understanding of many physiological features of the symbiosis, particularly of the mechanisms that account for mycorrhizal effects on plant growth and nutrition. Much earlier work was concentrated on the `big plant - small plant' syndrome using sterile soils with low P levels in pot experiments. This may have over-emphasised the potential importance of VAM fungi to plants in the absence of equivalent field plot inoculations under natural conditions to confirm results obtained in pots. Most mechanisms involved with the symbiosis remain poorly understood. Recent reviews by Smith (1980), Tinker (1980), Hayman (1982, 1983, 1984), Mosse *et al* (1981), Rhodes and Gerdemann (1980) and Gianinazzi-Pearson and Gianinazzi (1981) have thoroughly discussed many physiological aspects of the symbiosis including the nutritional relationship, and only the main points are summarised here.

1.2.1. VA mycorrhizas and P uptake

Phosphorus is by far the most important nutrient involved in the response of plant growth to VAM infection, and the uptake of P is consistently improved in mycorrhizal plants compared with non-mycorrhizal controls (see reviews). Nye (1977) concluded that the uptake of nutrients such as P is limited by the rate of diffusion through the soil and not by the ability of the root to absorb them from low concentrations in the soil solution. It seems likely, therefore, that VA mycorrhizas increase P uptake primarily by shortening the distance that

phosphate ions have to diffuse through the soil to the root. Hattingh *et al* (1973) found that VAM hyphae could intercept labelled phosphorus placed 27mm from a mycorrhizal root, whereas it remained unavailable to non-mycorrhizal roots. It is now fully accepted that hyphal uptake is the mechanism by which infection increases the uptake of P by the host plant. Phosphate ions, which are in low concentration in the labile pool (Bieleski, 1973), move slowly by diffusion since they are readily adsorbed to the soil colloids. Plants take up phosphate ions from the soil solution in and around the root rhizosphere at a much faster rate than they can diffuse to the root surface. Consequently, phosphate-depletion zones normally develop around the absorbing organs (roots or mycorrhizas) of the plant. This is especially so for coarse, sparse root systems that require high P inflow rates and absorb it from a relatively small proportion of the total soil volume. In very dense root systems the inter-root distance is very small, leaving little unexploited soil for hyphae to ramify into and the inflow of P into roots is much lower, reducing the likelihood of a response to VAM infection. The phosphate-depletion zones, 1-2mm wide, coincide with the rhizosphere and have been shown to be much larger around mycorrhizal onion roots compared with non-mycorrhizal onion roots (Owusu-Bennoah and Wild, 1979). Hence, the position of the extra absorbing mycelial network relative to the zones of P uptake by the plant roots, determines the degree to which the P nutrition of the host is improved.

Tinker (1980) suggested that responses to VAM infection can be expected whenever the maximum diffusive transfer of P to unit length of root (inflow) is appreciably less than that required to maintain the growth of the plant. It is therefore likely that P uptake by roots, dependant on diffusion of phosphate ions over relatively long distances, will be much more sensitive to the drying of soil than will mycorrhizal transport, which effectively provides a low resistance

transport pathway (Cooper and Tinker, 1981).

It has been shown that both mycorrhizal and non-mycorrhizal plants absorb P from the same soluble phosphate pool (Sanders and Tinker, 1971; Bolan *et al.*, 1984). The apparent solubilisation of poorly soluble sources of P, such as rock phosphates, is probably entirely due to the greater contact between the network of external hyphae and the surfaces of phosphate particles in soil where P is being physicochemically or biologically dissociated (Hayman, 1978). It has also been suggested that VA mycorrhizas reduce the threshold value for effective phosphate absorption from the soil (Mosse *et al.*, 1973). Cress *et al.* (1979) also claimed that mycorrhizal roots had a much higher affinity for P than non-mycorrhizal roots, based on a study of the kinetics of P uptake from solution, however the results are generally inconclusive.

1.2.2. VA mycorrhizas and P transfer to the host

There is now experimental evidence to show that P is translocated to internal fungal structures as polyphosphate granules contained inside vacuoles (Callow *et al.*, 1978). These are moved through the hyphal lumen by cytoplasmic streaming to the arbuscules, although bulk flow may also contribute to the translocation (Cooper and Tinker, 1981). The specific mechanism for polyphosphate synthesis, translocation and degradation is active and very efficient and the calculated inflow of P through external hyphae is approx. 1,000-fold faster than the phosphate diffusion rate through soil (Bieleski, 1973). Mycorrhiza-specific phosphatase activity has been identified in the young arbuscules of VAM infections suggesting that these phosphatases may play a key role in the active translocation and/or transfer mechanisms in VA mycorrhizas (see Capaccio and Callow, 1982).

The main site of phosphate transfer to the host, which occurs by an active mechanism across the membrane of both partners, seems to be the arbuscule (Cox *et al.*, 1980). This hypothesis is strengthened by the finding that the plasmalemma-bound ATPase activity of the host is concentrated around the arbuscles when VAM infection develops (Marx *et al*, 1982). It is also now accepted that the final breakdown of the arbuscules can account for only 1% of the P inflow to the host cells. Phosphate release by other structures such as internal hyphae or vesicles might also be involved, but the extensive increase of contact surface area makes the arbuscules the most likely sites for nutrient transfer between symbionts.

1.2.3. Absorption of other nutrients by VA mycorrhizas

It has often been reported that VAM infection also increases the concentration of nutrients other than P in plant tissues, but it is unclear if this uptake is merely a consequence of improved P supply. For example the translocation of calcium and sulphur (Rhodes and Gerdemann, 1978a) as well as zinc (Cooper and Tinker, 1978) by external hyphae of VAM fungi have been demonstrated. There are variable reports of effects of VAM infection on the uptake of nitrogen (N) by host plants and recent work has shown increased uptake and transport of N from two ^{15}N-labelled sources by *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe infection of celery (Ames *et al.*, 1983). There are also several reports of increases in concentrations of N in roots and shoots of legumes infected by VAM fungi (Smith and Daft, 1977). This effect has generally been attributed to increased nodulation and nodule nitrogenase activity, resulting from the improved uptake of P by mycorrhizal plants (Daft and El-Giahmi, 1975), since P is important for nodulation and fixation of N by *Rhizobium* spp. (Van Schreven, 1958).

An additional role for VAM fungi in improving plant water relations has been suggested recently. Nelsen and Safir (1982), for example, found that mycorrhizal onions were much more drought resistant than uninfected plants, and Allen (1982) concluded that the effect of infection on plant water relations was due to increased water uptake by the external mycelial network. Doubt about this theory, however, has been expressed (Fitter, 1985), although it is likely that hyphae can bind the rhizosphere soil to roots, so preventing the separation of root from soil during periods of low soil water potential, thereby accounting for the better water relations of VAM infected plants (Levy and Krikun, 1980; Dodd and Levy, unpublished observations).

1.2.4. Non-nutritional effects of VA mycorrhizas

Mycorrhizas can affect plant growth and vigour by mechanisms other than improved host nutrition. The production of substances with hormonal activity have been described for VAM fungi germinating and `growing' *in vitro* (Barea and Azcon-Aguilar, 1982b) and higher levels have been observed in VAM infected plants (Allen *et al*, 1980). These substances can alter plant morphology and physiology and some mycorrhizal effects such as earlier stimulation of flowering (Dodd *et al.*, 1983) may be mediated by changes in the hormonal balance.

A role for mycorrhizas in improving soil structure has also been suggested (Koske and Halvorson, 1981). This may be particularly significant in eroded soils and sand dunes, since VAM hyphae can bind soil particles into more stable aggregates (see above).

VAM infection can apparently also help plants withstand root diseases either by protecting the root system against pathogen attack or by compensating for root damage (Schonbeck, 1979). This, however, may largely be due to improved host-plant nutrition (Graham and Menge, 1982).

1.2.5. Carbon nutrition in VA mycorrhizas

Under low P conditions (optimal for the fungus), heterotrophic VAM fungi obtain carbon (C) compounds from their autotrophic hosts by means of biotrophic transfer across the living membranes of both partners (Smith, 1980). Direct evidence for such transfer was obtained by detecting ^{14}C-labelled compounds in fungal structures associated with plants that photosynthesised with $^{14}CO_2$ (Ho and Trappe., 1973; Cox *et al.*, 1975). The VA mycorrhizas do not appear to form trehalose or mannitol (Hayman, 1974) as in ectomycorrhizas and ericoid mycorrhizas (Harley, 1975) and lipids seem to be an alternative sink (Cox *et al.*, 1975). Bevege *et al.* (1975) recorded results that indicated that the VA fungus of an infected plant could represent a considerable drain on the supply of photosynthate of the host. Such a drain may explain the repeated observations of higher concentrations of P in mycorrhizal plants compared with non-mycorrhizal plants of equal size, since less photosynthate would be converted to plant dry matter production and so P concentrations expressed on a dry weight basis would appear higher (Stribley *et al.*, 1980). There are several reports of depressions of growth associated with VAM infection (Crush, 1976; Kiernan, 1983) which may have resulted from competition between fungus and host for a limited supply of photosynthate under high soil nutrient conditions or when host photosynthesis is reduced e.g. under low light intensities (Wang, 1984). Although Cooper (1975) considered that there was insufficient fungal biomass present to account for the depressions in growth she observed, measurements by Hepper (1977) and Bethlenfalvay *et al* (1982) show that up to 17% of the dry weight of roots in VA mycorrhizas may be fungal biomass, a much higher value than has been reported for other root parasites. Mycorrhizal infection may also increase the respiration of roots (Pang and Paul, 1980). The measured additional diversion of C to structures below the soil surface following VAM

infection was 10% of the total fixed in faba bean (Pang and Paul, 1980) and 8% in leek (Snellgrove *et al.*, 1982). Other biotrophic fungal infections, such as cereal rusts, also have the ability to alter the distribution of the photosynthate of the host, and redirect it to the infected zone (Lewis, 1973). Pang and Paul (1980), however, suggested that the drain of C to the VAM fungus did not have a deleterious effect on the growth of the host since mycorrhizal plants may fix more C to compensate for the 'loss' to the fungus. Such a compensation may be possible as a result of a lower percentage of dry matter in the leaves of VAM plants, which produces a greater assimilation rate per unit of dry matter in the leaf, though the net rate is the same (Snellgrove *et al.*, 1982). Recent work, however, on sunflower growing in a low P soil (5μg g^{-1}) and a moderate P soil (15μg g^{-1}) suggested that leaf expansion was a good indicator of mycorrhizal effects on plant growth, since there appeared to be differences in the leaf dry weight: stem dry weight ratio between mycorrhizal and non-mycorrhizal plants (Koide, 1985).

1.3. Factors affecting mycorrhizal establishment, development and function

The establishment of successful entry points on host roots, the internal and external development of the fungus (described earlier), and the resulting plant responses are all dependent on interactions between the fungus, plant and prevailing environmental factors. VAM fungi are present in virtually all soils, but population levels vary greatly for different ecological conditions. Indigenous VAM populations can be diminished by agricultural practices such as heavy fertilisation, fumigation and agrochemical treatments.

1.3.1. Mycorrhizal dependency

Certain plant species require VAM associations to a much greater extent than others, and this is usually referred to as ' mycorrhizal dependency' (MD),

which is defined as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield, at a given level of fertility" (Gerdemann, 1975). Generally, plants having coarse root systems (>0.5mm diam.) and lacking root hairs are highly dependent on VAM associations. Conversely, plants with a dense cover of long root hairs and root systems with fine rootlets (<0.1mm diam.) respond to mycorrhizas only in P deficient soils (St. John, 1980). However, differences in the relative MD between crop species or cultivars (Azcon and Ocampo, 1981) are also related to other inherent genetic factors which could affect the demand for P.

1.3.2. Fungal specificity

VAM fungi have very little host specificity and any given isolate can infect virtually any potential host plant (Hayman, 1982). Each isolate differs, however, in effectiveness, which appears to depend more on the specific soil-plant system that they colonise than on the host itself. The ability of an endophyte to develop a large amount of external mycelium has been suggested as being positively correlated with the enhancement of plant growth by VAM fungi (Graham *et al.*, 1982). The other critical factor for the effectiveness of VAM fungi is the rapidity of infection of the root system (Hayman, 1982), and Abbott and Robson (1981) found a close correlation between the efficiency of a fungal species and the percentage of root length infected at early stages of plant growth.

1.3.3. Soil conditions

Soil pH can influence the predominance of a given spore type, and some species are better adapted to infect in acid soils than alkaline or neutral soil and *vice versa* (Mosse, 1973a; Hayman and Tavares, 1985).

VAM fungi are sensitive to the soil moisture status (Saif, 1981) with waterlogging being particularly detrimental to mycorrhizal activity. Soil

temperature affects the preinfection stages of VAM development in that the number of appressoria increases as the temperature rises, although this may reflect increases in root biomass (Smith and Bowen, 1979).

The level and type of nutrients in the soil affect the formation of, and the response to, mycorrhizas (Smith, 1980). Generally a low to moderate soil P fertility enhances the degree of VAM development and plant response. It appears that the water-soluble P level in the soil and its buffering capacity may sometimes be a better indicator of the potential growth response to infection in a given soil, than bicarbonate-soluble levels, especially when drought conditions prevail (Dodd *et al.*, 1983). The general conclusion, however, is that P levels in the plant, rather than in the soil, control the establishment and functioning of mycorrhizas (Menge *et al.*, 1978), since recent work has also shown that P inhibition of VAM formation is associated with membrane-mediated decrease in root exudation (Ratnayake *et al.*, 1978).

1.3.4. Pesticide applications

Trappe *et al.* (1984) has recently reviewed the literature concerning the effects of agrochemicals on the VAM symbiosis. The information is fragmented and only highlights the problem that few comparisons can be made between results from different workers and even the relevance of such results to field application rates. It is, however, generally accepted that the fungicide benomyl is particularly inhibitory of the VAM symbiosis (Hale and Sanders, 1982). The effects of herbicides, nematacides and other chemicals on the symbiosis do not appear to adversly affect VAM fungi but there are many examples of conflicting results in the literature.

1.4. The role of indigenous VAM fungi in studies of plant growth responses to inoculation in natural soils

The contribution that naturally occurring VAM fungi make to the nutrition of agricultural plants is not known. Natural selection has usually not led to the dominance of the most effective strains of VAM fungi in a given soil since "the trend of evolution has been for survival, not high productivity [Bowen, 1980a)". Hence the indigenous populations have probably evolved to be compatible and adapted to their ecological niche, but may have evolved at the expense of their effectiveness. The indigenous strains of VAM fungi could, therefore, perhaps be replaced with more effective mycosymbionts by inoculation. Nevertheless, any beneficial effect of the indigenous VAM fungi on the nutrition of agricultural plants will depend on both the abundance and type of fungi present in the soil. Extensive mycorrhizas formed by ineffective fungi would be of little value, as would those formed by effective fungi at an inappropriate stage of plant development. For plant growth to respond to inoculation, therefore, there must be either an absence of inoculum or a low inoculum potential of indigenous VAM fungi.

The potential importance of the indigenous mycorrhizas for plant growth has been indirectly demonstrated by the effects of their elimination. Methyl bromide fumigation of the soil to control root pathogens, a common practice in intensive agriculture in semi-arid climates such as Israel, can cause stunting of crops such as citrus (Timmer and Leyden, 1978) and pepper (Krikun *et al.*, 1982). This phenomenon has been related to the severe reduction of the VAM population (Menge, 1982). Yost and Fox (1979) showed that at low levels of P in the soil, the growth of mycorrhizal crops was greatly reduced following fumigation, but that non-mycorrhizal crops (chinese cabbage) consistently grew better.

1.5. Factors determining plant benefits from VAM inoculation

There are several factors that can determine the success of VAM inoculation into field soils:

(a) Crop species

It is important to know which plant species derive more benefit from mycorrhizas (Pope *et al.*, 1983). For each plant there will be a level of available phosphate in the soil to which this plant will respond similarly whether or not it is mycorrhizal. Above this level inoculation is not necessary.

(b) Soil fertility

Positive responses are to be expected chiefly in low P soils. Olsens method for the estimation of the available P is usually used, but other factors like pH (Hayman and Tavares, 1985), organic matter, texture and buffering capacity of the soil should also be considered.

(c) Native VAM populations

The estimation of the number of VAM spores and of the amount of VAM infection in roots of preceding hosts has been used as a measure of the natural infectivity of a soil, but these data have not always correlated with infectivity (Hayman, 1983). For this reason, soil-dilution techniques have been developed (Wilson and Trinick, 1983) to assess the most probable number of viable VAM propagules (i.e. spores, hyphae or root fragments present in viable conditions). Nevertheless, as stated earlier, it is also necessary to estimate the effectiveness of the native fungi and the advantages of inoculation (Dodd *et al.*, 1983; Jensen, 1983). The relative efficiency of VA endophytes as inoculants could merely reflect a more rapid spread of the infection due to an increased inoculum level (Tinker, 1978), this may be important because the demand for P is higher at the earlier stages of plant growth in annual crops.

(d) Endophyte persistence

VAM fungi selected for inoculation into agricultural soils must be able both to enhance nutrient uptake by plants and to persist in soil. Inoculant fungi would be of little value in self-sown pastures if they were unable to survive beyond the year of inoculation.

Aims of the research

Insufficient attention has been paid to the functioning of VA mycorrhizas in natural ecosystems and in long term arable land, despite their ubiquity. It is often assumed that VAM fungi are uncommon in agricultural situations, nevertheless, an understanding of the contribution of VA mycorrhizas to the growth of winter cereals is imperative since it is known that they can readily form mycorrhizal associations. The work reported here, therefore, is an assessment of the levels of naturally occurring VAM fungi in low and high P soils sown to winter cereals in S.E England, and includes investigations of some factors affecting their development and role in cereal growth and nutrition.

In this thesis I will describe observations made over a three year period which suggest that VAM associations are common in commercial cereal fields and that substantial infection can occur early in the development of the crop. An ecological survey of species of VAM fungi at different field sites will be described and the relationship between temperature and rainfall and infection development in cereal roots in the field is investigated.

The initial step in comparing VAM fungi for their ability to increase plant growth and nutrient uptake is to establish `pure' pot cultures of single species or strains of fungi (Fang *et al.*, 1983). It is necessary to maintain isolates of the fungi in this way because they are biotrophic and cannot be grown in pure culture. The frequent incorrect identification and reporting of species (actually mixed cultures) in inoculation studies underlined a necessity to fully characterise the species successfully isolated from field sites. The subsequent bulking-up of sufficient quantities of high quality inoculum of the isolated endophytes for future inoculation experiments was made a prime objective.

Experiments will also be described which were carried out in controlled conditions and designed to assess the relative effectiveness of locally isolated

VAM fungi in stimulating the growth of winter wheat in pots and to investigate the effect of field rate pesticide applications on VAM infection development and *in vitro* effects on VAM spore germination. Results of investigations into the phosphorus nutrition of winter wheat plants infected by the isolated VAM fungi are also presented.

A field study assessing the relative efficiency of the indigenous VAM population in a commercial field with that of introduced endophytes infecting winter wheat will be described.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1. Growth Media for Pot Experiments

Coarse durite sand was used as a growth medium for all glasshouse pot experiments. This growth medium is widely used in glasshouses for raising seedlings because it is clean and free from contamination, and there is therefore little need for further sterilisation. Durite is the by-product of calcine flint stone put through a furnace. It consists of 97% silica with small percentages of iron oxides, calcium oxides and alumina, with a pH of 8.2-8.5 (measured in a slurry of 1:2 sand to distilled water). The phosphorus in the durite, extractable with 0.5M sodium bicarbonate at pH8.5 (Olsen *et al.*, 1954), was 2mg P L^{-1} The levels of exchangeable cations, potassium and magnesium, in the durite (measured in a 1N ammonium acetate extract) were 11mg K L^{-1} (by atomic emission spectrometry) and 10mg Mg L^{-1} (by atomic absorption spectrometry) respectively. The durite was obtained from Brett Robert & Sons Ltd., Brett H.O., Wincheap, Canterbury. The durite was steam sterilised at 80°C for 30 minutes or autoclaved for 1 hour at 120°C, prior to an experiment.

A coarse builders sand was used for initiation of pot cultures and small scale growth room experiments, where a slightly more water retentive and low phosphorus growth medium was required, and was sterilised as above before use.

2.2 Conditions of Growth for Pot Experiments

2.2.1. Greenhouses

Three greenhouses were used during the course of this study and descriptions of their light and temperature regimes will be given in the relevant Materials and Methods section of each chapter.

2.2.2. Growth Room

Several experiments were carried out in a constant temperature growth room 20 °C, with a relative humidity of 50-60%. Plants were grown under a maximum light intensity of 4.6Klx, grolux and warm white, and a 16/8 hour day/night photoperiod was employed.

2.3. Production of Inoculum

The obligately symbiotic nature of VAM fungi limits the techniques available to establish clean homogenous cultures. It was decided, therefore, to approach this problem by initiating single-spore pot cultures, (see Fang *et al*, 1983).

Apparently viable single spores of VAM fungi, isolated from the two field sites under study (see chapters 3 and 4), were used to inoculate the roots of one week old onion (*Allium cepa* L.) seedlings (cv. Ailsa Craig), which had been germinated in sterile vermiculite. Inoculated seedlings were transplanted into sterile coarse sand in 65mm diam. polythene grow bags (I.C.I.). Single spores were placed onto the roots using storkbill forceps (Gallenkamp) and roots were then covered carefully with the damp sand. The seedlings were then watered using a wash-bottle containing either distilled water or one-half strength Hoagland's solution (minus P) as required (Hoagland and Arnon, 1950). The plants were maintained in a growth room for three to four weeks. Successful infection of seedlings were usually obvious by their improved growth. Plants were, nevertheless, evaluated for VAM development by immersing the 3-4 week old

intact root systems in water in a Petri plate. This was then examined under a stereoscopic microscope (x50) for the presence of either external mycelium or sporulation. Where extensive mycelial growth had occurred plants were transfered to 75mm diam. grow bags containing 1.75kg of coarse sterile sand, and several fresh onion seeds were sown around this infected plant. These pots were replaced in the growth room and allowed to grow for a further three months being regularly irrigated with one-third strength Hoagland's solution (minus P). These "stock" cultures were subsequently used as inoculum for the "bulking" of inoculum, by chopping the infected onion roots into small sections (<2mm) and redispersing them through the sand from the same pot(which already contained detached VAM mycelium and spores). This was used to form layers of inoculum in 150mm diam. grow bags containing 4kg of sterile durite sand to a depth of 25cm, and sown with seeds of onion (cv. Ailsa Craig) or sweet corn (*Zea mays* L.) (cv. Kelvedon Glory). These plants were allowed to grow for up to 5 months in the greenhouse.

2.3.1. Inoculum for Pot Experiments

Bulk inoculum, consisting of infected roots segments, extramatrical hyphae and spores, was sieved (<2mm) for use in greenhouse and growth room experiments. The root systems remaining on the sieve were cut up into small segments and redispersed in the sieved durite sand. Individual samples of this inoculum were weighed out and applied as a layer of inoculum below germinated seeds to ensure rapid contact of emerging roots with the inoculum. Inoculum for control treatments consisted of autoclaved bulk inoculum.

2.3.2. Inoculum for Field Experiments

Bulk VAM inoculum from sweet corn pot-cultures was air-dried and sieved through a 5mm diam. sieve. Root systems, remaining on the sieve, were finely chopped and redispersed through the sieved durite. This mixture was applied below wheat seedlings at sowing (see chapter 7). Control inoculum consisted of sievings from non-infected sweet corn pot cultures.

2.4. Sampling of Pot Experiments

The root systems of plants in pot experiments were regularly sampled to monitor the progress of VAM infection. A hand corer of 3cm diam. was used to sample to the base of the pot and the subsequent sample used to estimate VAM infection and spore numbers. Excavated cores, from the sampled pot, were refilled using sterile durite sand or coarse sand. In the smaller growth room experiments whole pots were used as a sample and here entire root systems were removed and sub-sampled for estimation of infection. Replicate samples were taken in each case.

2.4.1. Sampling of Field Experiments and Field Survey

Roots were sampled regularly during the growth season to monitor VAM infection development. Field survey techniques are detailed in chapter 3 and are identical to the field experimentation sampling procedures. Evaluation of spore populations, at each field site, was performed by taking four random soil samples at each sampling in the field study. Four 50g sub-samples were taken immediately from the original samples (not mixed) and used to estimate viable spore numbers and species.

2.5. Assessment of VAM Infection

The percentage of fractional infection was assessed using a modification of the Hayman and Phillips technique (1970). Roots were cleared in 10% (w/v) KOH at 90°C for 1-2 hours (field material was cleared for longer periods), acidified in 1% HCl (v/v) and stained in 0.1% (w/v) trypan blue in lactoglycerol (lactic acid: glycerol: distilled water, 5:1:1 ratio). Chlorazole black E was also tested at 0.1% (w/v) and gave excellent definition of arbuscular structures, although staining took longer (Brundrett, Piche and Peterson, 1984). Trypan blue, however, was used routinely throughout this study. Cleared and stained root samples were then spread randomnly over a grid in a petri dish (9cm diam.) and examined under a Zeiss (W. Germany) stereoscopic microscope (x32). The proportion of intersects of roots and gridlines at which VAM infection was observed was used to determine the proportion of the sampled root system that was infected. Results are thus given as total percentage fractional infection. Giovannetti and Mosse, (1980) showed that this technique gave satisfactory estimates of VAM infection with a low standard error. A slide method was used for estimation of infection of field material under an Olympus BH binocular microscope and this is described and discussed in chapter 3.

2.6. VAM Spore Isolation Technique

Spores were isolated by wet-sieving and decanting field soil or pot experiment growth medium. 50g soil or sand was dispersed in 1 litre of tap water, allowed to settle for 10-15 secs. and sieved through 710, 300 and 106µm sieves. The fraction on the 106µm sieve was retained. The spores from field soil were then separated from heavier debris in this fraction by centrifugation in 50% sucrose solution for 2 mins. at 1700 r.p.m. The supernatant, containing spores, was decanted and washed three times with deionised water in a 37µm sieve, and resuspended in deionised water in a Doncaster nematode-counting

dish, for estimation of spore numbers. VAM spores were characterised by their size and colour (incident light), cytoplasmic appearence (transmitted light), wall structure and hyphal attachment (see chapter 4), using a stereoscopic microscope (x50). Spores from pots were isolated as described above from a weighed sub-sample of the core taken from the pot, and counted in a Doncaster dish.

2.6.1. Preparation of Spores for Germination Studies

Spores for germination studies were isolated from either field soil or stock pot cultures as described above. The spores from the 37µm sieve were filtered through a Whatman #1 filter paper (7cm diam.) to collect spores on a flat moisture retentive surface. The filter paper was then placed in a Buchner funnel (under low vacuum) and a second piece of filter paper, of the same size, placed on top, so forming a sandwich of spores. The spores were then surface sterilised with 0.5% sodium hypochlorite (NaOCl) for 3 mins. and flushed several times with sterile deionised water using the vacuum. The filter paper was transfered to a binocular dissecting microscope, situated in a clean air head. Spores were picked from the filter paper using sterile storkbill forceps and placed onto agar media in 9cm diam. petri plates or onto 5cm diam. Whatman 17 incubation pads in 5.5cm petri plates. These incubation pad plates were prevented from dehydrating by the regular application of an appropriate sterile deionised solution, every 5-6 days. The agar media used were either Difco or Oxoid products and are described in chapter 5. The pH of any solution was adjusted using either 1M HCl or 1M KOH before autoclaving. On agar media 10 spores were placed on each plate, whilst on the incubation pad plates 6-7 spores were used. Plates were subsequently wrapped in silver foil, to exclude light and control water loss, and incubated in a constant temperature growth room.

2.7. Analyses of Plants

2.7.1. Yields

Fresh weights of shoot material were measured in greenhouse and growth room experiments immediately after harvest. Dry weight values were obtained when small amounts of fresh foliage had been harvested. Shoot material was subsequently used for chemical analyses.

2.7.2. Concentration of Phosphorus in Shoot Material

Samples of plant material were wet digested in a 4:1 mixture of concentrated nitric/perchloric acid and dissolved in 6N HCl. The concentration of phosphorus in the solution was then determined spectrophotometrically as the yellow phospho-vanado-molybdate complex (analyses carried out by the analytical services, ADAS, Wye).

2.7.3. Concentration of Nitrogen in Shoot Material

The concentration of N in shoots was measured colorimetrically after Kjeldahl digestion (analytical service, ADAS, Wye).

2.8. Statistics

Effects of experimental treatments were statistically analysed using analysis of variance on the GENSTAT statistical computer program (Alvey *et al*, 1977). The statistical significance of treatments or interactions, independent of the scale of measurement, was determined using variance ratios, and of differences between means using the least significant difference (LSD) for yields and levels of VAM infection (Steel and Torrie, 1960).

2.9. Ultrastructural studies

All chemicals used in the preparation of material for electron microscopy were of an analytical grade.

2.9.1. Scanning electron microscopy

Spore samples were not fixed but air-dried and mounted directly on aluminium stubs using double-sided Sellotape, before being sputter-coated with gold for 2-4 mins at 40mA. Specimens were examined using a Cambridge Stereoscan 600 (accelerating voltage 15kV, at U.K.C.) or a Hitachi S-430 (accelerating voltage 15kV, at Wye College, University of London).

2.9.2. Transmission electron microscopy

(a) Preparation of material

Spores were individually picked out with storkbill forceps into fixative (0.5% (w/v) paraformaldehyde - 1% (w/v) glutaraldehyde in 0.1M cacodylate-HCl buffer (pH 7.2)) and gently punctured with a syringe needle (0.5x16mm) under fixative. These spores were fixed for 20-30 mins. They were subsequently washed, post-fixed in 1% (w/v) osmium tetroxide in 0.1M cacodylate-HCl buffer, washed and dehydrated through a graded ethanol-water series. Spores were then embedded in Spurr's resin (Spurr, 1969). The protocol used in the preparation of spores for T.E.M. is summarised in figure 2.1.

(b) Sectioning

Thick sections (1µm) for light microscopy were cut using a glass knife mounted on an LKB Ultratome III, and stained using 1% (w/v) methylene blue and 1% (w/v) sodium tetraborate (see next section). Stained sections were examined using light microscopy to facilitate the location of areas of infection for ultrastructural investigations. The face of the resin block was trimmed to form a

four-sided pyramid bearing the selected area of interest. Ultrathin sections of this selected region were cut using a fresh glass knife or a DuPont diamond knife. The sections were floated onto distilled water and collected on uncoated hexagonal mesh copper grids (300 mesh). After drying in air, sections were stained and examined using a Phillips 410 EM, at an accelerating voltage of 80kV.

(c) Staining Thick Sections for Light Microscopy

Thick sections (approx. 1µm) were cut using a glass knife, mounted on an LKB Ultratome III, and transferred to a drop of distilled water on a glass microscope slide. The sections were heat-fixed to the slide by warming over a bunsen flame. Two drops of 1% (w/v) aq. methylene blue and two drops of 1% (w/v) aq. sodium tetraborate were placed on the sections (borax raises the pH of the stain to approx. pH9). The slides were gently heated over a flame for 15 secs - it was important not to allow the stain to boil. The slide was allowed to cool and excess stain washed off with distilled water. The sections were blotted dry and a coverslip placed over them prior to examination.

(d) Staining Ultrathin Sections

Ultrathin sections, mounted on copper grids, were stained using uranyl acetate and lead citrate.

(i) Uranyl acetate was prepared as a 2% (w/v) aq. solution in distilled water and stored at 4°C.

(ii) Lead citrate was prepared using the method of Reynolds (1963). Grids bearing sections were placed on the surface of the uranyl acetate solution (sections in contact with the surface of the liquid) contained in small plastic embedding capsules. The grids were then removed, flushed with distilled water and blotted dry before being placed on the lead citrate solution, contained in similar vessels. Sections were incubated in lead citrate for 10

mins. at room temperature, and then flushed with distilled water and blotted dry on filter paper.

CHAPTER 3

PART 1

The early development of VA mycorrhizas in autumn-sown cereals.

3.1A. INTRODUCTION

It is well established that vesicular-arbuscular mycorrhizas (VAM) improve phosphorus uptake and growth in a wide range of plants (Hayman, 1983), and that VAM occur in many field crops under a range of environmental conditions (Mosse *et al.*, 1981), especially when the availability of phosphorus in the soil is limited. Phosphorus uptake in annual crops may be significantly affected only if the infection is well established shortly after seedling emergence (Jakobsen and Neilsen, 1983). Cereals, such as barley with a dense, fine root sytem, have been shown to respond less in the field to inoculation with VAM than crops such as lucerne or onion with a limited root system (Owusu-Bennoah and Mosse, 1979). Rapid development of a VAM infection could be a major determinant of response in annual crops. Sanders *et al.*, (1977) have, for example, presented data showing that low percentage root infections by VAM (<10%) can have a beneficial value to the host out of all proportion to their size, especially when the plants are very young and a well-developed external mycelium is present.

Most previous studies in temperate climates have suggested that infection of cereals by VAM does not reach appreciable levels (>30% root length) until late spring or even early summer. Asai (1934), for example, first observed a lack of VAM infection in winter wheat and other small grains until late April soon after grain heads had emerged. In the U.K. infection levels of winter wheat have been reported as sparse in May, but increasing to a peak in September (Hayman, 1970). In Denmark, very low infection by VAM (<5% root length) was noted in the

period up to April (Jakobsen and Neilsen, 1983) , whilst no VAM colonisation was detected until May in comparable studies in Kansas (Daniels Hetrick and Bloom, 1983). In contrast, more than 50% of root segments of winter wheat seedlings examined in West Pakistan were mycorrhizal a month after they were sown, (Saif and Khan, 1975) although this may reflect a much higher temperature regime, and the fact that large numbers of spores of Endogonaceous fungi were present in the soil. Higher levels of infection in December (7-8% root length) and March (15-16% root length) have been recorded in experimental plots of winter wheat in the U.K. (Buwalda, 1983) but this followed soil inoculation with VAM fungi. Such investigations have led to a general view that infection by VAM is unlikely to significantly influence the growth of winter cereals in temperate climates, at least until a period after flowering when any effects on grain yields would be much reduced.

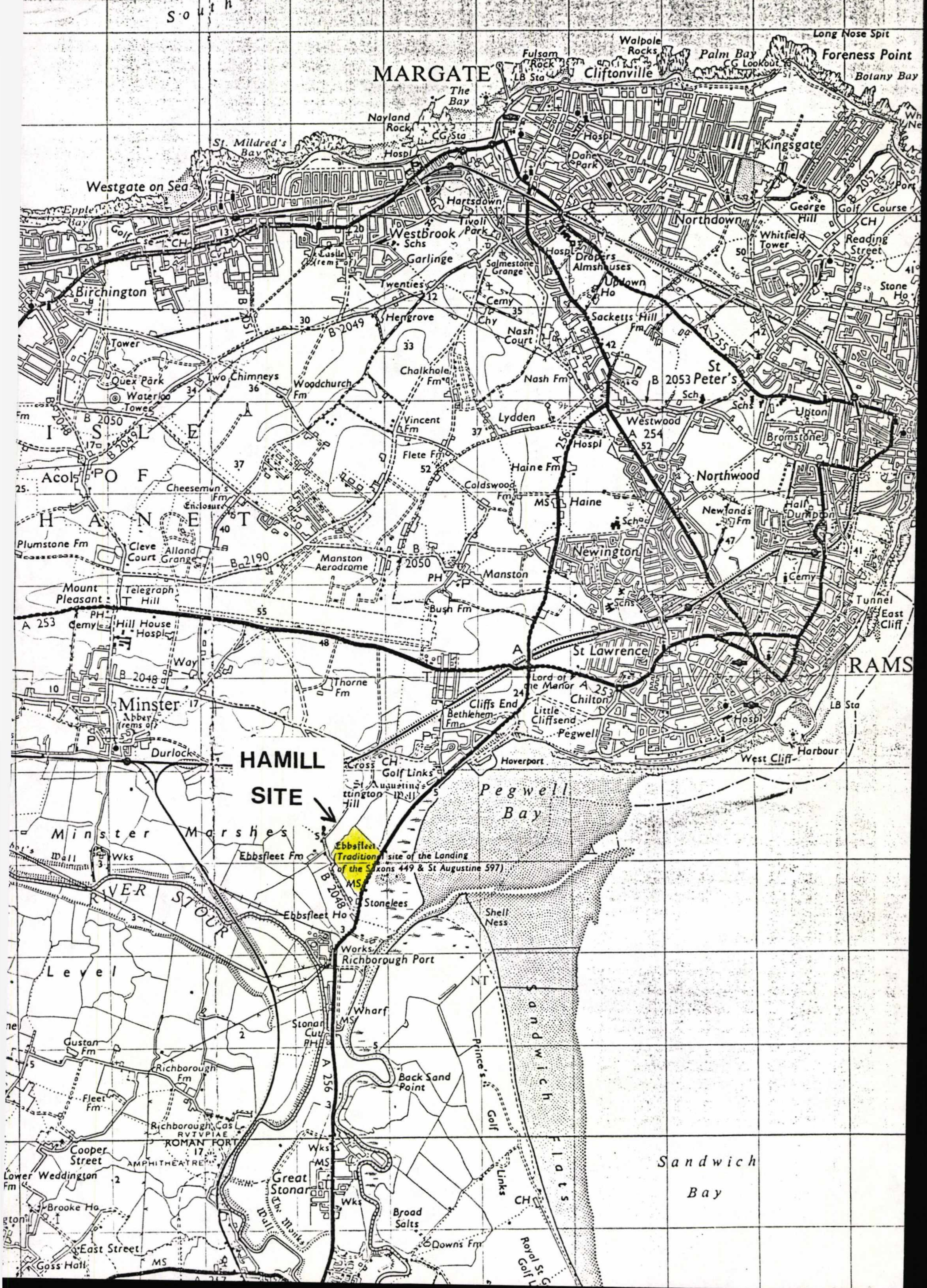
Most studies have tended to concentrate on spring sown crops (Black and Tinker, 1979; Jensen, 1983). Inoculation of spring barley in the field has shown that introduced VAM endophytes increased growth to a greater extent than the indigenous population (Clarke and Mosse, 1981). Other workers, however, have found that natural infection by indigenous endophytes was established too late to be likely to increase growth (Black and Tinker, 1979). The potential activity of a VAM infection may be gauged by estimating total arbuscular infection in addition to total infection (Hayman, 1974). In this study a survey of two commercial field sites was undertaken to investigate natural infection levels in winter wheat grown in monoculture over a three year period.

3.2A. MATERIALS AND METHODS

Soil samples, including cereal roots, were taken from three field sites, Gofflands 1 (G-1), Gofflands 2 (G-2), near Billingshurst, West Sussex, and Hamill, near Ramsgate, East Kent, through three consecutive winter cereal seasons 1982-1985. G-1, a clay loam soil, had been under wheat for four years prior to 1982 (Map reference TQ12 148240 and figure 3.1). G-2, another clay loam soil, had been permanent pasture for twelve years prior to being planted with winter wheat in 1982 (Map reference TQ12 140236 and figure 3.1). Hamill field, a silty clay loam soil, had been under continuous winter wheat since 1977 (Map reference TR 337630 and Fig. 3.2).

On each occasion eight samples were taken from the top 10cm of soil at approximately 10m intervals along a transect across the field. The line of transect was different at each time of sampling. The samples were bulked and eight subsamples consisting of several plants with seminal and nodal roots attached were obtained by washing them free of soil back in the laboratory. The roots were then chopped into 1-1.5cm segments and stained in trypan blue in a lactoglycerol solution (modified Phillips and Hayman, 1970). At least 80cm of root length per bulked sample were examined under the low power of the compound microscope (X100) and the percentage root infection calculated by noting the presence or absence of internal VAM structures in each field of view (approximately 1.5mm) and dividing by the number of fields of view examined. At the same time the quality of infection was assessed by recording, for each field of view, the proportion of infection due to arbuscules compared with that due to mycelium and vesicles, using the scale given in Table 3.1. Qualitative observations were also made of the relative amount of infection due to fine endophytes (*Glomus tenue*) in relation to that resulting from infection by hyphae of the coarse species.

re 3.1



South Channel
MARGATE
Walpole Rocks
Fulsam Rock
LB Sta
Cliftonville
Palm Bay
CG Lookout
Long Nose Spit
Foreness Point
Botany Bay
The Bay
Nayland Rock
CG Sta
Hospl
St. Mildred's Bay
Westgate on Sea
Epple Bay
Golf
CH
Dane Park
Kingsgate
Hartsdown
Tivoli Park
Westbrook
Schs
Garlinge
Castle (rem of)
Northdown
George Hill
Golf Course
Whitfield Tower
Reading Street
Birchington
Salmestone Grange
Drapers Almshouses
Updown Ho
Twenties
Cemy
Chy
Nash Court
Hengrove
Sacketts Hill Fm
Stone Ho
Tower
Quex Park
Waterloo Tower
Two Chimneys
Woodchurch Fm
Chalkhole Fm
Nash Fm
St Peter's
Sch
Schs
Upton
Bromstone
Vincent Fm
Lydden
Westwood
Hospl
ISLE OF THANET
Flete Fm
Haine Fm
Northwood
Acol
Cheeseman's Fm
Enclosure
Coldswood Fm
MS
Haine
Newlands Fm
Hall Dumpton
Plumstone Fm
Cleve Court
Alland Grange
Manston Aerodrome
Manston
Newington
Cemy
Mount Pleasant
Telegraph Hill
PH
Bush Fm
Tunnel
East Cliff
A 253
Cemy
Hill House Hospl
St Lawrence
RAMS
Way
Thorne Fm
Lord of the Manor
Chilton
Minster
Abbey (rems of)
Cliffs End
Bethlehem Fm
Little Cliffsend
Pegwell
LB Sta
Harbour
Durlock
West Cliff
Hoverport
HAMILL SITE
Cross
Golf Links
St Augustine's
ttington Hill
Pegwell Bay
Minster Marshes
Wall
Wks
Ebbsfleet Fm
Ebbsfleet (Traditional site of the Landing of the Saxons 449 & St Augustine 597)
Stonelees
RIVER STOUR
Ebbsfleet Ho
Shell Ness
Works
Richborough Port
Level
NT
Wharf
Stonar Cut
Sandwich Flats
Guston Fm
Richborough Fm
Back Sand Point
Prince's Golf Links
Fleet Fm
Richborough Cas
RVTVPIAE
ROMAN FORT
AMPHITHEATRE
Cooper Street
Lower Weddington
Great Stonar
The Monks Wall
Wks
Sandwich Bay
Brooke Ho
CH
Broad Salts
East Street
Downs Fm
Royal St G Golf L
Goss Hall

ure 3.2

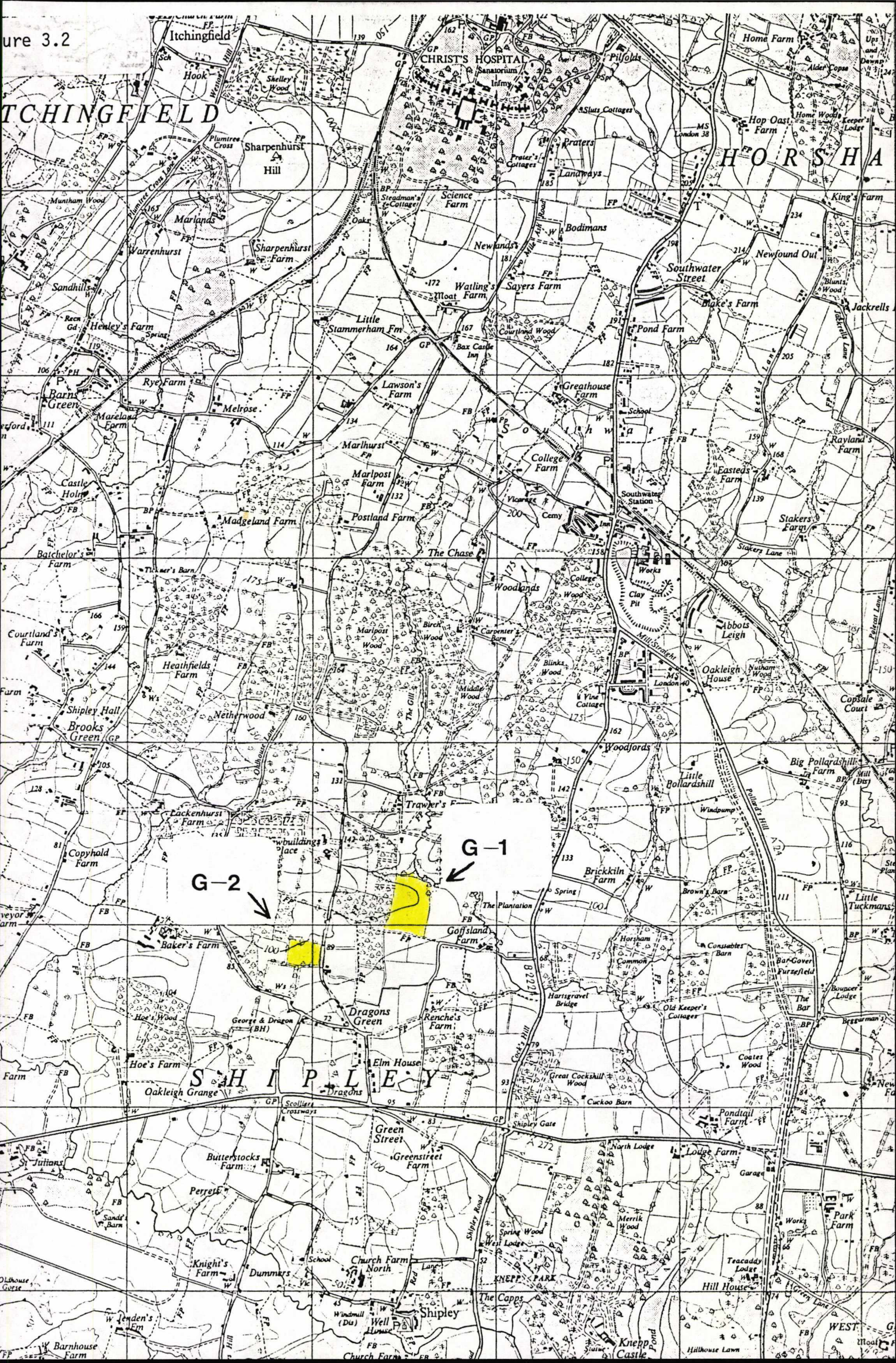

G-1
G-2
Itchingfield
TCHINGFIELD
CHRIST'S HOSPITAL
Sanatorium
Infmy
Pilfolds
Home Farm
Alder Copse
Hook
Shelley Wood
Sharpenhurst Hill
Plumtree Cross
Sluts Cottages
Hop Oast Farm
Home Wood
Keeper's Lodge
HORSHA
Praters
Praters Cottages
Lanaways
Steadman's Cottage
Science Farm
King's Farm
Muntham Wood
Marlands
Bodimans
Newlands
Warrenhurst
Sharpenhurst Farm
Southwater Street
Newfound Out
Sandhills
Watling's Farm
Moat
Sayers Farm
Blake's Farm
Jackrells
Henley's Farm
Little Stammerham Fm
Courtland Wood
Pond Farm
Bax Castle Inn
Barns Green
Rye Farm
Lawson's Farm
Greathouse Farm
School
Melrose
Marlhurst
College Farm
Rayland's Farm
Castle Holm
Marlpost Farm
Easteds Farm
Southwater Station
Madgeland Farm
Postland Farm
Vicarage
Cemy
Stakers Farm
Stakers Lane
Batchelor's Farm
The Chase
Works
Clay Pit
Woodlands
College Wood
Abbots Leigh
Courtland's Farm
Marlpost Wood
Birch Wood
Carpenter's Barn
Heathfields Farm
Blinks Wood
Oakleigh House
Nutham Wood
Copsale Court
Middle Wood
Vine Cottage
Shipley Hall
Brooks Green
Netherwood
Woodfords
Little Bollardshill
Big Pollardshill Farm
Trawler's Farm
Windpump
Lackenhurst Farm
Newbuildings Place
Copyhold Farm
Brickkiln Farm
The Plantation
Brown's Barn
Little Tuckmans
Baker's Farm
Goffsland Farm
Horsham Common
Constables Barn
Bar Gover Fursefield
Hartsgravel Bridge
Dragons Green
Renche's Farm
Old Keeper's Cottages
Bouncer's Lodge
The Bar
Hoe's Wood
George & Dragon (BH)
Elm House
Coates Wood
Hoe's Farm
SHIPLEY
Great Cockshill Wood
Oakleigh Grange
Scolliers Crossways
Dragons
Cuckoo Barn
Shipley Gate
Pondtail Farm
Green Street
Greenstreet Farm
North Lodge
Lodge Farm
St Julians
Butterstocks Farm
Garage
Perrett
Merrik Wood
Park Farm
Shipley Road
Spring Wood
West Lodge
Traccaddy Lodge
Knight's Farm
Dummers
School
Church Farm North
KNEPP PARK
Hill House
The Capps
Windmill (Du)
Well House
Shipley
Barnhouse Farm
Church Farm
Knepp Castle
Hillhouse Lawn
WEST

TABLE 3.1 - Scale for assessing the quality of VAM infection.

Score on scale	Amount of root cortex infected
0	Absence of VAM structure
1	0-15% of root cortex
2	15-45% of root cortex
3	45-75% of root cortex
4	75-100% of root cortex

TABLE 3.2 - Soil characteristics at each sample site

HAMILL			GOFFSLAND-1			GOFFSLAND-2		
Sample date	Soil* phosphorus	Soil pH	Sample date	Soil phosphorus	Soil pH	Sample date	Soil phosporus	Soil pH
9/1981	76	7.0	10/1982	16	6.9	10/1982	4@	6.4
11/1982	59	7.6	2/1983	13	7.0	2/1983	14	6.6
8/1983	39	7.6	11/1984	14	6.5	11/1984	30#	6.5
6/1984	35	7.5	3/1985	13	6.8	11/1984	40#	6.9
11/1984	62+	7.9				3/1985	76#	6.6

* = bicarbonate soluble P (Olsen) in mg L^{-1}

@ = pasture soil prior to fertilization

\+ = under Oil Seed Rape

\# = chicken manure added at 10 tonnes/hectare prior to sowing

TABLE 3.3 - Crop variety and sowing date at each field, 1982-1984

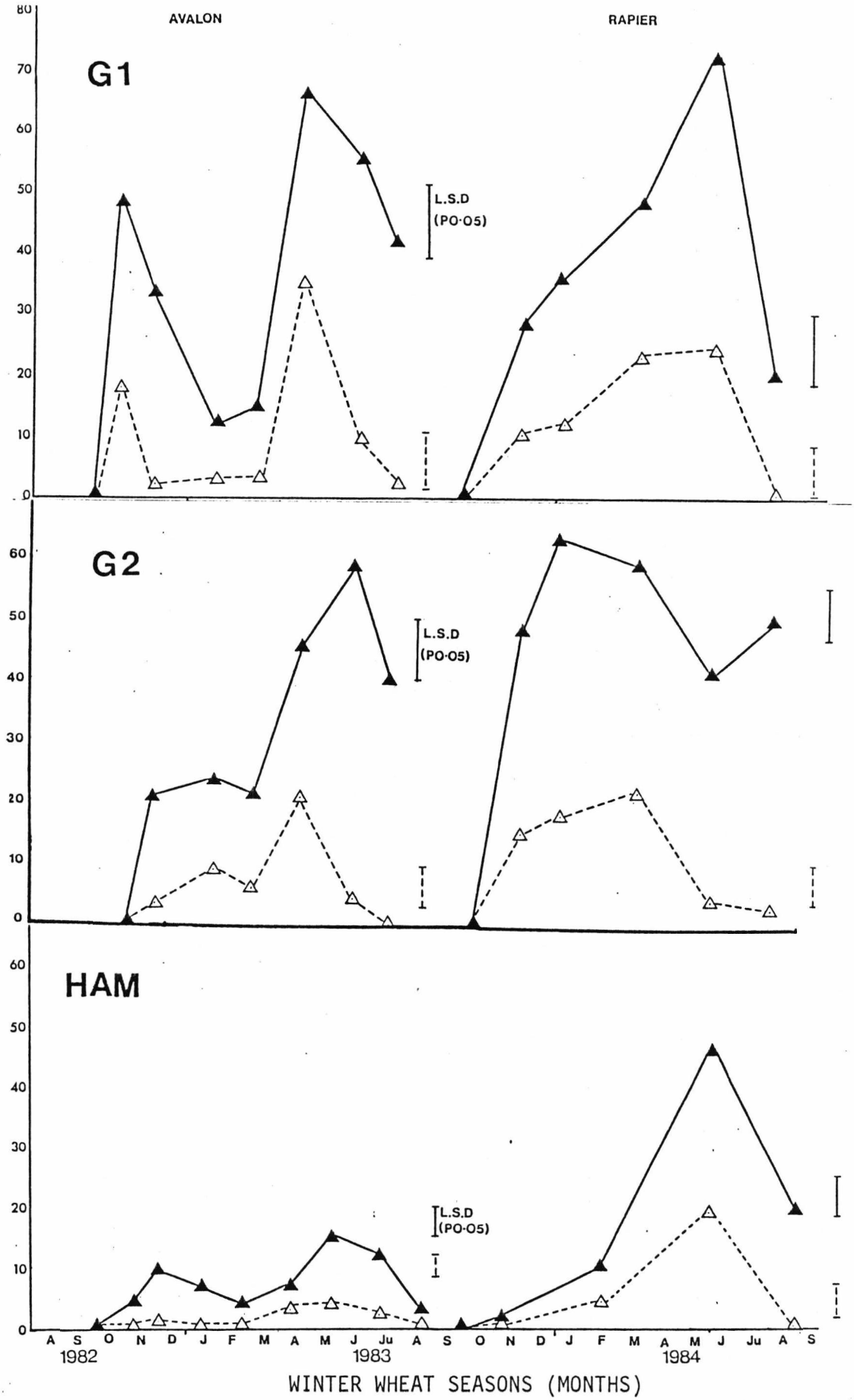
SITE	SEASON	CROP - VARIETY	SOWING DATE
G-1	1982/3	Wheat - Avalon	2/10/82
	1983/4	Wheat - Rapier	2/10/83
	1984/5	Barley	30/9/84
G-2	1982/3	Wheat - Avalon	5/11/82
	1983/4	Wheat - Rapier	15/10/83
	1984/5	Wheat - Rapier	21/10/84
	1984/5	Rye	14/10/84
HAMILL	1982/3	Wheat - Avalon	2/10/82
	1983/4	Wheat - Rapier	30/9/83
	1984/5	Oil seed rape	-

None of the sites were experimental plots but were in fields on commercial farms and were subjected to agricultural practices normal for intensively-grown cereals in North-West Europe. In the 1982-3 season for example, G-1 and G-2 received 100kg ha^{-1} of NPK fertiliser (0-22-22) prior to sowing and 50kg ha^{-1} of 'Nitram' (ICI Agrochemicals) at growth stage 7 (Feekes scale). The crop received a post-emergence herbicide application and was treated with a broad-spectrum fungicide (G-1 received a Focal application, G-2 received a Sportak application) in late spring. At Hamill the 'Nitram' application was given as 50kg ha^{-1} at growth stage 6 and 150kg ha^{-1} at stage 8. Three herbicides were applied between growth stages 3 and 6 (Dicurane (3), Avenge (5), Corridor (6)) and five fungicides were applied as three mixtures between growth stages 7 and 1 (Jupital + Bavistin (7), Jupital + Manzate (10.1), Corbel + Bavistin (10.1)). Soil pH and available bicarbonate-soluble phosphorus (Olsen *et al.*, 1954) were determined several times during the investigation and the results are given in Table 3.2. The dates of sowing and the respective crops and varieties sown at each field site during the three seasons are recorded in Table 3.3.

3.3A. RESULTS

Figures 3.3 and 3.4 show the % fractional infection of the roots by VAM during the three growing seasons. Fractional infection is the fraction of the length of host root containing mycorrhizal infection. During 1982/3 and 1984/5, the total infection levels increased rapidly at G-1 and G-2, and more gradually at Hamill, for the two months following sowing. A decrease in fractional infection then occurred over the subsequent two to three months. Infection levels then rose rapidly to a peak in late spring. These trends were reflected by the relative proportions of arbuscular infection, which dropped to zero by July or August (Figs. 3.3 and 3.4). In the 1983/4 season a different, yet consistent, pattern of

FIGURE 3.3 - Percentage fractional VAM infection in the roots of winter wheat varieties Avalon and Rapier at three sites (G-1, G-2 and Hamill) during the period 1982-1984.



▲= total infection (arbuscles, intercellular mycelium and vesicles); △= infection due to arbuscles alone. Bars indicate least significant differences at the 5% probability level (L.S.D PO.05).

Figure 3.3B - Changes in the relative proportions of fine and coarse endophytes within total VAM infection levels estimated in wheat root samples at the G-1 field site during the 1982-84 growth seasons.

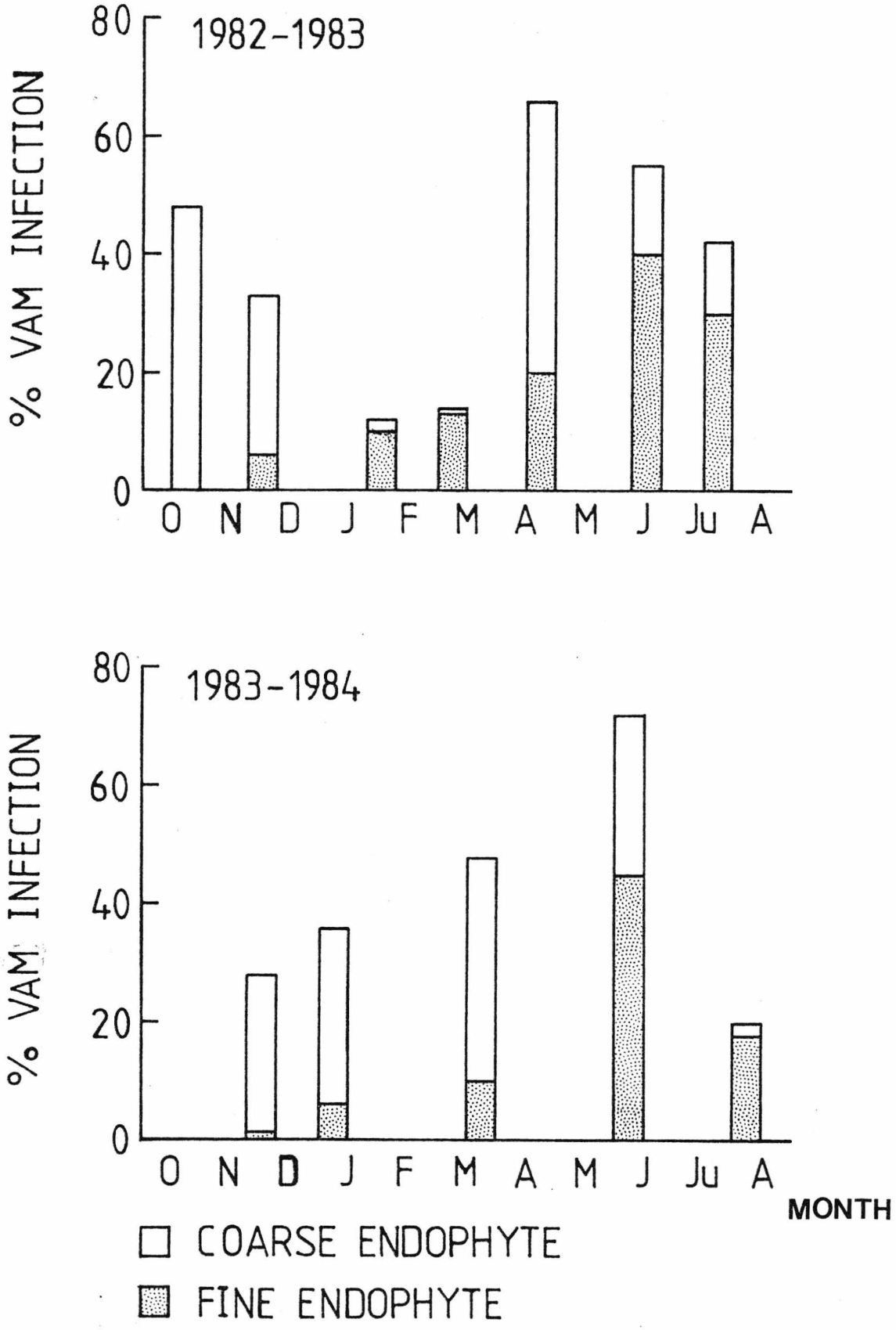


FIGURE 3.4 - Percentage fractional VAM infection in the roots of winter wheat var. Rapier and winter rye at G-2, and winter barley at G-1, during the 1984-85 season. ▲ = total infection; △ = infection due to arbuscles alone. Bars indicate the L.S.D. values at the 5% probability level.

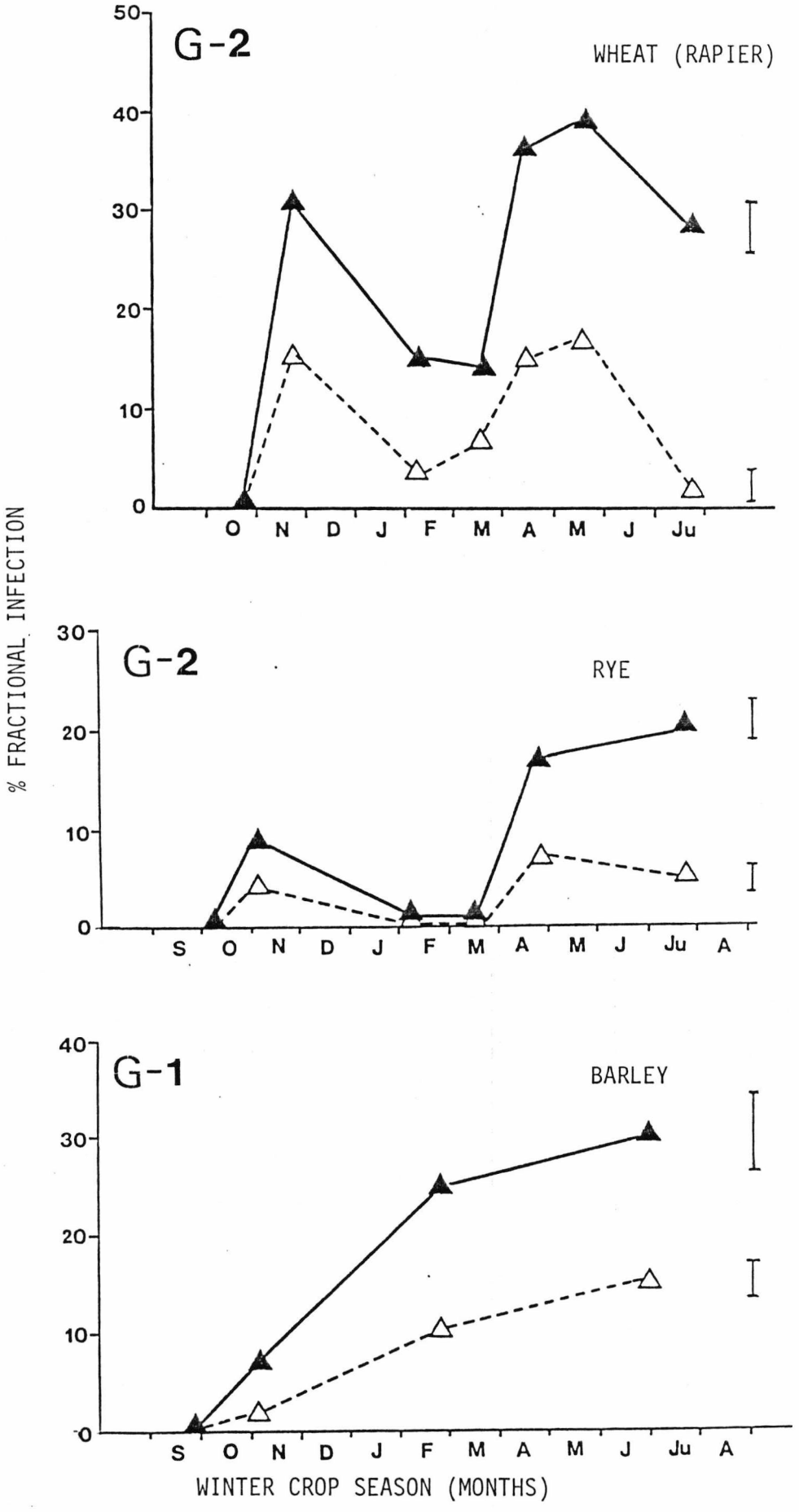
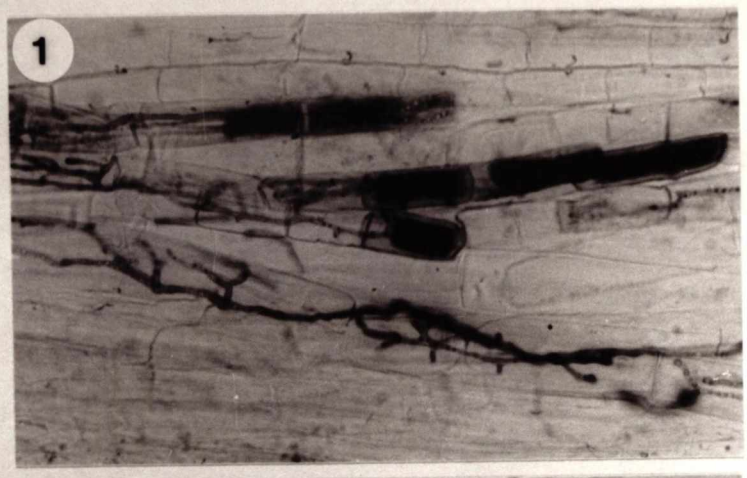


PHOTO 1 - Isolated arbuscular infection in roots of winter wheat from the Hamill site, November 1982 (x130, B.F.).

PHOTO 2 - Restricted intercellular spread of VAM hyphae, Hamill, November 1982 (x550, B.F.).

PHOTO 3 - Limited spread of VAM intercellular infection despite the presence of apparently normal appressoria, Hamill, January 1983 (x140, B.F.).

PHOTO 4 - Young arbuscular infection sites again with little lateral spread, Hamill, May 1983 (x140, B.F.).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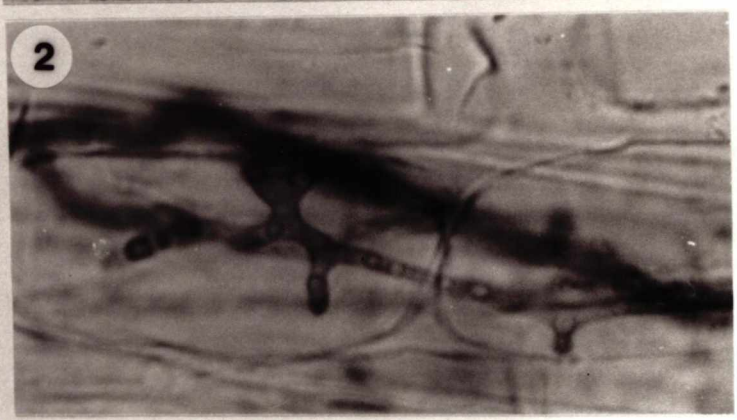
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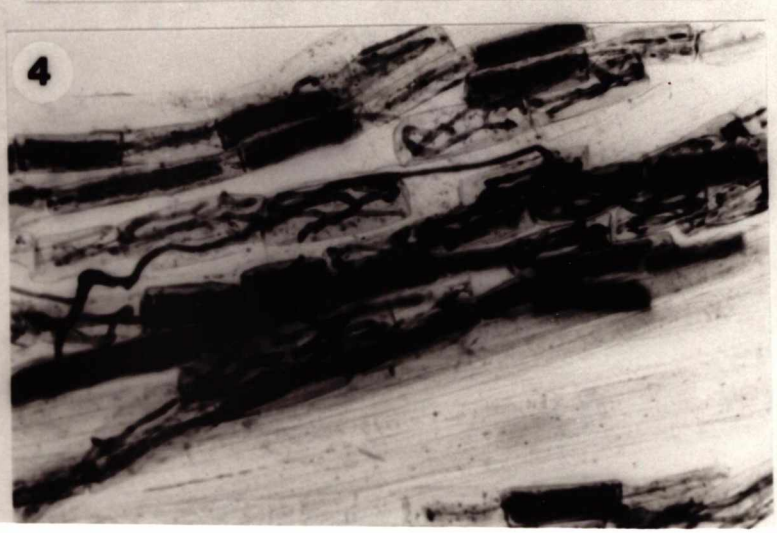

4

PHOTO 5 - The disappearence of infection sites in the overwinter decline of December 1982 at G-1 (x130, B.F.).

PHOTO 6 - Extensive arbuscular infection along wheat roots from the G-1 site in April 1983. Note the fine endophytic infection (x140, B.F.).

PHOTO 7 - Vesicular infection in wheat roots from the G-1 site in June 1983 (x150, B.F.).


5


6

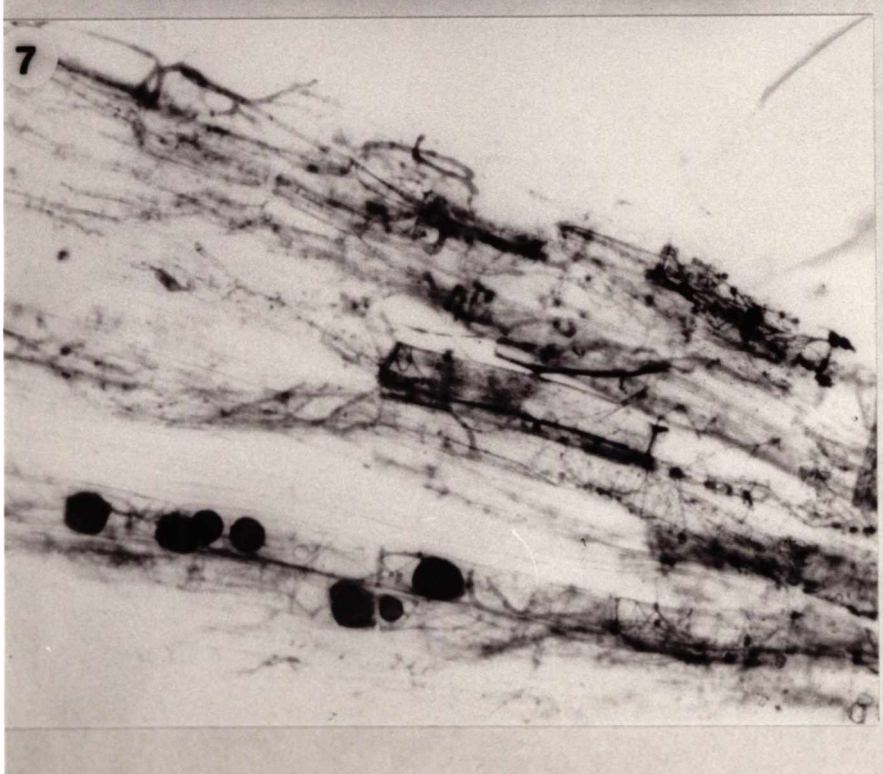
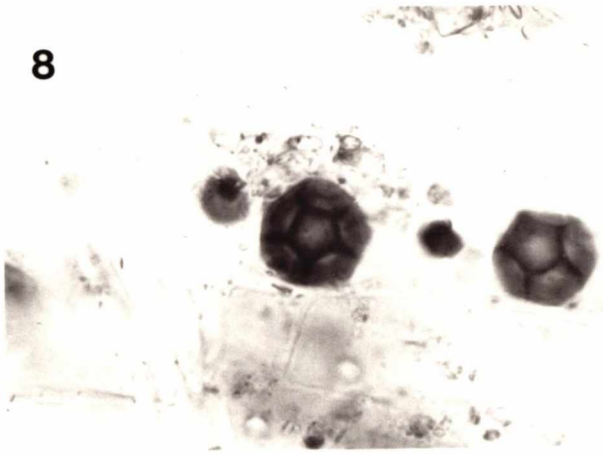

7

PHOTO 8 - Resting spores of Olpidium which resemble vesicles under the low power of a dissecting microscope (x500, B.F.).

PHOTO 9 - Moribund arbuscles (note the lack of branching) found in the overwinter decline at G-2 in February 1983 (x550, B.F.).

8



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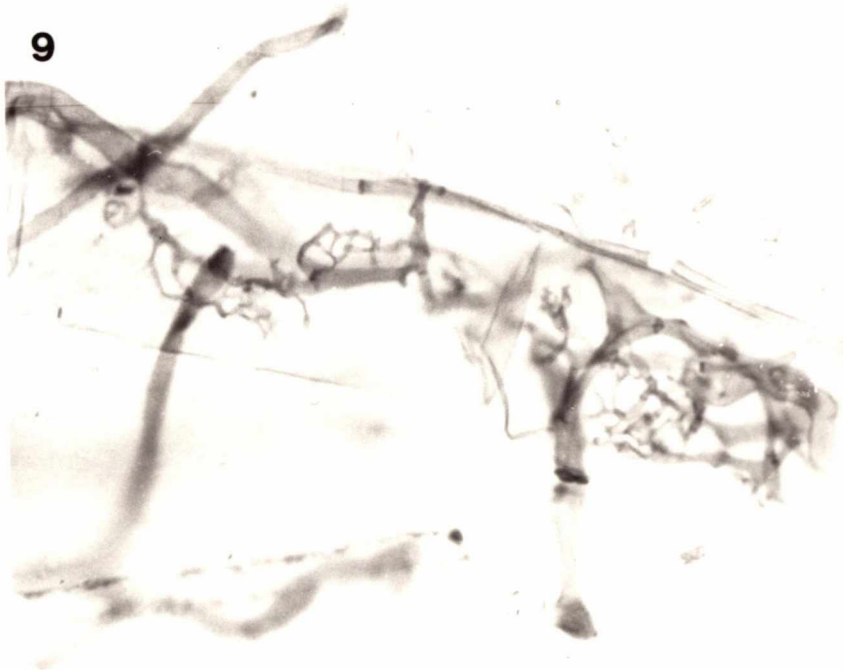


root colonisation was observed. There was no decrease in infection levels during the winter period and the mycorrhizal proportion of the root system increased steadily to a January (G-2) or June (G-1 and Hamill) peak. Infection levels at Hamill were consistently lower than those recorded at either of the Gofflands sites. In the 1984/85 season the two patterns of infection were observed together in G-1 and G-2. The early sown barley (late September) showed a gradual increase in infection through the winter period when the later sown (October) rye and wheat levels declined. Slightly lower peaks of infection in the wheat crop at G-2 in 1984/85 compared to previous years were observed which may have been due to the increased soil nutrient status (Table 3.2). Rye in the same field showed lower infection levels grown under the same field conditions.

The trend observed in the 1982/83 season can be clearly seen from photographs taken through the season (Photos 1-4) at the Hamill site. The photographs of November 1982 (Photos 1 and 2) show small patches of coarse arbuscular infection with little lateral spread. By January infections consisted of limited intercellular hyphal growth despite the presence of apparently normal appressoria (Photo 3). In May, however, coarse arbuscular infections reappeared but with little lateral spread of infection units (Photo 4). At the G-1 site the early October/November abundance of coarse endophytic appressorial formation and dense internal infection gave way to the December decline period (Photo 5). By April extremely well developed arbuscular infections occurred regularly along the roots examined (Photo 6). By June, infection consisted primarily of vesicles and intercellular hyphae (Photo 7). The microscopic appearance of the mycorrhizal infection also changed during the growing season and reflected changes in the relative proportion of infection due to endophytes with coarse hyphae relative to those with fine hyphae (Fig. 3.3B). Both types of endophyte were present in roots from all three sites when infection levels were high (Photo

6), but the proportion of roots infected by coarse hyphae dropped appreciably after late November in 1982 and 1984. During periods when infection levels were lowest (December to April, 1982/3 and 1984/5) colonisation was primarily due to the presence of the fine endophytes. Few coarse hyphae were observed during the overwintering period of these two growing seasons. Appressorial formation by hyphae of coarse endophytes was frequently noted, but lateral spread of the infection did not occur within the root cortex. The rise in infection levels noted during the spring periods was due to extensive mycelial and arbuscular growth of both types of endophyte within the root cortex. The observations during the 1983/4 (and for barley at G-2 in 1984-85) season differed in that the levels of infection due to coarse endophytes persisted overwinter. At the Hamill site 1983/84 the large increase in total infection (Fig. 3.3) consisted of both fine and coarse endophytic infections.

3.4A. DISCUSSION

3.4.1A. Early infection development

These results describe much more extensive colonisation of winter cereal roots in the pre-winter period than has previously been noted. It could be that this early peak in infection may have been overlooked in previous studies for two main reasons. Firstly, several reports (Hayman, 1970; Jakobsen and Nielsen, 1983) do not present data for the late autumn/early winter period, but give observations only from late winter onwards. Jakobsen and Nielsen (1983), however, took field samples of winter barley, wheat and rye in late November in Denmark, six weeks after seedling emergence. They sampled by extracting cores, but could not distinguish between fresh roots and the old roots of the previous barley crop, so no data were presented. Buwalda (1983) also took samples in late November but his results were obtained from a field that had been

fallow for seven years. In my study intact plants with attached root systems were removed from the soil for estimation of infection, using a trowel to a depth of 10 cms. Secondly, different techniques used for estimation of infection of stained field material may give rise to conflicting results. In particular, the use of the grid-line intersect method for assessment of early season field material under a low power dissecting microscope may result in both young infection sites and the presence of fine endophytes being overlooked. This would result in under estimation of fractional infection. In contrast, the cortex of roots collected later in the season is often colonised by other fungi which stain using the Phillips and Hayman method (e.g. *Olpidium* (Photo 8), *Polymyxa* and *Rhizoctonia*). These are sometimes difficult to distinguish from dense VAM infection under a low power dissecting microscope and hence their presence may lead to overestimation of fractional infection by VAM if this method of assessment is used. (as also noted by Miller *et al.*, (1983)). In addition, some staining of cortical cells of the root occurs regularly which may also appear similar to a dense arbuscular infection if a low resolution microscope is employed. In winter-sown crops, particularly towards the end of the season, much of the root material loses its cortical component during preparation. The vascular tissue remaining may subsequently be counted as non-mycorrhizal under a dissecting microscope. In this study such roots were not included in estimations of infection. A compound microscope (x100 magnification) has been used for estimation of infection in field material throughout these investigations in order to overcome these problems.

Early development of VAM infection in autumn-sown cereals is consistent with the view that arbuscular colonisation by VAM is favoured in the young, active regions of the root system. In my results the most extensive arbuscular phases in 1982/3 and 1984/5 occurred during periods when new root growth was likely to

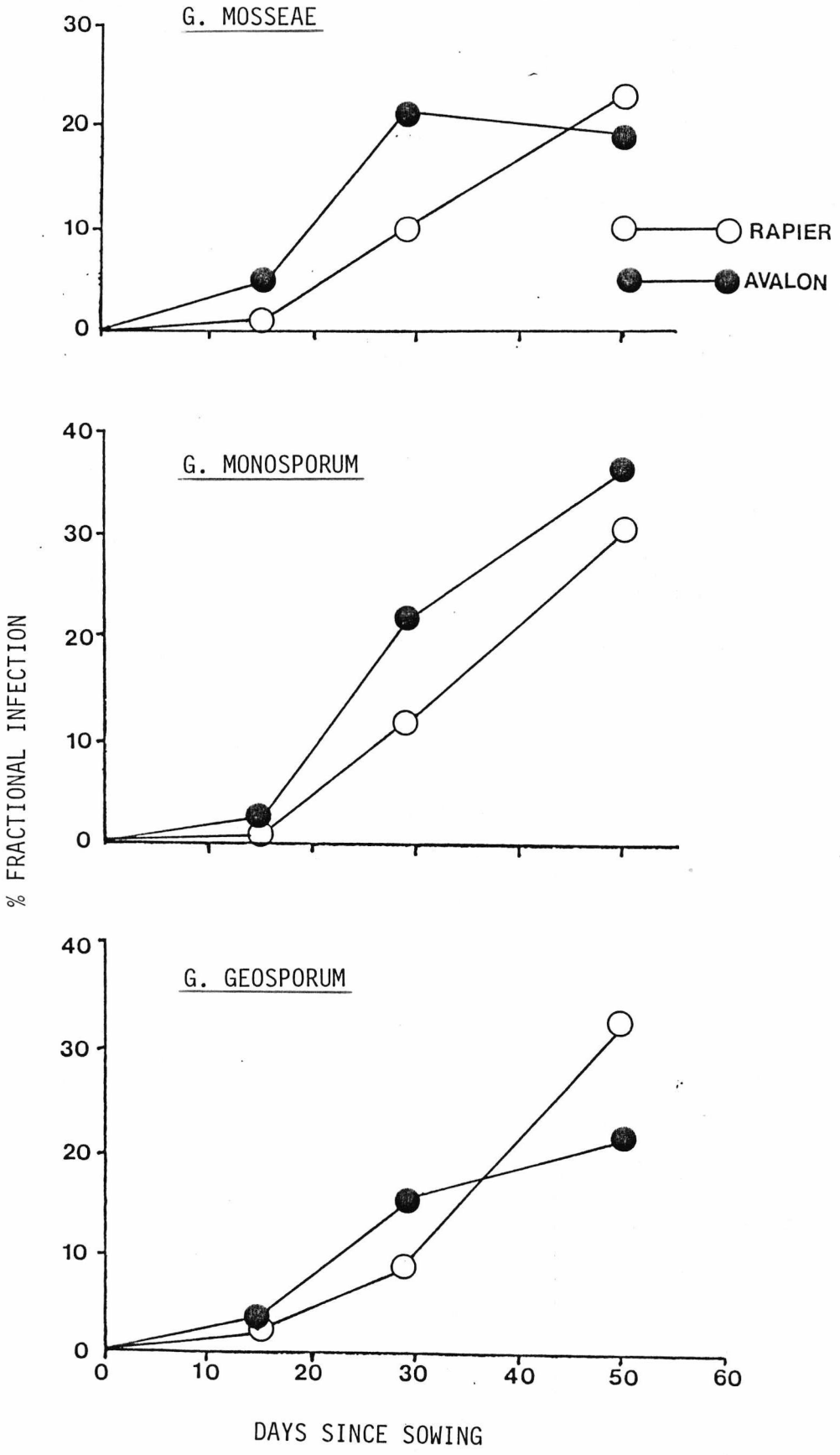
be most prolific, particularly during late October and March to May, prior to ear emergence (Barraclough and Leigh, 1984). Estimates of root extension rates for winter wheat gave figures of 5-6mm day^{-1} for the winter period compared to 18mm day^{-1} for spring (Gregory *et al.*, 1978). Again little data are available for early autumn. Barraclough and Leigh (1984) calculated the average growth rate between March and June for winter wheat, to be 1g m^2 day^{-1}. This figure was 5 times that calculated for December to March. In contrast, much of the infection recorded during summer (June to August) consisted of vesicles and intercellular mycelium with little of the arbuscular component remaining. Total fractional infection also decreased during the period after anthesis and it may be significant that Wolbank *et al.*, (1973) reported a decline in dry weight of cereal roots after this growth stage. Buwalda (1983), however, did not record this decline in fractional infection but noted instead an end of season plateau in the amount of infection present. This difference in observations may reflect differences in microscopic technique as discussed above.

In 1983/4 the two peaks in arbuscular activity were masked by a much higher carry-over of infection from the autumn 'flush' into the spring. One of the difficulties in interpreting these trends in infection, particularly in relation to the time course of development, is that % VAM infection is a ratio. Changes in fractional infection thus result from two separate processes, root growth and growth of the fungus. In this respect, Barraclough and Leigh (1984) showed that early-sown (September) crops made substantial root growth by December and continued to grow throughout the winter at a faster rate than later-sown crops. By March these early sown crops had over 4 times the root dry weight of late sown crops, and had produced 35% of their maximum root length (presumed to be in June) compared to only 10% for late sown crops. In September-sown crops roots were present at 1m depth by early December, yet roots of October sown crops

were not found at this depth until April. Barraclough and Leigh (1984) also found that there was substantial differences in root dry matter production at anthesis, between the two years studied, at both sites. It would appear that the observation of an overwinter decline in VAM infection in wheat in this study could be associated with the slower root growth rates of late-sown wheat during the period Dec-March noted by Barraclough and Leigh (1984). If the wheat crop can maintain a rapid root growth rate through this winter period, aided by earlier sowing, then it may be that VAM fungi, and particularly coarse endophytes, can establish infections and continue to spread within such an actively growing root system. For example in 1984/5, early-sown (30/9/84; Table 3.2) barley showed a steady increase in VAM infection, whereas late-sown (14/10/84) rye and wheat (sown 21/10/84) had the overwinter decline of VAM infection. The reduction in total fractional infection levels in G-2 in this same season, may have been due to the application of chicken manure and its effect on soil nutrients (Table 3.2), an effect also noted by Kruckelmann (1975) for farm yard manure.

Plants at both sites (approximately 250km apart) exhibited the same trends of infection in each season, so local factors were apparently of minor importance in establishing these trends. The difference between the results in 1982/3 and 1983/4 might have been due to the change of winter wheat cultivar, especially since different spring wheat cultivars are known to vary in susceptibility to VAM infection (Azcon and Ocampo, 1981). Daniels-Hetrick *et al.*, (1984) tested 9 winter wheat cultivars in three soils inoculated with indigenous and introduced endophytes in a glasshouse experiment. After 10 weeks all plants had become colonised and they noted significant differences in the intensity of infection between cultivars. However the cultivars supporting the greatest colonisation varied between the three soils that they tested. On this basis, though, the results for 1984/85 (Fig. 3.4) would have resembled those of 1983/84 and not those of 1982/83. In addition, the two wheat cultivars (Avalon, Rapier) used in our studies have performed similarly with respect to percentage fractional infection in pot experiments using pure inoculum of three VAM fungi. Figure 3.5 shows the results and it can clearly be seen that there was no difference between infection

FIGURE 3.5 - A comparison of the development of VAM infection with calendar time in the roots of two winter wheat varieties, Avalon and Rapier, when infected singly by each of three endophytes, Glomus geosporum, Glomus monosporum and Glomus mosseae.



progression in the two cultivars when colonised by those three coarse endophytes.

It is generally believed that the degree of mycorrhiza formation is inversely correlated with available soil phosphorus levels (Mosse *et al.*, 1981), and the lower infection levels recorded in Hamill soil are presumed to reflect the higher levels of bicarbonate-soluble phosphorus available at this site. It is notable, however, that over 40% of the root length was infected in May 1984, a value far higher than would be expected for a soil (Table 3.2) containing over 40mg L^{-1} phosphorus (ADAS rating 3). This peak in infection levels corresponded with a period of low rainfall, and it is likely that the drought conditions depressed the soil moisture content with a corresponding decrease in phosphorus mobility. The mobility of phosphorus and other mineral elements in the soil is lowered when soil water potentials decrease (Olsen *et al.*, 1961). This is known to lead to P-deficient plants during drought stress even though the soil phosphorus levels are adequate for greater growth under non-stress conditions (Nelson and Safir, 1982). Interestingly, infection by VAM tends to be stimulated in plants growing in conditions of drought-stress (Sieverding, 1981). We obtained our highest infection levels in a soil with comparatively low phosphorus availability and it would be unreasonable to compare these results with those obtained for a highly fertile soil. Several studies, however, which report negligible infection levels in the late winter period have been carried out on soils with similar low levels of phosphorus (e.g. Jakobsen and Nielsen, 1983; Daniels Hetrick *et al.*, 1984). Such soils are not necessarily marginal with respect to cereal cultivation, and large areas of winter wheat are grown each year under these conditions (Jarvis *et al.*, 1984).

3.4.2A. Occurrence of Fine Endophytes.

These observations also draw attention to the frequent occurrence of

infections due to fine endophytes in temperate agricultural soils, particularly during the winter months, and later towards the end of the cereal growth season, when these species make up the bulk of the mycorrhizal infection. Other workers have also noted a predominance of fine endophytic infection in crops during the winter months (Jakobsen and Nellsen, 1983). It has been suggested that the fine endophytes are less aggressive than the coarse with respect to infection ability from the growth medium (Wilson and Trinick, 1983), but once infection has occurred the mycelium of a fine endophyte is capable of producing many more secondary infection loci than that of a coarse endophyte. Furthermore, the fine endophyte was apparently slow to infect germinating seeds, but once established it was capable of a more rapid spread from plant to plant than other fungi investigated (Powell, 1979a). Elmes *et al.*, (1983) showed this same trend of slow initial establishment of indigenous fine endophytes infecting red clover in unsterile field soil, with a later rapid phase of spread of infection. The coarse endophyte introduced into the same soil, as a separate treatment, showed earlier colonisation of the red clover which subsequently led to a significant growth response over the control. This occurred despite the fact that final total fractional infections of the coarse and fine endophytes at the end of the growth period, were the same.

Fine endophytes are often the most abundant mycorrhizal types in natural soils (Fitter, 1985). These fungi rarely produce beneficial growth responses, even in infertile soils (Powell, 1979b), and, at least in the samples examined during the course of the present study, they seem to produce little external mycelium. It may be that these two observations are related as Sanders *et al.* (1977) reported that of four VAM isolates used to inoculate onion seedlings, it was the one that produced least external mycelium that was the least efficient in terms of stimulating growth of the host. However Abbott and Robson (1985)

showed that their fine endophytic isolate produced high levels of external mycelium yet infected plants had decreased shoot weights compared with control plants.

The inhibition of spread of coarse endophytic infection, seen over winter in 1982/83 and 1984/85 (Figs. 3.3 and 3.3B) within the roots, caused possibly by low temperatures (See next section), may not affect fine endophytes to the same degree. It is known that they have a different type of spread of infection (Wilson and Trinick, 1983). It is also known that fine endophyes are a major component of the all year round infection that occurs in natural grasslands (Read *et al.*, 1976). Coarse endophytes, apparently more aggressive, may therefore play a natural role in aiding seedling establishment in the Sussex fields investigated here, G-1 and G-2. The two peaks of coarse endophytic infection (Fig. 3.3) may, however, mask a general slower linear increase of fine endophytic infection through a season. The lower levels of infection consistently reported from temperate agricultural fields (Buwalda, 1983; Jakobsen and Neilsen, 1983; Mosse and Hayman, 1971) when investigating the natural VAM infection of crops in the field, may therefore consist chiefly of fine endophytes at certain times of the year. Rabatin (1979) reported seasonal differences in the percentage length of graminaceous host root infected with fine endophyte in three field sites in Pennsylvania, U.S.A. Highest levels were found in the late-winter and spring period in very P-deficient soils low in moisture. In roots sampled from soils with a high spring soil-moisture content fine endophytic infection increased to a maximum later in the growing season, a similar result to that observed here in the Sussex fields. She suggested that the fine endophytes were particularly adapted to penetrating host roots during brief seasonal fluxes of P availability found in spring.

CHAPTER 3

PART 2

The effects of environmental factors on seasonal differences in the pattern of VAM development in winter wheat root systems in the field.

3.1B. INTRODUCTION

Where soil temperatures vary considerably during the growth season of a crop such as winter wheat in temperate regions, it has been suggested (Buwalda, 1983; Mosse, 1981; Black and Tinker, 1979) that temperature may be the major rate-limiting factor involved in VAM infection spread. Buwalda (1983) suggested that low temperatures in winter contributed to the slower development of infection in winter sown cereals than in spring sown cereals. He observed a slow steady increase in infection during the winter crop season compared to a much faster increase in infection during the spring crop season, in inoculated and non-inoculated field experimental plots. He also suggested that annual differences in the rate of increase of soil temperatures in spring may explain annual differences in the rate of development of VAM infection in the roots of spring barley.

Temporal developmental events over different seasons have been related to cumulative temperature data using the concept of THERMAL TIME (Robertson, 1968). This concept has been used to help descibe more clearly the effects of temperature on leaf extension dynamics (Hay and Tunnicliffe-Wilson, 1982) and leaf appearance (Baker, *et al.*, 1980) in winter wheat. Buwalda (1983) was the first to apply this concept to the effects of temperature on VAM infection development in winter and spring sown cereals. He was successful in eliminating differences between seasonal variation by plotting infection progression against

thermal time using 5°C as a base.

The day-degree hypothesis basically states that for each day (p), the mean temperature, Tp, is measured and a temperature sum, h, formed according to.

$$h = \sum (Tp - Tc)$$

including those terms where Tp is above some threshold (base) value, Tc. The day-degree sum, h, essentially integrates some under lying temperature-sensitive process or processes. The approach is based on the notion of a developmental rate, whose response to temperature is approximately linear over a restricted temperature range. Therefore the thermal time (in degree-days) from sowing to each sampling date is estimated by summing the thermal time of each day. The use of a base temperature is arbitrary and it is not known if it has any physiological significance although Gallagher, *et al.*, (1979) found that leaf extension was temperature related and stopped around 0°C. This could relate to photosynthate being available to a VAM symbiont. Same, *et al.*, (1983) showed that decreasing root temperatures also decreased the concentrations of soluble carbohydrate within the roots. They further found a close correlation between root length infected and root concentrations of soluble carbohydrate.

3.2B. METHODS

Two calculations of thermal time were made. The first used a computer program written by J.R. Porter and L. Parry (1984) and kindly supplied by J. Rayner (Rothamsted Experimental Station) which integrated thermal time values in its calculation. The second involved a more simple calculation of,

$$(Tmax - Tmin / 2) - Tbase$$

for each day and the subsequent summing of these values for each day between sampling dates (Barraclough, personal communication). If the value calculated for each day is less than the Tbase then no thermal time elapses during that day. Base temperatures were set at 0°C, 1°C and 5°C in both cases. All calculations used daily maximum/minimum air temperatures above grass, obtained from the Metereological Office (Bracknell, Berks. U.K.) for data collected from Manston, Kent (TR 332664) and Plumpton, Sussex (National Grid Reference 53571136). Buwalda (1983) compared the use of air and soil temperatures and obtained similar relationships between thermal time and VAM infection.

3.3B. RESULTS

Figure 3.6 shows that there is little difference, initially, between the two methods of calculating thermal time. However, as the season progresses the computed calculation causes the accumulation of more thermal time than that of the simplified calculation. The simplified calculation was subsequently used to compare thermal time with calendar time and VAM infection development through the 1982-1985 winter wheat seasons.

Plots of calendar time and thermal time against fractional infection (Fig. 3.7) show that the apparent over winter decline in infection levels is reduced in the thermal time plots, at a base temperature of 5°C. It was particularly successful in eliminating the decline period at the G-2 site in 1982-83. Decline periods were not removed using thermal time plots at any of the other base temperature levels used, 0°C and 1°C (data are not presented here).

Plotting accumulated thermal time against calendar time for all crops at G-1 and G-2 covering the period September to March (Fig. 3.8), revealed that the earlier accumulation of thermal time could be a major factor in the elimination of

an over winter decline in infection. Two out of the top three thermal time accumulators showed a continued increase in infection through the winter. However there remains the anomaly of the wheat crop in G-2, 1983-84, which accumulated the second lowest thermal time sum but still showed the increasing trend of infection through the winter.

Closer examination of the data available for 1983-84, reveals that where no winter decline occurred (Fig. 3.7), there was a coincidental dry and mild autumn (Table 3.4), with a higher accumulation of thermal time than 1982-83 for the same period (October 1 - January 1). The 1984-85 period, however, had the highest thermal time accumulation of all three seasons. Table 3.4 shows the accumulated rainfall data for each autumn (Fig. 3.9 shows the total seasonal rainfall for each season studied). The 1983-84 season had the lowest rainfall (well below the yearly average) of all three seasons investigated, with 1984-85 being closest to the yearly average for this site. In the 1982-83 season the decline in infection (Fig. 3.7) began before the beginning of December and coincided with a period of negligible thermal time accumulation above the 5°C base (Fig. 3.8), coincident with a wet period (one third of the total autumn rain fell from mid-November to mid-December). In 1984-85 over one-quarter of the autumn rain fell in this same period and it accumulated more thermal time (base 5°C) than any of the other three seasons during this time (Table 3.4).

Analysis of the Hamill data revealed the same trends for the 1982-83 and 1983-84 seasons to those observed at the Sussex site, and results are therefore not presented here.

FIGURE 3.6 - A comparison of two thermal time calculations (base 5°C) using the VAM infection data from the G-2 site in the 1982-83 season.

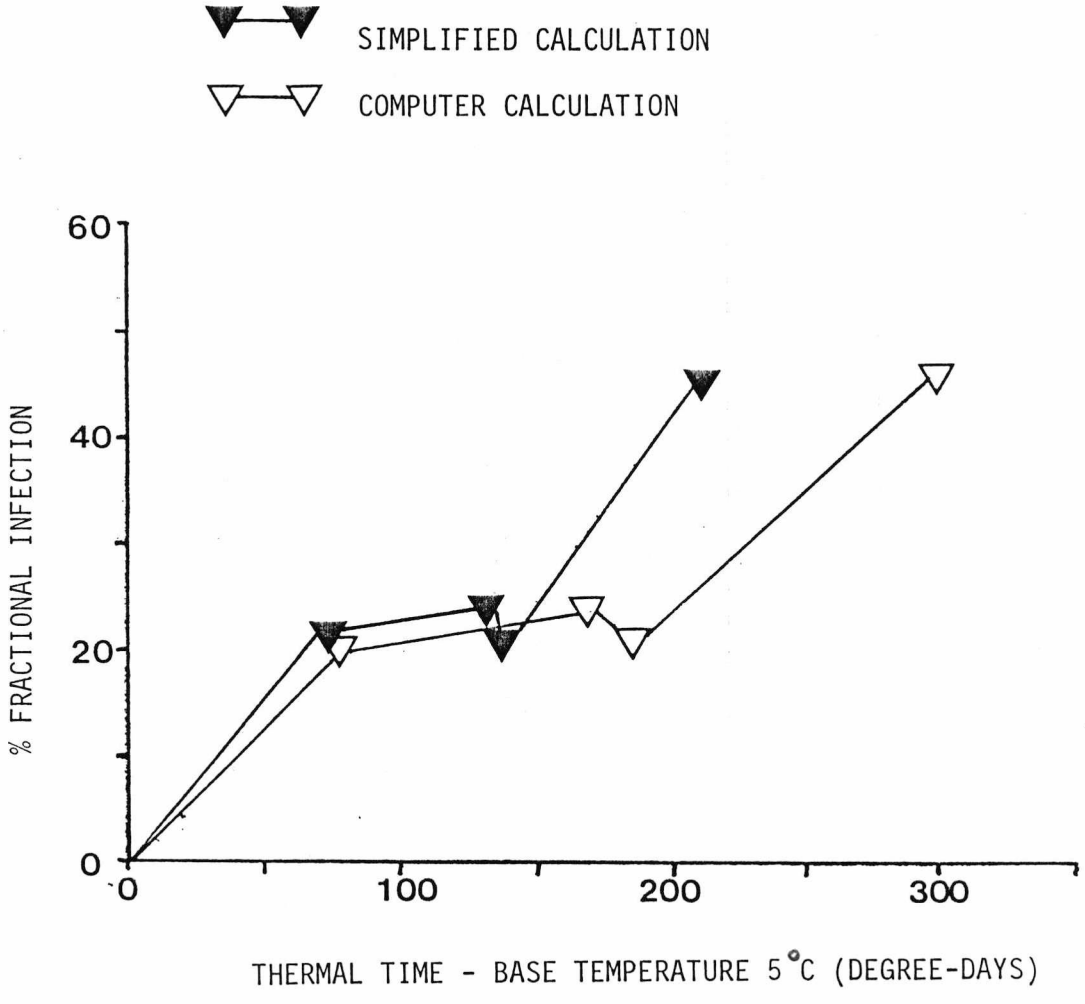


FIGURE 3.7 - A comparison of the relationships between the development of VAM infection in the roots of winter-sown barley and wheat when plotted against calendar time and thermal time (base 5°C), at G-1 and G-2 field sites 1982-1985.

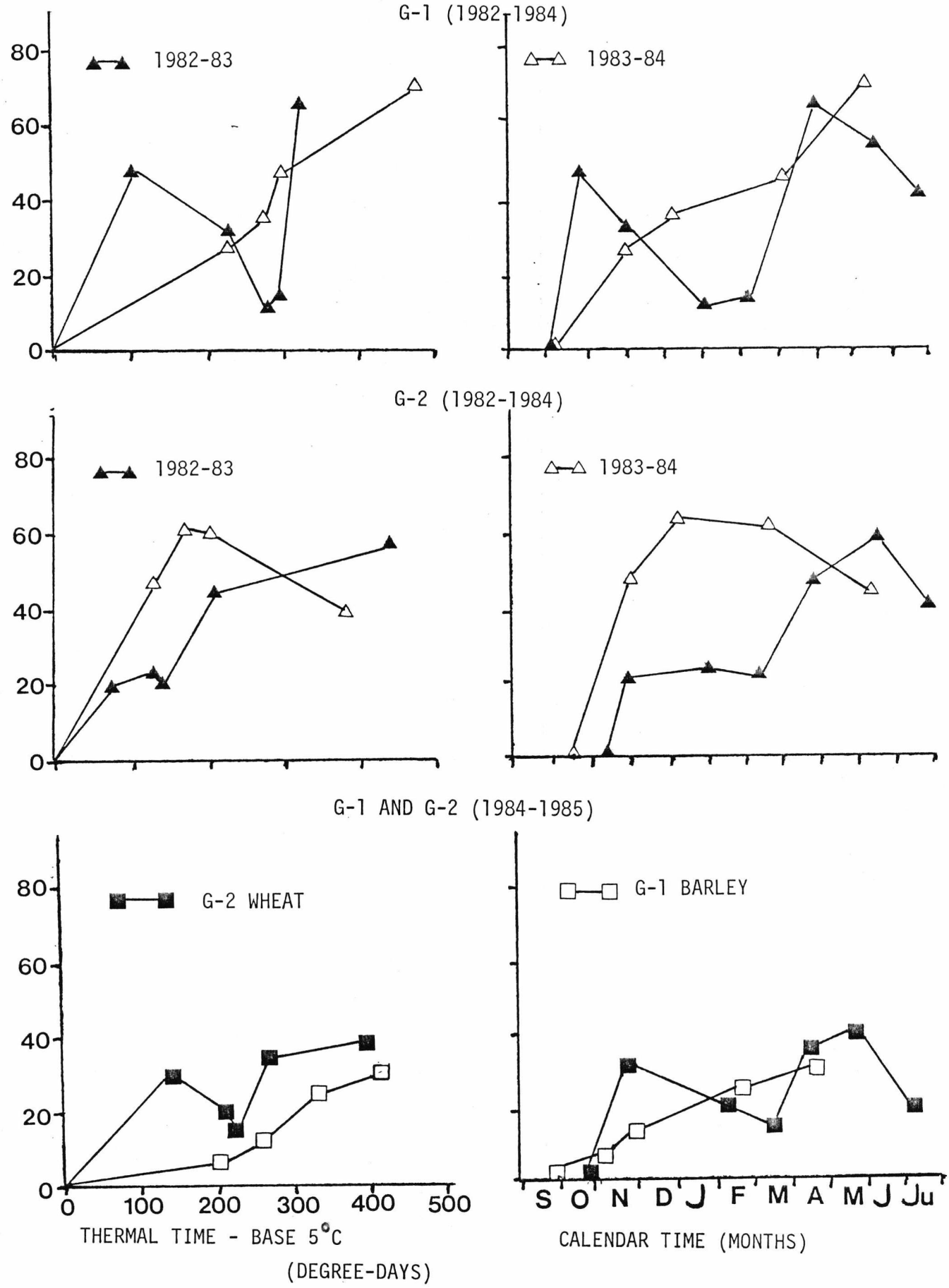


FIGURE 3.8 - A comparison of the accumulated thermal time (base 5°C) for each Sussex crop (1982-1985) with calendar time from sowing to April.

		OVERWINTER DECLINE (+ OR -)
A	- G-1 BARLEY 1984-85	(-)
B	- G-1 WHEAT 1983-84	(-)
C	- G-1 WHEAT 1982-83	(+)
D	- G-2 RYE 1984-85	(+)
E	- G-2 WHEAT 1984-85	(+)
F	- G-2 WHEAT 1983-84	(-)
G	- G-2 WHEAT 1982-83	(+)

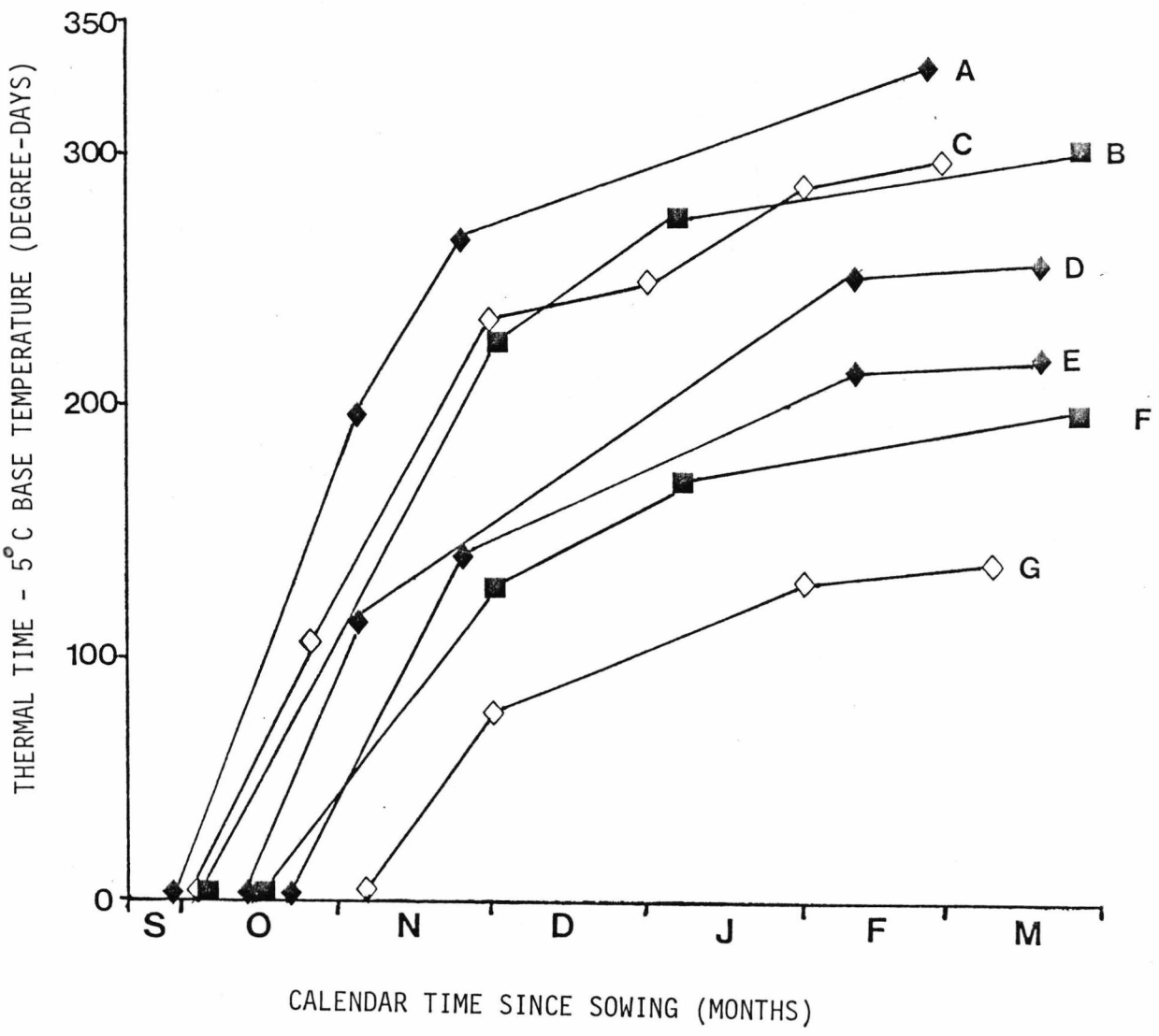


FIGURE 3.9 - The cumulative rainfall for each winter cereal season 1982-1985 at the Sussex field site.

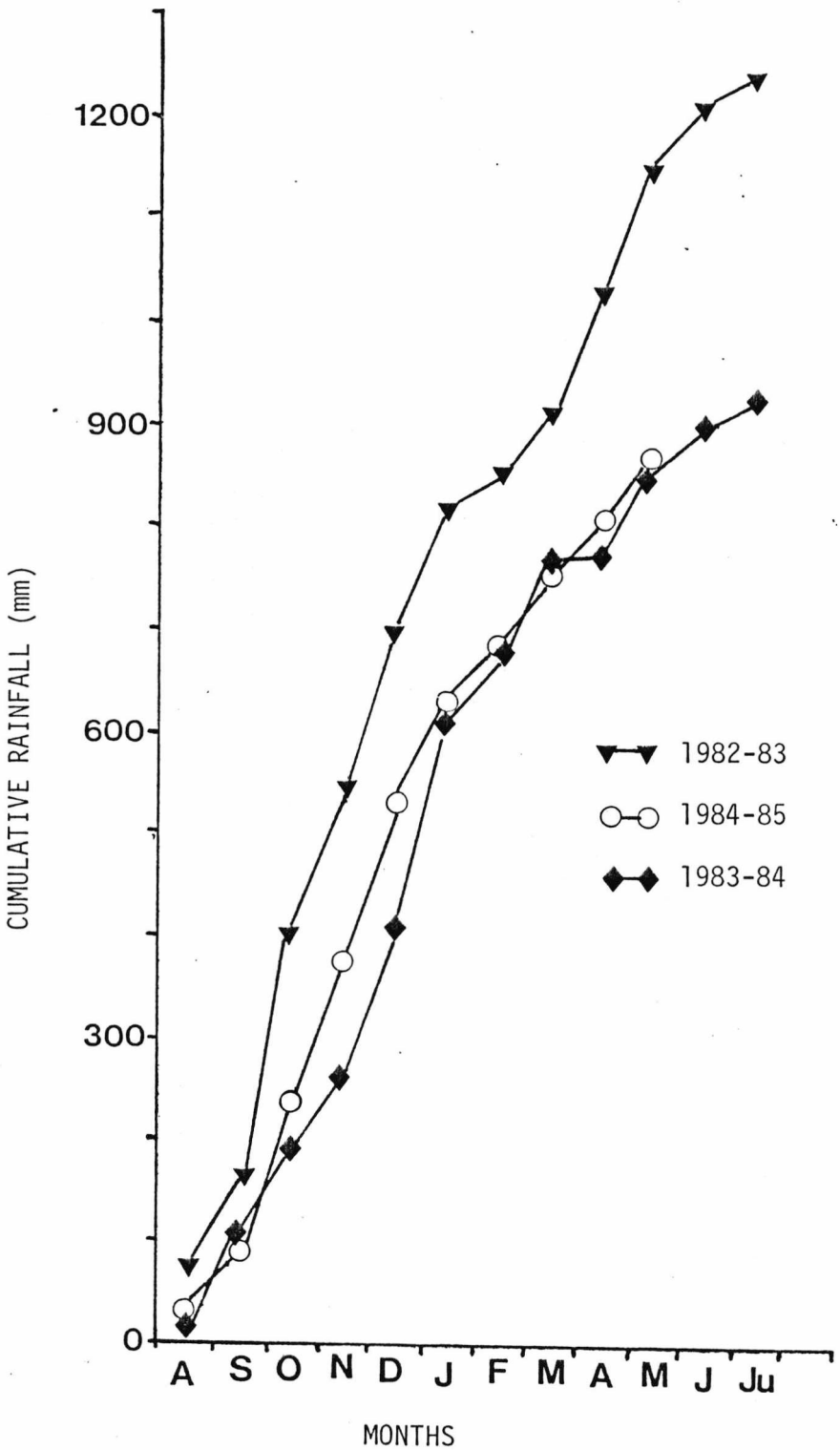


FIGURE 3.10 - The relationship of thermal time (base 5°C) to VAM infection development in the winter-sown crops of Sussex (G-1 and G-2) in the 1984-85 season. (Arrows indicate the thermal time accumulated up to January 1).

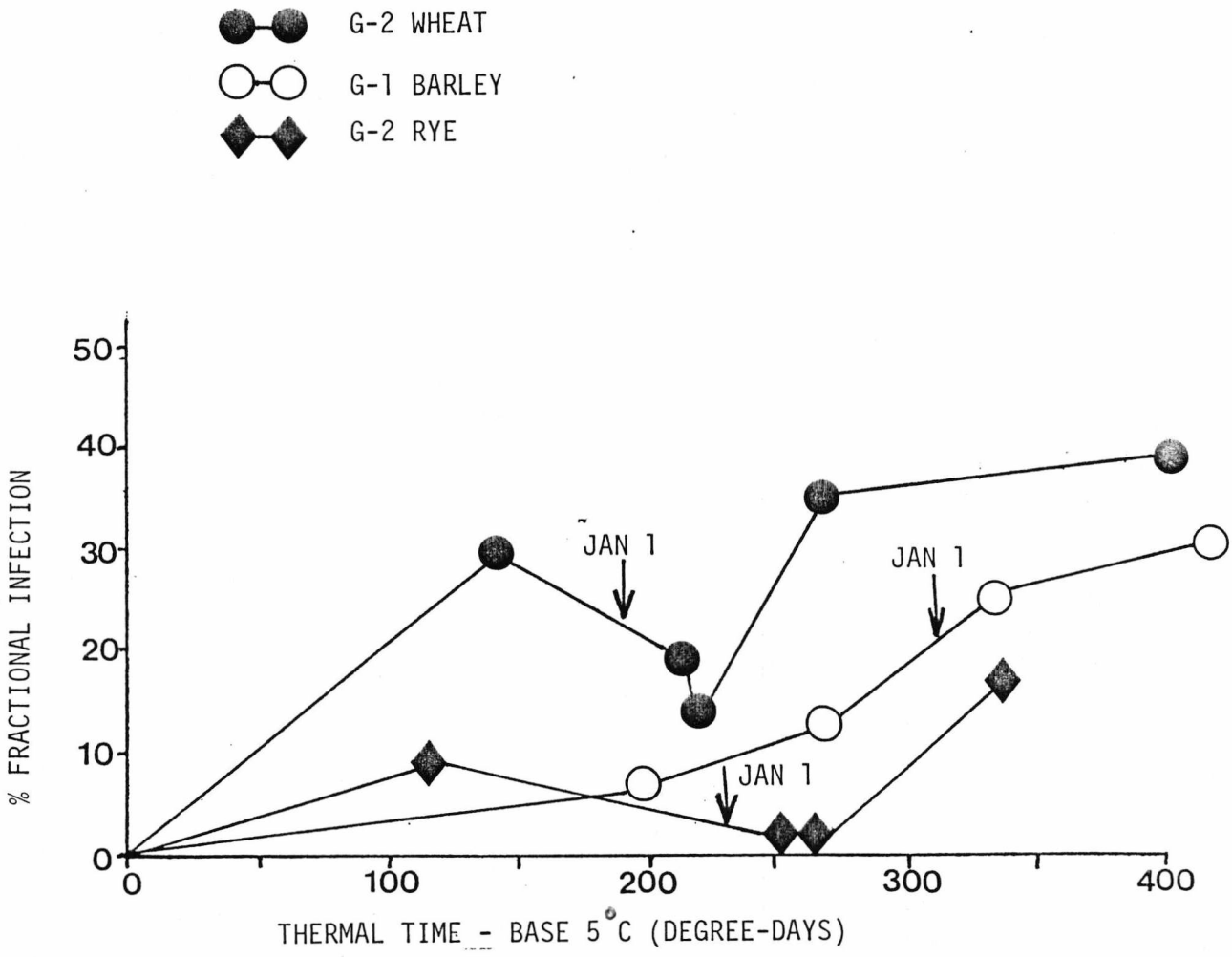


TABLE 3.4 - Total autumn rainfall and thermal time data for the Sussex site (G-1 and G-2), 1982-1985.

WINTER CROP SEASON	TOTAL RAINFALL BETWEEN SEPTEMBER 1 AND JANUARY 1 (mm)	ACCUMULATED THERMAL TIME (BASE 5°C) OCTOBER 1 TO JANUARY 1
1982-83	614	250
1983-84	388	278
1984-85	503	305

3.4B. DISCUSSION

Results shown here parallel work by Barraclough and Leigh (1984) with root dry weight accumulation of winter wheat through two successive seasons. They showed that plotting root growth data against thermal time eliminated some of the differences between root growth progression caused by early or late sowing of winter wheat crops at two sites investigated. However it did not eliminate differences observed between years. In my study the effect of rainfall on this Weald clay soil type seems to have had a complementary role to thermal time accumulation in affecting infection progression. This was probably due to the fact that close texture and high water content, in clay soils, can often restrict root development because of anaerobiosis. Trinick (1977) observed that high soil moisture reduced the mycorrhizal infection of lupins. Reid and Bowen (1979) showed that *Glomus mosseae* spore germination was sensitive to soil water content. These observations might indicate that oxygen content of the soil environment is important for normal functioning of mycorrhizas. Saif (1981, 1983) revealed that the mycorrhizal symbiosis functioned best when soil aeration was at a high level.

In 1984/85 the rainfall was approximately average for an autumn period (Table 3.4) but the highest accumulated thermal time (5°C) values occurred for the October to end of December period for the three seasons studied. In the three crops sown at the Sussex site, however, only the earliest sown (barley 30/9/85) showed the sustained trend of VAM development through the winter observed in 1983/84 at Sussex (Fig. 3.7) (Buwalda (1983) obtained his data from September-sown winter crops). The later sown winter wheat and rye crops at the same site showed an overwinter decline (Fig. 3.7). Figure 3.10 reveals that more thermal time (5°C) was accumulated by the early sown crop up to January 1 1985 than by the other two crops. Barraclough and Leigh, (1984)

showed that earlier sown crops (late September) made substantial root growth by December and continued to grow throughout the winter at a faster rate than late sown crops from mid-October onwards(see Discussion of previous section, 3.4A.). The roots of October-sown crops were slow to develop and their dry weight increased at a much slower rate over winter.

The effects of the soil water content on root growth may not have been as significant at the field sites studied by Barraclough and Leigh (1984), since their observations were made on winter wheat growing in better drained sandy/silty loams. It may be therefore that in this soil type the date of sowing could be of crucial importance to VAM development, in years where the effects of extreme fluctuations in environmental conditions (e.g. soil temperatures or soil water status) do not override it.

Results presented here (Figs. 3.7, 3.8, 3.9, 3.10) for thermal time calculations used a base temperature of 5°C only, since trends for 0°C and 1°C were similar. However Buwalda (1983) showed that there was no effect of season of sowing on the development of VAM infection when Tbase was set at 5°C, whereas at 1°C infection developed much faster in the spring-sown crops. This base level may be very close to the minimum temperature at which translocation of phosphorus in hyphae of VAM fungi occurs (Bowen *et al.*, 1975; Cooper and Tinker, 1981; Hayman 1983). Chilvers and Daft (1982) showed that the coarse endophyte *Glomus caledonicum*, in pot experiments, maintained its level of infection in onion roots through an extended cold period of 5°C, but had a distinctly moribund appearence (weak arbuscular structures and septation of intercellular hyphae - c.f. Photo 9 which shows arbuscles observed in the decline period of the 1983 winter). It was also noted that this same fungal isolate

could only infect Narcissus roots at 9°C. Thus temperatures below 5°C may be inhibitory to the spread of VAM infection inside the root. During pot studies on the effects of phosphorus on two VAM fungal isolates growing on winter wheat roots (Experiment 1 in Chapter 6) infection was also noted to decline to less than 5%, during the period when night temperatures consistently fell below 5°C. Although there appear to be no reports in the literature of the minimum temperature at which winter cereal root growth ceases, there seems little reason to assume that this would be different from that for leaf extension 0-1°C (Gallagher *et al.*, 1979). Barraclough and Leigh (1984) showed that the length of winter wheat roots increase through winter, implying that soil temperatures are rarely less than the minimum for root growth.

Buwalda (1983) concluded that plotting VAM infection against thermal time rather than chronological time indicated that temperature was the important factor in the development of VAM infection in roots of winter cereals. This is substantiated here by the reduction of the overwinter decline period (Fig. 3.7). Buwalda also stated that low temperatures during winter are responsible for the slow development of infection in roots of winter sown crops, whereas the results of this study would indicate that the development of VAM infection within winter cereal roots is probably determined by the effects of both soil temperature and soil water status directely on the host root system. As a consequence the root growth rate of the host and root longevity within a soil, may be the major rate limiting step for infection development. The soil type could, therefore, again be a cause of differences observed between results reported by workers involved in VAM research. It is interesting to note that in concluding his field experimentation, Buwalda (1983) sowed winter barley on the previous seasons fumigated plots (inoculated and non-inoculated). The inoculated plots had a much higher VAM soil presence than the previous season, due to the previous

crop finishing with about 40% of its root system infected, and here the earliest sown crop showed a more rapid rise in infection (by November 24) than seen in any of the other experiments. Fractional infection then levelled off over winter but increased again by March 27. The non-inoculated showed a continual steady rise, as noted in the previously experiments. It is possible therefore that the higher inoculum potential of his inoculated plots could be important in the development of an early autumn peak in infection, corresponding with that observed at the Sussex site investigated here, followed by a decline or levelling off of infection over winter. This effect is less dramatic in better drained soils, unlike those of the Sussex site.

A substantial body of evidence indicates that early sowing is critical to the achievment of high average yields (Batch, 1981). Mid-October had previously been considered to be an optimum sowing date for winter wheat but increasingly it appears that sowing should start earlier (e.g. late September). On commercial field sites such as those investigated in Sussex, where a high percentage VAM infection occurs, sustaining infection through winter in an apparently active state (as indicated by the proportion of infection as arbuscles) might be thought to be as highly beneficial, so contributing to the advantages of an early sowing date. This may be of interest to those involved in developing systems of sustainable agriculture. The results reported here do not quantify the role (stimulatory or not) of the VAM symbiosis. It is known that the inflow of phosphorus to roots of winter wheat has a large peak in mid-winter and a smaller peak in spring (Gregory et al., 1978), thus infection during late autumn and early winter could be extremely influential in optimising nutrient uptake in these plants in agricultural soils.

This work again highlights the number of factors that need to be considered simultaneously in order to obtain a clearer view of the role of VAM fungi in a

natural or agricultural situation. Relationships between soil inoculum potential, rate of spread and development of infection, and effects on phosphorus uptake and yield responses need to be investigated. These will obviously differ from soil to soil even for apparently similar investigations (Graham *et al.*, 1982; Hayman and Tavares, 1985), with the same endophyte. Confusion is often caused by the assumption that a given variable such as soil temperature or soil moisture will influence the host and its symbiont to a similar degree. This is not necessarily true and this problem will only be overcome by studying root and infection dynamics together in future studies. The common presentation of % infection must include data on root lengths in order that infected root length can be calculated. The laborious and tedious nature of this type of work would necessitate extensive cooperation between soil scientists and mycorrhizal workers in field investigations. For example, material from experiments dealing directly with root growth dynamics in the field such as those of Barraclough and Leigh (1983) could also have been assessed, at the same time, for mycorrhizal infection.

CHAPTER 4

The characterisation of VAM spores from Hamill and Sussex field sites.

4.1. INTRODUCTION

Many VAM fungi in the Endogonaceae produce abundant resting spores in soil (Gerdemann and Trappe, 1974) that may be important as propagules for initiating endotrophic symbioses (Manjunath and Bagyaraj, 1981; Mosse, 1982). VAM infected root fragments and hyphae found in soil can also initiate new infection sites on host roots (Powell, 1976), and spore numbers may therefore underestimate the potential infectivity of a soil in many cases. Knowledge of the diversity, distribution and spore population densities can, however, be valuable in understanding which endophytes are the chief colonisers of plant root systems in a particular soil. Other authors have analysed variations in spore densities in relation to season and soil environment (Giovannetti and Nicolson, 1983; Hayman, 1970; Walker *et al.*, 1982). As part of the experimental study of the ecological role of VAM fungal infection in winter wheat, at four field sites, (see chapter 3), a quantitative analysis of the occurrence of viable spore numbers and species was made, over a three year period. This was also carried out with the intention of isolating as many species of VAM fungi as possible as single spore 'pure' pot cultures for further physiological studies in later greenhouse experiments.

Recent descriptions of species in the Endogonaceae have increasingly relied upon wall structure of spores to separate species within genera (Walker and Trappe, 1982; Trappe and Schenck, 1982). Keys designed to aid identification (Hall and Fish, 1979; Trappe, 1982) use characteristics of wall structure using the light microscope only and are difficult to interpret by the non-specialist. This has also been due to a lack of photographic material available in the literature for the common species. The difficulty in allocating a species name, with certainty, to a particular fungal isolate, has caused many misidentifications in the literature. As a result, field researchers have tended to concentrate their studies on a few well-known and established isolates made available by the established taxonomic workers. The frequent use of `mixed' cultures in inoculation experiments under the auspices of a `pure' culture have also confused the literature. Recently, however, several workers have attempted to draw together the current taxonomic `melee' by the use of such aids as murographs (Walker, 1983) which are diagrammatic representations of wall structures, intended to be used in conjunction with a species description. Other workers have used electron microscopy as an aid to interpreting spore morphology and particularly wall ornamentation (Bonfante-Fasolo and Vian, 1984; Walker and Trappe, 1981). Finally biochemical and serological methods have also been used to aid the current anatomical (Abbott, 1982) and morphological classifications. The use of an indirect enzyme-linked immunosorbent assay (ELISA) aided the separation of four genera in the Endogonaceae (Aldwell *et al.*, 1985). Work by Sen and Hepper (1986) showed that the mobility of total proteins or specific enzymes, during electrophoresis, could be used to clearly distiguish between the resting spores of six *Glomus* spp., after subjecting spore extracts to polyacrylamide gel electrophoresis (PAGE) and selective enzyme staining. The banding patterns of six enzymes were diagnostic for each species.

In this study, therefore, a full characterisation of three pure isolates obtained from single-spore cultures, *G. geosporum*, *G. monosporum* and *G. mosseae* was attempted using light microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and selective enzyme staining of spore extracts after PAGE.

4.2. MATERIALS AND METHODS

4.2.1. Spore identification

Spore dimensions were measured using an ocular micrometer in the eyepiece of the compound microscope. The results are presented in Table 4.1 as a mean with maxima and minima noted either side. Confirmation of spore identity was carried out with the help of Dr. C. Walker based on morphological characteristics of the spore.

4.2.2. Biochemical Procedures

The three species of VAM fungi used - *G. geosporum*, *G. monosporum* and *G. mosseae* - were of similar colour and size and, therefore, distinction could pose problems for the non-taxonomist. They were obtained in pure culture after single-spore inoculations of onions. *G. monosporum* and *G. mosseae* were very similar morphologically in pot culture. All fungi were stored in durite at 4 °C prior to wet-sieving. 150-200 spores were examined for each sample to obtain a photographic record.

4.2.2.1. Sample Preparation

Immediately prior to electrophoresis, spores were placed in 5 μm of buffered sucrose (150g L^{-1}) containing bromophenol blue tracking dye (500mg L^{-1}).

The sample buffer contained:

(1). 20mM Tris-HCl

(2). 10mM $NaHCO_3$

(3). 10mM $MgCl_2$

(4). 10mM β-Morcaptoethanol

(5). 0.1mM EDTA

pH 8.0

The spores were crushed in the buffer and a further 5ul of buffered sucrose was added containing 0.6g L^{-1} Triton X-100. Samples were prepared rapidly and maintained at 6 ^{o}C between crushing and loading onto gels.

4.2.2.2. Electrophoresis

Samples were subjected to electrophoresis in vertical non-denaturing, polyacrylamide gels of either 65 x 110 x 1.5mm (Running time approx. 1 hour) or 120 x 110 x 2mm (Running time approx. 2-4 hours), without a stacking gel, on a Heath (Zenith) power unit.

RUNNING GEL:

	FINAL CONCENTRATION
(1) Tris-HCl (pH 8.8)	0.325M
(2) Acrylamide	80g L^{-1}
(3) Bis-Acrylamide	3.0g L^{-1}
(4) $(NH_4)_2S_2O_8$ (Fresh on day)	1.0g L^{-1}
(5) Temed	0.5g L^{-1}
(6) Distilled water	250mls L^{-1}

ELECTRODE BUFFER:

(1) Tris-base	0.025M
(2) Glycine	0.192M
	pH 8.3

The larger gel system was subjected to 25mA at 150V, whilst the smaller gels received 15mA at 100V.

4.2.2.3. Gel Staining

(a) Non-Specific Esterases

Gels were placed in 0.2M Tris-HCl buffer, pH 7.0, for 30 mins. and then transfered to a staining solution consisting of:

25mg Fast Blue RR diazonium salt

25ml 0.5M Tris-HCl, pH 7.1

0.75ml 1% a,B Naphthyl acetate in 50% acetone/water

Made up to 25ml with distilled water and filtered

(After Sen and Hepper, 1986)

(b) Acid Phosphatases

Gels were preincubated for 30 mins. in 0.2M acetate buffer, pH 5.0, and then transfered to a staining solution of:

1mg ml^{-1} B-naphthyl acid phosphate, sodium salt

1mg ml^{-1} Fast Blue RR diazonium salt

0.2M Acetate buffer, pH 5.0

(After Zuber and Manibhushanrao, 1982)

(c) Alkaline Phosphatases

The procedure that was used for detecting acid phosphatase was also used here to detect alkaline phosphatase activity, except that the acetate buffer was replaced by 0.2M Tris-HCl buffer, pH 9.0 and the substrate was sodium-1- naphthyl phosphate (after Zuber and Manibhushanrao, 1982).

4.2.2.4. Densitometer scans

After staining for enzyme activity, gels were washed with distilled water and stored in 7% acetic acid until they were photographed and scanned in a Biorad densitometer.

4.3. RESULTS

4.3.1. Spore Data Survey

Figures 4.1 and 4.2 show that counts of apparently viable spores were low in the cultivated fields investigated over the three year period. Soils from the Hamill (Avalon) site averaged approx. 10 spores per 50g of fresh soil whilst soils from Sussex averaged only 20 spores per 50g of fresh soil. The Hamill (pasture) site, however, did yielded consistently higher spore densities of 35-160 spores per 50g of fresh soil. Results also reveal a sporadic appearence of *Acaulospora* spp. and to a lesser extent *Gigaspora* spp. during the three years. The peaks of total spore numbers, particularly at the Hamill pasture, occurred around the peaks of rainfall at each site. It should also be noted that *Acaulospora* spp. and *Gigaspora* spp. also appeared around these peaks of rainfall, at both sites.

Figure 4.1 Spore numbers isolated from the Hamill site, in relation to peaks of rainfall during the period 1982-1985.

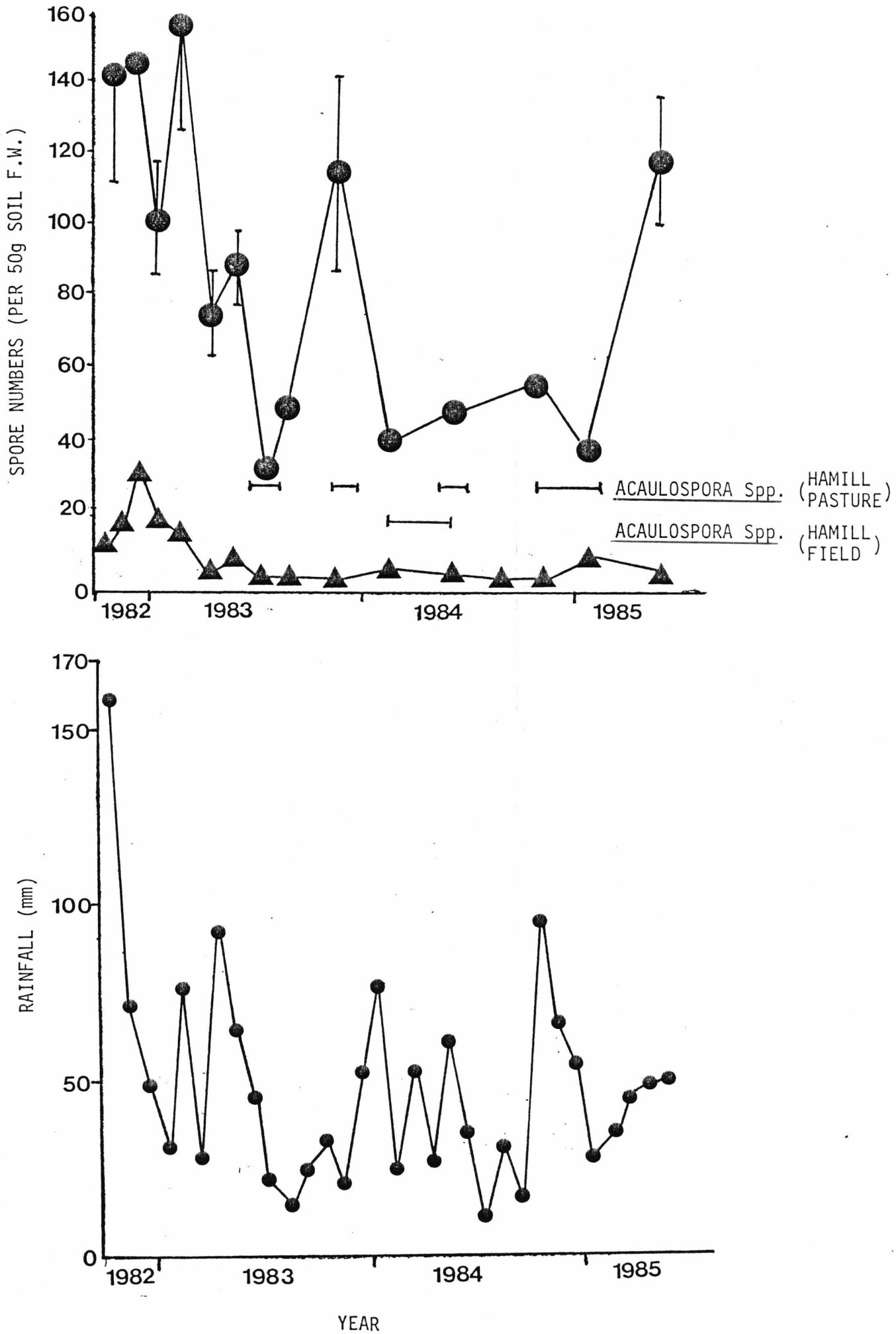
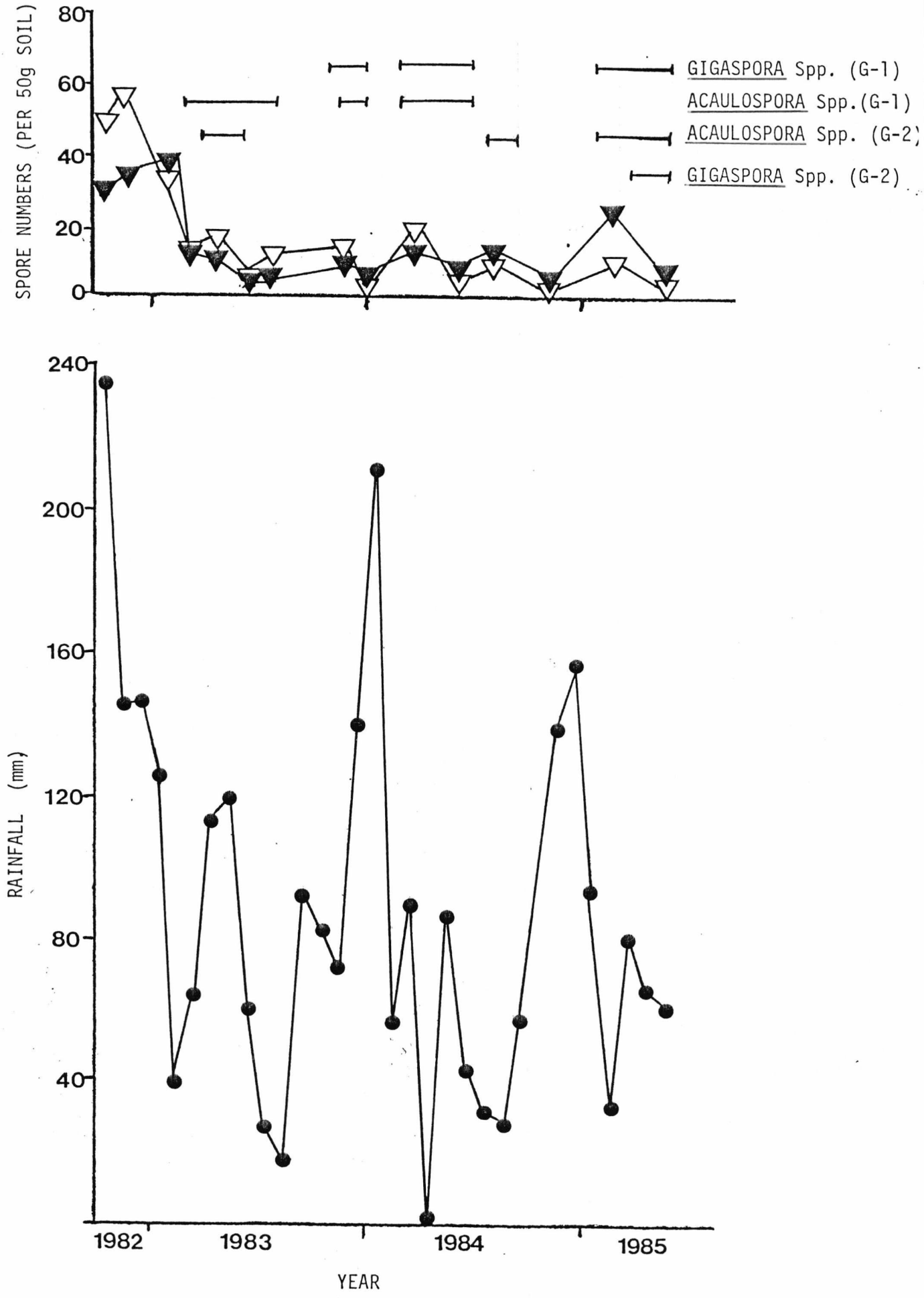


Figure 4.2 Spore numbers isolated from the Sussex field site (G-1 and G-2) in relation to peaks of rainfall in the period 1982-1985.



The fungi observed at each site are listed below:

	HAMILL	SUSSEX
G. constrictum	+	+
G. geosporum	+	+
G. macrocarpum(?)	+	+
G. monosporum	-	+
G. mosseae	+	-
G. tenue-Fine endophytes	+	+
Acaulospora sp. 1	+	-
Acaulospora sp. 2	+	-
Acaulospora sp. 3	-	+
Gigaspora sp. 1	-	+
Red-brown sporocarpic type	+	+

4.3.2. Species Descriptions

(a) *G. geosporum*

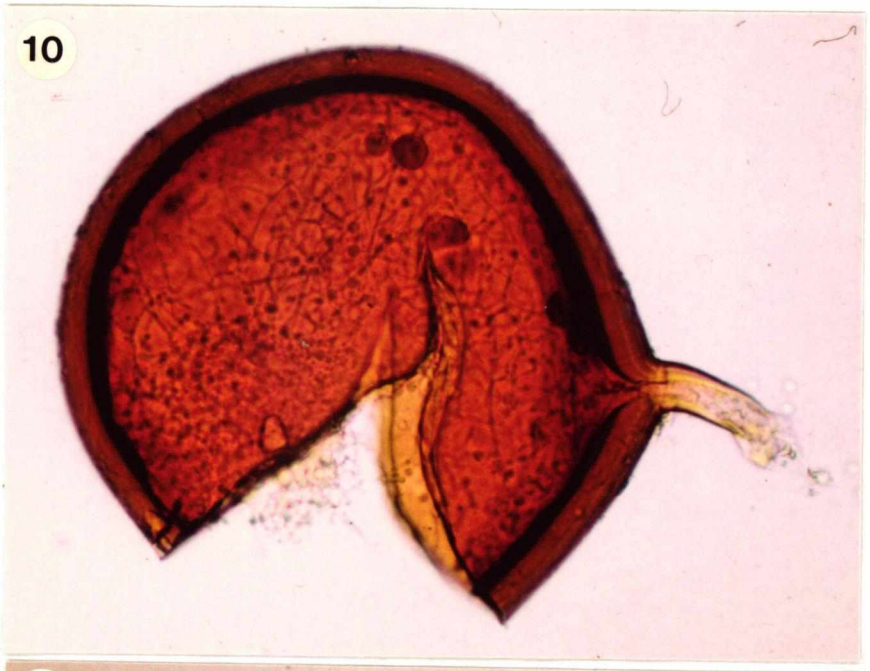
Original field spores used in the establishment of single-spore pot cultures were ectocarpic chlamydospores. They were honey-brown to red-brown, translucent, and globose to subglobose. The hyphal attachment was distinct in having thickened light brown pigmented walls (Photo 10). The wall was 6-15μm thick, with distinct laminations and possibly bilayered. The spore contents are closed off fom the attachment by an extension of the inner wall forming a curved septum (Photo 13, arrowed). Hyphal attachments are straight or recurved (Photos 14 and 15) and simple to slightly funnel-shaped at the spore wall.

Only ectocarpic spores were formed in pot cultures and these showed similar characteristics to the field spores i.e. a wall thickness of 6-12μm and hyphal attachments up to 150μm in length (Photo 14). The presence of a thin hyaline

Photo 10 Spore of G. geosporum isolated from field. Note single laminate wall and thickened wall of hyphal attachment. Spore has been parasitized by another fungus, (x 280, BF).

Photo 11 Spore of G. monosporum isolated from field showing relatively thick outer hyaline wall, thinner pigmented inner wall and hyphal attachment (x 350, BF).

Photo 12 Spore of G. mosseae isolated from field. Note long hyphal attachment with septum (arrowed) and single pigmented wall (x 300, BF).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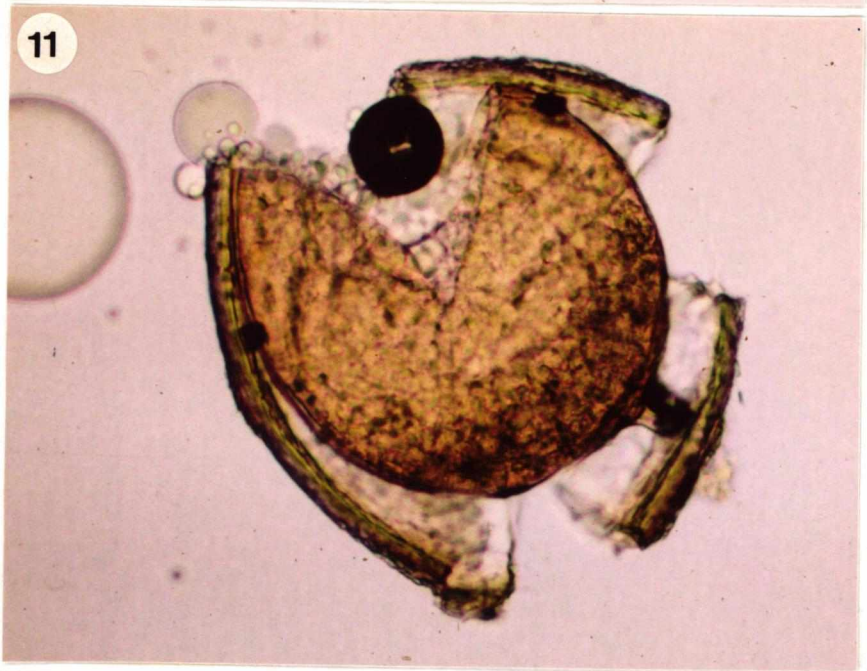
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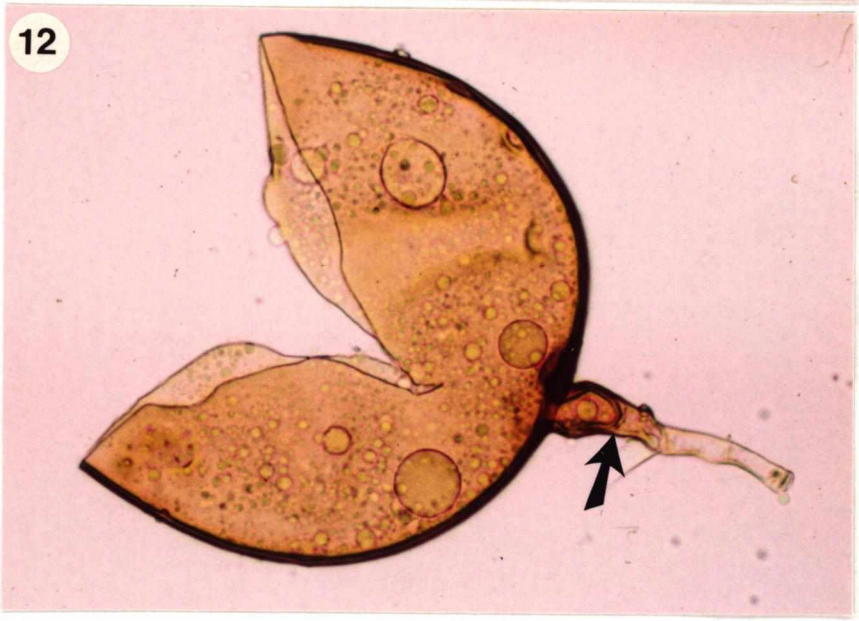

12

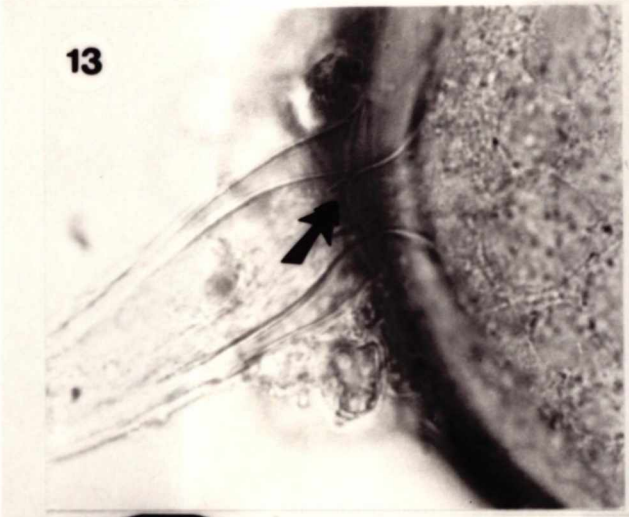
Photo 13 Hyphal attachment of pot-cultured ectocarpic spore of G. geosporum showing curved extension (arrowed) of inner wall to form a septum (x 1180, BF).

Photo 14 Pot-cultured ectocarpic spores of G. geosporum showing simple straight hyphal attachment (x 150, BF).

Photo 15 Section through hyphal attachment of a field spore of G. geosporum showing septum (arrowed) and recurved attachment (x 3000, SEM)

Photo 16 Surface texture of spore of G. geosporum isolated from field. (x 1000, SEM)

Photo 17 Spore of G. geosporum isolated from soil showing radial fissures through wall (arrows) and invasive fungus (x 840, BF)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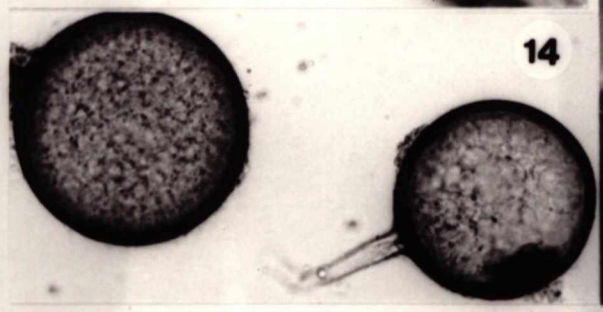
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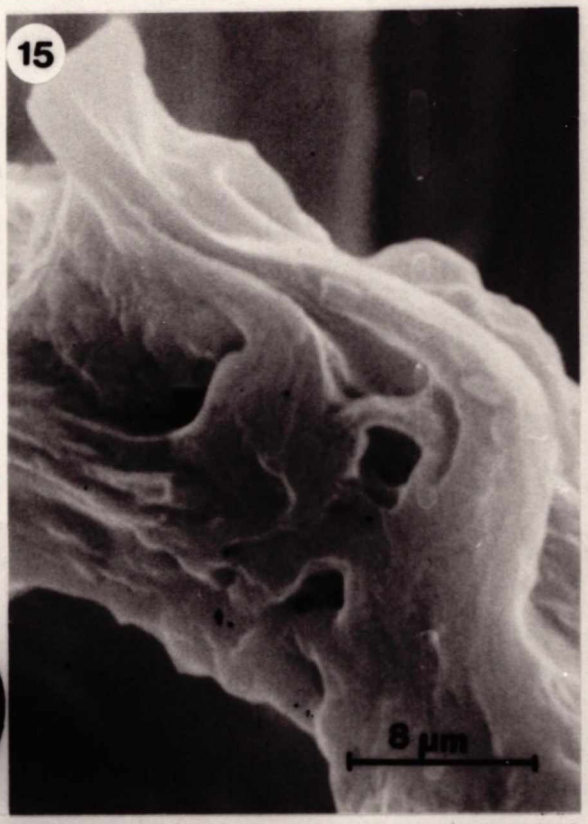
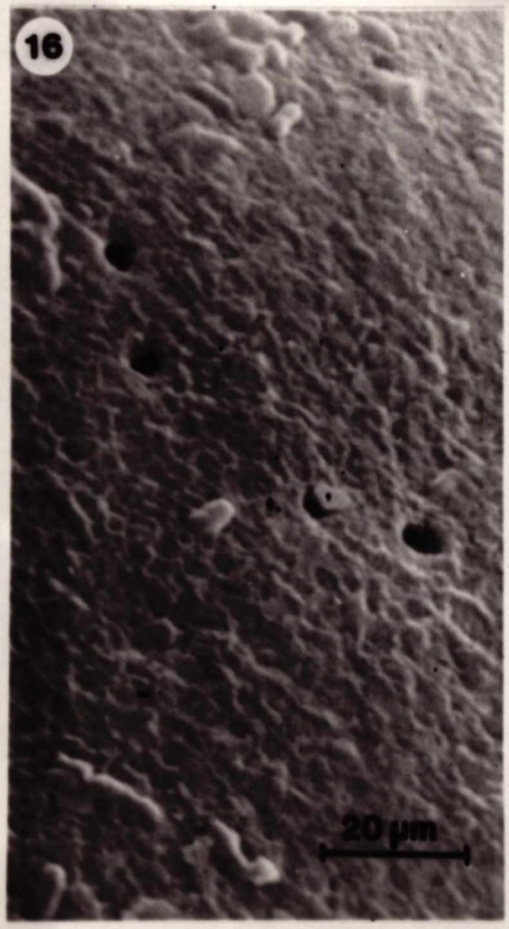

15
8 µm

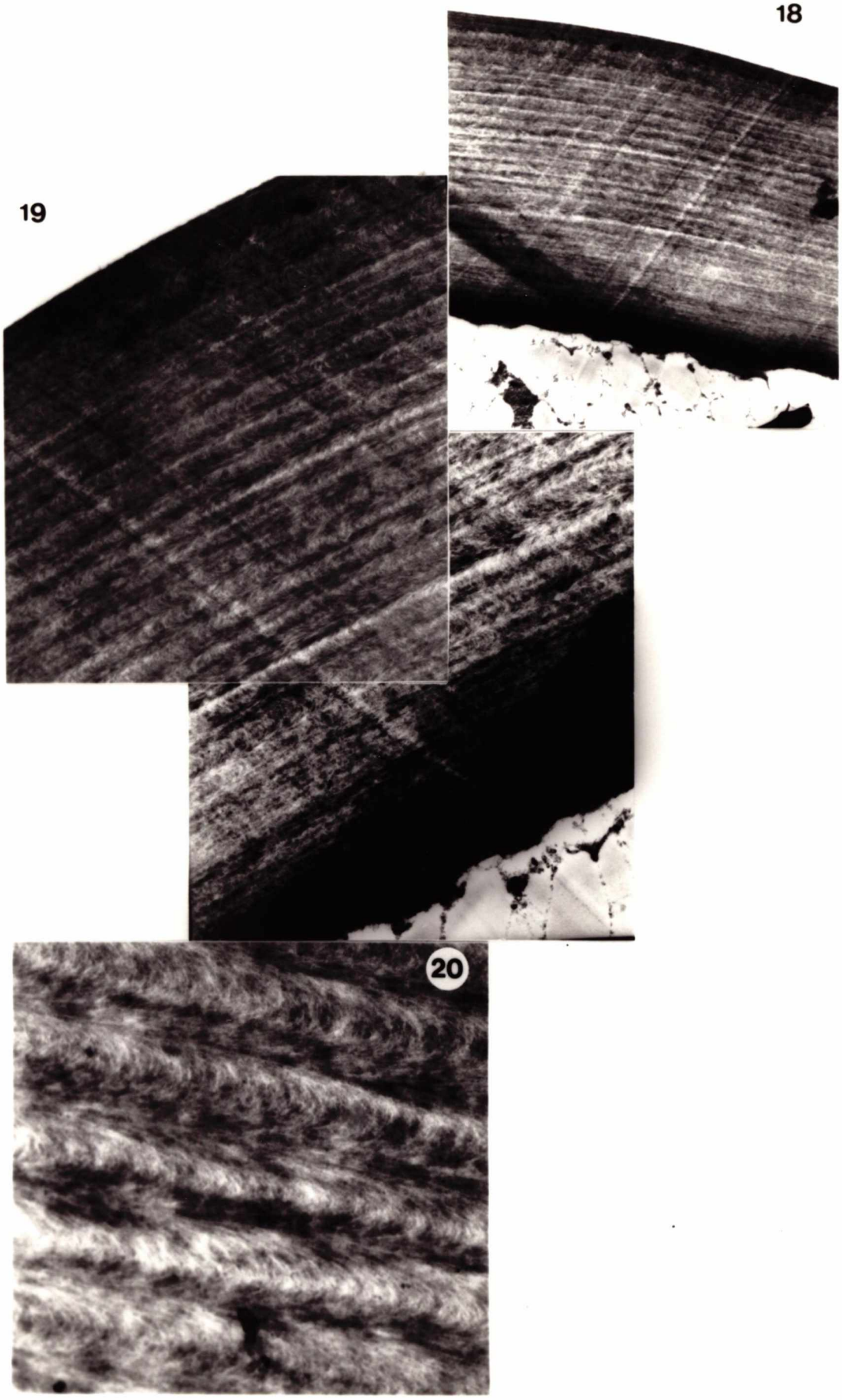

16
20 µm


17

Photo 18 Transverse section of wall of pot-cultured ectocarpic spore of G. geosporum showing laminations. Note innermost electron-dense layer (x 4300, TEM)

Photo 19 Transverse section of pot-cultured ectocarpic spore of G. geosporum showing layers of arcs of microfibrils and the inner electron-dense layer (x 9100,TEM)

Photo 20 Transverse section of arced-microfibrils and their bow-shaped orientation (x 29000,TEM)


18
19
20

outer wall as described by Walker, (1982) for this species (<1μm) could not be reliably detected. Older spores often split near the middle of the laminate wall when gently pressed under a coverslip, with the outer portion tending to 'slough', particularly in field spores. Field spores often contained invasive fungal structures (e.g. Photo 16). One such fungus, which occurred occasionally, is similar in appearence to *Anguillospora pseudolongissima*, also noted to have been a frequent hyperparasite of *G. fasciculatum* and *G. epigaeum* (Daniels and Menge, 1980).

SEM studies of the outer surface of field spores (Photo 17) revealed a slightly roughened texture but no other distinctive features such as 'overlapping plates'as noted by Tewari et al., (1982) for the closely related and morphologically similar *G. macrocarpum*.

In thin section (Photos 18-20), the wall of *G. geosporum* possessed a distinctly laminate wall with many layers (>20) of fibrillar subunits arranged regularly in 'arcs' of microfibrils and appearing similar to those described for *G. epigaeum* (Bonfante-Fasola and Vian, 1984) and *Acaulospora laevis* (Mosse, 1970). These bow-shaped structures (Photo 20) displayed a gradually decreasing radius of curvature from the outer to the inner wall, which may explain the apparent bilayering effect observed with the light microscope. The arc-like sub-structure of the outer wall gradually changed towards the innermost wall layer giving a disorganised appearence to the fibrils. The angle of arcing, however, changes with the angle of tilt of the specimen holder and thus is dependent on their relative orientation to the electron beam rather than being an inherent characteristic. The region of the wall adjacent to the protoplast stained darker and appears to consist of randomnly orientated microfibrils in a dark matrix. This has also been observed for *G. epigaeum* (Bonfante-Fasola and Vian, 1984).

(b) *G. monosporum*

The original field spores used in the establishment of single-spore pot cultures were ectocarpic chlamydospores. They were cream coloured with a green tinge, almost opaque, and globose to ovoid. The hyphal attachment was difficult to discern and slightly flared (Photo 11). Total wall thickness was 4-10um, composed of an inner pigmented layer (2-5μm) and a persistent hyaline outer layer (2-5μm). This outer layer had three distinct refractively different zones (Photo 21).

In pot cultured material two spore types were observed, ectocarpic and sporocarpic, the latter being the larger spore type (Table 4.1). Ectocarpic spores were smaller than those found in the field. There was a marked change in spore colour from field spore to pot cultured spore. The pot cultured spores becoming distinctly honey-brown, and shiny. The spore contents are separated from the subtending hyphae by a curved septum. Subtending hyphae are usually strongly recurved and appressed to the spore wall (Photo 22). During spore maturation a thick and more persistent hyaline outer wall develops (Photo 23; c.f. *G. mosseae*). The presence of a 'reticulate ridged' surface texture is observed in young spores (Photo 23) which seemed to correspond with the outer surface of the inner pigmented layer when observed by light microscopy. The outer hyaline wall of sporocarpic spores is very persistent, possibly due to the protection afforded it by the tightly interwoven hyphal peridium (Photos 24 and 28). Sporocarps may contain 1-3 spores (c.f. *G. mosseae*).

SEM studies confirm these observations. Photos 25 and 26 show the surface appearence of a pot culture spore and a field spore, respectively. Photo 26 shows the outer hyaline wall with smooth and soil 'weathered' regions. Photo 27 shows a separated outer wall and the 'reticulate ridges' below on the surface of the outer surface of the pigmented wall.

Table 4.1 Dimensions of spores of three VAM endophytes isolated from field samples and pure pot cultures

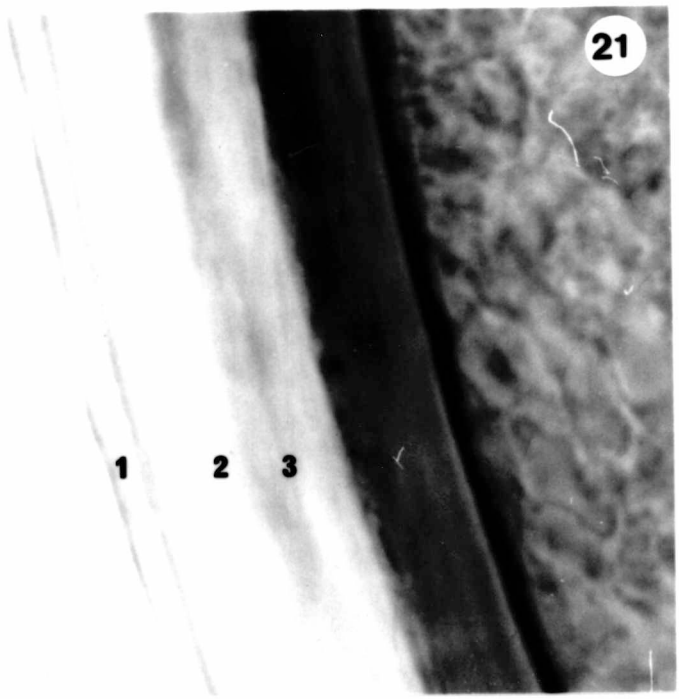
ENDOPHYTE	ORIGIN OF SPORE AND SIZE RANGE (um)		
	FIELD SPORE	ECTOCARPIC POT-CULTURE SPORE	SPOROCARPIC SPORE POT-CULTURE
G.geosporum	(160-)230(-290)	(136-)210(-264)	------
G.monosporum	(180-)250(-300)	(108-)130(-190)	(152-)204(-230)
G.mosseae	(160-)205(-304)	(98-)125(-176)	(144-)195(-225)

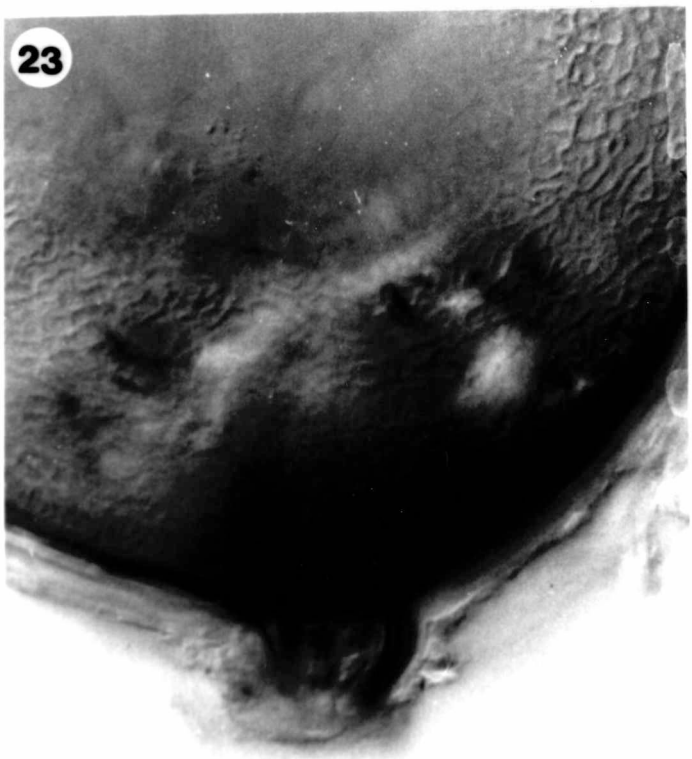
Photo 21 Wall structure of field spore of G. monosporum. Note tri-layered appearance of outer hyaline wall (numbered) due to different refractive properties (x 2500, NIC)

Photo 22 Pot-cultured ectocarpic spore of G. monosporum, showing recurved hyphal attachment (x 500, NIC)

Photo 23 Young pot-cultured ectocarpic spore of G. monosporum with thickened outer hyaline wall extending down along hyphal attachment. Note also 'reticulate-ridges' of spore surface beneath hyaline layer (x 760, NIC).

Photo 24 Pot-cultured sporocarp of G. monosporum containing two spores inside a tightly interwoven hyphal peridium (x 190, BF)





24

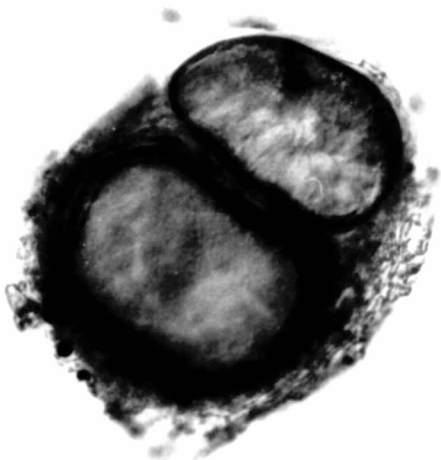
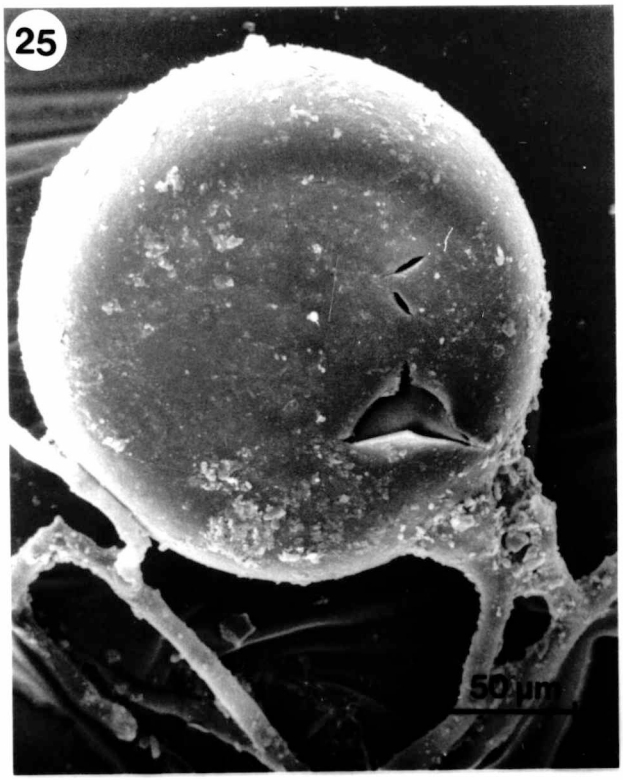


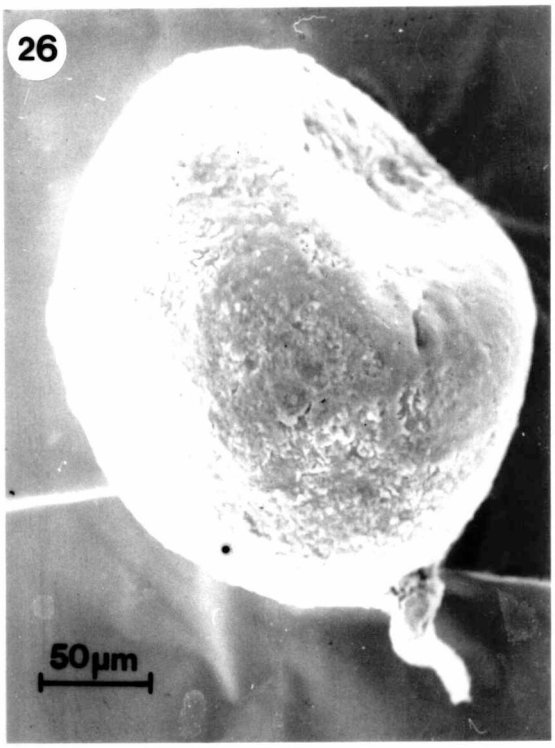
Photo 25 Pot-cultured ectocarpic spore of G. monosporum showing a split in a hyaline outer wall and revealing the inner layer. Note the flared hyphal attachment (x 350, SEM)

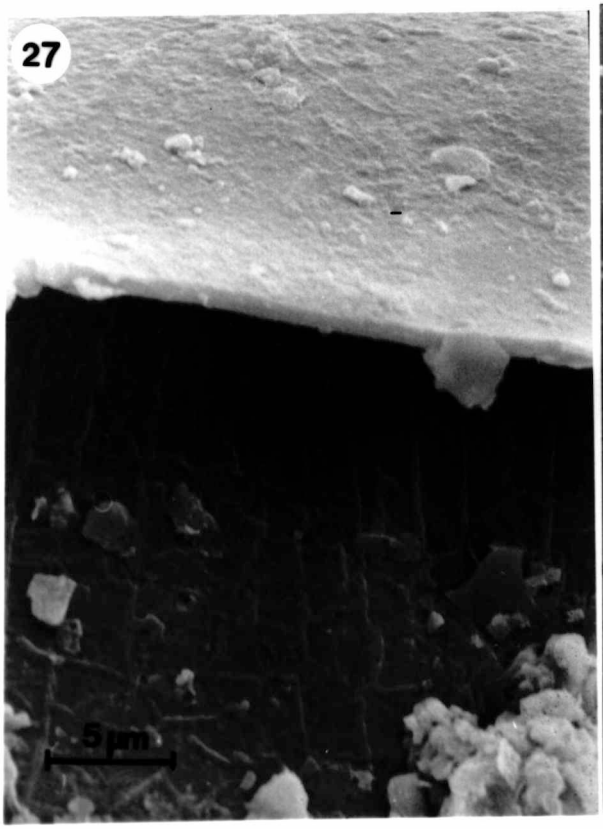
Photo 26 Spore of G. monosporum isolated from field showing short hyphal attachment and zones of rough and smooth hyaline outer wall surface (x 300, SEM)

Photo 27 Outer wall of pot-cultured ectocarpic spore of G. monosporum showing edge of split wall and 'reticulate ridging' on the outer surface of the pigmented wall (x 3500, SEM)

Photo 28 Pot-cultured sporocarp of G. monosporum. Note the tightly interwoven thin hyphae enveloping the spore (x 200, SEM)


25
50µm


26
50µm


27
5µm

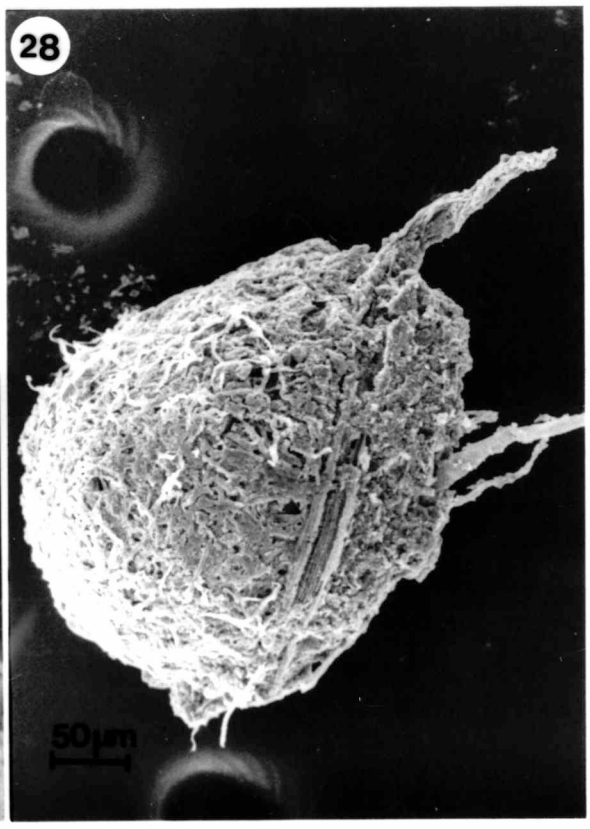
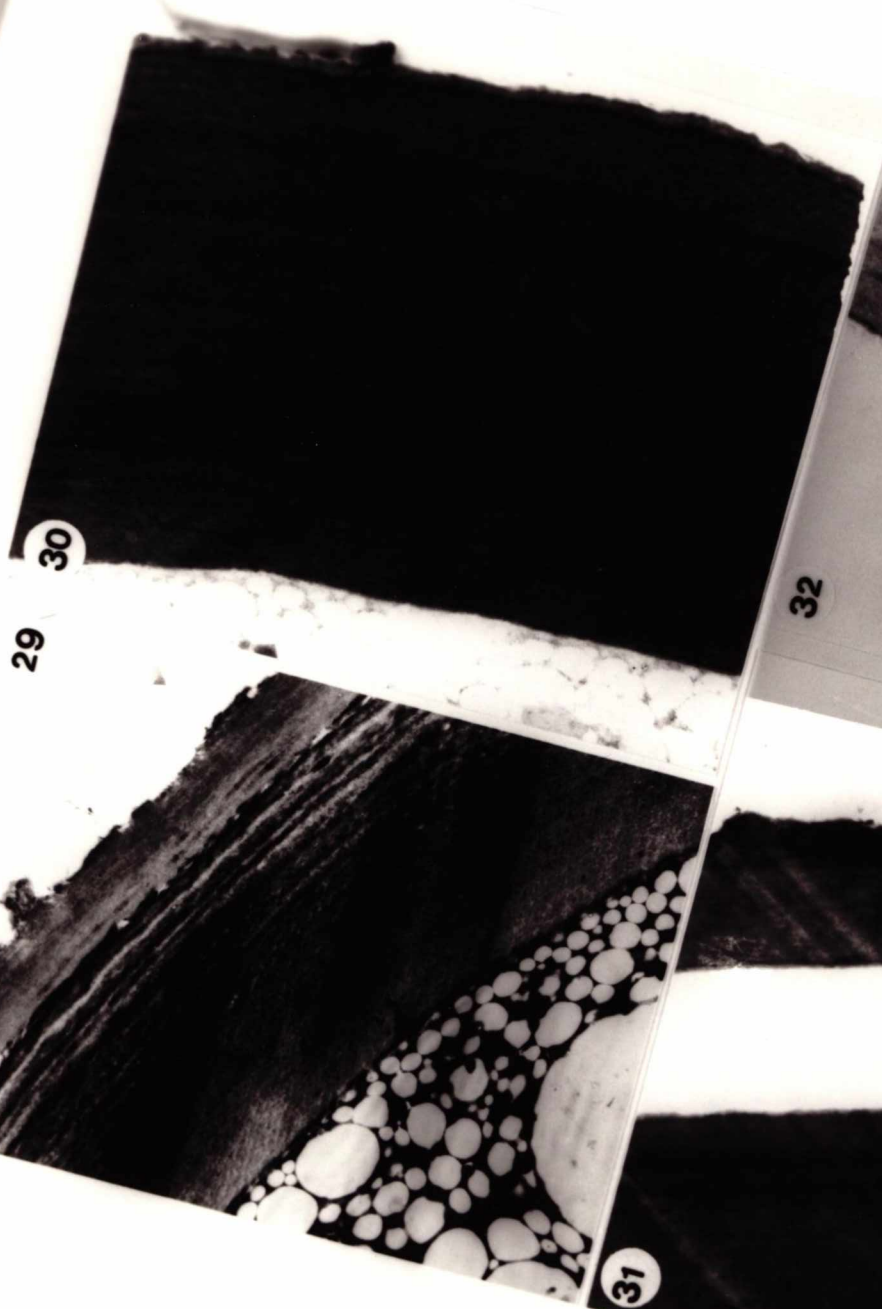

28
50µm

Photo 29 Transverse section of ectocarpic pot-cultured spore of G. monosporum showing thick pigmented inner wall and a thinner, more electron-lucent outer wall (arrow) with a roughened surface (x 6300, TEM)

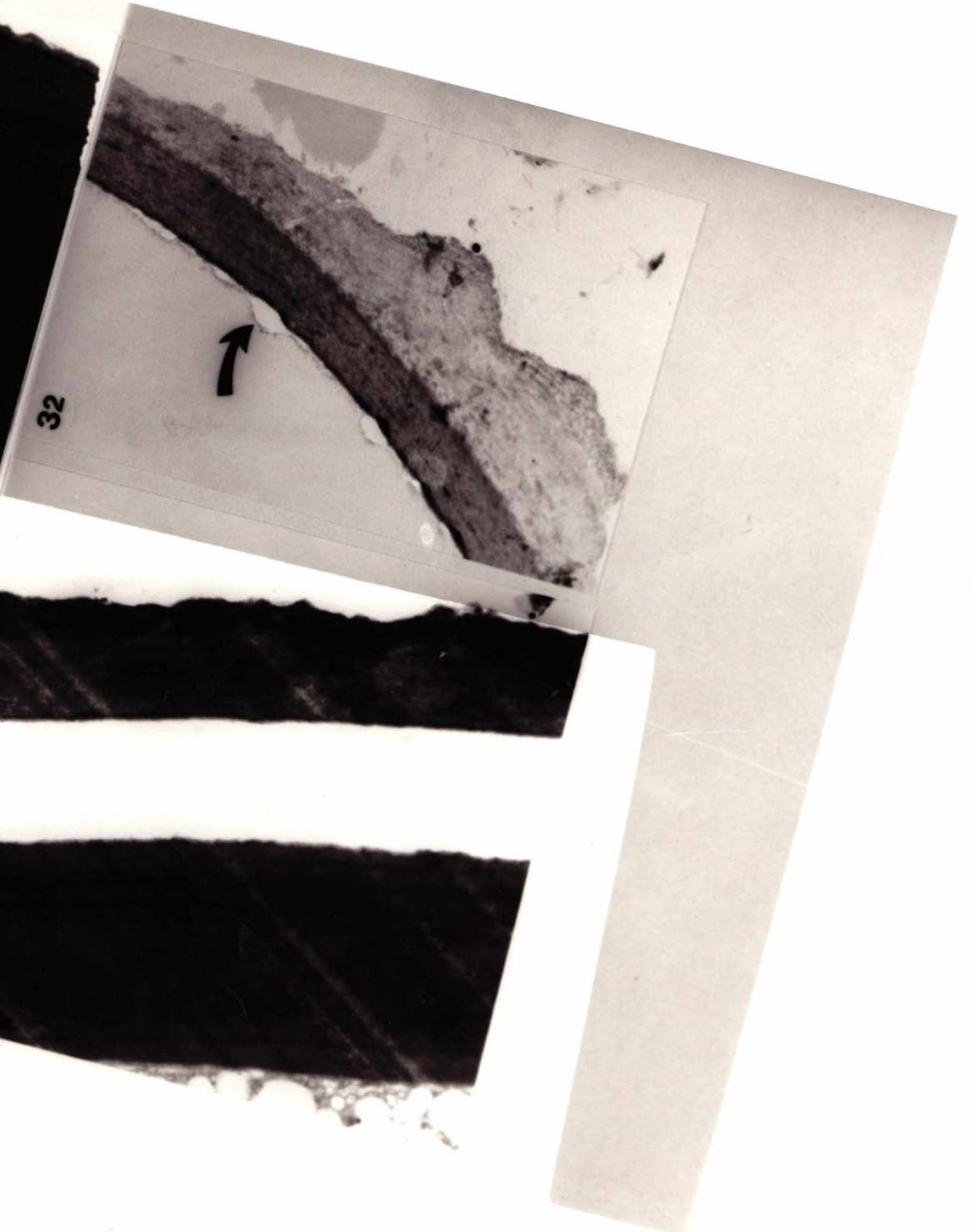
Photo 30 Transverse section of inner wall of pot-cultured ectocarpic spore of G. monosporum. Wall laminations are evident. (x 19500, TEM)

Photo 31 Transverse section of separated walls of pot-cultured ectocarpic spore of G. monosporum with faint laminations in the inner pigmented wall (x 10400, TEM)

Photo 32 Transverse section of outer wall of pot-cultured ectocarpic spore of G. monosporum showing two zones of different electron-density and appearance. Note thin layer (arrow) on the inner surface of the wall. (x 13900, TEM)


29
30

31


32

TEM of thin sections of spores reveal the structures of inner and outer walls (Photos 29 and 30). The inner pigmented wall is shown as a more electron-dense, homogenous wall with faint laminations (Photo 31). Unlike *G. geosporum*, however, there does not appear to be a dark staining zone near the protoplast. There are no clear indications of an arc-like sub-structure to the fibrillar wall but sufficient spores have not been sectioned to confirm their presence or absence. They are certainly less distinct than those of *G. geosporum* but a detailed study is necessary to discover their exact nature. The outer wall appears more irregular and electron-lucent with two distinct zones (Photo 32), the outer zone having been apparently degraded in places. There appears to be a dark staining thin layer Between the inner and outer walls (arrowed Photo 32) which may be equivalent to the 'reticulate-ridged' layer observed with the light microscope and the SEM.

(c) *G. mosseae*

The original field spores used in the establishment of single-spore pot cultures were ectocarpic chlamydospores. They were cream, translucent, globose to ovoid with a flared hyphal attachment (Photo 12) up to 150μm in length. Only a single pigmented wall could be discerned (2)-3-(4)μm in diam.

In pot culture three spore types were observed: ectocarpic, endocarpic and sporocarpic, the latter being the largest spore type. Ectocarpic spores were smaller than those found in the field (Table 4.1). As was observed for *G. monosporum*, spore colour of pot-cultured spores had changed to a honey-brown colour. The spore contents are divided from the subtending hypha by a curved septum which extends for up to 70μm down the attachment (arrowed Photo 33; see Dodd and Krikun, 1984). Examination of spores in various stages of maturation in pot culture revealed the initial appearence of a relatively thick

Photo 33 Pot-cultured ectocarpic spore of G. mosseae showing relatively long hyphal attachment with septum (x 420, BF)

Photo 34 Young chlamydospore of G. mosseae showing early hyaline wall (x 1050, NIC)

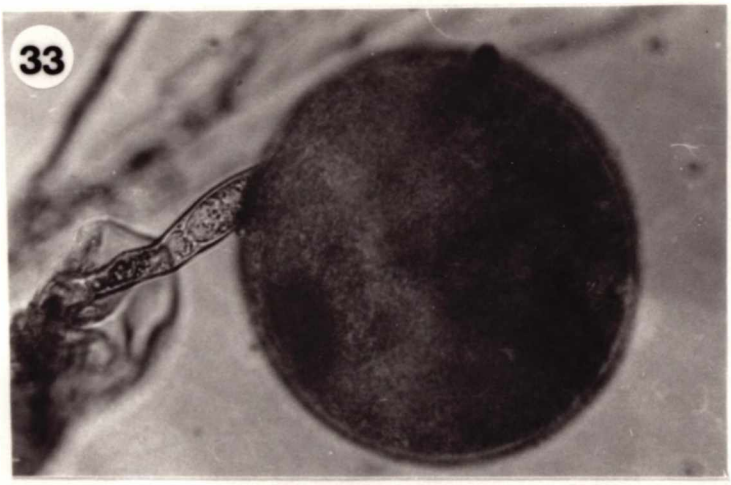
Photo 35 Maturing pot-cultured ectocarpic spore of G. mosseae showing bi-layered structure, inner pigmented wall and outer hyaline wall (x 1260, NIC)

Photo 36 Pot-cultured sporocarp of G.mosseae showing split in outer hyaline wall. Note peridium of thick loosely interwoven hyphae in contrast to the sporocarp of G. monosporum (x 350, SEM)

Photo 37 Optical section of spore of G. mosseae from field, showing 'lignituber-like ingrowths' through the wall caused by parasitic attack (x 500, BF)

Photo 38 'Cracks' which appear to traverse the wall of a pot-cultured ectocarpic spore of G. mosseae. This artifact is caused by fragmentation of the inner interstitial layer between the outer hyaline wall and the inner pigmented wall (x 1680, BF)

Photo 39 Field spore of G. mosseae showing 'reticulate-ridging' on the surface of the spore (x 350, SEM)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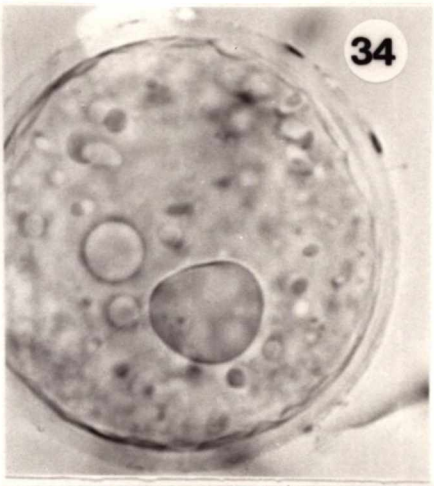
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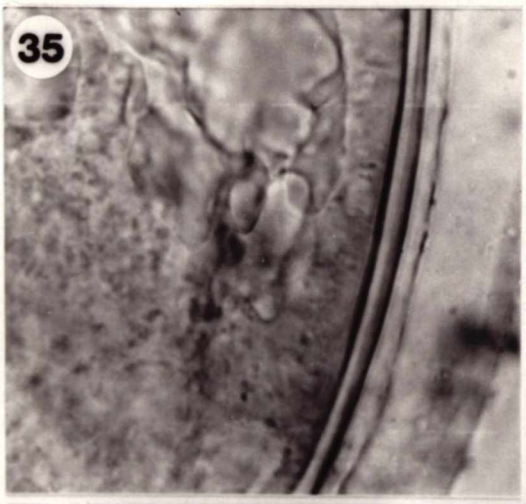
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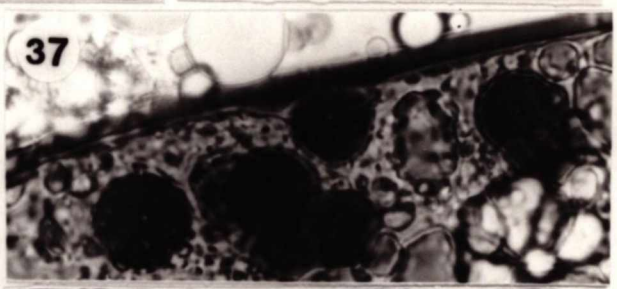
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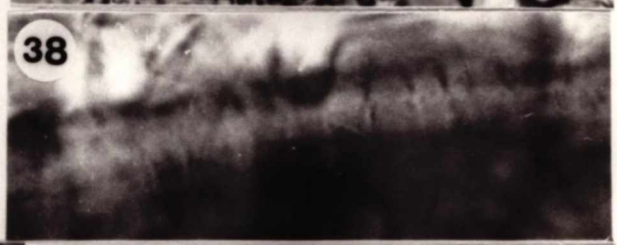
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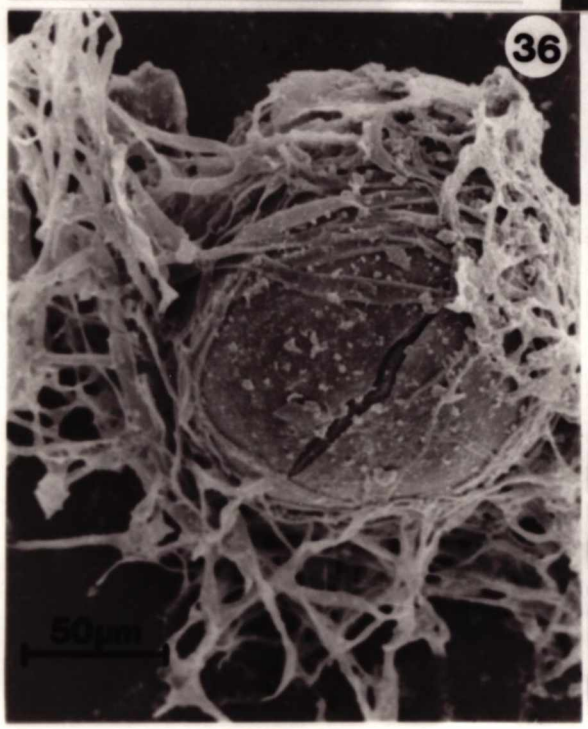

36
50µm

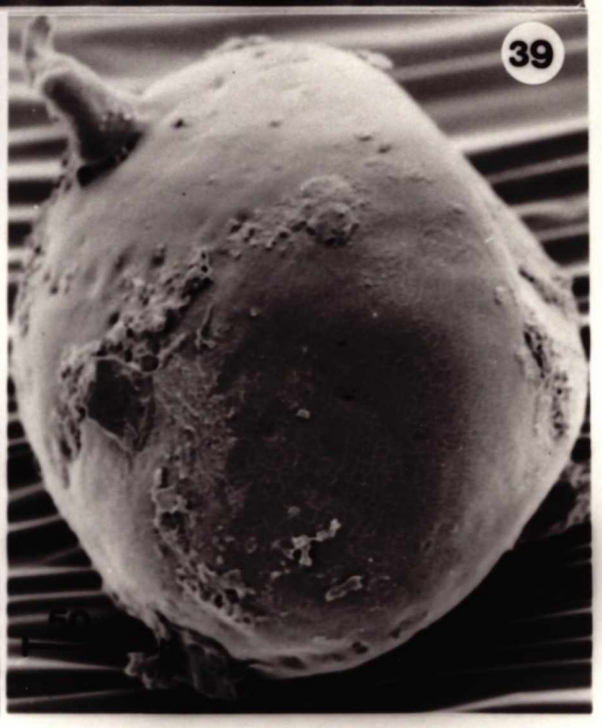

39

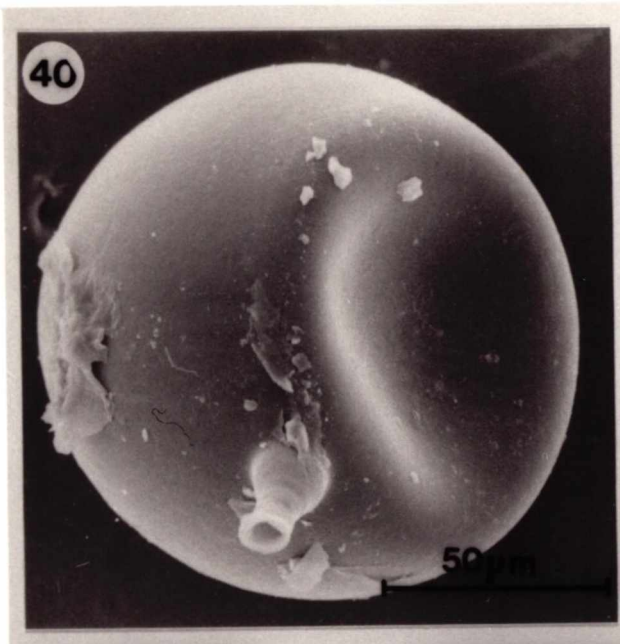
Photo 40 Surface of pot-cultured ectocarpic spore of G. mosseae lacking an outer hyaline wall (x 600, SEM)

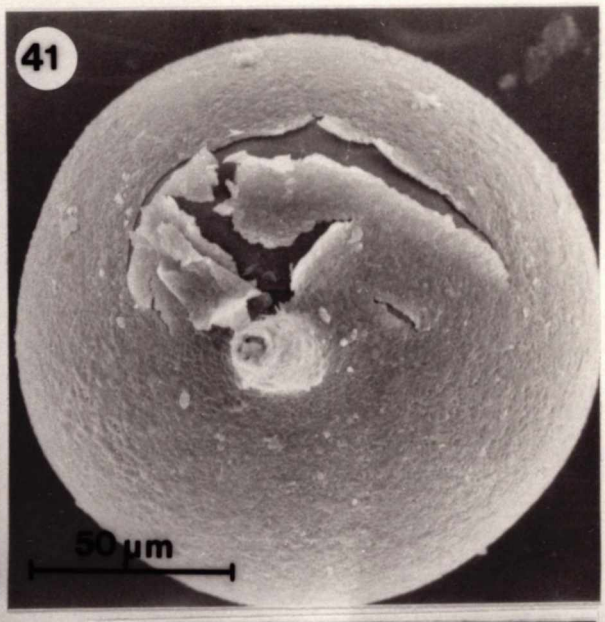
Photo 41 Surface of pot-cultured ectocarpic spore of G. mosseae with split hyaline outer wall (x 550, SEM)

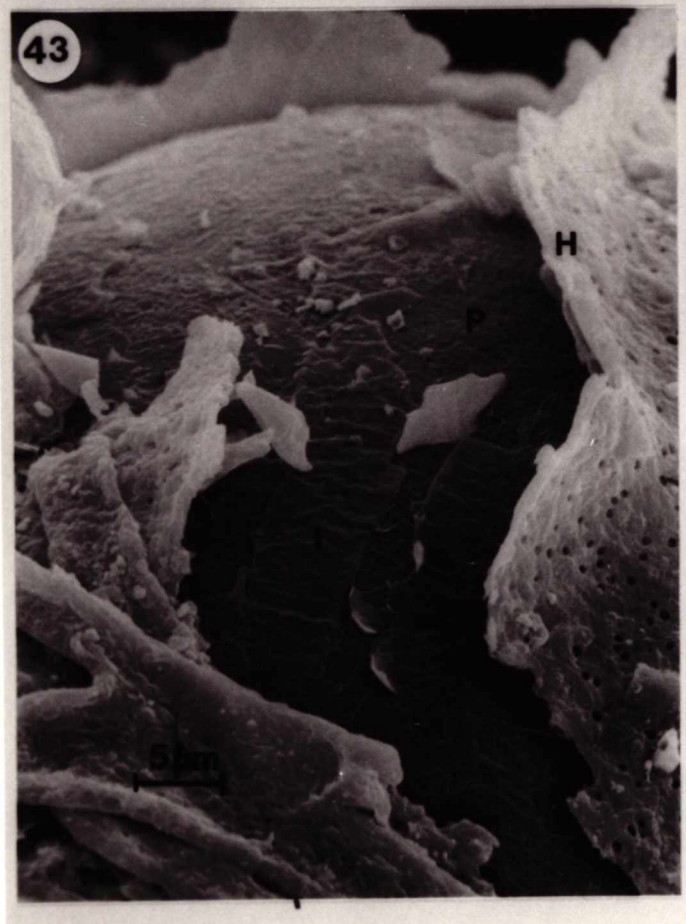
Photo 42 Surface of the hyaline outer wall of a pot-cultured ectocarpic spore of G. mosseae showing rough texture (x 10000, SEM)

Photo 43 Surface of pot-cultured sporocarpic spore of G. mosseae showing the outer hyaline wall (H) split revealing the thin inner 'reticulate-ridged' interstitial layer (I) overlaying the outer surface of the pigmented (P) wall (x 2100, SEM)

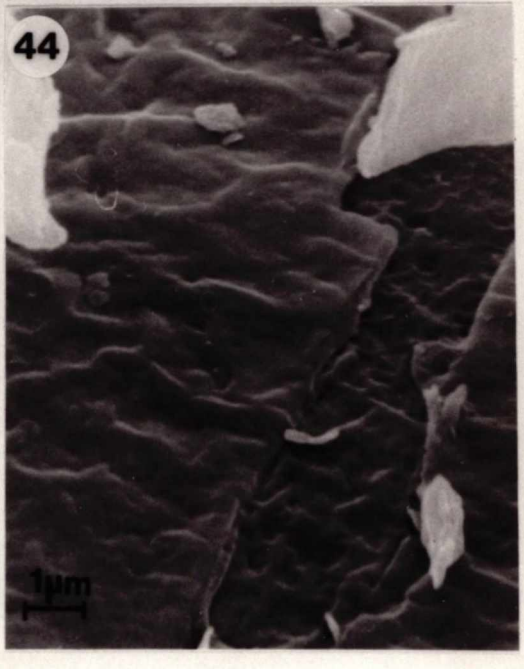
Photo 44 Higher magnification of a region from Photo 43 showing the reticulated area (x 8000, SEM)


40
50 µm


41
50 µm


43
H
P
5 µm


42


44
1µm

(1-2μm) outer hyaline layer with a faint inner pigmented wall developing inside this (Photo 34). The hyaline wall then tends to become evanescent as the inner wall develops (Photo 35) and when the spore is mature it may be absent altogether (this absence is not so frequently observed with ectocarpic pot culture spores of *G. monosporum*). Sporocarpic spores tend to retain their outer wall more consistently up to maturity as was the case in *G. monosporum*. The peridium of *G. mosseae* sporocarps consists of loosely interwoven thick walled hyphae (in contrast to the tightly interwoven thin walled hyphal peridium of *G. monosporum*) (Photo 36). Sporocarps may contain from 1-6 spores. Field spores frequently have 'lignituber-like ingrowths', presumably synthesised during parasitic attack. These appear as dark brown and give the field spores a spotted appearence under the low power of the dissecting microscope (Photo 37). Using Nomarski interference microscopy, ectocarpic spores from pot culture are sometimes observed to have a very thin outer hyaline wall which fragments under slight pressure of a coverslip. This can occasionally lead to a 'cracked' appearence as if fissures are present through the pigmented wall (Photo 38). The cracks are, however, an artifact and may lead to misidentification of species e.g. *G. monosporum*, and are equivalent to the 'reticulate ridged' layer observed clearly under SEM of *G. monosporum* spores (see previous section).

Examination of spores using the SEM reveals evidence to substantiate the presence of an interstitial layer between outer and inner walls. Photos 39-41 show ectocarpic spores with and without the hyaline outer walls. Photo 39 is a field spore and note the area of 'reticulate-ridges' on the spore surface. The outer surface of the hyaline wall does not have any distinct reticulation (Photo 42). Photo 43, however, clearly shows the presence of a thin layer between outer and inner wall surfaces and it appears to be peeling away in certain areas.

Figure 35 shows a close-up of this layer overlaying the outer surface of the inner pigmented wall. It appears that this reticulate layer fragments as the spore matures and especially where the outer hyaline wall is weathered away. *G. mosseae* spores seem to have a more evanescent outer wall than *G. monosporum* spores in pot culture.

(d) *Acaulospora* spp.

These spores appeared in spore sievings at irregular intervals through the study period (see Figs. 4.1 and 4.2), in all four fields. Two distinct types were observed amongst the few spores available from the Hamill site. Photos 45-47 show details of *Acaulospora* sp. 1 from Hamill pasture. Photo 45 shows the diagnostic collapsed mother vesicle (arrowed) still attached to the red azygospore. The two structures are held together by the occluded pore (arrowed Photo 47). The wall consisted of a hyaline outer layer overlying an inseparable inner pigmented wall. Note the inner membranous layer, a further characteristic of *Acaulospora* and *Gigaspora* spp. (arrowed Photo 46). This spore type most closely resembles *Acaulospora laevis*.

Another spore type which appeared sporadically in the Hamill avalon field was *Acaulospora* sp. 2. This was honey coloured, but its outer hyaline wall was ornamented and there were 2-3 membranous walls situated on the inside of the pigmented wall (arrowed Photo 48). SEM observations showed the outer surface to be ornamented with short protuberances (Photos 49 and 50). This spore most closely resembles *Acaulospora spinosa* (Walker and Trappe, 1981).

All attempts to pot-culture both spore types using single and multiple spore inoculations failed. Other *Acaulospora* spp. were observed at the Sussex field site but spores were too few to investigate morphology in detail and attempts at initiating pot cultures were unsuccessful.

Photo 45 Azygospore of Acaulospora sp. from Hamill Pasture with attached collapsed mother vesicle (x 180, BF)

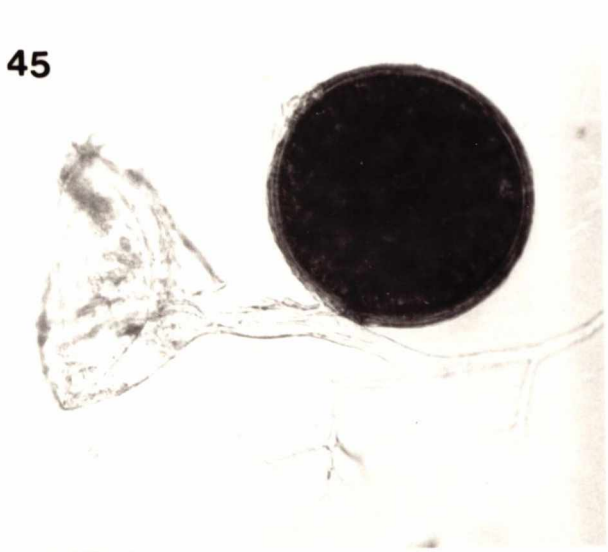
Photo 46 Wall structure of Acaulospora azygospore showing a thin outer hyaline wall (H), an inner red pigmented wall (P) and a detached inner membranous (M) wall (x 1470, BF)

Photo 47 Details of the occluded pore attachment formed between the mother vesicle and azygospore (x 1250, SEM)

Photo 48 Wall structure of honey-coloured Acaulospora spore from Hamill Avalon field, showing the complexity of the wall. The arrows indicate the inner membranous walls (x 1090, BF)

Photo 49 Surface morphology of honey-coloured Acaulospora spore showing the presence of short 'spines' (x 500, SEM)

Photo 50 Close-up view of spines on surface (x 2000, SEM)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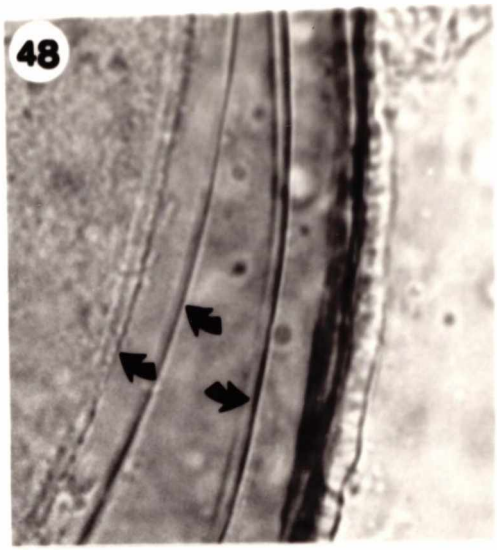
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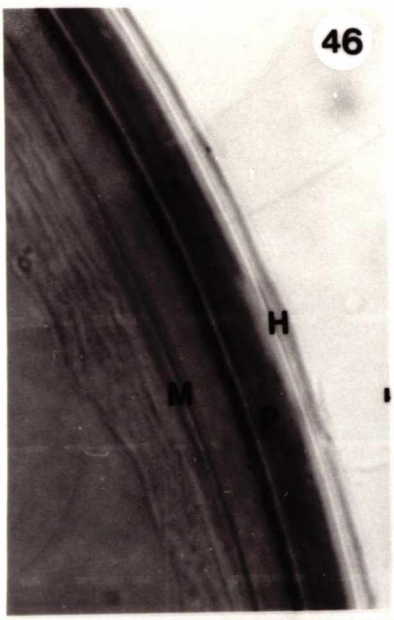
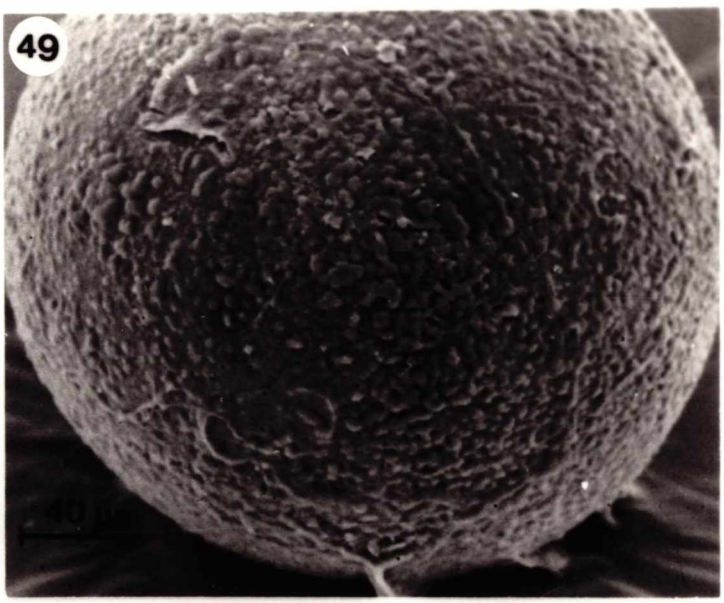
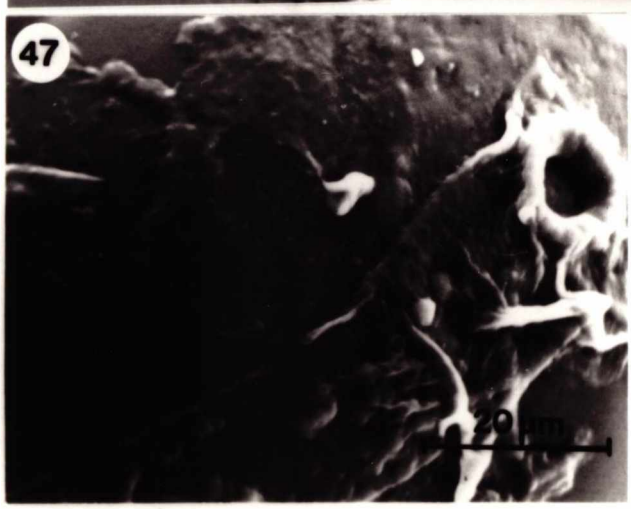
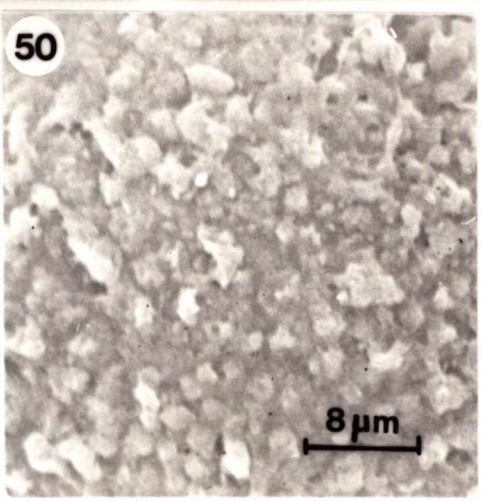

46
H
M


49
40 µm


47
20 µm


50
8 µm

(e) Fine Endophytes (*G. tenue*)

The presence of these has only been detected after staining of field root material at each field site (see chapter 3). Photo 51 shows typical internal root mycelium with 'attached vesicles' (Hall, 1984). These are likely to be small appressoria aiding spread of infection from cell to cell, and from this point of view are not 'vesicles'. This view is supported by work done by Trinick *et al.*, (1983) on the spread of fine endophytes within roots. Photo 52 shows another structure occasionally seen within stained roots. Such fan-like structures have been called 'anomalous' hyphae by Hall, (1984).

(f) Other Species

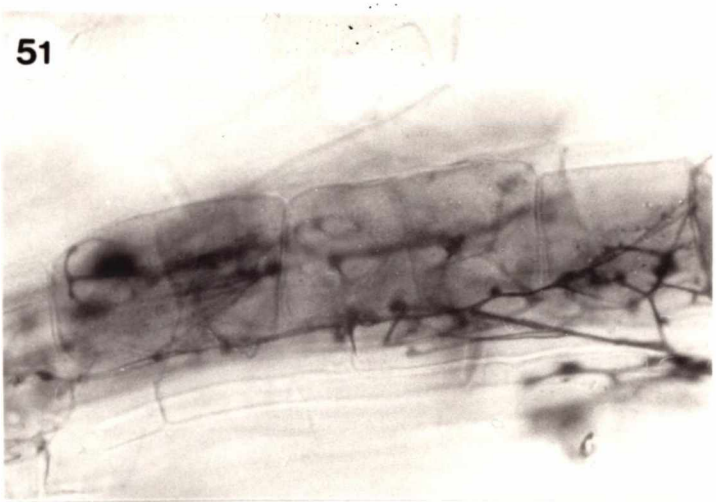
Spores observed in field sievings, from all sites, have included empty spores of a type resembling either *G. macrocarpum* or *G. fasciculatum*. It has not been possible to identify these on the basis of available keys. *G. constrictum* has also been observed regularly, but failed to initiate pot cultures.

A common spore type which was found in large numbers in all fields, but especially in the disturbed sites, was a red-brown sporocarpic spore These spores, approx. 100μm in diam., have been seen in groups of 1-6. The spores are not covered by a peridium, but are attached to a yellow weft of coarse hyphae (Photo 53). They are commonly observed with radial fissures through their walls and have been seen to contain zoosporangia inside the spore. These spores consistently failed to initiate pot cultures despite their numbers and their relevance to the Endogonaceae is uncertain and needs further study.

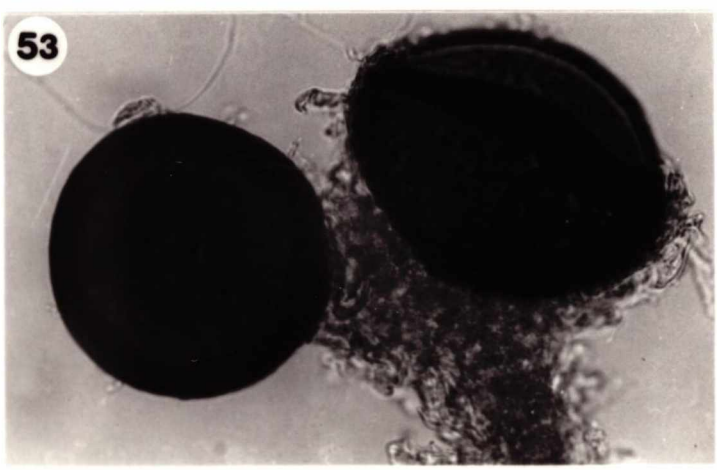
Photo 51 Fine endophyte infection in the roots of winter wheat from the Sussex field site. Note the presence of 'vesicles' or appressoria (x 500, BF)

Photo 52 Fan-like fine endophyte structure occasionally observed in root infections from the field (x 670, BF)

Photo 53 Dark red-brown sporocarpic spore type present in large numbers in the cultivated field sites. Note the thick walls and pigmented hyphal matrix (x 500, BF)


51


52


53

4.3.3. Electrophoretic Study

Photo 54 shows a photograph of the banding patterns obtained for esterase activity following electrophoresis of the spore contents of *G. geosporum*, *G. monosporum* and *G. mosseae*. Two banding types could be distinguished, black bands (due to hydrolysis of 1-naphthyl acetate) and red bands (from 2-naphthyl acetate; Sen and Hepper, 1986). Faint bands (1) could not be placed with certainty in either category. *G. geosporum* produced only one black band (4B), whilst *G. monosporum* and *G. mosseae* produced two common bands (3B and 5B). *G. mosseae*, however, could be distinguished from *G. monosporum* by the production of a red band (2R) which was the fastest to develop on the gel. An extra fainter band (1) could also be detected for *G. mosseae*. Densitometer scans of the gel confirmed the banding patterns observed and indicated relative intensities of bands (Fig. 4.3). The scan of the *G. monosporum* track indicated the vague presence of a band equivalent to (1), which was observed strongly for *G. mosseae*.

No bands of activity were obtained from alkaline phosphatase staining. One band was detected on gels stained for acid phosphatase activity but only for *G. mosseae* samples. This was white with a yellow background and ran at $E_f = 0.61$. It should be noted, however, that this result was inconsistent during six gel runs. A photographic record was not obtained since the band quickly disappeared even when fixed in 7% acetic acid.

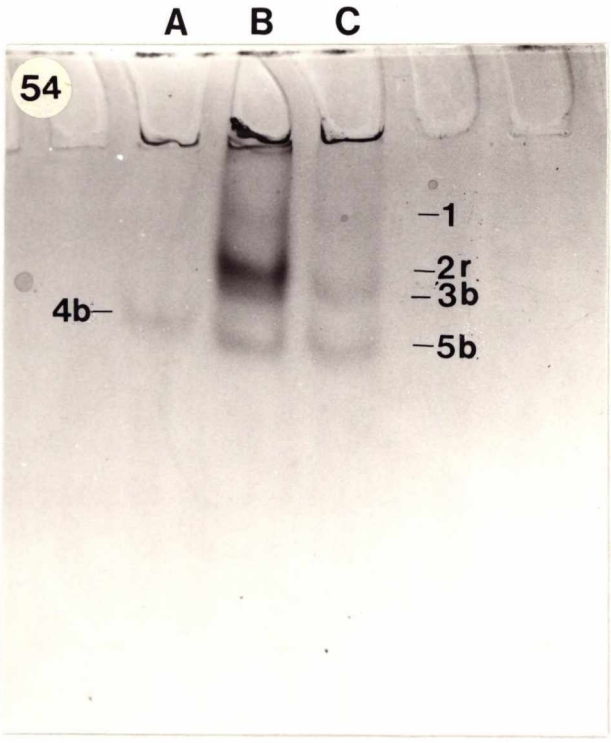


Photo 54 Banding patterns of esterase activity following polyacrylamide gel electrophoresis

A = G. geosporum
B = G. mosseae
C = G. monosporum
r = red band
b = black band

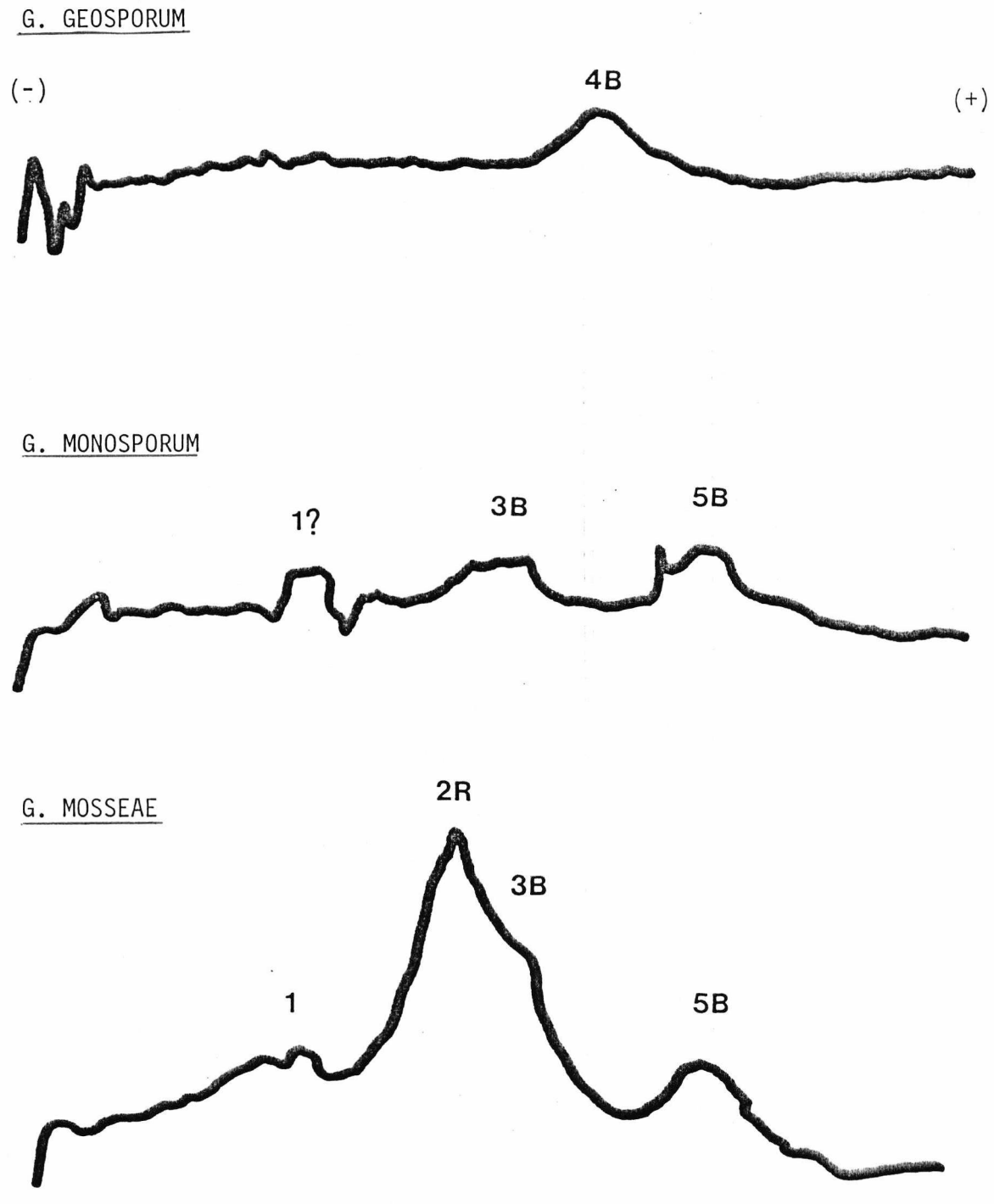


Figure 4.3 Densitometer scannings of esterase bands from three VAM endophytes

4.4. DISCUSSION

4.4.1. Spore Survey

Results of the spore survey revealed low numbers of apparently viable spores in cultivated fields compared to an adjacent undisturbed pasture. Allen and Boosalis (1983) also noted higher spore numbers in a grassland adjacent to disturbed fields growing wheat. Spore numbers decreased further with continued wheat production. Other work, however, has suggested that soils from perennial (undisturbed) ecosystems contain fewer VAM spores than do cultivated fields supporting annual crops, due to the potential for easier root to root infection in grasslands containing many species of the Gramineae (Hayman, 1982; Sward *et al.*, 1978). Previous ecological studies in New Zealand grasslands, scrub and forests have yielded spore densities in the range 6-1590 per 100g of fresh soil (Hayman, 1978). Sward *et al.*, (1978) found spore numbers in similar quantities to those observed in this study, as did Hayman (1970) who followed spore numbers through a winter wheat season and found 58-189 per 50g of soil. Hayman (1970), however, found that spore numbers increased in the summer months and were much lower in the winter period. The extremely high spore numbers (72-180 g^{-1} soil) quoted in the literature, for natural ecosystems, (Read et al., 1976) compared with these lower values may be due to the inclusion of the many dead empty spores present in sievings in counts. I have only counted apparently viable spores (cytoplasm visible). I have also omitted the red-brown spore type from counts since most of these spores appeared empty and parasitised. The inclusion of these spores in the final spore totals would have dramatically increased the levels reported in the cultivated fields, probably above that of the pasture site. Read et al., (1976) also noted this spore type and its predominance in natural ecosystems in Eastern England, and this might help explain the figures of 72-180g^{-1} obtained. These spores have not

been shown to form mycorrhizas, at least in this study, so their relevance to mycorrhizal ecology is questionable. It should also be noted that the smallest sieve mesh size used when wet sieving spores was 106μm , and therefore small-spored species may have been overlooked.

Changes in spore numbers did occur through the study of the Hamill pasture site, and to a lesser degree at the Sussex sites. The peaks in spore densities, occasionally observed, appear to be coincident with or immediately following rainfall peaks for that area (Figs. 4.1 and 4.2). Sward *et al.*, (1978) also noted this correlation in three disturbed Australian heathland sites. Walker et al., (1982) revealed a statistically significant correlation between spore numbers and mean gravimetric soil moisture content at their two field sites in a two year survey. It has also been inferred that soil water status plays a role in sporulation of VAM spores in other arid and tropical climates (Dodd and Krikun, 1984; Giovanotti, 1985; Redhead, 1977). Reports from the literature (Hayman, 1970; Roldan-Fajardo et al., 1982; Walker et al., 1982) have stated that there is a general trend for spore populations to be least in spring and greatest in early autumn. These trends may be related to root senescence. The results presented here do not follow this trend and again underlines the difficulties in predicting VAM populations in soil. Factors affecting distribution include soil fertility, soil moisture, soil depth, soil disturbance, plant susceptibility.

Examination of levels of individual VAM species sporulating through the study period showed different patterns. Whilst levels of *Glomus* spp. followed a similar trend to that of the overall spore population, the intermittent appearance of spores of *Acaulospora* and *Gigaspora* spp. (Figs. 4.1 and 4.2) deserves further study, since other workers have also noted differences in sporulation with time for their VAM species investigated (Walker et al., 1982). It is known that spores of *Acaulospora* spp. have a long constitutive dormancy period prior to quiescence

(Tommerup, 1983a). A quiescent spore fails to germinate unless physical and chemical conditions are favourable, and germination is only prevented because the environment is unsuitable i.e. exogenous dormancy. A constitutively dormant spore is one that fails to germinate although it is exposed to physical and chemical conditions that will support germination and hyphal growth of apparently identical, non-dormant spores of the same species. In the same experiments spores of *Glomus* spp. had short constitutive dormancy periods of 6 weeks in wet soil, which was reduced to one week in dry soil. Soil moisture had apparently no effect on the change to quiescence of spores of *Acaulospora* spp. Tommerup (1983) suggested that short dormancy periods, such as those of *Glomus* spp., would prevent the spores germinating immediately after formation thereby aiding the survival of inoculum in moist field soils. Tommerup, (1985) also reported that the failure of quiescent spores to germinate in moist soils was due to fungistasis. A long dormancy period, such as that of *Acaulospora* spp. would have advantages for survival through diverse soil environmental conditions over a longer time period. The full complement of VAM species in a particular soil would, therefore, include individual species capable of immediate spore germination and root infection with the onset of conditions suitable for host development (*Glomus spp.*) and complementing these would be species from the *Acaulospora* genus, capable of long term dormant survival prior to quiescence and potential germinability. These fungi would, therefore, not react to a temporary onset of favourable soil conditions in an otherwise extended period of unfavourable conditions. The role of different VAM genera within the overall endophytic population of a soil obviously requires further study since it is known that *Acaulospora* and *Glomus* spp. are prevalent in these grassland ecosytems (Crush, 1973).

The low spore numbers found in the cultivated fields is an example of the regularly observed lack of correlation between spore numbers and root infection levels in soils (Allen and Allen, 1980; Dodd *et al.*, 1983; Hayman and Stovold, 1979). Winter wheat root systems became heavily mycorrhizal in the Sussex soils soon after seedling emergence (chapter 3), despite low numbers of viable spores. The inability of dormant spores to germinate and infect plant root systems may be one major factor contributing to the lack of such a relationship. (Tommerup, 1983a). It should be stressed that direct spore counts ignore the contribution of soil hyphae, infected root fragments and root vesicles (Tommerup and Abbott, 1981; Powell, 1976), to mycorrhizal infectivity. The most probable number (MPN) method attempts to quantify the total number of VAM infective propagules in a soil (Porter, 1979), and would have probably given an estimate of the relative proportion of fine endophytic infectivity in this study. Attention, however, should also be drawn to the fact that certain species, such as *G. occultum*, Walker, stain lightly, or not at all, in the period shortly after root penetration (Schenck and Smith, 1982). It has recently been shown that MPN counts alone are inadequate in soils containing endophytes such as this (Morton, 1985), and it was suggested that spore numbers should be included with MPN counts to give a more accurate assessment of soil infectivity.

The large standard errors observed for samples at the Hamill pasture site (Fig. 4.1), highlight the variation within individual samples. This could well be important in natural, undisturbed sites, with a large variety of plant species, rather than in cultivated sites with its intensive monoculture.

4.4.2. Spore Identification and Taxonomy

The range of morphological types of VAM species at four field sites has been described in this chapter and a thorough microscopical study has been carried out of three spore types obtained in pure culture, *G. geosporum*, *G.*

monosporum and *G. mosseae*. Two morphologically different spore types from two different soils, and isolated from their field characteristics *G. monosporum* (Sussex) and *G. mosseae* (Hamill), became very similar morphologically in pot culture. The use of interference microscopy along with SEM and TEM studies revealed only small differences between the two pot-cultured isolates. More significant differences were detectable in the morphology of the sporocarps. The change in morphology was most dramatic for *G. monosporum* and was consistently observed with each of six single-spore cultures established. Pot cultured spores have now been placed into sterile soil from where the spore was isolated to show if the original field morphology can be thus reestablished, demonstrating the potential importance of edaphic factors on spore morphology. The validity of the current taxonomic systems rests on a basic assumption that spore morphology is not influenced by interactions with hosts or soil environments. To this extent, recent work by Morton, (1985) revealed that this does hold true for *G. occultum* and *G. diaphanum*. (Morton and Walker). In this study, pot culture spores of *G. monosporum* and *G. mosseae* showed considerable similarities in wall structure under the light microscope.

The taxonomic descriptions (with photographs) of *G. monosporum* in the literature (e.g. Gerdemann and Trappe, 1974; Hall, 1984), have shown it to have apparent 'echinulations' present between the outer and inner walls. This has been consistently used as a distinguishing feature of *G. monosporum*. It has also been noted that this is not a constant feature and apparently only observed in a minority of spores in a population (Nemec et al., 1981; Walker for this isolate - personal communication). SEM and TEM studies of *G. monosporum* and *G. mosseae* in this work have shown them to have similar wall ultrastructure. *G. monosporum*, however, has a more persistent outer hyaline wall than *G. mosseae*. In both cases a thin layer was observed between the outer walls with a

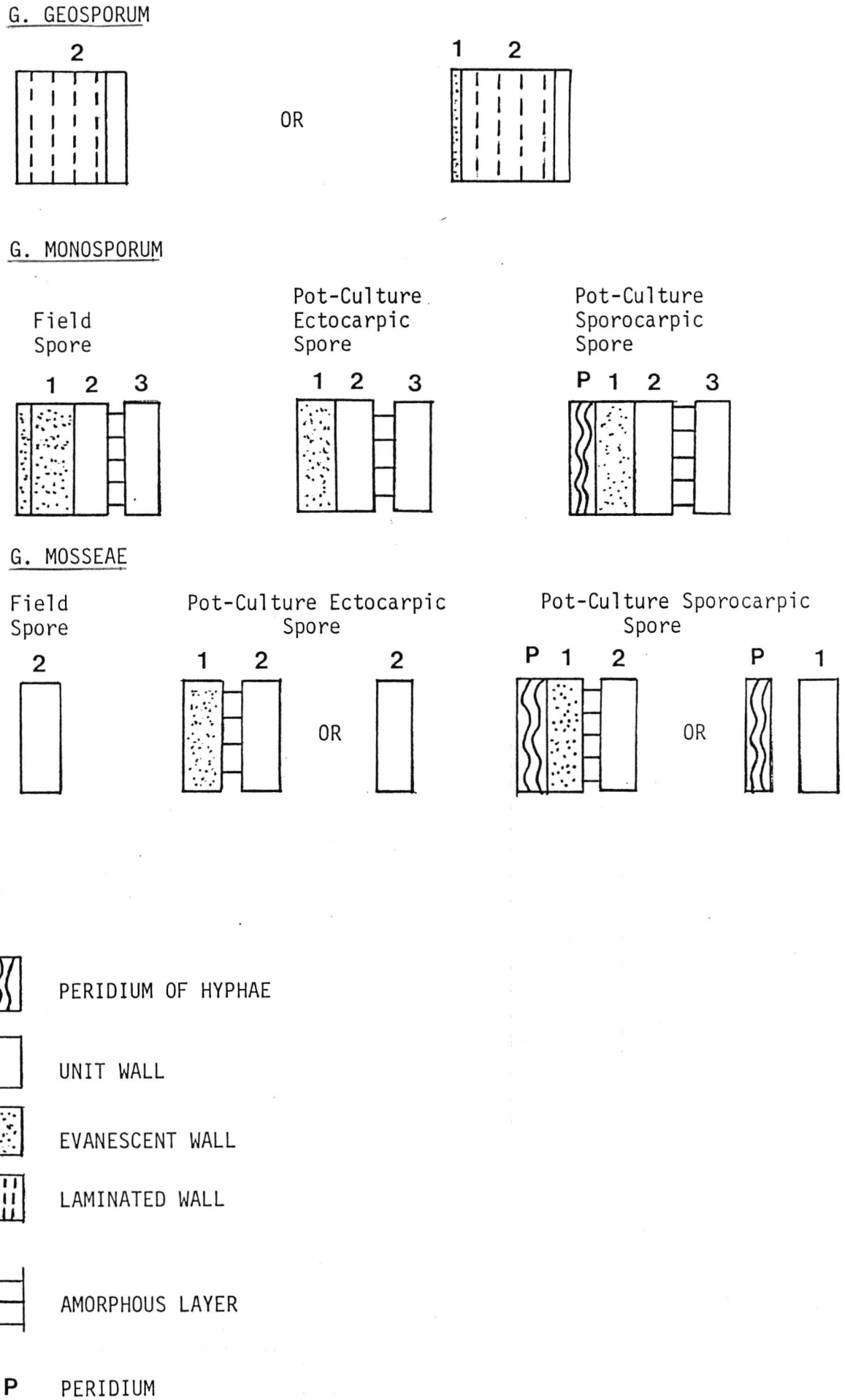
'reticulate ridge' surface morphology with the light microscope and SEM. This has been seen to fragment in such a way as to give the impression of thin 'fissures' running between the two walls (Photo 38) which have an echinulate appearence. No true echinulations were ever observed using SEM or TEM. The relationship of this layer in respect to the inner and outer walls is clearly seen in the ectocarpic *G. monosporum* spore shown in Photo 55. It may be that the frequent report of a thin outer hyaline wall for *G. mosseae* at maturity (Nemec *et al.*, 1981) may represent this interstitial layer and not the more evanescent outer wall, which is usually only observed in younger spores. Preparations of *G. geosporum* showed no similar architecture under the SEM, which suggests that these wall features are not artifacts of the technique.

TEM studies of *G. geosporum* revealed finer details of the wall laminations observed under the the light microscope. *G. geosporum* possesses an arced organisation of the fibrils of the fungal wall similar in appearence to those described by Bonfante-Fasolo and Vian, (1984) for *G. epigaeum*, and by Mosse, (1970) for *Acaulospora laevis*. This organisation has already been described for chitin fibrils in the cuticle of crabs and other Crustacea (Livolant, Giraud and Bouligand, 1978) and in the cuticle of insects (Neville, 1975). Similar bow-shaped structures have been described in algal chitinous walls, but had not been previously recorded in fungal walls (Pearlmutter and Lembi, 1978). In a primary wall of a plant such arced patterns are transient and become dispersed by extension during morphogenesis, a mechanism possibly controlled by an endogenous 'clock' (Roland *et al.*, 1983). In the mature VAM spore wall, however, where there is no more extension, the arced structure remains and the number of layers could be used to 'date' the periods of wall deposition, in a way analogous to that of ageing trees by counting 'tree-rings'. The increase in arc curvature going from the outer older arcs to the inner younger ones suggests a

Photo 55 - Pot-cultured ectocarpic spore of G. monosporum, showing a flaking interstitial layer (I) underneath the hyaline outer layer (H) and overlaying the inner pigmented (P) layer (x 600).


55
H
I
50µm

Figure 4.3 - Murographs of G. geosporum, G. monosporum and G. mosseae.



progressively accelerated rhythm of wall synthesis leading to the dispersed nature of the innermost layer of the *G. geosporum* wall (Photos 18 and 19).

In summary, the combined use of light microscopy, SEM and TEM can help the taxonomist to study spore architecture and morphology, aiding taxonomic identification. With the subsequent use of murographs (Fig. 4.3) in published material (Walker, 1983), the establishment of a standardised taxonomical grouping of the Endogonaceae could be possible. Current leading taxonomists should collaborate to formulate a unified and yet flexible key for the Endogonaceae, utilising the results of light and EM procedures along with data from new techniques like ELISA, gel electrophoresis and monoclonal antibodies. I suggest that this should be made a major priority which will help to stop the present emphasis on creating and publishing `new' species on the basis of slight morphological differences.

4.4.3. Electrophoretic Taxonomy

The results of staining spore contents for esterase activity following PAGE were successful in differentiating between the three VAM fungi tested. This indicates that such a chemotaxonomic method can be used to help distinguish between VAM fungi at the species level. Sen and Hepper (1986) showed that this method is reproducible between assays, and for one VAM species maintained under a range of conditions, such as on different hosts in a variety of soils. Their results indicated that the mobility of six enzymes, including esterases, was not influenced by the environmental or physiological conditions under which the spores used had been raised. Using esterase staining, all six of the *Glomus* spp. that they tested could be separated. They subsequently gave recommendations for the enzyme stains, needed to differentiate between any particular pair of species. The methods of enzyme staining are so sensitive that only relatively few spores are needed, much less than that needed for the production of antibodies

(Kough et al., 1983). The method can therefore be used to check homogeneity of 'pure' stock cultures. Sen and Hepper (1986) stated that it would be preferable to run authenticated fungi as standards on the same gel when unknown strains are being identified rather than relying on absolute mobilities. In this study, however, the pattern of esterase banding obtained for *G. mosseae* was easily identified by Sen and Hepper (pers. comm.) as equivalent to their *G. mosseae* isolate banding pattern, even though I had modified the gel technique that they had used considerably (the stacking gel was removed, an 8% running gel used compared with the 6% gel used by Sen and Hepper (1986) and thinner gels were also used). It may thus be possible to provide a directory of banding patterns, especially esterase, which could be useful in aiding the current taxonomic problems.

From this work it has been possible to separate two species with very similar morphologies using enzyme staining. The problems of distinction caused by two intergrading morphological species like *G. monosporum* and *G. mosseae* can be solved conclusively. Species designations have recently been made using electrophoretic patterns for proteins in *Rhizopus* spp. (Seviour et al., 1985), *Botrytis* spp. (Backhouse et al., 1984) and within *Sclerotinia* (Tariq *et al.*, 1985) where fewer interspecific differences were observed. The future use of protein banding patterns as an aid to taxonomy in the Endogonaceae should be investigated and implemented as soon as possible, since it requires the use of a simple biochemical technique which rapidly gives results.

The absence of any particular enzyme activity, like acid and alkaline phosphatases in this study, does not imply that these enzymes are missing merely that they could be labile and become inactivated during separation, or that the detection method used for the enzyme may be inappropriate for this

fungus.

The potential application of this technique in helping to differentiate between several VAM endophytes within a single root system (Sen and Hepper pers. comm.) could provide a long needed breakthrough in identifying species in mixed infections.

CHAPTER 5

The effects of pH, temperature and pesticides on spore germination of *G. geosporum*, *G. monosporum* and *G. mosseae*.

5.1. INTRODUCTION

No VAM endophyte has yet been maintained axenically. Spores, however, germinate readily and have limited hyphal growth on water agar (Mosse, 1959), but fail to germinate on high nutrient agars. Low nutrient concentrations, however, greatly improve hyphal growth (Daniels and Graham, 1976). Spore germination of various species has also been reported to be stimulated by dark storage of spores at 6-10^{o}C (Hepper and Smith, 1976), addition of dialysed soil extract (Daniels and Graham, 1976), and particular optimum pH levels (Green *et al.*, 1976). It has also been suggested that other microorganisms stimulate germination by metabolising self-inhibitors contained in the spores (Daniels and Trappe, 1980), since improved germination and hyphal growth has frequently been noted when contaminated spores are incubated on agar media (Dodd and Krikun, 1984; Photo 59), yet inhibition of spore germination is commonly recorded when spores are incubated in sterilised soils (Wilson, 1984).

Considerable variation has been found in the germination of spores isolated from either stock pot cultures or directly from agricultural soils (Daniels and Duff, 1978). This is especially marked following storage of spores in soil, on damp filter paper or in sterile solutions (Sward *et al.*, 1978). The inhibition of spore germination of spores of several species following formation indicates an

initial constitutive period of dormancy (Tommerup, 1983). This period was several weeks for *Glomus* spp., and up to six months for *Acaulospora laevis*. It has also been noted recently that ectocarpic and sporocarpic spores of the same species differed in their ability to germinate with the increasing age of the stock pots from which they were isolated (Hardie, 1984).

The addition of pesticides to the soil may also affect spore germinability. The use of pesticides to control plant pathogens and pests is commonplace. Concern has developed recently amongst agriculturalists about the effects of pesticide usage on non-target beneficial mycorrhizal fungi. Diverse groups of fungicides and herbicides are frequently used in wheat cultivation in temperate zones and the widespread and intensive application of these toxicants may result in indiscriminate killing of both pathogenic and non-pathogenic beneficial microorganisms (Domsch, 1964). Evidence indicates that some systemic fungicides, such as benomyl, are capable of eliminating VAM fungi from the soil (Menge, 1982; Jalali and Domsch, 1975). Little work, however, has been conducted on the effects of such chemicals on the ability of VAM spores to germinate following exposure to such pesticides, particularly herbicides (Tommerup and Briggs, 1981).

The development of a VAM symbiotic relationship with a host can be divided into four stages (Tommerup and Briggs, 1981):

(i) spore germination, or initiation of hyphal growth from infected root inoculum.

(ii) growth of hyphae through the soil to roots.

(iii) penetration and successful initiation of infection in roots.

(iv) spread of infection, development of a mycorrhizal relationship with roots and spore production.

Many previous investigations on the effects of agricultural chemicals on VA mycorrhizas, reviewed by Trappe (1984), have assessed final changes in the percentage of infected roots, and spore production. Thus, changes measured are the result of the response of each of the four stages to the added chemicals (this aspect is covered in detail in the following chapter). It was decided to carry out parallel experiments to the pot experiments (following chapter), to test the effects of four herbicides and three systemic fungicides on the germinability of spores (stage i) of three VAM endophytes. The herbicides chosen are used intensively in winter wheat cultivation in S.E. England and were all used during the 1982-85 period on the fields at Hamill and Sussex. In advance of these experiments, several tests were necessary to select the best axenic system to use to test the pesticides in the concentrations relevant to normal field rate applications. This involved the testing of the three VAM endophytes to find the temperature and pH optima necessary to achieve maximum germination.

5.2. MATERIALS AND METHODS

5.2.1. Experiment 1

This experiment involved the preliminary investigation of germinability of spores isolated from field and stock pot cultures on water agar (WA) and WA amended with 0.1% (w/v) nutrient broth (NB), over a range of pH 6-9. Temperature screening, 5-25^{o}C, was carried out for all three endophytes on the incubation pad plates (see chapter 2). The duration of tests varied between 15-21 days and were carried out at 25^{o}C unless stated otherwise. The incubation pad plate technique was used because it eliminates the difficulty of incorporating pesticides into molten agar (50^{o}C is the temperature at which most active pesticide moieties become unstable). 50 spores per treatment were incubated for agar tests, whilst 35 were used on the incubation pad plates. Field spores

were isolated from their original moist field soils after storage at 4°C in a cold room for two months. For temperature studies, all three endophytes were incubated at pH 7.5, chosen after by the preliminary study outlined above. Spores for this test were isolated ftom stock pots stored for approx. 6 months in a cold room at 4°C.

5.2.2. Experiment 2

This experiment compared stock pot culture age with the ability of spores to germinate on incubation pad plates at a range of pH values 6-9. Tests were performed on batches of spores which had been stored for between 3-24 months in a cold room at 4°C. Final checks on germination were made after 20 days, following incubation at 25°C.

5.2.3. Experiment 3

Batches of spores, of each endophyte, capable of the highest germination rates were selected after preliminary experiments. The incubation pad technique was then used to study the effects of several pesticides on spore germination. The following four post-emergence herbicides were used in Experiment 3:

(i) Avenge (Cyanamid Ltd.)

(ii) Ceridor (Elanco Ltd.)

(iii) Dicurane (Ciba-Geigy Ltd.)

(iv) Harrier (I.C.I. Plant Protection)

These are all used in the autumn and spring at times when infection of the wheat root systems by VAM, initiated by germinating spores and infected root fragments, appears to be important (chapter 3). It was decided to attempt to closely approximate the concentration of residual soil herbicide in the top 2-3 cms of soil after spraying. Nicholls and Buxton (1982) simulated the movement

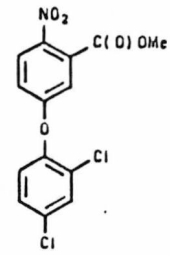
and degradation of two active herbicide moieties, one of which (chlortoluron) is the active ingredient of Dicurane. During the period 1974-1981 they used a computer to try to estimate the level of residual herbicide activity likely to remain in the upper soil layers, taking into account the weather data. Their results indicated that, on average, 50% of applied chlortoluron was likely to be recovered from the top 2-3 cms of soils in the spring following an autumn application. This figure was used as a basis to set for the concentrations that a VAM spore would be likely to encounter during this period. The field rate application in kg or L per hectare was reduced to the amount applied to a 10 cm^2 surface area (the approx. area of the incubation pad petri plate). This was considered as 100% of the applied herbicide and a second concentration of 50% was used to simulate the maximum average concentration likely to be encountered in the soil through the autumn period following Nicholls and Buxton's hypothesis (not accounting for the adsorption properties of pesticide or soil). It should be noted, however, that the average soil temperatures in the autumn in the field range from 6^{o}C in September to 3^{o}C and below in February (Nicholls and Buxton, 1982).

(i) Avenge 630

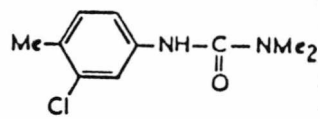
This is a wild oat herbicide for use as a post-emergence spray on cereals from the two leaf stage (1 on Feekes scale) to end of tillering growth stages (3). It is translocatable and is applied at a rate of 1.58kg $hectare^{-1}$ with a wetting agent. It is sold in solid form and its active ingredient is difenzoquat methyl sulphate (1,2-dimethyl-3,5-diphenylpyrazolium methyl sulphate; Fig. 5.1). It is also known to control early mildew on winter barley.

Figure 5.1. - Chemical formulae of active moieties of herbicides.

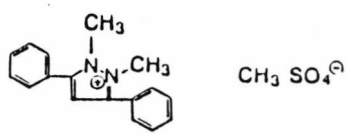
Herbicide **bifenox**



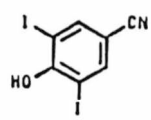
Herbicide **chlortoluron**



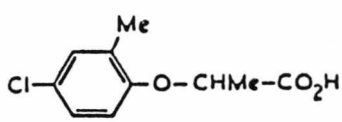
Herbicide **difenzoquat methyl sulphate**



Herbicide **ioxynil**



Herbicide **mecoprop**



(ii) Coridor

This post-emergence herbicide is a suspension concentrate containing:

(a) 18.75% (w/v) bifenox (methyl 5-(2,4 dichlorophenoxy)-2-nitrobenzoate; Fig 5.1)

(b) 46.25% (w/v) mecoprop (2-(4-chloro-2-methylphenoxy) propionic acid; Fig. 5.1)

It is used for the control of broadleaf weeds in cereals. Its recommended application rate is 3-4L hectare^{-1} applied between the three leaf (2) and second node (7) stage. Mecoprop is a translocatable post-emergence herbicide of the chlorophenoxy group, weed death resulting from extreme hormone-like responses giving rise to distorted tissues. It is thought that phenoxy herbicides may act on the cell nucleus by interfering with protein synthesis.

(iii) Dicurane 500L

This is a a pre- or post-emergence herbicide in a liquid formulation containing 50% (w/v) active ingredient chlortoluron (3-(3-chloro-p-tolyl)-1, 1-dimethylurea It controls black-grass, wild-oats, othet annual grasses and many broad-leaved weeds in most major varieties of winter wheat and barley. The recommended application rate is 7L hectare^{-1}.

(iv) Harrier

This liquid formulated, post-emergence herbicide contains:

(a) 45% (w/v) mecoprop

(b) 1.5% (w/v) clopyralid

(c) 5% (w/v) ioxynil (4-hydroxy-3,5-duodobenzonitrile; Fig. 5.1).

It kills broad-leaved weeds and is a non-ester water based formulation. It is

applied at a field rate of 5L hectare^{-1}.

5.2.4. Experiment 4

Three current systemic fungicides, for winter cereals, were used to test the effect of estimated residual soil concentrations of fungicide on VAM spore germination:

(i) Bavistin FL (BASF Ltd.)

(ii) Calixin (BASF Ltd.)

(iii) Tilt Turbo (Ciba-Geigy Ltd.)

(I) Bavistin FL

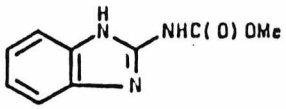
This is a flowable formulation containing 50% (w/v) carbendazim (methyl-2- benzimidazole carbamate; Fig. 5.2). It is a broad spectrum fungicide with systemic, protectant and curative properties but can be used only up to full ear emergence in cereals. It is, however, recommended to be used from when leaf sheaths become erect (5) to first node (6) at a field rate of 0.5L hectare^{-1}.

(II) Calixin

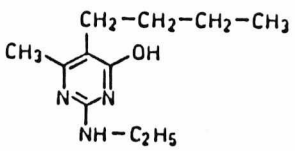
This is a liquid formulation containing 75% (w/v) tridemorph (N-tridecyl-2,6-dimethylmorpholine; Fig. 5.2). It is applied to control the cereal diseases powdery mildew, *Rhynchosporium* and yellow rust. It is systemic with eradicant and prophylactic properties. Application usually occurs between the leaf sheath erect stage (5) and the appearence of the flag leaf (9) to control mildew, at a field rate of 0.7L hectare^{-1}.

Figure 5.2. - Chemical formulae of active moieties of fungicides.

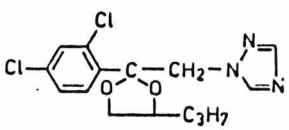
Fungicide **carbendazim**



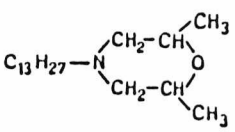
Fungicide **ethirimol**



Fungicide **propiconazole**



Fungicide **tridemorph**



(iii) Tilt Turbo 375

This is an emulsifiable concentrate containing:

(a) 12.5% (w/v) propiconazole

(b) 25% (w/v) tridemorph

It has contact and systemic action against a wide range of cereal diseases including, *Rhynchosporium*, net blotch, rusts, mildew and septoria. Eyespot is also suppressed at low levels. Clearence has been given for applications of three sprays per crop at 1L hectare^{-1}, with provision that one is made in the autumn.

The spores used in these fungicide experiments were isolated from Experiments F1 and F2 in the following chapter. One batch (O) was taken from the control treatment where no fungicides had been applied to the wheat crop grown in pots in the greenhouse. The second batch (M/S/B) was a treatment which had received three sprays in each of the two experiments:

(a) Milgo E (a.i. ethirimol; Fig. 5.2)

(b) Sportak (a.i. prochloraz)

(c) Bavistin FL (a.i. carbendazim; Fig. 5.2)

The spores had been stored in their respective durite environments (air-dried) for 3-4 months at 10^{o}C. This experiment was designed to compare the effects of a fungicide on two types of VAM spore populations; one which had allowed formation of spores in a fungicide free environment, and one which had been exposed to several fungicides, by residual activity in the soil and via the systemic activity of the fungicide within the host.

5.3. RESULTS

5.3.1. Experiment 1

pH tests using field spores of *G. mosseae* and *G. monosporum* on WA alone indicated a pH optimum of 7 for the former endophyte and 8 for the latter (Fig. 5.3). *G. mosseae* spores incubated on WA+NB had reduced germinability (9-23%), whilst those of *G. monosporum* had higher germination percentages (39-52%) over the pH range 6-8. Germination of *G. monosporum* spores at pH 9 on both media was low. Although germination percentages for *G. monosporum* were consistent over the pH range 6-8 on WA+NB, most hyphal growth occured at pH 7. More extensive hyphal growth was also noted on WA+NB compared with WA alone (Fig. 5.4). Field spores of *G. geosporum* were frequently contaminated (see chapter 4 and Photo 57) therefore no reliable data were recorded. It should be noted that no vesicles formed on WA treatments of either endophyte but were observed on WA+NB at pH 7-8 for both endophytes.

Spores of *G. mosseae* from a pot-culture stored at 4^{o}C for 6 months did not germinate on WA alone, but showed a peak of germination at pH 7-8 on WA+NB of 43-45%.

5.3.2. Experiment 2

Table 5.1 shows the effect of temperature on the spore germination of endophytes. All three species have highest germination percentages at 25^{o}C (Photo 58 shows *G. monosporum* on SW+NB at 25^{o}C). No germination was observed on incubation pads with sterile deionised water (SW) alone and at 5^{o}C and 10^{o}C with the SW+NB solution. As a consequence of this and the data from Experiment 1, further experiments were carried out on incubation pad plates moistened with sterile deionised water containing 0.1% NB (SW+NB), at pH 7.5 at 25^{o}C in the dark. It was noted during these studies that some erratic

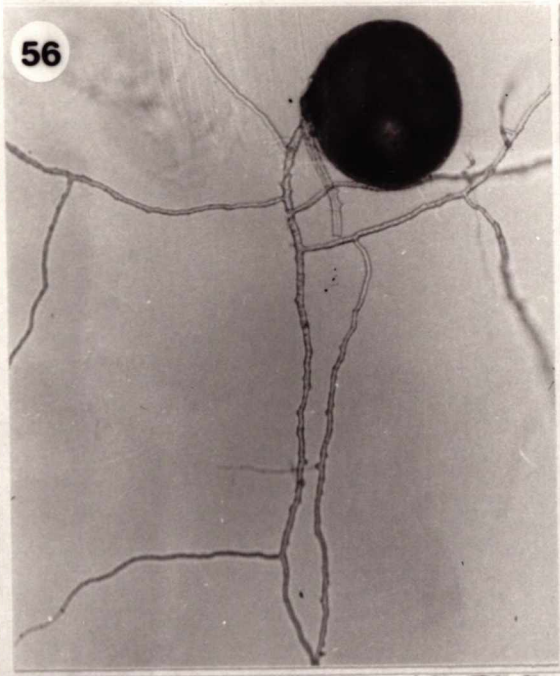
PHOTO 56 - Field spore of G. monosporum germinating on WA+NB agar at pH 8.0 showing extensive hyphal growth (x 150, B.F.).

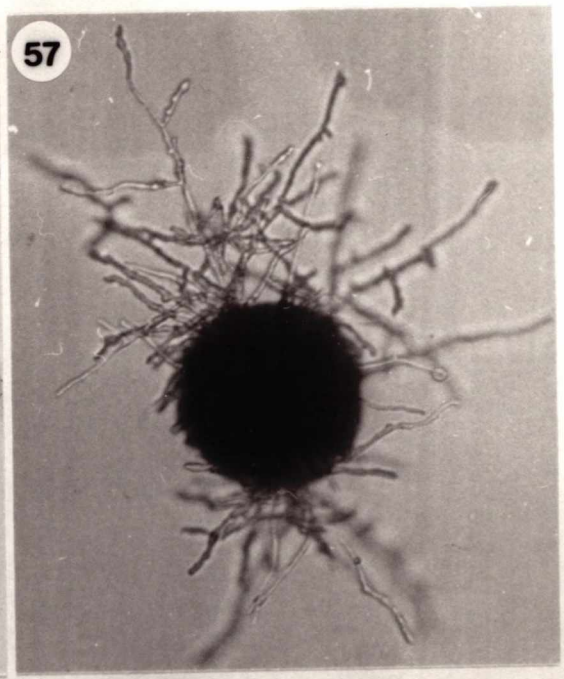
PHOTO 57 - Field spore of G. geosporum incubated on water agar at pH 7.0 showing fungal colony originating from within the spore (x 160, B.F.).

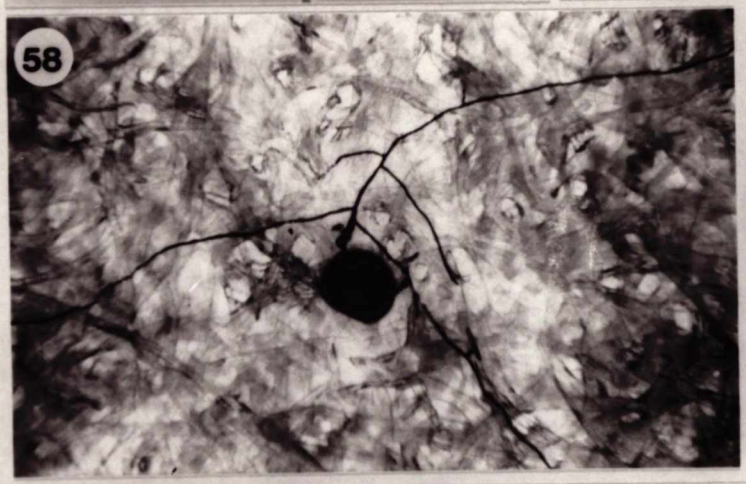
PHOTO 58 - Germinating pot-cultured spore of G. monosporum on an incubation pad plate containing a solution of SW+NB at pH 7.5 (x 100, B.F.).

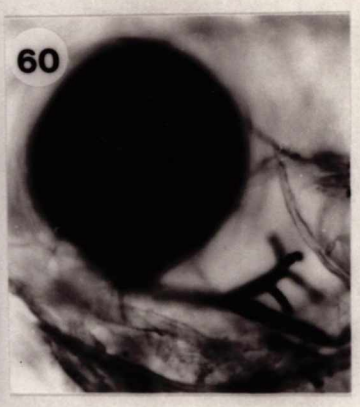
PHOTO 59 - Bacterially contaminated field spore of G. monosporum germinating on water agar at pH 7.0. Improved hyphal growth is observed with increased frequency of secondary spores (vesicles) on the mycelium (x 150, B.F.).

PHOTO 60 - Short, swollen germ tube of a pot-cultured spore of G. monosporum exposed to Tilt Turbo on an incubation pad plate at pH 7.5. (x 150, B.F.).


56


57


58


60

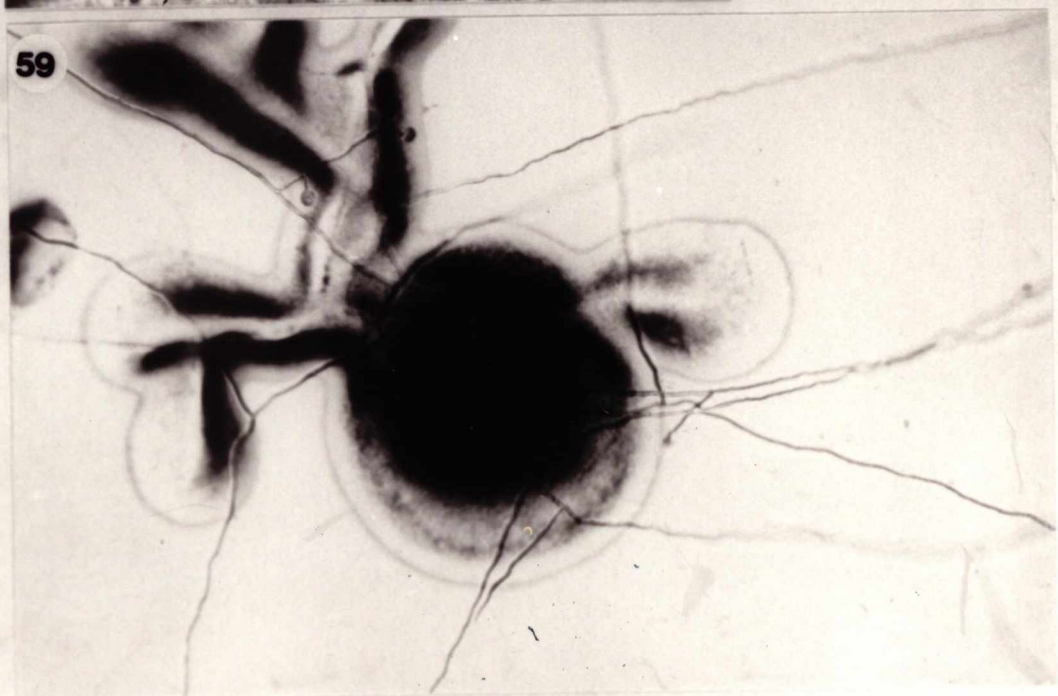

59

Figure 5.3. - Field spore germination of G. monosporum and G. mosseae on water agar (WA) and water agar with 0.1% nutrient broth (WA+NB) at different pH's (germination after 20 days).

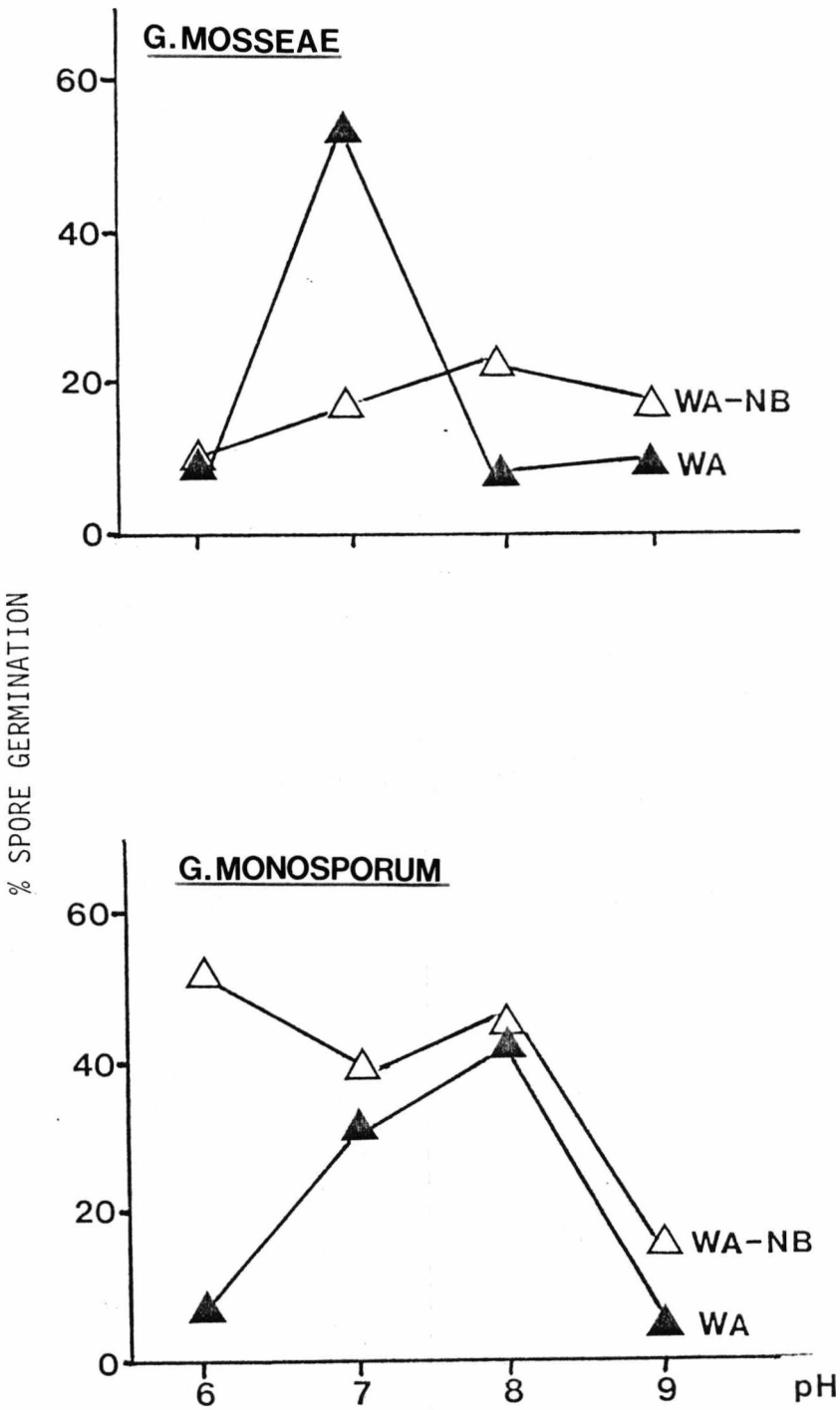
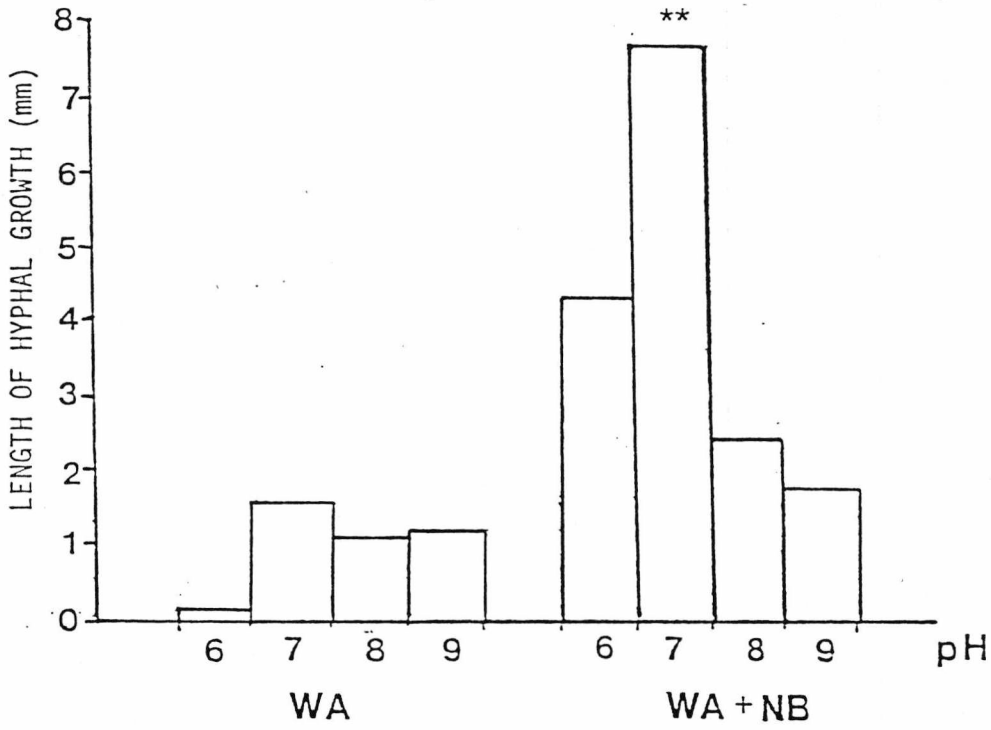


Figure 5.4. - Hyphal growth from field spores of G. monosporum incubated on water agar (WA) and water agar amended with nutrient broth (WA+NB).

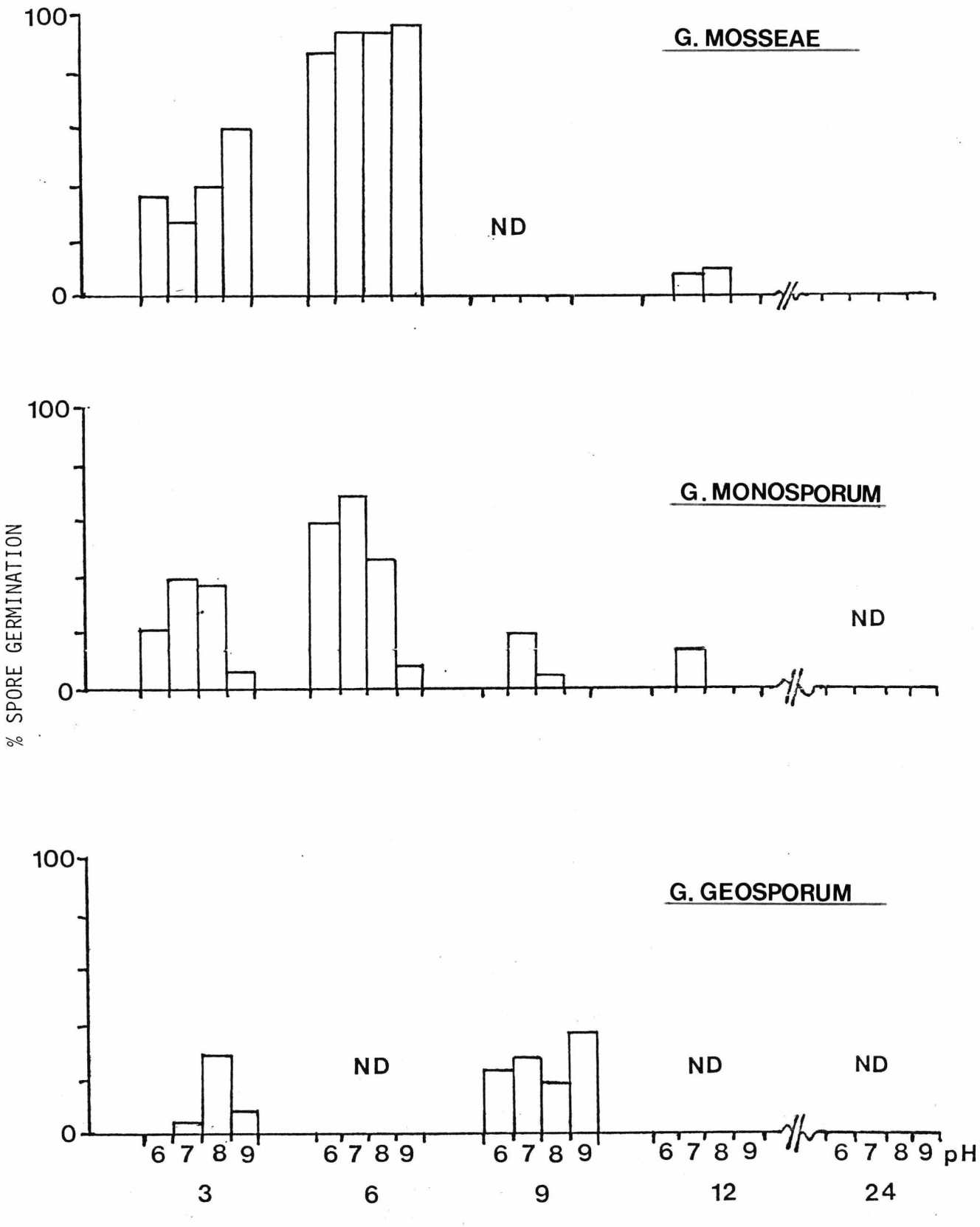


(** = significantly different at P=0.05)

Table 5.1. - Effect of temperature on the spore germination of three VAM fungi (from pot cultures) on incubation pad plates. Germination after 20 days.

INCUBATION TEMPERATURE °C	% SPORE GERMINATION					
	G.GEOSPORUM		G.MONOSPORUM		G.MOSSEAE	
	SW	SW+NB	SW	SW+NB	SW	SW+NB
5	0	0	0	0	0	0
10	0	0	0	0	0	0
20	0	10	0	19	0	27
25	0	23	0	53	0	93

Figure 5.5 . - Effect of age of cold stored ($4^{o}C$) pot cultures on the spore germination of G. geosporum, G. monosporum and G. mosseae on incubation pad plates at different pH's (germination after 20 days).



ND = NO DATA

germination of spore batches occured which seemed to be linked to the age of the stock pot culture from which they were obtained. Results from studies on the effect of cold ($4^{o}C$) storage of spores (Fig. 5.5), indicate a trend towards zero after periods of one year in storage for *G. monosporum* and *G. mosseae*. There appears to be a storage time for these two endophytes of around 3-6 months, at which time high percentage germination can be attained under optimal conditions. Results for *G. geosporum* showed that moderate levels of germination could still be attained after storage for 9 months. Germination of *G. monosporum* at pH 9 was again low as in Experiment 1.

5.3.3. Experiment 3

Table 5.2 shows the results of herbicide application on spore germination. Avenge totally inhibited germination of spores of all three endophytes. Ceridor and Harrier behaved similarly to each other in apparently stimulating germination at the higher concentration, whilst preventing germination at the lower concentration. This is noteworthy since both contain tridemorph as the active herbicide moiety. Dicurane had little inhibitory effect on germination and appeared to stimulate germination of *G. monosporum* and *G. mosseae* above control levels. A preliminary test (data not presented here) using spores of *G. monosporum* and *G. mosseae* exposed to higher higher concentrations of Ceridor ($5\mu l\ ml^{-1}$) and Dicurane ($9\mu l\ ml^{-1}$) strongly inhibited germination. Both herbicides reduced germination of *G. mosseae* from control levels of 91% (Ceridor 23% and Dicurane 37%). *G. monosporum*, however, whose control germination was 67% was completely inhibited by both herbicides (0%). Plates had been incubated at 25C, pH 7.5 for 20 days.

Table 5.2. - Effect of four herbicides (highest concentration is equivalent to field application rate) on the spore germination of three VAM fungi on incubation pad plates (SW+NB). Germination after 20 days.

HERBICIDE TREATMENT	PESTICIDE RATE	% SPORE GERMINATION		
		G MOSSEAE	G MONOSPORUM	G GEOSPORUM
CONTROL	0	20	8	0
AVENGE	0.8µg ml^{-1}	0	0	0
	0.4µg ml^{-1}	0	0	0
CERIDOR	2µl ml^{-1}	58	25	26
	1µl ml^{-1}	0	0	0
DICURANE	3.5µl ml^{-1}	25	21	4
	1.75 µl ml^{-1}	26	38	33
HARRIER	2.5µl ml^{-1}	22	22	50
	1.25µl ml^{-1}	0	0	0

5.3.4. Experiment 4

An initial test of equivalent field rate applications of Bavistin and Tilt Turbo on spore germination of *G. mosseae* completely inhibited germination. So, for the purposes of the main screening experiment, the concentrations were reduced by a factor of 10. Work by Carr and Hinkley (1985) had indicated that concentrations of 10-100 ng ml^{-1} of benomyl covered a range of response to application, i.e. from normal germination rates to inhibition of spore germination of *G. caledonicum*.

Table 5.3 shows that Bavistin completely inhibited germination of spores of *G. monosporum* and *G. mosseae* from both (O) and (M/S/B) stock cultures. *G. geosporum* (O) spores did germinate in the presence of Bavistin (15-16%) but this was lower than control *G. geosporum* spores (41%). *G. geosporum* (M/S/B) spores did not germinate when exposed to Bavistin. Calixin appeared to have little effect on germination of spores from control cultures (O), it did, however, appear to inhibit spores of *G. geosporum* from the (M/S/B) stock cultures at the higher concentration. Tilt Turbo appeared intermediate between Bavistin and Calixin in inhibiting germination on spores of all three endophytes from control (O) cultures, but did have a noticeable inhibitory effect on *G. mosseae* spores from the (M/S/B) pot cultures at both concentrations. *G. geosporum* germination was also inhibited at the higher concentration.

Table 5.3. - Effect of three systemic fungicides (highest concentration is ten times below normal field rate application rate) on the spore germination of three VAM fungi from two pot culture sources placed on incubation pad plates (SW+NB). Germination after 20 days.

PESTICIDE TREATMENT	PRIOR SPRAY SERIES +/-	PESTICIDE RATE	% SPORE GERMINATION		
			G.MOSSEAE	G.MONOSPORUM	G.GEOSPORUM
CONTROL	-	0	33	37	41
	+	0	43	9	50
BAVISTIN	-	25nl ml^{-1}	0	0	16
	-	12.5nl ml^{-1}	0	0	15
	+	25nl ml^{-1}	0	0	0
	+	12.5nl ml^{-1}	0	0	0
CALIXIN	-	50nl ml^{-1}	33	29	24
	-	25nl ml^{-1}	13	40	29
	+	50nl ml^{-1}	26	6	0
	+	25nl ml^{-1}	38	17	41
TILT TURBO	-	35nl ml^{-1}	10	27	15
	-	17.5nl ml^{-1}	17	14	7
	+	35nl ml^{-1}	4	13	0
	+	17.5nl ml^{-1}	0	7	28

5.4. DISCUSSION

The best temperature for the germination of spores of three *Glomus* endophytes was revealed as 25°C in this investigation, which corroborates work done previously on *G. epigaeum* (Daniels and Trappe, 1980; Graham, 1982) and *G. mosseae* (Schenck *et al.*, 1975), both in soil and on agar media. Tommerup (1983b), however, achieved germination of spores of *G. caledonicum* at 5°C and 10°C using spores sandwiched between membrane filters and buried in a Lancelin sand. There was, however, a lag in the onset of germination below 20°C for all three endophytes tested by Tommerup (1983b), but all species did eventually reach the same germination percentages found at 25°C. This apparent lag at lower temperature may explain low or negligible germination found in this study and in other studies using short spore incubation periods of around two weeks (Daniels and Trappe, 1980; Koske, 1981; Graham, 1982).

All three endophytes in this study appeared to have an optimal pH range for germination between 7-8 when incubated on weak nutrient media. This correlates well with other *Glomus* spp. studied (Daniels and Trappe, 1980; Green *et al.*, 1976). Field spores of *G. monosporum* and *G. mosseae* did show an optimal pH value when incubated on water agar, but the relevance is unclear since pot-cultured spores did not germinate under these conditions, so further investigation was impeded. It was noted, however, that germination of *G. monosporum* was reduced at pH 9 in both field and pot culture spores throughout the studies, whilst *G. geosporum* and *G. mosseae* were unaffected. This may reflect an adaptation to the local edaphic factors from where spores were originally isolated, since *G. monosporum* was isolated from a soil of pH 6.5, whilst *G. geosporum* and *G. mosseae* were isolated from a soil of pH 7.5. This preference for a lower pH on agar media has been noted for two *Gigaspora* spp.

which were common in more acid soils (Green *et al.*, 1976). Whilst the same workers found that *G. mosseae*, more common in alkaline soils, had a more alkaline pH optimum for germination. It is noteworthy that in Experiment 1 the field spores of *G. monosporum* showed significantly higher hyphal growth (Table 5.4) at pH 7 on WA+NB and the effect of pH on hyphal growth may be a more important factor. Carr and Hinkley (1985) also noted that whilst spore germination of *G. caledonicum* was unchanged over the range of pH 5.3-7.4, a significant optimum of pH 6.6 was recorded for hyphal growth. This aspect is worthy of further investigation. (see also Abbott and Robson, 1985).

Considerable variability has been found in the germination of spores isolated from stock pots and directly from agricultural soils (Mosse, 1959; Daniels and Graham, 1976; Hepper and Smith, 1976; Daniels and Duff, 1978). This may be explained, to some extent, by periods of constitutive dormancy in VAM fungal spores (Tommerup, 1983a). In this study, however, there was a trend towards reduced or inhibited spore germination with spore age (Fig. 5.5) for *G. monosporum* and *G. mosseae*, despite storage at low temperatures (4^{o}C) and subsequent incubation on weak nutrient media. This result is consistent with work reported by Hardie (1984) for two isolates of *G. mosseae*, which noted the same trend for ectocarpic spores stored for up to a year. Certain VAM fungi, like those studied by Hardie (1984), and *G. monosporum* and *G. mosseae* in this study, produce both ectocarpic and sporocarpic spores and in these examples ectocarpic spores have been used in experimentation. Sylvia and Schenck (1983b) also noted that maximum germination of spores of three *Glomus* spp. was relatively low and possibly due to storage of the VAM inoculum in refridgerated soil over a long period affecting spore viability. Tommerup (1985) found that germination of quiescent spores of three VAM endophytes was prevented in soils under crops or pastures and pot cultures of VAM fungi under

long term storage. Water-soluble, heat-labile compounds which were extracted from the crop and pot culture soils prevented germination of spores. Self-inhibitors were also implicated in regulating germination. Most of the early germination studies, however, were conducted on sporocarpic spores (Mosse, 1959; Hepper and Smith, 1976). Hepper and Smith (1976) reported that storage of *G. mosseae* sporocarps at 6^{o}C for several weeks enhanced germination. In other studies, however, ageing of spores of *Gigaspora* spp. in the cold had a detrimental effect on germination (Sward *et al*, 1978; Kosko, 1981). It may be that ectocarpic spores, when newly formed, are quiescent but have only a limited period of time in which to germinate before long term storage in the same soil causes spores to become dormant under the influence of exogenous factors i.e. mycostasis. Tommerup (1985) defined mycostasis "as a form of exogenous dormancy associated with the biological properties of the soil, resulting in the absence of spore germination when physico-chemical properties of the soil are otherwise conducive to germination". Daniels and Trappe (1980) have suggested that after a time, certain microorganisms or plant exudates are needed to either metabolize self-inhibitors in the spore or produce chemicals that can stimulate germination. Spores of other biotrophic fungi are known to produce self-inhibitors (Macko, 1981). This may be important since it has been shown that the absence of a bacterial population can reduce VAM spore germination (Wilson, 1984). For the two endophytes studied here, *G. monosporum* and *G. mosseae*, a period of between 3-6 months storage would appear to be necessary to obtain maximum spore germination using the incubation pad system described. Daniels and Graham (1976) also showed that spores stored at 10^{o}C for 4 months germinated more readily than freshly isolated spores.

Results of the herbicide tests, at concentrations equivalent to those found in the top 2-3 cms of soil after field applications, showed that individual herbicides affected spore germination differently. Avenge totally inhibited spore germination of all three endophytes. It is known to have the ability to control early mildew infections on barley and its a.i. DMS (Fig. 5.1), may therefore possess more general fungicidal properties. It should be noted that Avenge was the only herbicide tested used as the solid form with a wetting agent, and this might have been a factor. Although the low levels of germination observed in the controls (Table 5.2) hindered interpretation of the results, Ceridor and Harrier did appear to behave similarly, in that spore germination was apparently inhibited at the lower concentration used, whilst a neutral to stimulatory effect was noted at the higher concentration (particularly for *G. geosporum*). Both these herbicides contain the a.i. mecoprop in the same concentrations in their formulations (approx. 45% w/v), which apparently causes weed death by extreme hormone-like responses of the weed leading to distorted tissues. Previous work has shown that plant hormones, along with amino acids, vitamins and other organic substances present in the plant rhizosphere, are capable of being produced by common soil microorganisms and that they may enhance the development of VAM fungi in the absence of any host plant (Barea and Azcon-Aguilar, 1982; Hepper, 1979; Hepper and Jakobsen, 1983). Thus the hormone-like activity of mecoprop could indicate a type of chemical which could aid spore germination and development of hyphal growth at a particular concentration, since extensive hyphal growth was noted but not quantified. The inhibition at lower concentrations cannot be easily explained. Dicurane generally produced neutral to stimulatory responses, particularly at the lower concentration. Tommerup and Briggs (1981) tested three herbicides on the spore germination of two *Glomus* species and *Acaulospora laevis*. Herbicides were incorporated into soil at

equivalent field rates and up to ten times this concentration. No inhibition of germination or hyphal growth was observed even at the higher concentrations. It has, however, been noted that a number of herbicides have stimulated growth of some ectomycorrhizal fungi in axenic culture at low concentrations (Trappe *et al.*, 1984).

Results of the fungicide tests were clearer and effects of low concentrations of fungicides on spore germination were demonstrated. Bavistin inhibited germination of spores of *G. monosporum* and *G. mosseae* in both stock culture batches. Spore germination of *G. geosporum* was only inhibited completely in the (M/S/B) cultures, which had experienced two Bavistin sprays. This would indicate that the levels of 12-25ng ml^{-1} of Bavistin used may represent the concentration at which it becomes inhibitory to spore germination of *G. geosporum* under axenic conditions. Carr and Hinkley (1985) also noted that 10ng ml^{-1} of benomyl inhibited spore germination and hyphal growth of *G. caledonicum* on water agar, but below this concentration germination and normal hyphal growth was observed. The inhibition was attributable to the activity of carbendazim, one of the hydrolysis products of benomyl, and the a.i. of Bavistin. Carbendazim is believed to disrupt microtubule formation, affecting nuclear division and hyphal growth (Howard and Aist, 1980). Tommerup and Briggs (1981), however, showed no effect of carbendazim on spore germination and hyphal growth when incorporated into soil with concentrations as high as 100μg g^{-1} of soil. Carr and Hinkley (1985) suggested that this was probably due to carbendazim being adsorbed onto the sandy soil used, decreasing the amounts to which the spores were exposed below inhibitory levels. This, however, may reflect the natural field situation since carbendazim is known to have a higher adsorption coefficient than benomyl in soil (Bateman and Nicholls, 1982). Carr and Hinkley (1985) also noted that spores of *G. caledonicum* could

germinate when removed from media containing at least 100ng benomyl ml^{-1}, suggesting a fungistatic rather than a fungicidal effect. This may help explain the temporary effects of benomyl soil drenches on the development of VA mycorrhizas (Boatman *et al.* 1978; Spokes *et al.* 1981).

Calixin had little effect on spore germination when compared with controls in both (0) and (M/S/B) cultures tested, except at the higher concentration used for *G. geosporum* (M/S/B) spores, where no germination occurred (Table 5.3). A similar result was noted for *G. geosporum* (M/S/B) spores incubated with Tilt Turbo at the higher concentration. Both fungicides contain tridemorph as an a.i., whilst Tilt Turbo also contains propicanazole. Both these active moieties are known to inhibit sterol biosynthesis in fungi (Davidse and de Waard, 1984), particularly in the Ascomycotina, Basidiomycotina, and Deuteromycotina. Tilt appeared to reduce spore germination relative to control levels, but but to a lesser extent than Bavistin. Propicanazole was shown to be the most inhibitory to VAM infection of citrus roots of four sterol inhibiting fungicides investigated by Nemec, 1985. Sterol inhibiting fungicides have been shown not to have a major effect on spore germination of their target fungi, but to cause germ tubes and hyphae to become swollen and/or excessively branched (Davidse and de Waard, 1984). Swollen ends of short germ tubes were observed in these studies, particularly in the Tilt and Calixin treatments (Photo 60).

A reduction in spore germination of the (M/S/B) spores of *G. monosporum* was noted compared with (0) germination levels. This was not noted for the other two endophytes tested. Germination of the (M/S/B) spores of *G. geosporum* also appeared to be inhibited by the higher concentrations of Calixin and Tilt as well as both concentrations of Bavistin. These, (M/S/B), spores had formed in an environment which had been exposed to both Bavistin and Sportak, another sterol inhibiting fungicide similar in activity to Calixin and Tilt. It may be

that the presence of such toxicants in the local environment, at the same time as these spores were formed, produced a spore population different from that found in the absence of these chemicals, with regards to germinability. Prior exposure of spores to these chemicals in the original pot would poten tially have been over a long period since such pesticides are known not to be easily leached from pots during normal irrigation, and to be in fact 'cycled' around the pot due to the effects of evaporation of water from the pots (P. Nicholls, Rothamsted, pers. comm.). Short term adaptation can occur in VAM fungi since it is already known that a strain of *G. mosseae* tolerant to heavy metals was isolated from a heavily polluted spoil site (Gildon and Tinker, 1981).

Most work on the effect of fungicides on mycorrhizas in axenic culture has been carried out on ectomycorrhizas and results showed that they tended to depress growth (Trappe *et al*, 1984). It was particularly noted that when several species of fungi were exposed to a given fungicide in a culture medium, they tended to differ in response. This appears to be the case in this study.

As yet, it is difficult to translate results in axenic conditions to natural field conditions for VAM fungi, and even ectomycorrhizas, due to the problems of complex microorganism interactions in the plant rhizosphere and soil properties. The differential sensitivity of different species to edaphic factors and pesticide applications, however, may be useful for controlling mycorrhizal formation in experiments. This series of screening experiments has determined the effects of temperature, pH and pesticide applications on spore germination of several different VAM isolates.

CHAPTER 6

The effects of pesticides and phosphorus applications on the VAM symbiosis of winter wheat grown in pots.

6.1. INTRODUCTION

The application of systemic fungicides to field crops to control cereal diseases, is now common practice (ADAS, 1984). Annual surveys have shown that the proportion of winter wheat fields receiving fungicide applications has increased from 1% in 1974 to 59% in 1980 (Priestley and Bayles, 1982). If these applications were to have adverse effects on VAM fungi, phosphorus uptake by the crop could be affected with a consequent effect on yield. It is known that benomyl, which rapidly breaks down to form carbendazim, significantly decreased P uptake by VAM infected plants compared to unsprayed controls (Boatman *et al*, 1978). Bailey and Safir (1978) found that VAM infection of soybeans was reduced and yields decreased by benomyl application. They postulated that reduced germination of propagules, hyphal growth or reduced host-fungus compatibility could account for the effects of benomyl application. They did not, however, consider possible effects on P uptake. Many other reports of the inhibitory action of of benomyl have been covered in a recent review by Trappe *et al* (1984). Bavistin FL is a systemic fungicide currently in widespread use on U.K. cereal crops and has carbendazim as its active moiety.

Recent trends in the development of new fungicides for the control of diseases of small grain and forage crops have been towards systemic, sterol-

inhibiting, triazole-based products with broad spectrum activity against powdery mildews, rusts and leaf spots. The goal of new fungicide development has been to formulate products that are effective against a wide range of plant pathogens when applied at low rates and preferably with only one application needed. Tilt Turbo (a.i. propicanazole) and Sportak (a.i. prochloraz) have been two such fungicides, widely used on cereals in S.E England. Nemec (1985) has recently screened four such sterol-inhibitors and found propicanazole to be the most inhibitory of VAM infection of Citrus roots.

Two members of two other systemic fungicides (grouped according to their mode of action) were chosen to be screened for any inhibitory effects on VAM infections, namely Milgo E (a.i. ethirimol) and Calixin (a.i. tridemorph). These have been previously tested on the VAM symbiosis via soil application, as indeed have most fungicides in VAM studies, and found to have inhibitory effects on VAM infection and sporulation (Jalali and Domsch, 1975). Tridemorph is a fungicidally active morpholine derivative, giving broad spectrum control along with curative and eradicant properties.

Besides fungicides, most agricultural crops are also treated with herbicides. Herbicides by their nature, are toxic to a wide range of plants, many of them potential hosts to VAM fungi. The possibility therefore exists that these chemicals could inhibit the effectiveness of VAM fungi by preventing infection processes, subsequent sporulation and P uptake. The published literature on herbicide/VAM interactions is much sparser (Trappe *et al.* 1984), although studies using normal field rate applications have indicated that they may have little obvious effect on the symbiosis. It is, however, noteworthy that VAM infection of a *Chenopodium* sp. was promoted by a simazine application, possibly correlated with an apparent increase in the release of amino acids and sugars from the roots (Schwab *et al.* 1982). It may be, therefore, that the host plant is

more sensitive to increased herbicide dosage than its VAM partner (Smith *et al*, 1981, Ocampo and Barea, 1982). Smith *et al* (1981) tested six herbicides incorporated directly into soil in pot and field experiments and found no effect of normal application rates on VAM infection and sporulation.

This study, as a follow up to work described in the previous chapter, was an assessment of the effects of such pesticides on the four stages of the development of a VAM relationship with its host, outlined in the introduction of the previous chapter. Four herbicides were, therefore, chosen to cover a range of modes of action, Avenge 630, Ceridor, Dicurane and Harrier, as well as five fungicides, Bavistin FL, Calixin, Milgo E, Sportak and Tilt Turbo, all of which have widespread use in S.E. England on cereals. It must be acknowledged that pesticide screening such as this should really be performed in a range of soils and under conditions as near to those pertaining at the time that the pesticide is applied in the field. In my work I have attempted to apply sprays at the recommended crop growth stage used in intensive cereal agriculture in the U.K.. Previous studies have not always related the time of application or the concentration of pesticide used to that expected in the field.

6.2. MATERIALS AND METHODS

6.2.1. Experiment 1

The effect of infection of two VAM fungi, *G. geosporum* and *G. mosseae*, on winter wheat var. Avalon was investigated in durite at three different phosphorus levels. The fungi were both originally isolated from the Hamill field site and were pure cultures raised from single-spore inocula. This initial experimentation was designed to identify the conditions needed for maximum VAM infection of both isolates and to study the effects of added superphosphate on growth of host plant and its symbiont.

Four kg per pot of steam sterilised durite was used amended with three superphosphate levels: 0g (0P), 2.3g (2P), and 4.6g (4P) per pot. This gave a range of phosphorus levels equivalent to the Olsen P levels found in field soils used for cereal crops (Table 6.1). The superphosphate was thoroughly mixed into the durite. Inoculation was carried out by the addition of 100g of finely chopped infected roots, from pot cultures of either endophyte, as a band approx. 8 cms below the durite surface. Control pots received a similar 100g aliquot of autoclaved inoculum along with 100ml of a filtrate of the pot culture of both endophytes. Each pot was sown with seven seeds, thinned to six plants if necessary after 14 days. There were 8 replicate pots per treatment arranged in a randomised design. Plants were initially grown in an unheated glasshouse. During the period 25/10/83 (sowing date) to 11/1/84, the minimum nightime temperatures regularly fell below $5^{o}C$ and it was noticed that VAM infection was inhibited. As a result a greenhouse heater was installed to maintain night temperatures above $9^{o}C$. Plants received a half strength Hoaglands nutrient solution (-P) each week, whilst in the warmer early summer period, between nutrient applications, plants were also watered daily with tap water. Root infection levels, foliage fresh weights (including separate ear and straw fresh weights at harvest) and internal shoot N,P,K levels (pooled data), were measured at 42, 70, 110 and 170 days.

6.2.2. Experiment H1

After consideration of the results of Expt. 1, no superphosphate was added to durite in order to achieve high root infection levels. This would allow inhibitory effects of pesticide application on VAM infection levels to be monitored most effectively.

Table 6.1 Chemical characteristics of the durite growth medium in Experiment 1.

PHOSPHORUS TREATMENT	pH	N(mg L^{-1})	P(mg L^{-1})	K(mg L^{-1})
0P	8.5	5	7	88
2P	7.6	0	52	86
4P	7.0	0	117	74

Fungal treatments were as follows:

(a) *G. geosporum*

(b) *G. monosporum*

(c) *G. mosseae*

(d) non-inoculated control

(e) non-inoculated control +P

the latter treatment receiving 2.3g of superphosphate dispersed through the pot. Six seeds of Avalon were sown per pot, thinned to 5 plants after 14 days. Five herbicide spray treatments were used at day 107 at equivalent field rates (calculated on an area basis), Avenge (1.58 kg ha^{-1}), Cerdor (4L ha^{-1}), Dicurane (7L ha^{-1}), Harrier (5L ha^{-1}) and a water sprayed control. Each concentration was applied to five replicate pots of each fungal treatment until initial runoff. Irrigation was as for Expt. 1 and infection levels were measured after 49, 101 and 146 days. Results of total and ear fresh weight yields and foliar NPK levels (pooled) at harvest are presented. Chlamydospore numbers were determined by wet-sieving four replicate 10g samples obtained from pooled sub-samples at harvest.

6.2.3. Experiment H2

In order to investigate the effect of potential soil herbicide residues and a second herbicide application on a subsequent wheat crop, the same durite was re-used for similar treatments after dry sieving through a 4mm sieve to remove most of the old root system. No additional inoculum was added but 2.3g of superphosphate was added to the control +P treatment. Sprays were applied as before at day 50 and irrigation was as for Expt. 1. Infection levels were measured after 43, 65 and 98 days. Results of total fresh weight yields and foliar NPK levels (from pooled data) at harvest are presented. Chlamydospore spore numbers

were determined as for Expt. H1. Both these experiments, Expt. H1 and H2, were carried out in the same greenhouse at different times (University of Kent) in a randomised block design and statistically analysed as 5x5 factorials. Expt. H1 covered the period from November to April, whilst Expt. H2 occupied the period from mid-June to mid-September. These seasonal differences should be noted when comparing the results of these experiments.

6.2.4. Experiment F1

Four fungal treatments were included in this experiment:

(a) *G. geosporum*

(b) *G. monosporum*

(c) *G. mosseae*

(d) non-inoculated control

all were inoculated as in Expt. 1. Six seeds of Avalon were sown per pot and thinned to five after 14 days. Three fungicides were tested separately along with a triple programme of each fungicide and a water sprayed control treatment. The sprays were applied at the recommended cereal growth stages at equivalent field rates (calculated on an area basis). Milgo E (1.25L ha^{-1}) at day 38, Sportak (1.0L ha^{-1}) at day 56 and Bavistin FL (0.5L ha^{-1}) at day 75, the triple spray programme (M/S/B) received each of these sprays on the same days. There were five replicates of each treatment arranged in a randomised block design and results were statistically analysed as a 4x5 factorial. Infection levels were measured after 38, 75, 104 and 135 days (harvest). Fresh weights of shoot material were determined after 75, 104 and 135 days. Internal foliage NPK levels (pooled) were obtained for water sprayed control treatments after 75, 104 and 135 days and for the M/S/B treatment at 104 and 135 days. Chlamydospore numbers were determined as for Expt. H1.

6.2.5. Experiment F2

As for the herbicide experiment, Expt. H2, it was decided to re-use the same durite to assess the potential effects of any residues along with a second application of the same spray on a subsequent wheat crop. The durite from Expt. F1 was dry sieved through a 4mm sieve to remove the old root systems, and all pots received 3g of KNO_3 and 1g of superphosphate to improve plant growth. The control +P treatment received an extra 2.5g of superphosphate. Six Avalon seeds were sown per pot and thinned to five plants after 14 days. Milgo E was sprayed after 86 days and Sportak after 128 days. The experiment was harvested after 186 days. The Bavistin FL spray treatment was not assessed due to an apparent plant disorder causing a loss of plant material. Infection levels were measured after 86, 128 and 186 days (harvest). Final total and ear fresh weight yields are presented. Foliar NPK levels for water sprayed control treatments after 86, 128 and 186 days along with levels for all treatments at harvest. Chlamydospore numbers were determined as for Expt. H1. Both these experiments were carried out in a temperature controlled greenhouse at Wye College, Wye. Expt. F1 covered the period April to August whilst Expt. F2 covered the period October to May.

6.2.6. Experiment F3

This experiment was designed to screen two different systemic fungicides and Bavistin FL for effects on a young rapidly spreading VAM infection of wheat. These two new fungicides Calixin and Tilt Turbo were tested because of their recent widespread use in cereal production in the S.E. of England and more particularly on the two farm sites studied here (see chapter 3). Four fungal treatments were set up:

(a) *G. geosporum*

(b) *G. monosporum*

(c) *G. mosseae*

(d) non-inoculated control

prepared as in Expt. 1. Fresh durite was used for this experiment with 3g of KNO_3 dispersed through each 4kg of durite per pot. Five pre-germinated Avalon seeds were sown per pot and four fungicidal treatments were used at equivalent field rate applications: Bavistin FL (0.5L ha^{-1}), Tilt Turbo (1.0L ha^{-1}), Calixin (0.7L ha^{-1}) and a non-sprayed control. These were applied until runoff at day 56. A randomised block design was used and results statistically analysed as a 4x4 factorial. Infection levels were measured at day 26, 56, 74, and 88 (harvest) whilst final fresh weight yields and shoot NPK levels (pooled) were obtained at harvest. This experiment was carried out in an unheated glasshouse at the Estate Centre, University of Kent during the period July to October.

6.3. RESULTS

6.3.1. Experiment 1

An initial development of infection was inhibited during the early winter period (December-January), when temperatures consistently fell below $5^{o}C$ causing fractional infection levels to fall to less than 5% (Fig. 6.1). Temperatures increased towards the end of January, due to the use of a greenhouse heater, and infection increased sharply in the 0P treatments of both endophytes (Fig. 6.1). Infection increased steadily but slowly in the 2P treatments, and there was little infection in the 4P treatments. A similar temperature-related decline overwinter was also noted for field VAM infections of winter wheat in the same season (see chapter 3). Despite this decline period, *G. geosporum* at 0P significantly increased yields ($P<0.01$) over the 0P control at

Figure 6.1 Effects of superphosphate addition on development of VAM infection in Experiment 1. Error bars show levels of significance (LSD, P = 0.05).

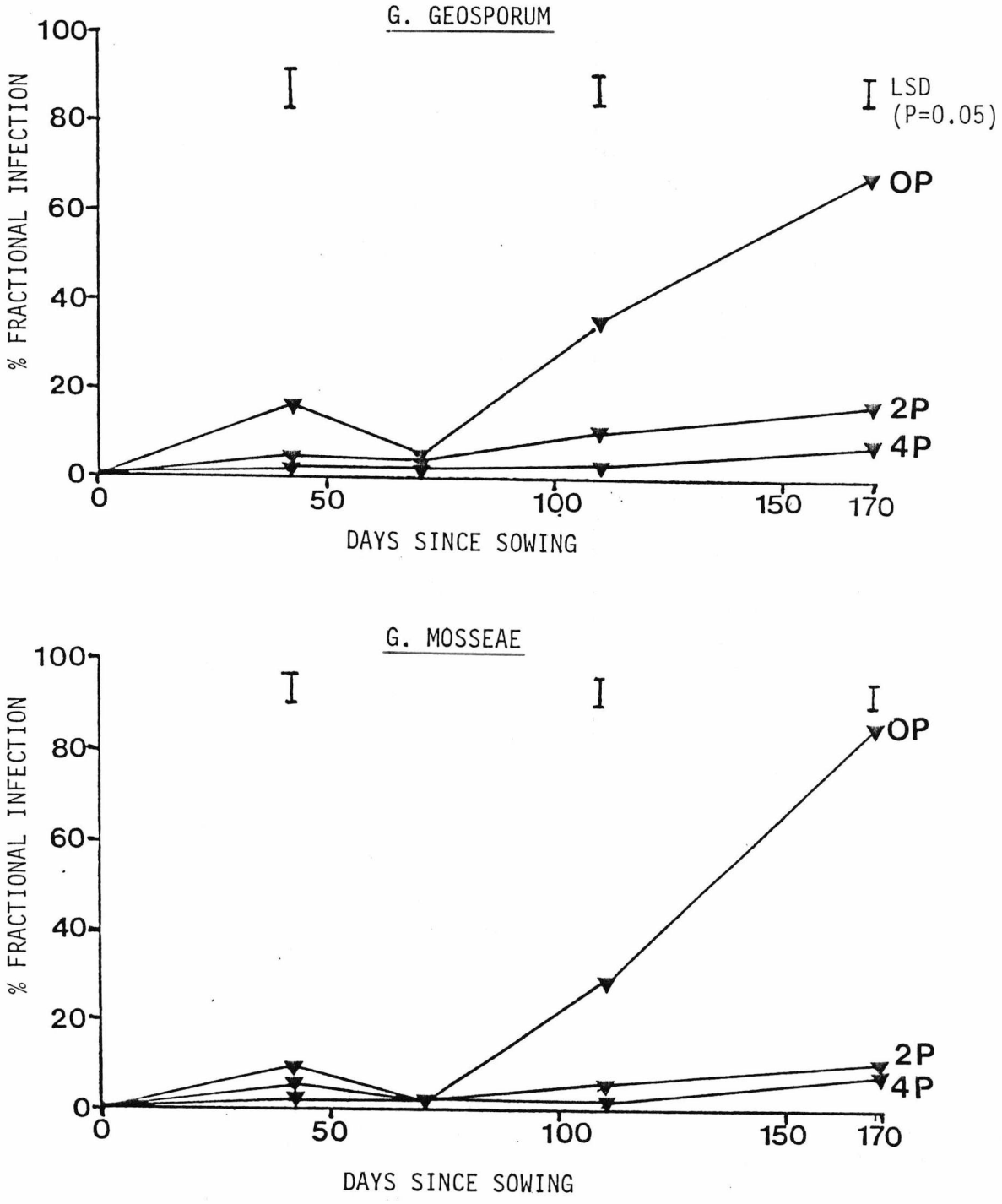


Table 6.2 Fresh weight yields and shoot concentrations of nutrients of wheat plants in Experiment 1 (day 70). Mean values from each treatment are given, with levels of significance.

	F values	Significance
Phosphorus	61.84	***
Fungi	21.09	***
Phosph X Fungi	40.03	***

Treatment	0P	2P	4P	LSD Value		
				P05	P01	P001
Phosphorus	2.783	3.492	4.042	0.25	0.33	0.44
Treatment	Control	G.geosporum	G.mosseae			
Fungi	3.600	3.992	3.475	0.25	0.33	0.44
Treatment Interaction	Control	G.geosporum	G.mosseae			
0P	1.975	4.175	2.200			
2P	4.962	3.775	3.087	0.44	0.58	0.75
4P	3.862	4.025	4.237			

Phosphorus Treatment	Fungal Treatment	% Element (DW) Foliage		
		N	P	K
0P	Control	3.51	0.21	5.50
	G. geosporum	3.68	0.37	5.07
	G. mosseae	3.63	0.23	5.34
2P	Control	4.01	0.54	5.52
	G. geosporum	3.06	0.47	5.40
	G. mosseae	3.88	0.45	5.35
4P	Control	3.59	0.47	5.33
	G. geosporum	4.14	0.51	5.49
	G. mosseae	3.85	0.50	5.45

Table 6.3 Fresh weight yields and shoot concentrations of nutrients of wheat plants in Experiment 1 (day 110). Mean values from each treatment are given, with levels of significance.

	F values	Significance
Phosphorus	16.43	***
Fungi	2.76	NS
Phosph X Fungi	3.93	**

Treatment	0P	2P	4P	LSD Value		
				P05	P01	P001
Phosphorus	3.30	6.40	5.72	1.1	1.5	2.0
Treatment	Control	G.geosporum	G.mosseae			
Fungi	4.64	5.90	4.89	NS	NS	NS
Treatment Interaction	Control	G.geosporum	G.mosseae			
0P	2.19	5.57	2.15			
2P	6.10	5.69	7.42	2.0	2.6	3.4
4P	5.62	6.44	5.10			

Phosphorus Treatment	Fungal Treatment	% Element (DW) Foliage		
		N	P	K
0P	Control	3.36	0.20	5.00
	G. geosporum	3.46	0.31	4.90
	G. mosseae	3.58	0.23	5.20
2P	Control	3.86	0.46	4.50
	G. geosporum	3.92	0.44	4.80
	G. mosseae	ND	ND	ND
4P	Control	4.03	0.52	4.50
	G. geosporum	3.98	0.46	4.90
	G. mosseae	ND	ND	ND

Table 6.4 Ear yields of wheat plants in Experiment 1 (day 170). Mean values from each treatment are given, with levels of significance.

	F values	Significance
Phosphorus	13.65	***
Fungi	1.83	NS
Phosph X Fungi	6.10	***

Treatment	OP	2P	4P	LSD Value		
				P05	P01	P001
Phosphorus	2.40	3.77	3.81	0.6	0.9	1.2
Treatment	Control	G.geosporum	G.mosseae			
Fungi	3.42	3.57	3.00	NS	NS	NS
Treatment Interaction	Control	G.geosporum	G.mosseae			
OP	1.36	3.32	2.52			
2P	4.84	3.45	3.01	1.2	1.8	2.7
4P	4.05	3.92	3.92			

Phosphorus Treatment	Fungal Treatment	% Element (DW) Foliage		
		N	P	K
OP	Control	1.80	0.18	1.20
	G. geosporum	2.40	0.33	1.40
	G. mosseae	1.90	0.23	1.20
2P	Control	2.60	0.38	1.10
	G. geosporum	2.30	0.33	1.30
	G. mosseae	2.40	0.34	1.50
4P	Control	2.60	0.41	1.30
	G. geosporum	2.50	0.39	1.50
	G. mosseae	2.30	0.35	1.30

Table 6.5 Straw yields (F.W.) of wheat plants in Experiment 1 (day 170). Mean values from each treatment are given, with levels of significance.

	F values	Significance
Phosphorus	12.64	***
Fungi	4.83	*
Phosph X Fungi	1.65	NS

Treatment	0P	2P	4P	LSD Value		
				P05	P01	P001
Phosphorus	9.87	15.28	14.64	2.4	3.1	4.1
Treatment	Control	G.geosporum	G.mosseae			
Fungi	11.38	15.03	13.37	2.4	3.1	4.1
Treatment Interaction	Control	G.geosporum	G.mosseae			
0P	6.55	13.60	9.40			
2P	13.09	16.04	16.71	NS	NS	NS
4P	14.51	15.45	13.94			

day 70, presumably due to the increased internal P levels observed (0.37% against 0.21%; Table 6.2B). It was, however, interesting to note that the 2P control plants had significantly higher yields (P0.001) than either of the endophytic 2P treatments, and slightly higher internal P levels (0.54% against 0.47% and 0.45%, Table 2). Both these significant growth effects had also been noted at day 42 when plant heights were measured (data not presented).

By day 110, infection levels of *G. geosporum* and *G. mosseae* at 0P had increased to 36% and 29% respectively (Fig. 6.1), and plants infected with either endophyte had higher internal P levels than the 0P control (Table 6.3C). Total fresh weight yields of *G. geosporum* at 0P were significantly higher (P0.001) than the other two 0P treatments (Table 6.3B). Control plants at 2P produced a higher yield than the equivalent endophytic plants, but this effect was not significant at P0.05. Control plants at 4P produced significantly lower yields than control plants at 2P.

At day 170, high infection levels were observed in the 0P treatments (*G. geosporum* 68% and *G. mosseae* 85%; Fig. 6.1). Infection levels were much lower in the 2P treatments (*G. geosporum* 16% and *G. mosseae* 10%) and extremely low in the 4P treatments. Treatments at 2P and 4P produced significantly higher ear and straw yields (P0.001) than 0P treatments overall (Table 6.4B and 6.5B) in non-inoculated and inoculated plants. The control treatment and *G. geosporum* inoculation resulted in higher ear yields than *G. mosseae* inoculation, but this effect was not significant. *G. geosporum* at 0P produced significantly higher (P0.01) ear yields than the 0P control. Inoculation with *G. mosseae* at 0P also resulted in higher yields than the 0P control, but this was not significant at P>0.05. Table 6.4C shows that plants infected with *G. geosporum* contained almost twice (0.33%) the internal P of the control at 0P (0.18%), with *G. mosseae* between the two (0.23%). The concentration of

shoot P in plants at 0P inoculated with *G. geosporum* was practically the same as that observed in the *G. geosporum* treatment at 2P. There was also no significant difference between the ear yields of these two treatments (Table 6.4B). *G. geosporum* treatments produced significantly higher straw yields (Table 6.5B) than the control treatments (P0.01) and *G. mosseae* treatments (P0.05). The interaction of phosphorus x fungi was not significant at P0.05 despite the apparent differences between the 0P treatments, and the fact that there had been a significant effect noted at the previous harvest (day 110). To eliminate this masking effect, the 0P and 2P treatments were extracted from the table and analysed individually by one way analysis of variance. Although both endophyte treatments at 2P produced higher straw yields than the 2P control(Table 6.5B), this was not significant at P0.05. *G. geosporum*, however, at 0P produced significantly higher straw yields than the equivalent control (P0.001) and plants inoculated with *G. mosseae* (P0.01).

6.3.2. Experiment H1

Infection in the roots of all plants inoculated with one of the three endophytes was slow to develop and spread in this experiment (up to 80 days). At the time of herbicide application (day 107), therefore, fractional infection levels were less than 20% for all endophytes, with *G. monosporum* levels especially low (<10%). Table 6.6 shows final infection levels which were still low. Any effects of herbicide application on VAM levels were consequently on a well developed root system with a late infection and spread of the endophytes.

Table 6.7 shows the effect of the four herbicides on total final fresh weight yields. The highly significant effect of overall herbicide application (F value 20.35), was entirely due to the lowering of yields by Dicurane. This may have been a phytotoxic effect on wheat. The same effect was noted on final ear yields (Table 6.8B) which were also significantly reduced (P0.001). Ceridor and

Harrier significantly increased ear yields (P0.01) over water-sprayed control yields. All three VAM fungi and the control +P treatment significantly increased total yields (Table 6.7B) over the non-inoculated control treatment (P0.05), whilst both *G. geosporum* and *G. mosseae* treatments were significant at P0.001. These inoculant effects were consistent in each individual herbicide treatment including Dicurane. Significantly increased ear yields over water-sprayed control levels (Table 6.8B) were noted for *G. geosporum* (P0.01), *G. mosseae* (P0.05) and control +P (P0.05). All three endophytes significantly increased ear yields (P0.05) over non-inoculated control yields when sprayed with Harrier (*G. geosporum* at P0.001, *G. mosseae* at P0.01 and *G. monosporum* at P0.05). These increased yields produced by *G. geosporum* and *G. mosseae* were apparently related to their higher infection levels within the root systems unlike those of *G. monosporum* at harvest (Table 6.6). Infection by *G. monosporum* and *G. mosseae* was unaffected by herbicide application, but *G. geosporum* infection was significantly higher in the Harrier treatment than the water-sprayed control *G. geosporum* treatment (P0.05).

Control +P treatments resulted in higher internal shoot P levels than the other treatments (Table 6.6), but only plants infected by *G. geosporum* had higher levels than the non-inoculated control. It should be noted that although *G. mosseae* infection had little effect on internal P levels, it was consistent in producing plants with the highest internal N and K levels in all herbicide treatments (Table 6.6). This might explain the increased yields observed despite low internal P levels.

6.3.3. Experiment H2

During the course of this experiment the non-inoculated control treatments, became infected and were therefore omitted from any analyses and as a result only data for the three endophytes are presented. The phytotoxicity (apparently

cumulative) of Dicurane had severely stunted plant growth by the 50 day sampling and so these treatments were also eliminated. It was, however, noted that there had been little effect of Dicurane on VAM infection levels in roots of *G. geosporum* and *G. mosseae* plants at this stage (Fig 6.2), but levels were lower in *G. monosporum* plants compared with its water-sprayed control. This may, however, been due to the poor condition of these plants at the sampling time.

Overall, earlier infection, and therefore, higher final infection levels were observed in this experiment compared with Expt. H1. Herbicide application did not have a significant effect on infection by *G. geosporum*, at harvest, but Ceridor had significantly reduced (P0.05) infection at day 65 (Fig 6.2) relative to the other treatment levels. Ceridor had a similar effect on infection of plants by *G. mosseae* at harvest. Avenge significantly reduced *G. mosseae* infection at day 65 and at harvest relative to water-sprayed control plants and those treated with Harrier. Ceridor and Harrier significantly reduced infection levels of *G. monosporum* below water-sprayed control levels at day 65, but this effect had diminished by harvest.

Table 6.9B shows that, at harvest, Avenge (P0.001), Ceridor (P0.05) and Harrier (P0.001) had significantly reduced yields relative to water-sprayed controls. Plants infected by *G. geosporum* (P0.001) and *G. mosseae* (P0.01) significantly outyielded those infected by *G. monosporum*. This effect was consistent in all herbicide treatments, except in the case of Avenge which significantly (P0.05) reduced yields of plants infected by *G. mosseae* relative to those infected by *G. geosporum*. Yields of all plants inoculated with endophytes and also receiving an Avenge application were reduced relative to water-sprayed controls, and this was significant at P0.001 for the *G. geosporum* and *G. mosseae* treatments.

Inoculation with *G. geosporum* and *G. monosporum* consistently resulted in

Table 6.6 The effect of herbicide applications on VAM infection levels and shoot nutrient concentrations (DW) of wheat at harvest in Experiment H1.

Pesticide Treatment	Fungal Treatment	% Element (DW) in Shoots			% Final VAM Infection*
		N	P	K	
Control	Control (+P)	0.85	0.17	1.39	0
	Control	0.78	0.10	1.34	0
	G.geosporum	0.74	0.11	1.37	31
	G.monosporum	0.74	0.11	1.32	10
	G.mosseae	1.02	0.09	1.89	36
Ceridor	Control (+P)	0.77	0.16	1.43	0
	Control	0.88	0.09	1.42	0
	G.geosporum	0.83	0.10	1.58	23
	G.monosporum	0.02	0.09	1.34	8
	G.mosseae	1.03	0.09	1.78	36
Dicurane	Control (+P)	1.53	0.28	2.48	0
	Control	1.39	0.18	2.54	0
	G.geosporum	1.61	0.22	3.07	37
	G.monosporum	1.36	0.19	2.33	9
	G.mosseae	1.61	0.14	2.83	35
Harrier	Control (+P)	0.85	0.16	1.33	0
	Control	0.76	0.10	1.22	0
	G.geosporum	0.87	0.12	1.58	46
	G.monosporum	0.82	0.09	1.23	14
	G.mosseae	0.99	0.09	1.62	32
Avenge	Control (+P)				0
	Control				0
	G.geosporum	ND*	ND	ND	26
	G.monosporum				12
	G.mosseae				33

*LSD (P0.05) for significant difference between *G. geosporum* treatments = 6.0. Differences between treatments of the other two endophytes were not significant.

ND= DATA

Table 6.7 - Effect of herbicide application on total fresh weight (FW) yields of shoots of wheat at harvest of Experiment H1. Mean values of results are quoted with levels of significance.

	F Value	Significance
Herbicide	20.35	***
Fungi	6.41	***
Herb X Fungi	1.70	NS

Treatment	Control	Dicurane	Ceridor	Avenge	Harrier	LSD		
						P05	P01	P001
Herbicide	24.96	18.59	24.84	24.61	24.74	1.73	2.29	3.0
Treatment	G. mosseae	G. geo-sporum	G.mono-sporum	Control	Control (+P)			
Fungi	25.05	24.81	23.46	21.14	23.38	1.73	2.29	3.0
Treatment Interact'n	G. mosseae	G. geo-sporum	G.mono-sporum	Control	Control (+P)			
Control	25.76	25.36	23.48	22.98	27.24			
Dicurane	20.12	19.82	18.58	15.70	18.74			
Ceridor	27.38	25.16	27.14	22.82	21.70	NS	NS	NS
Avenge	25.00	24.50	24.32	24.20	25.04			
Harrier	26.98	29.22	23.80	20.02	23.70			

Table 6.8 - Effect of herbicide application on ear yields (F.W.) of shoots of wheat at harvest of Experiment H1. Mean values of results are quoted with levels of significance.

	F Value	Significance
Herbicide	106.54	***
Fungi	3.55	*
Herb X Fungi	3.52	***

Treatment	Control	Dicurane	Ceridor	Avenge	Harrier	LSD		
						P05	P01	P001
Herbicide	3.336	0.964	3.796	3.448	3.832	0.33	0.43	0.56
Treatment	G. mosseae	G. geo-sporum	G.mono-sporum	Control	Control (+P)			
Fungi	3.156	3.336	2.996	2.748	3.140	0.33	0.43	0.56
Treatment Interact'n	G. mosseae	G. geo-sporum	G.mono-sporum	Control	Control (+P)			
Control	2.580	3.540	3.340	3.300	3.920			
Dicurane	1.340	1.100	0.780	0.760	0.840			
Ceridor	3.960	3.760	4.360	3.340	3.560	0.73	0.97	1.25
Avenge	3.520	3.640	2.660	3.460	3.960			
Harrier	4.380	4.640	3.840	2.880	3.420			

Table 6.9 Effect of herbicide application on total shoot yields (F.W.) at harvest in Experiment H2. Mean values of results are quoted with levels of significance.

	F values	Significance
Herbicide	18.35	***
Fungi	11.63	***
Herb X Fungi	3.70	**

Treatment	Control	Avenge	Harrier	Ceridor	LSD Value		
					P05	P01	P001
Herbicide	16.38	10.19	12.69	14.04	1.7	2.3	3.0
Treatment	G. monosporum	G. mosseae	G. geosporum				
Fungi	11.32	13.88	14.76	-	1.49	2.0	2.6
Treatment Interaction	G. monosporum	G. mosseae	G.geosporum				
Control	13.56	17.70	17.88	-	3.0	4.0	5.3
Avenge	10.66	8.18	11.72				
Harrier	8.64	13.92	15.50				
Ceridor	12.44	15.74	13.94				

Table 6.10 Effect of herbicide application on the % fractional infection and concentrations of nutrients in shoots of VAM-infected wheat plants at harvest in Experiment H2.

Herbicide Treatment	Fungus	Percentage Element (DW) in Shoots			Final % VAM Infection
		N	P	K	
Control	G.geosporum	2.09	0.12	4.27	72
	G.monosporum	2.17	0.11	4.24	72
	G.mosseae	1.96	0.09	3.29	76
Avenge	G.geosporum	2.83	0.17	4.93	76
	G.monosporum	2.69	0.18	5.18	64
	G.mosseae	2.26	0.10	4.10	43
Ceridor	G.geosporum	2.39	0.10	3.79	63
	G.monosporum	2.17	0.11	4.55	47
	G.mosseae	2.25	0.09	4.21	41
Harrier	G.geosporum	2.57	0.14	4.99	73
	G.monosporum	2.48	0.12	5.03	56
	G.mosseae	2.24	0.10	4.67	65

Table 6.11 Effect of herbicide applications on spore production of VAM fungi at harvest in Experiment H1 (A) and H2 (B), with levels of significance and means of treatments.

(A)

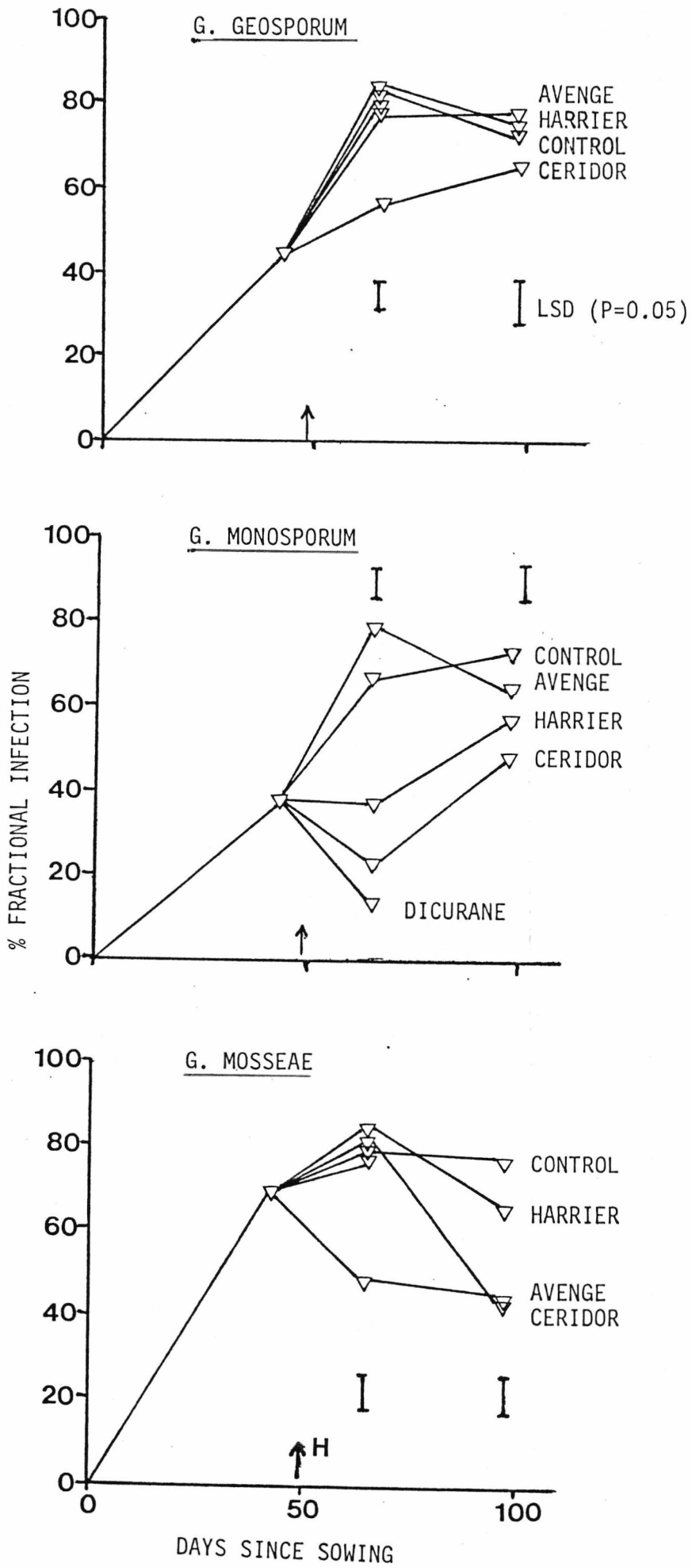
Fungi	Herbicide Treatments with Spores per 10gm (FW) Soil					LSD
	Control	Avenge	Ceridor	Dicurane	Harrier	P05
G. mosseae	1*	1	4	1	3	NS
G. monosporum	0	2	0	1	1	NS
G. geosporum	14	11	8	9	9	NS

(B)

Fungi	Herbicide Treatments with Spores per 10gm (FW) Soil					LSD
	Control	Avenge	Ceridor	Dicurane	Harrier	P05
G. mosseae	18*	6	10	ND	32	12
G. monosporum	12	6	2	ND	12	7
G. geosporum	66	24	30	ND	28	21

* means of four replicates

Figure 6.2 Effects of herbicide applications on VAM infection development in Experiment H1 with levels of significance. Arrows (H) show time of addition of herbicides.



plants with higher shoot P concentrations than plants with *G. mosseae* in all the herbicide treatments (Table 6.10). The higher overall P concentrations observed in the Avenge treatments were probably due to their reduced growth relative to water-sprayed controls (seen in Dicurane treatments in Expt. H1). N/P/K levels of plants infected by *G. mosseae* and sprayed with Avenge were lower than those in both of the other endophytes, and this should be noted with the fact that Avenge also significantly reduced *G. mosseae* infection levels immediately following its application (Fig. 6.2).

In terms of spore production, only low spore numbers were isolated at the end of Expt. H1 in all treatments including controls, and therefore no significant effects of herbicide application were observed (Table 6.11A). At the end of Expt. H2, however, differences were noted between herbicide treatments (Table 6.11B). Harrier significantly increased (P0.05) *G. mosseae* spore numbers relative to controls, whilst Avenge significantly reduced (P0.05) spore numbers. Ceridor significantly reduced (P0.05) spore numbers of *G. monosporum*. All three herbicides significantly reduced spore numbers of *G. geosporum*.

6.3.4. Experiment F1

Table 6.12 shows the results after 75 days, the time of Bavistin application, but after Milgo and Sportak sprays. Overall, shoot fresh weights were unaffected by fungicide application, but yields of the Milgo/Sportak (M/S) treatments were noticably lower than the water-sprayed controls. Both *G. geosporum* (P0.05) and *G. monosporum* (P0.01) significantly increased yields over *G. mosseae* and non-inoculated control treatments. This trend was reflected in the shoot P concentrations (Table 6.12C) where plants infected by *G. geosporum* and *G. monosporum* had twice that of the non-inoculated control levels. It should be noted that *G. mosseae* plants were much slower to develop internal infection than the other two endophytes up to day 39 and this may have had an effect on

reducing any response to infection. *G. geosporum* and *G. mosseae* infection levels were unaffected by fungicide applications at this stage, but *G. monosporum* infection levels were significantly (P0.05) lower than the water-sprayed controls after Milgo application and in the M/S treatments (Fig. 6.3).

Table 6.13B shows results after 104 days revealing no significant effect of fungicide application on yields, however, Sportak and M/S/B treatments had noticably lower yields than water-sprayed controls. All three endophytes significantly increased yields relative to non-inoculated control. There were no significant interactions. The infection levels of all three endophytes were significantly lower (P0.05) in the M/S/B treatment compared with the water-sprayed controls (Fig. 6.3). Infection was also significantly lower in the Bavistin sprayed treatments of *G. monosporum* and *G. mosseae* and in the Sportak treatment on *G. mosseae*. All VAM infected plants contained higher internal shoot P concentrations than uninfected controls (Table 6.13C). The effect of the M/S/B treatment in reducing infection levels of *G. geosporum* was reflected in the lower internal P concentration recorded in comparison with that of the other two endophytes. It was, however, still higher than the uninfected control treatment. Of note, again, is the slightly higher levels of N and K recorded in *G. mosseae* infected plants.

At harvest, day 135, there was still no overall effect of fungicide application nor significant interactions (Table 6.14). Plants infected with *G. geosporum* (P0.01) and *G. monosporum* (P0.001) produced significantly higher yields than non-inoculated controls. *G. monosporum*-infected plants also significantly outyielded those infected with *G. mosseae* (P0.001). Plants infected with *G. mosseae* outyielded the non-inoculated control but this effect was not significant. The M/S/B treatment reduced or delayed ear formation compared with the water-sprayed controls, and this effect is reflected in the internal shoot P

concentrations observed (Table 6.14C), with only plants infected with *G. monosporum* having a higher internal P concentration than the non-inoculated control.

6.3.5. Experiment F2

Infection of plants in this experiment was slow, especially for *G. mosseae* (Table 6.15) and as a result spraying was delayed. Subsequently a plant disorder causing foliar discolouration of the leaf tips and poor plant vigour curtailed the experiment and caused the elimination of the planned Bavistin application.

Infection levels increased little after 86 days, reaching a maximum of between 30-40% for the three endophytes (Table 6.15). The control +P treatment did not reduce infection levels of *G. geosporum* and *G. mosseae* but caused a reduction in plants infected with *G. monosporum* after 86 days. The spray applications had little effect on infection levels. All plants infected by the endophytes had higher levels of N/P/K than non-inoculated controls (Table 6.16) and also higher than the control +P treatment at harvest (Table 6.16C). There was no effect of fungicide application or fungal treatment on the ear yields (Table 6.17), but there were significant effects on total yields (Table 6.18). Both control +P and Milgo treatments significantly increased total yields (P0.05) relative to control levels. *G. monosporum*, however, was the only endophyte to significantly increase yields relative to non-inoculated control treatment levels. This was unexpected since all three endophytes contained higher internal shoot N/P/K levels than control treatments at harvest (Table 6.16C) with *G. monosporum* the lowest of the three. This anomaly may relate to the plant disorder noted.

With respect to spore production, individual applications of Sportak, Bavistin and the M/S/B treatment had all significantly decreased the spore numbers of *G. geosporum* and *G. mosseae* during Expt. F1 (Table 6.19A). By the end of Expt. F2 Milgo, Sportak and M/S treatments had all significantly decreased spore levels of *G. geosporum* (Table 6.19B). In both Expt. F1 and Expt. F2 Milgo significantly increased spore production of *G. monosporum* whilst spore numbers in the Sportak and M/S/B treatments were lower than control levels. The addition of phosphorus in Expt. F2 significantly reduced spore production of *G. mosseae*.

6.3.6. Experiment F3

Infection spread, particularly by *G. geosporum* and *G. mosseae*, was slow up to the time of spraying at 54 days. Plants receiving the Calixin application produced the highest final infection levels with all three endophytes, and these were significantly higher than the water-sprayed controls for *G. geosporum* and *G. mosseae* (Figs. 6.4 and 6.5A). Bavistin significantly reduced infection relative to water-sprayed control levels at all samplings for all three endophytes. Tilt Turbo significantly reduced infection of *G. geosporum* and *G. monosporum* relative to water-sprayed control levels at harvest, and *G. mosseae* after 75 days. These trends, at harvest, were reflected by the internal N/P/K shoot concentrations (Table 6.21 and Fig. 6.5B) since all three endophytes had slightly higher levels of P than non-mycorrhizal controls in the water-sprayed control and Calixin treatments but not so in the Bavistin and Tilt treatments. Indeed, Bavistin reduced internal N/P/K levels relative to non-mycorrhizal control levels, whilst Calixin increased them. There was no overall effect of fungicide application on final yields of wheat (Table 6.20) but Calixin yields were the highest. *G. geosporum* and *G. monosporum* significantly increased ($P<0.01$) yields above non-mycorrhizal controls despite the slow infection. Few spores were noted at harvest so spore counts were not made.

Table 6.12 Effect of fungicide applications on shoot yields (F.W.) of wheat and shoot nutrient concentrations after 75 days in Experiment F1 with levels of significance and means of treatments.

	F values	Significance
Pesticide	1.37	NS
Fungi	4.91	**
Pesticide X Fungi	1.56	NS

Treatment	Control	Milgo	Sportak	M/S	LSD Value		
Pesticide	7.10	6.15	6.78	5.96	NS	NS	NS
Treatment	G. mosseae	G.monosporum	G. geosporum	Control	P05	P01	P001
Fungi	5.64	7.66	7.02	5.66	1.3	1.7	2.2
Treatment Interaction	G. mosseae	G.monosporum	G.geosporum	Control			
Control	6.44	6.42	8.82	6.72			
Milgo	5.16	7.66	5.58	6.20	NS	NS	NS
Sportak	5.26	9.44	7.58	4.84			
M/S	5.70	7.12	6.12	4.90			

Fungicide Treatment	Fungal Treatment	% Element (DW) in Shoots			% VAM Infection
		N	P	K	
Control	Control	1.53	0.06	2.61	0
	G.geosporum	1.41	0.11	2.78	57
	G.monosporum	1.41	0.12	2.37	65
	G.mosseae	1.51	0.07	2.81	70

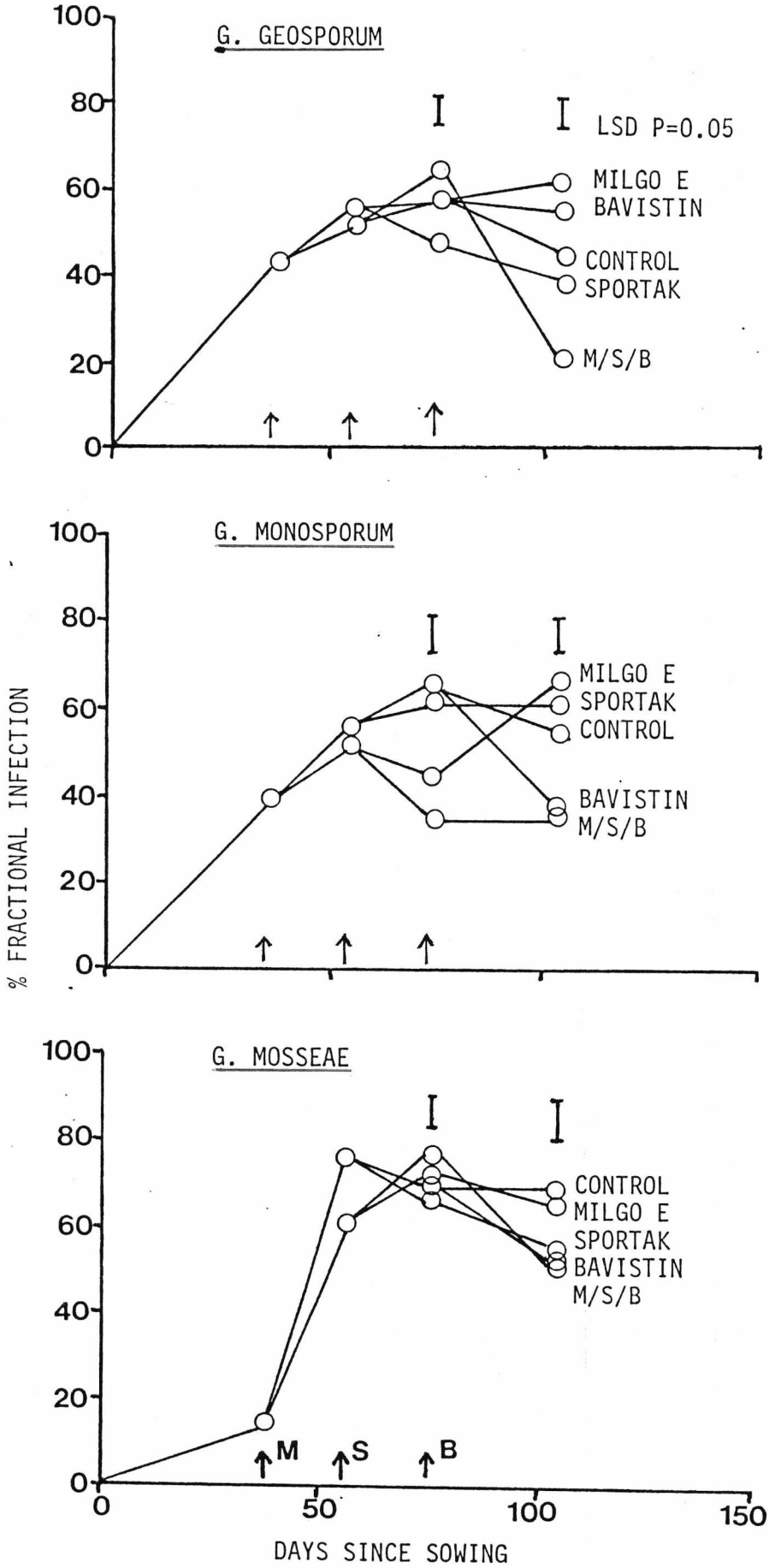


Figure 6.3 Effects of fungicide applications on development of VAM infection in Experiment F1. Error bars show levels of significance (LSD, P = 0.05). (M = Milgo, S = Sportak, B = Bavistin applications).

Table 6.13 Effect of fungicide applications on shoot yields (FW) of wheat and shoot nutrient concentrations after 104 days in Experiment F1 with levels of significance and means of treatments

	F values	Significance
Pesticide	2.08	NS
Fungi	16.10	***
Pesticide X Fungi	1.76	NS

Treatment	Control	Milgo	Sportak	Bavistin	M/S/B	LSD	
Pesticide	10.34	9.76	7.97	10.85	8.69	NS	
Treatment	G.geo-sporum	G.mono-sporum	G. mosseae	Control	P05	P01	P001
Fungi	10.07	13.28	8.45	6.30	2.1	2.7	3.5
Treatment Interaction	G.geo-sporum	G.mono-sporum	G. mosseae	Control			
Control	13.10	14.56	7.36	6.36	NS	NS	NS
Milgo	8.46	14.42	9.14	7.04			
Sportak	8.08	8.68	8.68	6.44			
Bavistin	10.36	18.36	7.98	6.70			
M/S/B	10.36	10.36	9.10	4.96			

Pesticide Treatment	Fungal Treatment	% Element (DW) in Shoots			% VAM Infection
		N	P	K	
Control	Control	2.30	0.07	4.09	0
	G.geosporum	2.36	0.12	3.21	44
	G.monosporum	2.04	0.09	3.17	54
	G.mosseae	2.30	0.09	4.21	69
M/S/B	Control	2.07	0.05	3.47	0
	G.geosporum	1.98	0.07	3.26	21
	G.monosporum	2.19	0.12	2.68	36
	G.mosseae	2.25	0.08	3.81	52

Table 6.14 Effect of fungicide applications on shoot yields (FW) of wheat and shoot nutrient concentrations after 135 days in Experiment F1 with levels of significance and means of treatments.

	F values	Significance
Pesticide	0.91	NS
Fungi	14.75	***
Pesticide X Fungi	1.22	NS

Treatment	Control	Milgo	Sportak	Bavistin	M/S/B	LSD	
Pesticide	12.63	13.96	11.62	11.69	14.41	NS	
Treatment	Control	G. mosseae	G.geo-sporum	G.mono-sporum	P05	P01	P001
Fungi	8.17	10.86	13.47	18.95	3.4	4.5	5.8
Treatment Interaction	Control	G. mosseae	G.geo-sporum	G. mono-sporum			
Control	9.68	10.02	11.22	19.62			
Milgo	6.92	12.82	17.90	18.20			
Sportak	6.64	11.34	12.08	16.42	NS	NS	NS
Bavistin	9.96	10.62	12.10	14.10			
M/S/B	7.66	9.52	14.06	26.40			

Pesticide Treatment	Fungal Treatment	% Element (DW) in Shoots		
		N	P	K
Control	Control	2.18	0.07	3.47
	G.geosporum	2.40	0.10	3.58
	G.monosporum	2.22	0.10	2.91
	G.mosseae	2.36	0.10	3.83
M/S/B*	Control	2.08	0.14	0.94
	G.geosporum	2.09	0.14	1.34
	G.monosporum	2.13	0.18	1.16
	G. mosseae	2.11	0.14	1.07

Table 6.15 VAM infection development in the fungicide treatments of Experiment F2 with levels of significance and means of treatments

Fungal Treatment	Pesticide Treatment	Days since Sowing		
		86	128	186
G. geosporum	Control	39	33	39
	Control (+P)	44	ND	33
	Milgo E	30	28	41
	Sportak	16	42	30
	M/S/B	10	ND	34
LSD (P05)		8.0	6.0	6.0
G. mosseae	Control	15	22	23
	Control (+P)	17	ND	36
	Milgo E	13	21	20
	Sportak	8	19	24
	M/S/B	11	ND	30
LSD (P05)		NS	NS	7.0
G. monosporum	Control	32	26	24
	Control (+P)	8	ND	20
	Milgo E	24	13	34
	Sportak	29	21	32
	M/S/B	25	ND	32
LSD (P05)		7.0	5.0	5.0

Table 6.16 Concentrations of nutrients in shoots of wheat after 86(A), 128(B) and 186(C) days of Experiment F2.

(A)

Fungal Treatment	% Element (DW) in Shoots		
	N	P	K
Control	2.62	0.24	5.45
G.geosporum	3.17	0.30	6.16
G.monosporum	3.13	0.33	6.34
G.mosseae	3.20	0.30	6.34

(B)

Fungal Treatment	% Element (DW) in Shoots		
	N	P	K
Control(+P)	1.44	0.39	3.30
Control	1.47	0.20	3.45
G.geosporum	2.44	0.29	4.83
G.monosporum	1.86	0.27	4.05
G.mosseae	1.92	0.26	4.14

(C)

Pesticide Treatment	Fungal Treatments	% Element (DW) in Shoots		
		N	P	K
Control	Control	0.73	0.11	1.63
	G.geosporum	1.16	0.29	2.30
	G.monosporum	0.96	0.16	2.08
	G.mosseae	1.21	0.22	2.32
Control (+P)	Control	0.70	1.15	1.38
	G.geosporum	0.81	0.20	1.55
	G.monosporum	0.69	0.16	1.46
	G.mosseae	0.81	0.19	1.58
Milgo E	Control	0.78	0.11	1.61
	G.geosporum	1.27	0.24	2.89
	G.monosporum	0.99	0.16	1.86
	G.mosseae	1.11	0.22	2.50
Sportak	Control	0.73	0.11	1.64
	G.geosporum	1.27	0.22	2.64
	G.monosporum	1.08	0.18	2.11
	G.mosseae	1.03	0.16	2.18

Table 6.17 Effect of fungicide application on ear yields (FW) of VAM-infected and uninfected wheat plants at harvest of Experiment F2. Mean values from each treatment are given with levels of significance.

	F Values	Significance
Pesticide	0.91	NS
Fungi	2.31	NS
Pesticide + Fungi	2.02	NS

Treatment	Control	Control (+P)	Milgo	Sportak	LSD
Pesticide	2.98	3.14	2.90	2.65	NS
Treatment	*G. mosseae*	*G.geo-sporum*	*G.mono-sporum*	Control	
Fungi	2.52	3.16	3.20	2.77	NS
Treatment Interaction	*G. mosseae*	*G.geo-sporum*	*G.mono-sporum*	Control	
Control	2.50	3.74	2.60	3.08	NS
Control (+P)	2.42	3.82	3.68	2.64	
Milgo	2.36	2.90	3.98	2.36	
Sportak	2.82	2.20	2.56	3.02	

Table 6.18 Effect of fungicide application on total yields (FW) of VAM-infected and uninfected wheat plants at harvest of Experiment F2. Mean values from each treatment are given, with levels of significance.

	F values	Significance
Pesticide	3.42	*
Fungi	3.67	*
Pesticide X Fungi	1.21	NS

Treatment	Control	Control (+P)	Milgo	Sportak	LSD Value		
					P05	P01	P001
Pesticide	16.02	19.74	19.71	17.21	2.9	3.8	5.0
Treatment	G. mosseae	G.geo-sporum	G.mono-sporum	Control			
Fungi	16.18	18.22	20.77	17.42	2.9	3.8	5.0
Treatment Interaction	G. mosseae	G.geo-sporum	G.mono-sporum	Control			
Control	13.84	18.48	17.36	14.42			
Control (+P)	17.84	20.32	20.54	20.26	NS	NS	NS
Milgo	15.44	19.20	26.00	18.20			
Sportak	17.60	14.88	19.20	16.80			

Table 6.19 Effect of fungicide application on spore production of VAM fungi in Experiments F1 (A) and F2 (B). Mean values from treatments with levels of significance.

(A)

Fungi	Fungicide Treatments with Spores per 10g (FW)					LSD
	Control	Sportak	Bavistin	Milgo E	M/S/B	P05
G. geosporum	147*	62	89	139	77	42
G. monosporum	25	13	25	67	13	13
G. mosseae	58	25	16	20	6	12

(B)

Fungi	Fungicide Treatments with Spores per 10g (FW)					LSD
	Control	Control +P	Milgo E	Sportak	M/S	P05
G. geosporum	200*	218	128	27	100	28
G. monosporum	22	13	43	25	16	11
G. mosseae	37	14	15	20	90	23

* means of 4 replicates

Table 6.20 Effects of fungicide application on total yields (FW) of wheat at harvest in Experiment F3. with levels of significance and means of treatments

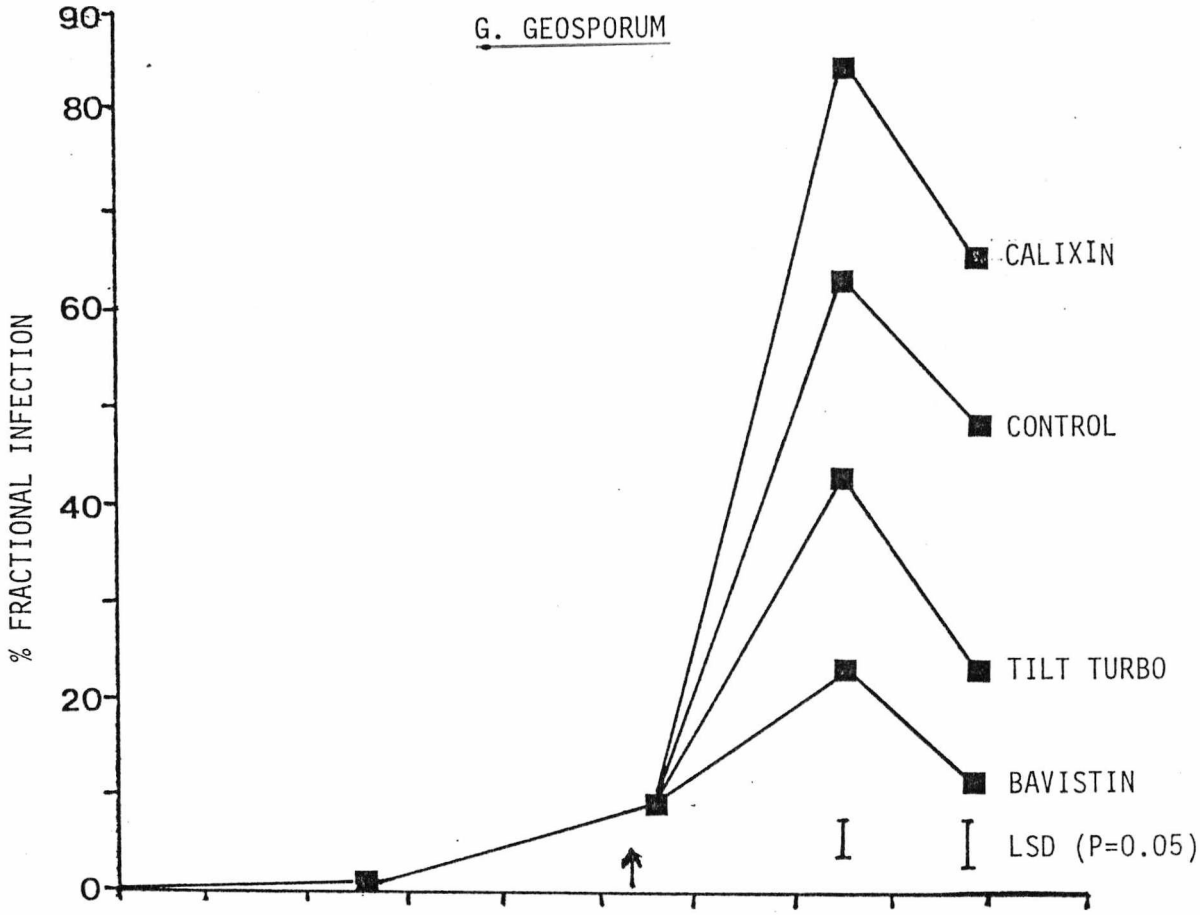
	F value	Significance
Pesticide	1.08	NS
Fungi	5.42	**
Pesticide + Fungi	0.18	NS

Treatment	Tilt	Bavistin	Calixin	Control	LSD		
					P05	P01	P001
Pesticide	11.82	11.04	12.64	10.64	NS	NS	NS
Treatment	G. mosseae	G. monosporum	G. geosporum	Control			
Fungi	10.99	13.44	12.72	8.99	2.4	3.2	4.3
Treatment Interaction	G. mosseae	G. monosporum	G. geosporum	Control			
Tilt	10.87	13.65	13.75	9.00			
Bavistin	10.12	12.72	12.85	8.47			
Calixin	13.17	14.95	12.95	9.50	NS	NS	NS
Control	9.80	12.45	11.32	8.97			

Table 6.21 Concentration of nutrients in shoots of wheat at harvest in Experiment F3.

Pesticide Treatment	Fungal Treatment	% Element (DW) in Shoots		
		N	P	K
Control	Control	1.95	0.06	4.21
	G.geosporum	2.06	0.08	4.56
	G.monosporum	1.86	0.08	4.23
	G.mosseae	2.17	0.07	4.18
Bavistin	Control	2.37	0.07	4.33
	G.geosporum	1.83	0.06	3.72
	G.monosporum	1.90	0.06	4.13
	G.mosseae	1.93	0.06	3.70
Tilt Turbo	Control	1.85	0.06	3.75
	G.geosporum	1.96	0.07	3.88
	G.monosporum	1.83	0.07	3.46
	G.mosseae	1.94	0.06	4.03
Calixin	Control	1.98	0.06	4.35
	G.geosporum	2.08	0.08	4.46
	G.monosporum	2.15	0.09	4.82
	G.mosseae	2.19	0.09	4.86

Figure 6.4 - Effects of fungicide applications on development of VAM infection in Experiment F3. Error bars show levels of significance (LSD, P=0.05). Arrows (F) show time of addition of fungicides.



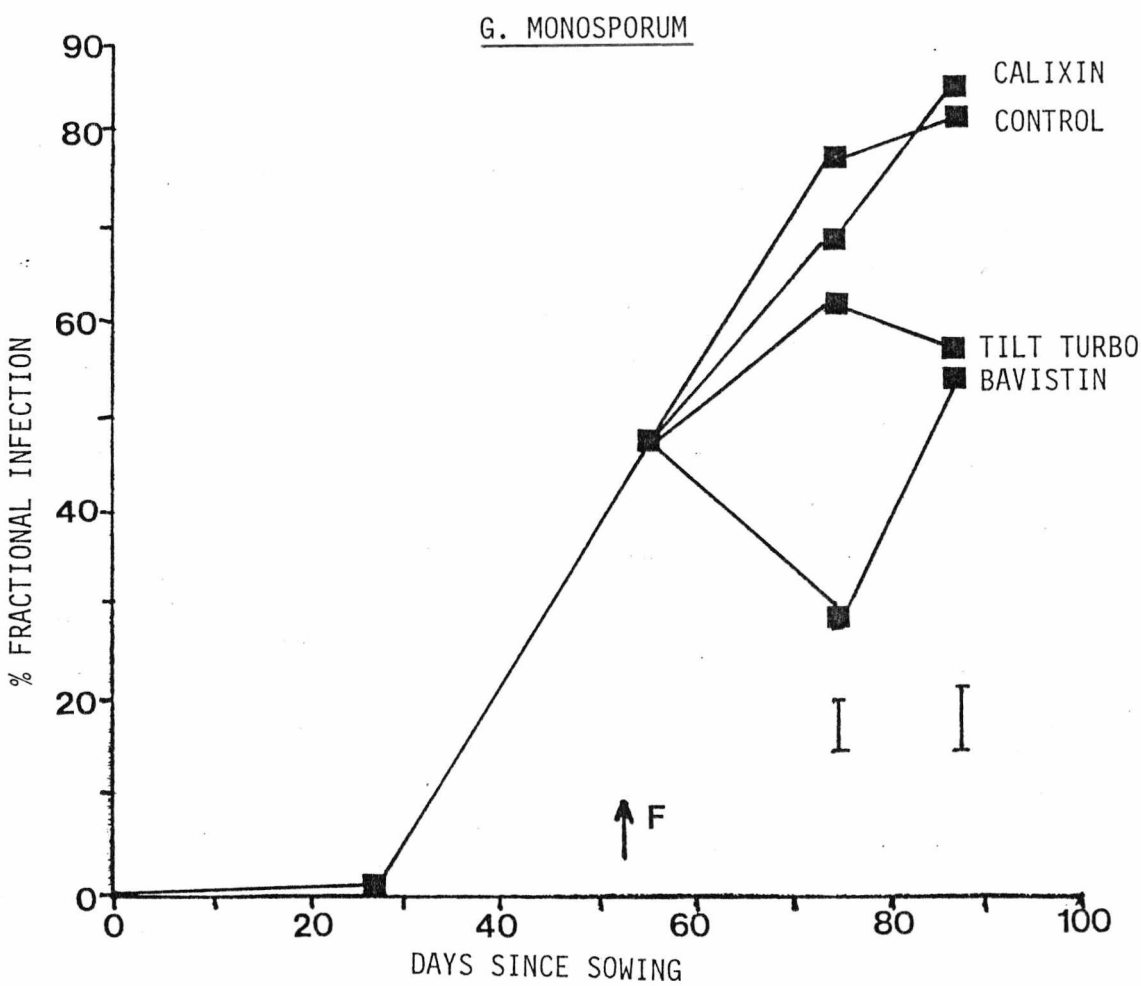
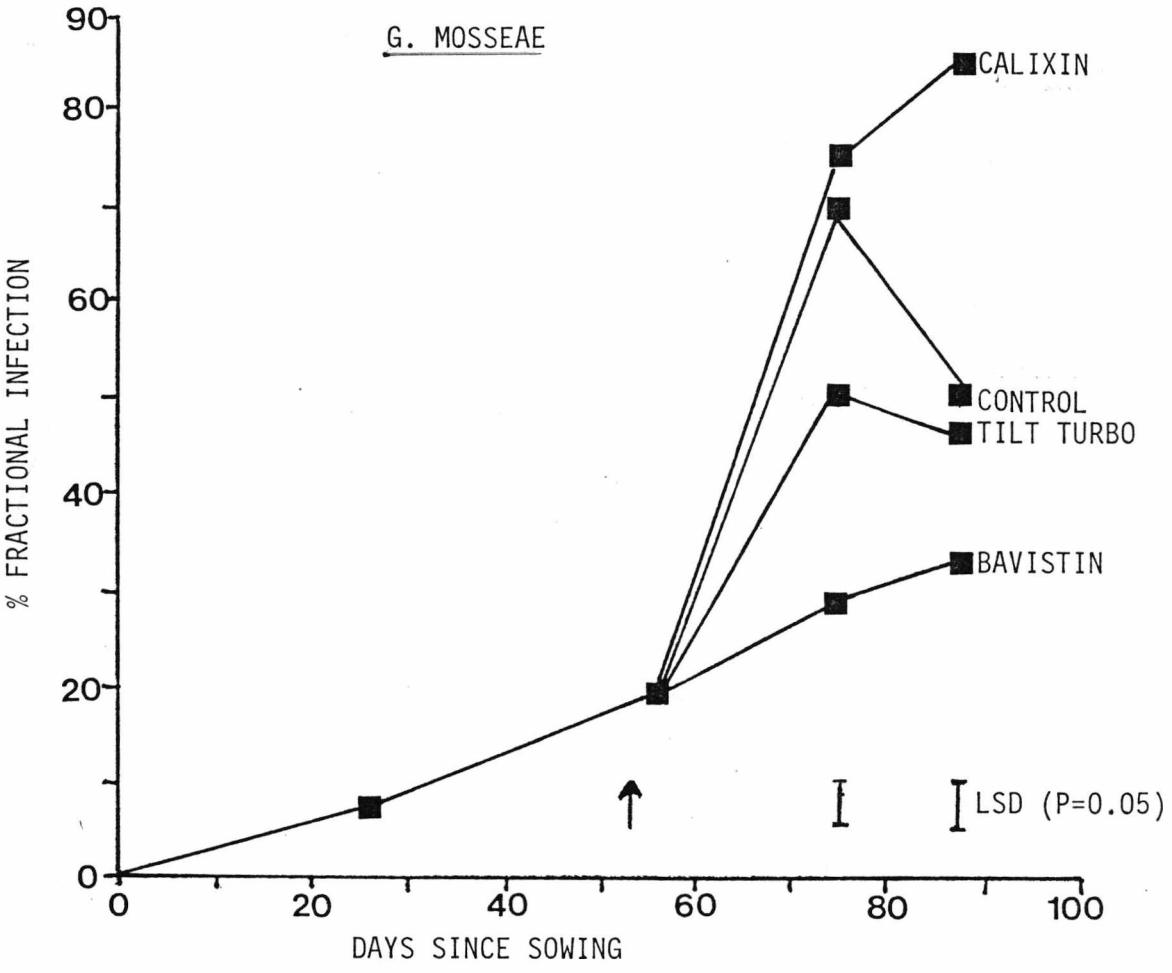


Figure 6.5A - Effects of fungicide applications on development of VAM infection in Experiment F3 (continued).



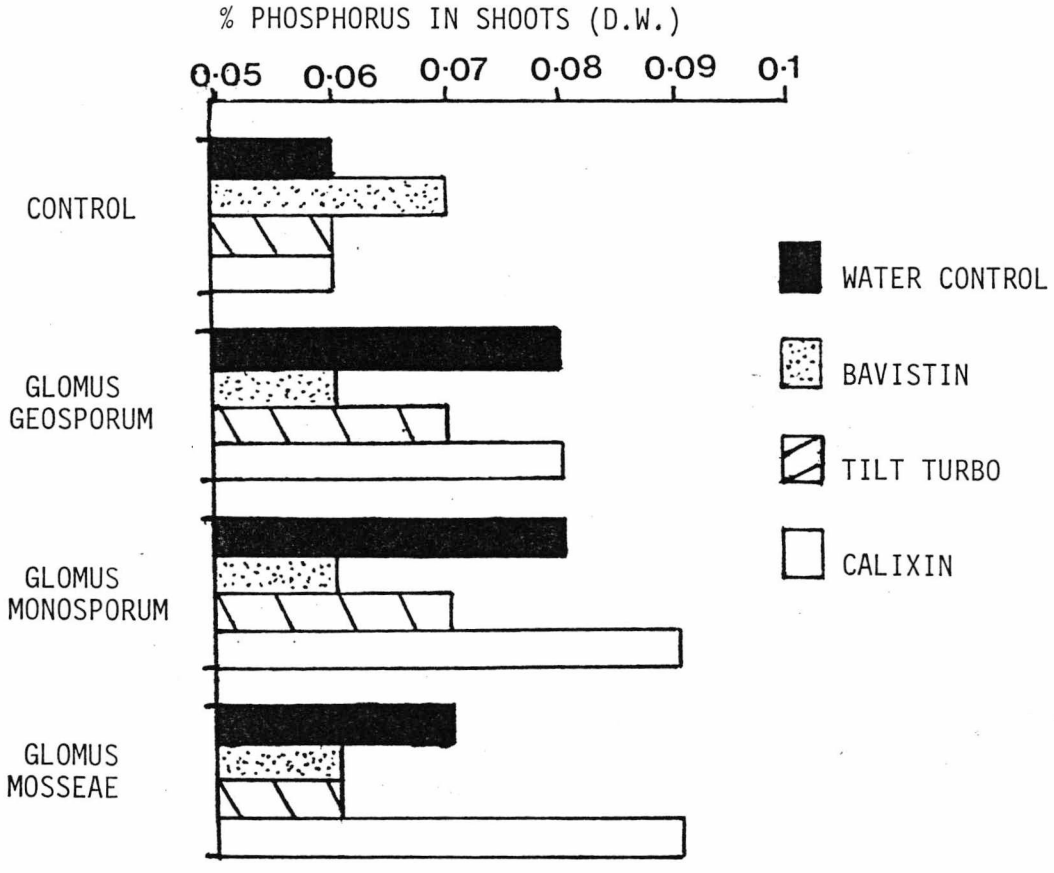


Figure 6.5B - Effects of fungicide applications on final shoot phosphorus concentrations at harvest of Experiment F3.

6.4. DISCUSSION

6.4.1. Experiment 1

As in previous studies (e.g. Kiernan *et al.* 1983), increasing soil phosphorus was correlated with decreased infection levels of both VAM endophytes tested, in the root systems of winter wheat (Fig. 6.1). It was noted in this study, however, that the 2P phosphorus level (approx. 50ppm Olsen extractable P) in the growing medium was adequate for non-mycorrhizal wheat growth but led to a significant reduction in both VAM infection and ear yields in mycorrhizal plants (Table 6.4). The final infection levels of *G. geosporum* and *G. mosseae* in these two treatments were 16% and 10% respectively, and the infection did not appear abnormal (i.e. there were arbuscules present). Crush (1976) frequently noted that VAM fungi were deleterious to legume crops which were heavily fertilised (high soil P), and apparent pathogenicity has also been noted with sparse VAM colonisation (Crush, 1976; Hall *et al.* 1977). Davis and Young (1985) showed that 10% infection levels of *Gig. margarita* depressed wheat growth in pots. Kiernan *et al* (1983), however, observed that several VAM endophytes, tested over a range of soil P levels, inhibited plant growth.

This occurred at different P levels and with different infection levels of various endophytes (4-59%). Furthermore, Koide (1985) noted a permanent decrease in the leaf area of VAM-infected sunflowers when grown in a soil of moderate P availability (15$\mu g\ g^{-1}$), whilst such an effect was transient in a soil of low P availability (5$\mu g\ g^{-1}$). These observations can be explained by considering the nutrient drain imposed by the presence of the symbiont. Snellgrove *et al* (1982) showed that a VAM endophyte increased transport of fixed carbon to the roots of leek plants by about 7% of the total and suggested that about 10% may be an average value for well-infected mycotrophic species under steady growth conditions. This loss of photosynthate maybe compensated for in VAM-infected

plants (growing in low soil P conditions) by increased uptake of nutrients stimulating plant growth. If, however, the plant can obtain sufficient P from the soil solution without the aid of a VAM symbiont, it could be that infection would be essentially parasitic. Bethlenfalvay *et al* (1982) showed that the early plant growth inhibition of soybean infected by *G. fasciculatum* was a result of carbohydrate demand on the host by the endophyte. It is known that the carbon requirement for growth of grain is met largely by photosynthesis carried out after anthesis, whilst the number of grains are determined before anthesis. The nitrogen requirement can on the other hand be provided to a large extent by nitrogen taken up before anthesis (Spiertz and Vos, 1985). Since grain analysis of the VAM infected plants in the 2P treatments also revealed lower N concentrations, it may be that a combination of carbon drain and reduced transfer of N to the grain could have inhibited ear development. In this case, the fungal infection sites could have acted as an opposing metabolic 'sink' to the grain, throughout plant growth. The presence of a fungal-specific (VAM) alkaline phosphatase localised in the mature arbuscles of *G. mosseae* infecting onion has been shown (Gianinazzi *et al*, 1979). This phosphatase activity was inhibited by increasing phosphate concentrations in the incubation medium of onion root extracts (Gianinazzi-Pearson and Gianiniazzi, 1978). It is believed, therefore, that this enzyme could aid the transfer of P from the polyphosphate granules, localised in the same vacuoles as this alkaline phosphatase activity (Cox *et al*., 1975), which the VAM fungus moves from its external hyphal network into the root cortex (Capaccio and Callow, 1982). If the inner root cortical cells have adequate levels of P, this could halt the transfer of P across the arbuscular/host plasmalemma interface. In reacting to such a situation it has been shown that the fungus is capable of rapid polyphosphate synthesis, possibly via an increase in the activity of polyphosphate kinase (Capaccio and Callow, 1982). If arbuscles

are active without transfering such nutrients as P it may be acting as a type of haustorium and drain photosynthate from the host. F. Amijee (Rothamsted Experimental Station) has also observed growth depressions in *G. mosseae* infected leeks grown at high soil P levels (pers. comm.). This aspect of VAM/host interactions needs further study since high soil P levels are commonly found in intensive agricultural systems, correlated with low VAM infection levels (Black and Tinker, 1979; Dodd and Jeffries, 1986).

A second point derived from this experiment is the potential efficiency of the two endophytes in relation to their effects on plant yields and nutrient uptake. *G. geosporum* (0P) significantly increased yields over non-mycorrhizal controls (Tables 6.2-6.5) at all samplings, whilst plants infected with *G. mosseae* (0P) had higher yields than the control plants only at harvest. This may relate to its initial slow infection spread up to day 70. It should be noted that the growth response in *G. geosporum* infected plants (0P) noted at harvest was evident as early as the first sampling, indicating an early stimulatory effect (Table 6.2). Plants infected by *G. geosporum* already had twice the internal P concentration and fresh weight of the non-mycorrhizal control (0P). This almost certainly indicates the the that rapid early infection of young root systems is necessary for a beneficial growth response to occur. In Expt. F1 (Fig. 6.3) *G. mosseae* was again slow to infect and at the end of the experiment the same trend of 'efficiency' was observed as this experiment, with *G. monosporum* giving even higher final yields than *G. geosporum*. It should, however, be noted that this trend in 'efficiency' also occurred in Expts. F2 and F3 where all three endophytes were slow to infect and it might therefore be assumed that under these growth conditions *G. mosseae* is less efficient than the other two endophytes in promoting plant growth and P uptake. In contrast *G. mosseae* appeared the most efficient of these three endophytes in a sand of pH8.0 used in

Experiment 1 of chapter 8 and was also the only endophyte to infect peppers grown in a high P soil (Olsen P 76 mg L^{-1}) at a pH of 7.3 in a commercial greenhouse at Wye College, with 30% of the roots infected (Dodd and Jeffries unpublished data).

Significantly increased yields of VAM-infected plants relative to non-mycorrhizal controls were still revealed after late infection with *G. mosseae* producing overall the highest plant growth (Table 6.7). A feature of this late stimulation of growth by *G. mosseae* was not higher shoot P levels but higher internal N concentrations (Table 6.6). This has also been noted with *G. caledonium* infection of lucerne (Nielson and Jensen, 1983). As a comparison, Ames *et al* (1983) observed that celery infected by *G. mosseae* took up more ^{15}N from two ammonium nitrogen sources (organic and inorganic), than non-infected plants. Their plants, however, had very low infection levels (<7%) and there was no significant growth response. The occurrence of late infection in Expt. H1 did not eliminate a growth response as seen by increased ear yields of VAM-infected plants (Table 6.8). It may therefore be that late season infection of winter wheat, in low soil P conditions, could have a beneficial role to play in the pre-anthesis period, since yields of *G. mosseae* plants were signifcantly higher than control +P plants (Table 6.7) despite having lower foliar P. It is probable that deficiencies of other nutrients like N and K helped to highlight the beneficial effects of VAM infection.

6.4.2. Herbicides

The main effect noted in **H** 1 was the apparent phytotoxic action of Dicurane (a.i. chlortoluron) when applied as a foliar spray. It caused a significant reduction (P0.001) in final ear and total yields associated with higher internal nutrient levels (Tables 6.6-6.8). Spokes *et al* (1981) also noted that chlortoluron incorporated directly into the soil medium, reduced germination and

prevented normal growth of onion in a glasshouse experiment at a a field rate equivalent to 4kg ha^{-1}. Potential phytoxicity problems of chlortoluron should be noted when used in pot studies since it would appear to have a powerful residual action. Soil taken from a field in S. Kent, which had received a Dicurane application three months previously, totally inhibited normal radish growth when used in a pot study (Dodd, personal observation). Dicurane, however, had no effect on VAM infection levels and little effect on spore germination (see previous chapter). Plants infected with *G. mosseae*, in Expt. H1, had improved final ear and total yields relative to non-inoculated control levels and may, therefore, have helped alleviate the phytotoxic effects of chlortoluron. Ocampo and Barea (1985) also noted that VAM fungi seemed to aid wheat plant recovery from the phytotoxic low doses of a carbamate herbicide (a photosynthesis inhibitor).

There were transitory or no inhibitory effects of the other herbicides tested in Expt. H1 on VAM infection levels. Ceridor and Harrier did tend to increase the total yields of VAM-infected plants and significantly increased ear yields (Table 6.8). Both these herbicides contain the translocatable hormone-like active moiety mecoprop, along with a contact herbicide ingredient, as outlined in the previous chapter. It is interesting to note that Nilsson (1973) showed that mecoprop enhanced 'take-all' disease caused by *Gaeumannomyces graminis* in pot experiments at low concentrations. It was suggested that susceptibility of the host to infection was increased due to the malformation of the wheat roots after mecoprop application, allowing a more rapid invasion of the fungus than into healthy roots. Since root parameters were not measured in this study it is not clear if these herbicides increased the length of infected roots causing changes in efficiency of nutrient uptake or whether the hormone-like activity had a beneficial effect on the fungi directly. It is known that some VAM fungi can produce substances with hormone-like activity under axenic conditions (Barea

and Azcon-Aguilar, 1982) and within the plant (Allen *et al.*, 1980), it may therefore be that a synergistic hormonal effect is affecting plant growth. The role of hormones in VAM associations requires further study. The low levels of infection observed in *G. monosporum* treatments in Expt. H1 (<14%) were probably responsible for the observed reduction in the beneficial effect on plant growth compared with the other two endophytes.

Expt. H2 revealed the effects of a second spray treatment on the same growth environment. Allowing for the problems of contamination of the controls, the reduced plant vigour caused by these herbicide applications was probably due to phytotoxicity problems caused by the accumulation of residues in the durite. Dicurane treated plants died 15 days after spraying and yet only plants infected with *G. monosporum* showed significantly lower root infection levels than the water-sprayed treatments (Fig 6.2). Avenge significantly reduced (P0.001) overall total yields and yet had no effect on the root infection levels of either *G. geosporum* or *G. monosporum*, and *G. mosseae* levels were significantly reduced only at the time of harvest. Ceridor generally lowered the infection levels of each endophyte at 15 days after application, and also at harvest but was the least inhibitory of plant growth.

Spore levels were generally reduced or unaffected over the duration of the two experiments following herbicide applications. This would accord with previous work reporting that herbicide application had little effect on VAM sporulation when equivalent field rates were used, although occasional reductions may be observed at higher doses (Pope and Holt, 1981; Smith *et al.* 1981; Sylvia and Schenck, 1983).

Although herbicide applications seem to have had few apparent direct effects on VAM infection parameters, it is noteworthy that recent work has indicated depressed metabolic activity of *G. mosseae* colonising alfalfa and wheat root

systems following spraying with a photosynthesis inhibitor herbicide. This was recorded 48 hours after application and may be short term since Santakunari and Das (1972) concluded that plants recover their normal metabolic activity and growth 72 hours after herbicide application.

6.4.3. Fungicides

Results of these three experiments affirmed the fungitoxic effect of foliar applied Bavistin (a.i. carbendazim) on VA mycorrhizas in pot experiments, at equivalent field rate applications. The thiazoles (benomyl, carbendazim and thiabendazole) are particularly suppressive of the Zygomycotina but less effective with most members of the Ascomycotina and Basidiomycotina. The Endogonaceae have been placed in the Zygomycotina, albeit in a separate order, the Endogonales, (Benjamin, 1979) and the effects of benomyl on the VAM symbiosis support this placement. Benomyl, which breaks down to give the active moiety carbendazim, has frequently been incorporated into the soil of VAM-infected plants and has subsequently been shown to reduce both infection and sporulation of several *Glomus* spp. (Bailey and Safir, 1977; Boatman *et al*, 1978; De Bertoldi *et al*, 1977; Jalali and Domsch, 1975; Nemec, 1980; Ocampo and Hayman, 1980; Parvathi *et al*, 1985; Spokes *et al*, 1981; Verkade and Hamilton, 1983). This method of application is, however, not widely used commercially, the more common practice being foliar application of fungicides to cereal crops. Much of the previous work has ignored this along with the fact that timing and rates of applications of fungicides normally occur at specific growth stages of the particular crop and not at sowing. The active moieties of all the fungicides tested in this study remain localised within the treated leaves following foliar application and are not translocated downwards within the plant to the roots (see Davidse and de Waard, 1984). Only 5% of the active moiety applied to a plant in a foliar spray passes through the leaf cuticle into the plant. It is,

however, known that these compounds are readily taken up by roots and rapidly translocated to the leaves in the transpiration stream. Since only about 20-40% of the surface of the pot was covered by wheat foliage in these experiments, 60-80% of the applied spray would have reached the surface of the growth medium directly or as 'run-off' (P. Nicholls, Rothamsted Exp. Sta., pers. comm.). It is also known that fungicides (more than herbicides) have fairly high adsorption coefficients in soils. Bateman and Nicholls (1982) used a computer simulation to attempt to predict the movement of benomyl in soil in pots and in the field. They used it to estimate the upward movement of water and reversibly-adsorbed fungicide during periods of soil drying, and downward movement after watering. They concluded that a cycling of the fungicide within the pot would occur, creating an environment whereby roots would be continually exposed to the applied chemicals. The inhibitory and stimulatory effects of the pesticides on the VAM symbiosis in this study would therefore appear to be as a result of either direct contact with the VAM mycelial network in the pot or their uptake by hyphae (or root) and subsequent effect on fungal metabolism. Fungicide movement in field soil would also be greatly influenced by soil conditions, rainfall and time of application. A cereal crop, for example, receiving an early autumn application of Calixin to control mildew would have only a small percentage of 'ground-cover' and The cycling of fungicides in pots is likely to be less applicable in the field situation, particularly for crops grown in temperate regions where soils are at or near field capacity for much of the growing season. Expts. F1, F2 and F3 showed the negative effects of spraying Bavistin on a well-developed VAM infection (Expt. F1) and on a rapidly spreading young infection (Expt. F3). In Expt. F1 the later application of Bavistin alone and as a third spray in a simulated typical intensive spray program (R. Chapman, personal communication), reduced overall VAM infection levels (particularly in the M/S/B treatment). The

most dramatic effect was noted with the M/S/B treatment on *G. geosporum* (Fig. 6.3). Hale and Sanders (1982) applied a soil drench of benomyl on a well established VAM infection of red clover. It halted further infection and reduced phosphorus inflow by an order of magnitude compared with untreated controls. They also showed a decrease in the rate of plant growth coincident with this reduction of phosphorus inflow, implying that the VAM fungus was the major factor involved in phosphorus uptake in the low soil P conditions used. They further suggested that the mechanism of this inhibition was interference with the polyphosphate containing vesicles in the external hyphae via direct action on cytoplasmic microtubule formation. This effect was more clearly observed in Expt. F3 where all VAM-infected plants had reduced internal N/P/K levels compared with their water-sprayed controls (Table 6.21). The absence of a corresponding growth depression in these treatments may be explained by the fact that wheat is not a wholly mycorrhizal dependant plant under low P conditions compared with plants like cassava (Howeler *et al*, 1982), onion (Dodd and Krikun, 1983) or soybean (Bailey and Safir, 1978). The infection levels of all three endophytes were significantly reduced relative to water-sprayed control treatment percentages at both samplings after Bavistin application in Expt. F3 (Fig. 6.4), revealing the strong inhibitory effect of this fungicide on an actively spreading VAM infection.

Tilt Turbo and Sportak sprays belong to a different systemic fungicide group, the Triazole derivatives, which are effective in controlling plant pathogens in the Ascomycotina, Basidiomycotina and Deuteromycotina (Rathmell and Skidmore, 1982). Sportak (a.i. prochloraz) was used in Expt. F1 and F2 and appeared to have inhibitory effects on *G. geosporum* and *G. mosseae* infections (Fig. 6.3) in the former experiment. It also reduced yields following application in Expt. F1 but these had recovered at harvest. Effects in Expt. F2 were masked by reduced

plant vigour, but, Sportak treatments produced the lowest ear yields at harvest. This was most evident in the VAM treatment yields (Table 6.18) whilst it also reduced spore numbers of *G. geosporum* over both experiments. The application of Tilt Turbo (a.i. propicanazole) in Expt. F3 had a less inhibitory effect than Bavistin on VAM infection levels (Fig. 6.4), causing a slight reduction in the internal nutrient status of the shoots of VAM-infected plants compared with water-sprayed controls (Table 6.21). Nemec (1985) tested four such sterol inhibiting fungicides including propicanazole, and found it to be the most inhibitory against citrus plants infected by two *Glomus* spp. These sterol inhibiting fungicides are known to inhibit the biosynthesis of ergosterol in fungi and is the suggested mechanism of action in mycorrhizal species. This inhibitory trend of Tilt was also observed in the spore germination studies in the previous chapter.

Calixin has tridemorph as an active ingredient, as does Tilt Turbo, and is used as an eradicant of powdery mildews (Ascomycotina). Its precise mode of action is unclear, but, it is believed to act on the respiratory chain. Calixin treatment increased infection levels, internal nutrient levels of the shoot, and yields of all three VAM treatments at harvest in Expt. F3 (Fig. 6.4 and Table 6.21). It also appeared to stimulate germination of spores of all three endophytes at certain concentrations (see previous chapter). These responses are difficult to explain but it has previously been noted that single sprays of tridemorph applied in October on winter barley have caused increased tillering and root growth with associated improved crop vigour (Lester, 1971). If such an increase in root length also increased the length of root with VAM infection under low soil nutrient conditions a plant growth response might ensue due to more efficient uptake of nutrients. Similar responses following pesticide application have been reported in other crops e.g for sour orange plants inoculated with G.

mosseae and subjected to varying concentrations of sodium azide (Nemec, 1980), for white ash seedlings inoculated with *G. fasciculatum* subjected to 0.5kg ha^{-1} of paraquat (Pope and Holt, 1981), for VAM-infected soybeans subjected to metalaxyl (Groth and Mortinson, 1983) and for fosetyl-Al on mycorrhizal leeks (Jabaji-Hare and Kendrick, 1985). The latter two interactions are particularly interesting since both these two fungicides exhibit highly specific fungitoxicity towards Oomycetes (Bruin and Edgington, 1984) with no effects reported towards the Zygomycotina. Menge *et al* (1979) showed that Terrazole, as a soil drench, significantly increased root colonisation and spore production by *G. fasciculatum* in sorghum and this is also effective against Oomycetes. Fosetyl-Al is also a downward translocated fungicide within the plant whilst the systemic fungicides used in this study are all translocated upwards.

Milgo E application (a.i. ethirimol) had no effect on VAM infection of wheat roots even when applied in the early stages of endophyte colonisation (c.f. Bavistin). In Expt. F2 it significantly increased final total yields (Table 6.18). Brookes (1972) also noted increased vigour and weight of pots produced by ethirimol treated plants and this may indicate the presence of a disease problem in Expt. F2 which was relieved by the Milgo application (there was no evidence of mildew pustules on the leaves). Ethirimol is normally used as a seed coated treatment and in such a form has been shown even more deleterious to VAM infections than benomyl (Jalali and Domsch, 1975). Ethirimol is known to be more effective against its target fungi (mildews) when applied as a soil treatment six weeks after sowing, since it is taken up by the roots and readily metabolised to release a fungicidally active derivative (Erwin, 1973). Milgo had little effect on *G. monosporum* spore production over two experiments but did reduce spore levels of *G. mosseae* at both harvests.

6.5. CONCLUSIONS

In conclusion to these studies, it should be emphasised that results of greenhouse and laboratory screenings require verification in the field before information for or against pesticide application is disseminated. With this in mind, results of the four herbicides tested indicate that normal field application rates are unlikely to have any serious adverse effects directly on VAM infection, sporulation and spore germination. This conclusion was also arrived at by Smith *et al* (1981) following field studies with six herbicides. Potential problems could only arise from the phytotoxicity of such chemicals as Avenge and Dicurane when used in pot studies.

The effects of the systemic fungicides tested on VAM infections ranged from inhibitory (Bavistin) through less inhibitory (Sportak and Tilt Turbo) to stimulatory (Calixin), a trend paralleled in the spore germination studies of the previous chapter. The site and mode of action of these fungicides in the plant would seem to be the major determinants of any effects on the VAM association. Another important point arising from these studies is the possibility of using pesticides to help elucidate certain aspects of VAM fungal metabolism, presently made difficult due to their obligate nature. The use of different sterol inhibitor pesticides like Sportak, Calixin and Tilt Turbo could be used to investigate sterol metabolism. Since the modes of action of many systemic fungicides are unclear, however, explanations of the results presented here are difficult. There does, however, appear to be consistency between the pesticides grouped together because of their similar active moieties (Ceridor/Harrier and Sportak/Tilt Turbo). The new fungicide Tilt mbc deserves to be studied in the light of these findings since it contains both carbendazim and propicanazole as active moieties, both of which appear inhibitory to VAM fungi.

CHAPTER 7

The effect of inoculation of VAM fungi on winter wheat grown in fumigated and non-fumigated soil in the field.

7.1. INTRODUCTION

The beneficial influence of VAM fungi on plant growth in pots under low soil-P conditions was reaffirmed in the preceding chapter. Similarly most of the published results of the effects of VAM fungi on plant growth are also from pot experiments and there is a distinct lack of investigations on mycorrhizal effects in the field (Fitter, 1985). The ultimate test of the potential beneficial role of VAM inoculation on the growth of a crop is whether it can increase yields under a typical arable field system. Results from pot experiments often do not match those from field work (Hayman and Mosse, 1979). This may be due to differences in the environment i.e. light, temperature, soil water status, pH and restricted root growth. Nevertheless there have been pot experiments which have paralleled field work (e.g. Dodd *et al.* 1983; Krikun *et al.* 1982). In field experiments using cereal crops, Khan (1975) and Saif and Khan (1977) reported that inoculation of seedlings prior to transplanting into the field increased growth of wheat by about 20 and 150% and that of barley by about 30 and 300% with and without added P respectively. Inoculation of a non-sterile seed bed gave growth increases in the range 0-133% in studies on winter barley (Clarke and Mosse, 1981; Owusu-Bennoah and Mosse, 1979; Powell, 1981). Jakobsen (1983b) found that inoculation of fumigated plots increased the dry-

matter yields of spring barley by 20%, and increased P uptake by approx. 100%. Buwalda *et al.* (1985b) also found that artificial inoculation with *G. mosseae* increased the yields of both spring and winter barley and wheat sown on fumigated plots by 17-25%, but little effect was observed in non-fumigated plots. Mycorrhizal infection also increased the P concentration in the shoots at very early stages of growth, an effect which declined with time.

In my investigations, the three year study of the Sussex site, described in chapter 3, revealed high levels of naturally occurring VAM infection in the roots of winter wheat crops throughout the season. It is, however, very difficult to define the role of such infection because of problems in manipulating the soil inoculum potential of the indigenous population without drastically changing other soil properties. Such information may be obtained indirectly by comparing the growth and P uptake in plants from fumigated plots with those from non-fumigated plots (Dodd *et al.* 1983; Plenchette *et al.* 1983a,b; Yost and Fox, 1979). A more direct method is the inoculation of soil in which the indigenous VAM population have been suppressed by fumigation (Buwalda *et al.* 1985a, b).

This study was, therefore, designed to investigate the effect of inoculation of several isolates of VAM fungi on growth and nutrient uptake of a winter wheat crop grown in fumigated and non-fumigated soil at two levels of added superphosphate. The purpose being to indicate the relative efficiency of the indigenous VAM population compared with other single species inoculants.

7.2. MATERIALS AND METHODS

7.2.1. Experiment 1

This experiment was set up near the headland end of the Sussex Ex-pasture field at Goffsland Farm, West Sussex. The remainder of the field had been sown to rye on 14/10/84.

7.2.1.1. Experimental Design

Two plots covering a 320m^2 area (32x10m) were staked out each with 18 whole plots which were sub-divided into two. Whole plots measured 4.5x3.5m and sub-plots 3x2m with 0.5m paths around each sub-plot. The intention was to harvest a 2m^2 area within each sub-plot. Roots were sampled throughout the duration of the experiment, up to day 196, by removing plants (as outlined in chapter 3, section 3.1.2.) with their root systems attached. The plants were taken from rows situated on either side of the central harvest area. Treatments on whole plots comprised, two levels of superphosphate application in factorial combination with fumigation and non-fumigation, with each whole plot sub-divided into an inoculated and non-inoculated sub-plot. There were three fungal treatments:

1. *G. geosporum*
2. *G. monosporum*
3. Mixed (*G. geosporum* and *G. monosporum*)

Each of the six treatments was replicated three times in a completely randomised design within each of the two large plots. P was applied on 24/10/84 to both large plots after fumigation. This was broadcast as granular superphosphate (18%P) on the soil surface at rates of 0 and 120 kg P ha^{-1} and raked into the surface layer.

7.2.1.2. Fumigation

The fumigation treatment was designed to eliminate the indigenous VAM population in one of the two large plots (Photos 61 and 62) on 16/10/84. Methyl bromide was the fumigant used at a rate of 125g m^{-2} using the hot gas method of application. The liquid methyl bromide flowed initially into a copper heating coil and was volatilised. The gaseous fumigant was then dispensed into perforated layflat tubes (Photo 61) as a measured dose using weighing scales (Photo 62). The whole area was covered under a heavy sheet of polythene which was dug into the soil to a depth of 20cm around the perimeter of the large plot, thereby allowing diffusion of the gas into the soil. The sheet of polythene was removed from the plot 72 hours after the fumigant was applied and vented for 6 days prior to sowing. After fumigation great care was taken to avoid contaminating the fumigated plots with non-fumigated soil.

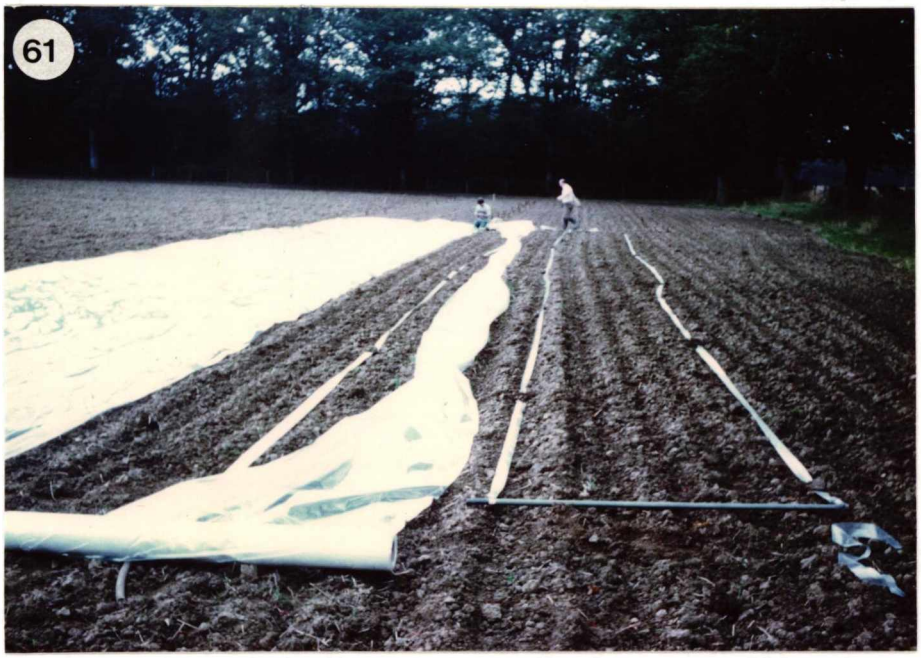
7.2.1.3. Inoculation

Inoculum of each of the VAM fungal treatments was applied at 500g m^{-2} to the inoculated sub-plots. Non-inoculated sub-plots received an equal aliquot of non-infected medium as a control. The inoculum was applied along furrows 6-10cms deep, originally marked out by a tractor-drawn seed drill, giving 13cm gaps between rows across the plot.

7.2.1.4. Sowing

The experiment was sown with winter wheat var. Rapier on 22-23/10/84 at a rate of 350 seeds m^{-2}. The seed was sown directly on top of the inoculum using a single-row manual seed drill. The sowing date was later than anticipated due to a very wet October causing a delay in fumigation and venting. The crop received a herbicide application of Dicurane (7L ha^{-1}) 5 weeks after seedling emergence. Unfortunately fresh chicken manure had been applied to the entire

Photos 61 and 62 - Methyl Bromide fumigation of Sussex (G-2) field site showing application of heated gas under polythene tarps.



field by the farmer in September 1984 at a rate of 10 tonnes ha^{-1}. This fact was not discovered until mid-November 1984 following receipt of the results of soil analysis.

7.2.1.5. Measurements

In order to assess the effects of fumigation on the availability of nutrients in the soil samples were taken for analysis on 14/11/84. Roots were sampled at regular intervals during the experiment for the estimation of VAM infection levels. Plant foliage was sampled and analysed for internal element concentrations twice during the experiment(on 14/11/84 and 5/5/85). Severe lodging of the crop growing on the fumigated plot occurred during the wet summer months and an attempt to obtain yield data was unsuccessful.

7.2.2. Experiment 2

In order to maximise the use of the fumigated soil, this experiment was designed to test the ability of three VAM fungal isolates, *G. geosporum*, *G. monosporum* and *G. mosseae*, to improve the early season nutrient uptake of a winter wheat crop allowed to grow for 56 days.

In July 1985 a section of the fumigated plot was scythed down and cleared, leaving a total plot of 3x8m. This area was extensively rotavated to plough stubble into the soil and create a fine soil texture suitable for sowing. This plot was further sub-divided into 8 sub-plots of 1.5 x 2.5m separated by 0.5m wide paths. There were four fungal treatments:

1. *G. geosporum*
2. *G. monosporum*
3. *G. mosseae*
4. Non-inoculated control

Each treatment was replicated twice and randomnly arranged. The inoculum of each endophyte was applied at a rate of 5kg m^{-2} and controls received a similar aliquot of non-infected material. This was broadcast over the the soil surface and rotivated into each sub-plot to a depth of 10cms. Sowing occurred on 26/7/85 using the winter wheat var. Rapier and the experiment was allowed to run for 56 days. Root samples were taken after 20, 38 and 56 days for estimations of VAM infection. Foliage from each plot was sampled at day 56 and analysed for internal nutrient levels. There was insufficient material to calculate yield data.

7.3. RESULTS

7.3.1. Experiment 1

Table 7.1 shows the results of soil analysis performed 24 days after sowing (14/11/84). The levels of nutrients in the soil were extremely variable between samples and (much higher than in previous years) in the case of Olsen P on the untreated NF (0) plots (c.f. Table 3.3, chapter 3). It was discovered that the farmer had applied fresh chicken manure at a rate of 10 tonnes ha^{-1} in September prior to the sowing of his rye crop in the other section of the field. This treatment had also been applied to the experimental area of the field. It had not, however, been spread uniformly across the field and clumps of manure were evident in the upper surface layers of the soil. Soil samples taken for analysis containing a high proportion of this manure would have resulted in the high values of P, in particular, observed in the NF (+P) sample (Table 7.1). The Dead Sea Bromine Co., Israel, advises that a manure dressing of $200m^3$ ha^{-1} should be the maximum application before first cultivation in a soil about to be fumigated. When larger dressings or lumpy manures are used, these must be applied at least 3 months before fumigation to allow decomposition and thorough mixing with the soil. The high levels of nutrients which were subsequently released as a result of this manure application would have created a soil environment totally unsuitable for VAM infection. High levels of ammonium-nitrogen are known to be released after fumigation under normal fumigation conditions anyway (Rovira and Simon, 1985). This effect was reflected in the foliage analysis after 196 days (Table 7.2), which revealed significantly higher shoot P, K and Br^- concentrations in plants sampled from fumigated plots. Internal N levels were also higher. It was subsequent to this sampling that severe lodging of the crop growing on the fumigated plot occurred. This was almost certainly due to a combination of wet and windy weather at the time and excessive

vegetative growth in the high soil nutrient (especially N) environment. It was decided, therefore, to abandon plans to obtain harvest yield data from plots and to initiate another small-scale experiment on the fumigated plot alone.

A comparison of the foliar phosphorus concentrations in the winter wheat crops in 1982-83 (G-1, G-2, and Hamill) and the crop from Experiment 1 at G-2 showed that the latter, in 1984-85, had higher shoot P levels than the two Sussex crops in 1982-83 (Fig. 7.1). The foliar P concentration, however, was still lower than that observed in the Hamill crop of 1982-83.

7.3.2. Experiment 2

Results of soil analysis from the cleared fumigated plots (Table 7.1) indicated that soil P levels had fallen appreciably over the season to 21mg P L^{-1}, and consequently VAM inoculation would be expected to produce infected wheat plants if sufficient inoculum could be incorporated into the plots.

Results in Fig. 7.2 show the progression of infection over the 56 day growth period. At the first sampling time only, plants infected with *G. mosseae* had significantly higher infection levels. However, by day 56, plants infected singly by each of the three introduced endophytes had significantly higher infection levels and higher shoot P concentrations than the non-inoculated control plants (Fig. 7.3).

Table 7.1 Soil analyses of Sussex G-2 site on three occasions after soil fumigation.

SAMPLE DATE	TREATMENT	pH	P	K	Mg	NO_3^-	Br^-
14/11/84	NF (0)	6.9	40	136	92	17	6
	NF (+P)	6.2	159	139	94	14	6
	F (0)	7.1	129	395	202	35	12
	F (+P)	6.1	71	129	100	14	12
7/3/85	NF (0)	6.6	76	328	138	--	--
27/9/85	F (0)	7.0	21	188	104	--	--

Table 7.2 Concentrations of nutrients in shoots of winter wheat grown in Experiment 1 after 196 days, with levels of significance and means of treatments.

TREATMENT	% ELEMENT IN SHOOTS* (DW)				%
	N	P	K	Br	VAM
NF (0)	2.79	0.42	3.92	19	45
NF (+P)	2.46	0.39	3.60	32	51
F (0)	2.90	0.52	4.50	320	0
F (+P)	3.30	0.61	4.91	320	0
LSD (P05)	NS	0.08	0.67	55	8

Fig. 7.1 Comparison of concentrations of P in shoots of winter wheat grown at Hamill or Sussex in 1982-83 with that grown at Sussex G-2 in Experiment 1 in 1984-85.

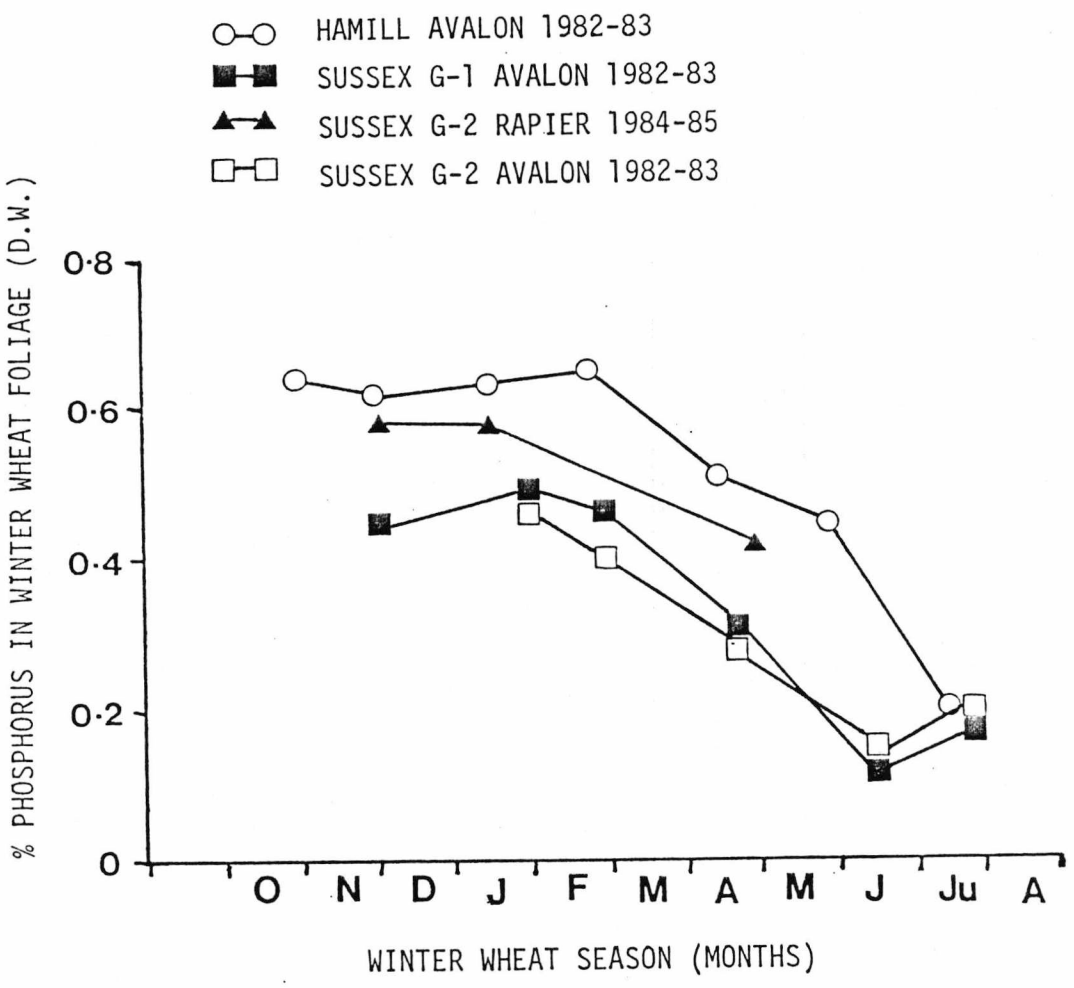


Fig. 7.2 Development of VAM infection of introduced endophytes and the indigenous endophyte population in Experiment 2. Error bars show levels of significance at P = 0.05.

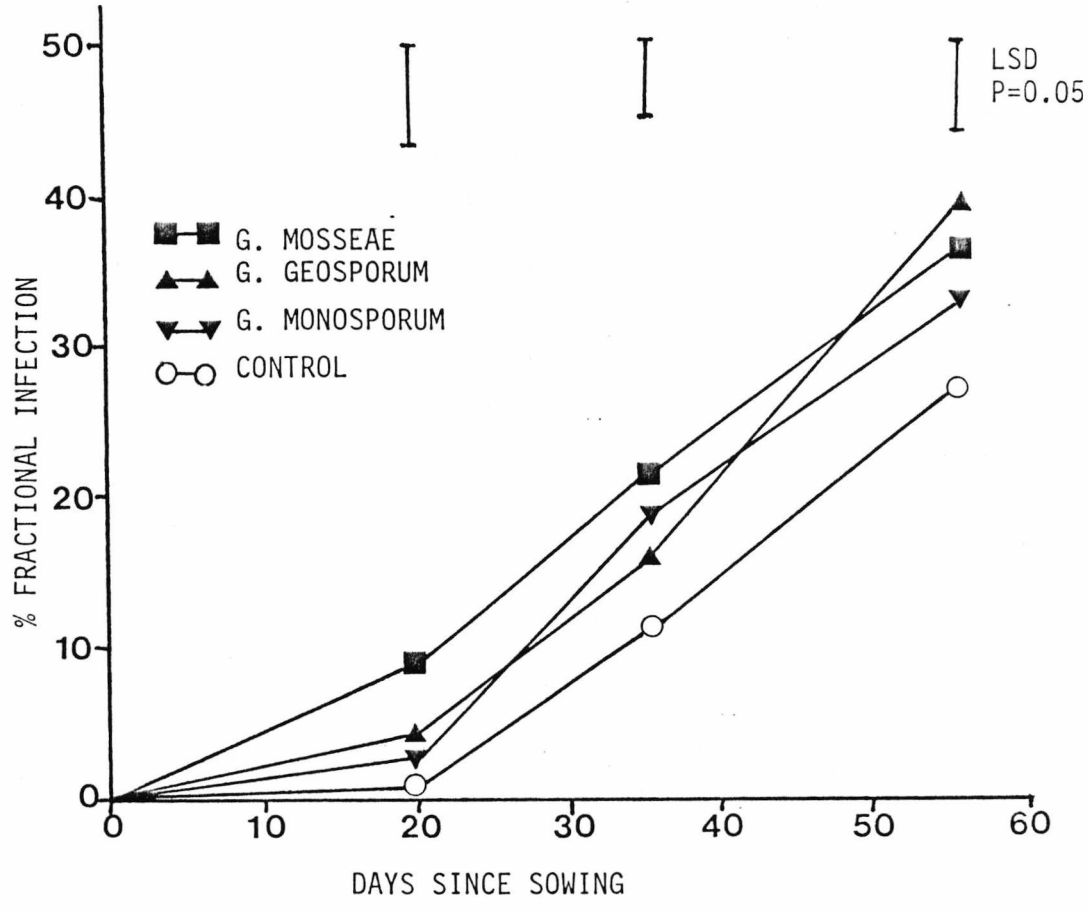
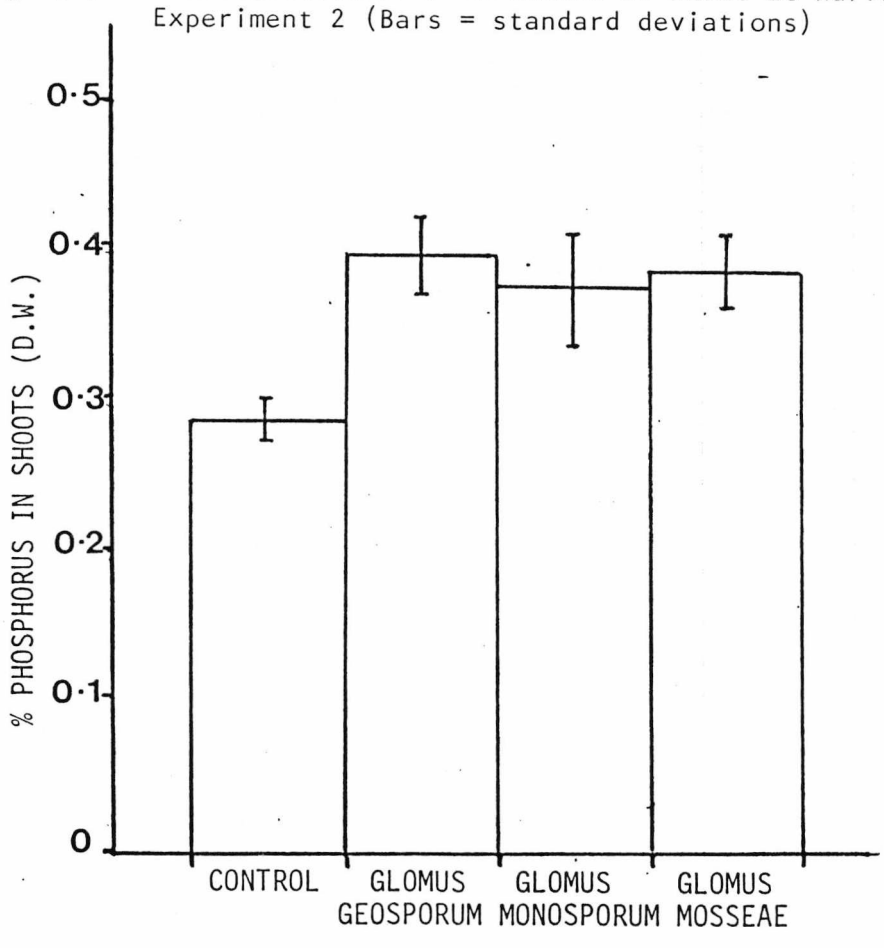


Fig. 7.3 Concentration of P in shoots of wheat at harvest of Experiment 2 (Bars = standard deviations)



7.4. DISCUSSION

Attempts to inoculate a wheat crop growing in fumigated soil with VAM fungi failed in Experiment 1, probably due to a massive release of nutrients from the freshly applied chicken manure. As a consequence, vegetative growth of wheat on this plot visibly better and greener in appearence than on the non-fumigated plot (see also Rovira, 1976). Fumigation of calcareous soils in Australia increased wheat plant dry matter and grain yields at harvest (Rovira and Simon, 1985). Fumigation caused the release of ammonium-nitrogen in the soil and led to higher nitrate-nitrogen 15-25 weeks later, whereas addition of nitrogen fertilizer did not increase yields on non-fumigated plots. Rovira and Simon (1985) concluded that better growth and higher yields were attributable both to control of a root disease complex and to release of ammonium-nitrogen. Rovira (1976) studied the effect of soil fumigation on nutrient levels in the field and found that the bicarbonate-extractable phosphate doubled following fumigation. He also showed that root growth and phosphate uptake by the roots increased. Inoculation of the non-fumigated plots in this study did not increase VAM root infection levels in the wheat crop during the experiment. There was also no significant difference in infection levels between plots receiving P and those without added superphosphate (Table 7.2). This is interesting since the soil samples taken from the NF (+P) plots on 14/11/84 had four times the Olsen P level of the NF (0) plots (Table 7.1). High soil P is believed to greatly inhibit VAM infection (Abbott and Robson, 1982) and yet it has also been observed that a highly calcareous soil in Israel which received very high levels of broadcast superphosphate (up to 3,000kg ha^{-1}) did not suppress VAM infection levels in celery, melon, onion or pepper root systems (Dodd *et al.*, 1983 and unpublished data and observations). It was discovered in the latter study that this phenomenon was due to the low levels of water-soluble P compared with other

sands and sandy-loams. Further laboratory work showed that 70% of the added P to this soil was irreversibly bound within 10 days of application, as estimated by adsorption isotherms (Yarmitsky and Dodd unpublished data). Heavy clay soils are also known to have similar phosphorus-binding properties and may help explain this anomaly (Bowman and Olsen, 1985). Recent work has also shown that wheat did not respond to added VAM inoculum in non-fumigated soils, whereas there was a large beneficial effect on wheat growth after inoculation of G. *mosseae* in fumigated soil (Buwalda *et al.* 1985a, b). It was suggested, that this was due to a greater development of the external mycelial network in fumigated soils due to the elimination of predatory fauna such as springtails which feed on hyphae (Warnock *et al.* 1982). Since uptake of P and other nutrients occurs via this external mycelium, (as described in detail in the Discussion of chapter 6) infection would be less efficient in non-fumigated soil. Thus any reduction in the absorptive surface area or damage of the major nutrient translocating hyphae would result in a reduced growth-promoting role for the VAM infection.

Soil analysis prior to Experiment 2 (Table 7.1) revealed the dramatic drop in soil P levels to 20mg L^{-1} over a 10 month period, and all three VAM fungi were successful in establishing infections in the root systems following the July inoculation. Each VAM fungus introduced increased internal shoot P concentrations above non-inoculated control plant levels at day 56 (Fig. 7.3). The root systems of control plants also contained substantial infection by day 56, but this chiefly comprised fine endophytic infection. This rapid reinfestation by a natural VAM species was not unexpected since Menge (1982) also found that levels of the indigenous VAM population returned to normal six months after fumigation of a similar clay-loam soil. He suggested that this was due to a poorer fumigation of a heavy moist soil type, which would have allowed survival of VAM

propagules at all depths. Reinfestation of fumigated sandy and sandy-loam soils took longer than 13 months to recover due to better fumigation. Mosse *et al* (1982) and Jakobsen (1983b) and have also noted that horizontal spread of an introduced endophyte in a soil was considerable within a single growth season of a crop. Warner and Mosse (1980) showed that VAM fungi are also capable of limited independent spread in soil, probably due to rudimentary saprophytic growth from an organic base.

Since data on the numbers of infective propagules in each plot at the start of Experiment 2 are not available, it is not possible to decide whether there was slower build-up of infection in control plant roots due to less initial inoculum or whether this was a characteristic of the infecting endophytes. Results from chapter 3 of this thesis indicated that fine endophyte infection was a major component of the total VAM infection observed in this Sussex site. It appeared to constitute most of the infection detected in wheat roots growing in the control plots of Experiment 2, although this was not quantified. Fitter (1985) highlighted the common observation that fine endophytes, although most abundant in temperate natural soils, have rarely been shown to produce growth responses even in infertile soils (Powell, 1979). High levels of fine endophyte infection were observed at the end of the winter wheat seasons studied in 1982-85 (chapter 3) in Sussex fields, whereas coarse endophyte infection was prevalent in the early autumn and late spring peaks of arbuscular activity. The results of Experiment 2 may therefore indicate the relative ineffectiveness of the fine endophytic infection in this field site, since all the other three introduced coarse endophytes increased shoot P levels by 25% (Fig. 7.3) with only about 10% higher infection at day 56 (Fig. 7.2). This could also indicate the importance of the presence of coarse endophytic infection to a wheat crop at a time when P inflow is at a maximum (e.g. early autumn) in a low P soil.

Plenchette *et al* (1983a,b) showed the potentially damaging effect of fumigation on mycotrophic crops like leek growing in pots and in the field. They revealed, however, that oat and wheat grew equally well in fumigated and non-fumigated soil with a P-fertility of 100µg g^{-1} (considered sufficient for good growth and yields of both cereals). Both crops had well developed root infections (>50%) but mycorrhizal efficiency was very low. Baylis (1975) stated that plants with a graminoid root system were less dependent on mycorrhizae for their supplies of phosphorus. Plenchette *et al* (1983b) also noted that wheat is a plant requiring less P for growth than mycorrhizal-dependent species like carrot, leek or onion. The field work of Buwalda *et al* (1985a,b) was carried out on a low P silty-clay loam soil, originally with 5mg P kg^{-1} which was increased to 28mg P kg^{-1} by the highest superphosphate application. The site had also been fallow for 5 years prior to their first experiment. They found that yields at harvest continued to respond to applied P even when uniformly high levels of infection (40-50%) had been established over several seasons. They therefore suggested that the ability of the cereal root system to absorb P was not greatly increased by mycorrhizal infection. This beneficial response to added P was also noted in the levels of shoot P concentrations from the first wheat crop grown in the Sussex G-2 field in 1984/85 when compared with levels observed in the 1982/83 crop when the soil P value was much lower (Fig. 7.1). Although there is a difference in the cultivar used, the sowing dates were similar for the Sussex Ex-pasture crops. It should also be noted that maximum infection levels were lower in the 1984/85 crop than in either of the two previous seasons when the soil P level was much lower (see chapter 3). The conclusion reached by Buwalda *et al* (1985b) was that VAM infection was useful but not essential to cereals in the field even under low soil P conditions. This was also the conclusion on the role of VAM on wheat growth in the desert of S. Israel (Krikun, personal communication). My

results do not challenge this conclusion but highlight the fact that the role of natural VAM infections, (both fine and coarse endophytic infections) needs further investigation to assess the contribution of high infection levels of each type to cereal growth and nutrition. This would be important since VAM infections are known to drain carbon from the host (Snellgrove *et al.* 1982).

CHAPTER 8

Phosphatase activity in the roots and rhizosphere of plants infected with the VAM fungi *Glomus geosporum*, *Glomus monosporum* and *Glomus mosseae*.

8.1. INTRODUCTION

The beneficial growth responses conferred on mycotrophic host plants by vesicular-arbuscular mycorrhizal (VAM) fungi have been explained primarily in terms of improved uptake of phosphate (Nye and Tinker, 1977; Hayman, 1983; Mosse *et al.*, 1983). The chief attribute of the VAM fungi is their apparent ability to tap soluble phosphate beyond the phosphate-depletion zone that develops around the root surface in soils of low P status. This zone arises because of the poor mobility of phosphate ions in the soil (Tinker, 1980; Hayman, 1983).

The external hyphae of VAM fungi can absorb nutrient ions, and translocate them to the root (Pearson and Tinker, 1975). Phosphate, in particular, is taken up and translocated as polyphosphate granules by protoplasmic streaming before being hydrolysed in the arbuscles prior to transmembrane transfer into the host cell (Capaccio and Callow, 1982). These workers showed the presence of both polyphosphate-synthesizing and -degrading enzymes within the fungal network, whilst Glaninazzi *et al.* (1979) showed the presence of fungal-specific alkaline phosphatase activity within the vacuoles of mature arbuscles and intercellular hyphae. Polyphosphate granules have also been shown to be present within these vacuoles by Cox *et al.* (1975). The role of internal fungal acid

phosphatases is unclear but they are also present and may be associated with the growth and development of the fungus within the host tissue (Gianinazzi *et al.*, 1979).

The efficiency of phosphorus utilisation has been inversely linked to the presence of root phosphatases (McLachlan, 1976; 1980a,b), since acid phosphatase activity was found to be lower in plant species or cultivars that were more efficient at P uptake grown under P-deficient conditions. Increased uptake of P by ectomycorrhizal trees has been attributed to the increased root surface area but, in addition, phosphatase activity has been implicated (Bartlett and Lewis, 1973; Alexander and Hardy, 1981; Antibus *et al.*, 1981). Acid phosphatases released by the ectomycorrhizal fungal mantle and attached mycelium ramifying through the soil are believed to catalyse the hydrolysis of complex (organic) phosphorus compounds into more readily absorbed forms (Ho and Zak, 1979). Ridge and Rovira (1971) indicated that root surface phosphatases acting in the immediate root environment may be more important in the organic P mineralisation than soil bound enzymes in the surrounding soil. Antibus *et al.* (1981) showed that the acid phosphatase activity of ectomycorrhizal roots of *Salix rotundiflora* and *Entoloma sericeum* was 40 times as great as non-mycorrhizal roots on a surface area basis. Dighton (1983), on the other hand, showed a decrease in phosphatase activity produced by mycorrhizal Birch roots compared with non-mycorrhizal controls. There appears to be little other evidence that plant and microbial extracellular phosphatases play a prominent role in the mineralisation of soil organic phosphorus compounds and it has been suggested that phosphatase activity only becomes important after initial breakdown of soil organic compounds catalysed by a host of other microbial enzymes (Speir and Ross, 1978).

Extracellular phosphatases have not been found specifically associated with

the VAM external mycelial network in the root rhizosphere and there is little published information concerning root phosphatase activity involving a VAM symbiosis. Azcon *et al.* (1982) compared the effects of VAM infection and phytate addition on the growth, P uptake and root phosphatase activity of wheat and lavender (high mycorrhizal dependency under low P conditions). VAM infection decreased root phosphatase activity of lavender compared with non-mycorrhizal control plants, but had no effect on wheat.

An investigation of acid phosphatase activity in the root and rhizosphere of VAM infected and uninfected plants was therefore carried out in sterile sand culture in an attempt to discover if phosphatase activity is related to mycorrhizal dependency under low nutrient conditions. The experiment was also designed to determine if the "efficiency" of a particular VAM isolate is related to its ability to alter rhizosphere levels of phosphatases. Three plants were used to cover the range of mycotrophic responses, namely rape (a non-mycorrhizal species which has been shown to be efficient in absorbing soil P (Nye and Baldwin, 1976)), wheat (low mycorrhizal dependency in low P soils) and onion (high mycorrhizal dependency in low P soils).

8.2. MATERIALS AND METHODS

8.2.1. Plant inoculation and growth

Rape (*Brassica napus*), onion (*Allium cepa*, var. Ailsa Craig) and wheat (*Triticum aestivum*, var. Avalon) seeds were germinated on glass paper (Whatman GF/A) moistened with sterile distilled water. After 2 days four germinated seeds with similar radicle development (5-10mm length) were planted 2cm below the surface of the growth medium. The plants were grown in a constant temperature room at 20°C having a 16h day/8h night cycle. Relative humidity and light intensity were maintained at approximately 60% and 4.6Klx respectively. The plants were irrigated with one third strength Hoaglands nutrient solution containing no orthophosphate (Hoagland and Arnon, 1939).

In Experiment 1, pregerminated seeds of rape, onion and winter wheat were sown in polythene pots containing 1.75kg builders sand (pH 8.0) and 7.0mg L^{-1} $NaHCO_3$-soluble P (Olsen *et al.*, 1954). Inoculum, from maize-grown pot-cultures of three VAM fungal isolates *Glomus geosporum*, *Glomus monosporum* and *Glomus mosseae*, was thoroughly mixed into the sand at a rate of 150g dry weight per pot. Controls received an equal amount of autoclaved inoculum from each isolate soaked in a filtrate of the inoculum of all three endophytes but which was free of any infective VAM propagules. Six replicates were used for each treatment. Plants were thinned after one week to three per pot for rape, 2 per pot for onion and 4 per pot for wheat. Sampling occurred after 53, 72 or 98 days.

In Experiment 2 pregerminated seeds of winter wheat (Avalon) were sown in a coarse builders sand of pH 7.5. Inoculum (300g) of each of the three VAM fungal isolates were thoroughly mixed into each pot as above. Six replicates were again used for each treatment. Plants were thinned to 4 per pot after seven days. Samples were taken after 21, 51 and 74 days.

8.2.2. Assessment of plant growth and VAM infection.

After harvesting plant shoots and roots, fresh weights were determined. In addition shoot dry weights were recorded in both experiments ($80^{o}C$ for 24 hours). Roots were washed, laid flat and the central 1cm of each root was taken and stained for VAM infection followed by estimation of levels of infection using the grid-line intersection method.

8.2.3. Rhizosphere sampling procedure.

Plants were carefully removed from the bags and the root systems with adhering sand were excised from the shoots. The roots were then shaken by hand until most of the sand was detached (outer rhizosphere sample) and then washed with sterile distilled water (10-100 ml depending on the weight of the root-sand mass) to remove the small amount of strongly adhering sand (inner rhizosphere sample). Sand without plants, but otherwise treated similarly to that in which plants were grown, served as the non-rhizosphere control. Samples were taken with a 1 cm soil-borer at the same time as rhizosphere samples.

8.2.4. Assay of Phosphatase activity.

Phosphatase activity was measured using a modification of the method of Tabatabai and Bremner (1969). Samples (1g dry weight) of the inner and outer rhizosphere and control sand were incubated with 1ml 50mM-p-nitrophenyl phosphate (PNP) and 4ml 0.1 M-sodium acetate buffer, pH 5.2, for one hour at 25 ^{o}C in a shaking water bath (60 rev min^{-1}). The reaction was terminated by the addition of 5ml 0.5M- NaOH and the samples were centrifuged at 2,500xg for 10 min. The optical density of the supernatant was measured in a spectrophotometer (LKB ultraspec 2000) set at 400 nM. Phosphatase activity was expressed as the amount of (spectrophotometrically determined) p-nitrophenol released during incubation. Controls were assayed by adding 1ml PNP to a

suspension containing sand and acetate buffer before the addition of NaOH. The standards were prepared in acetate buffer solution containing 0, 10, 20, 30, 40 or 50 mg p-nitrophenol. Intact root phosphatase was measured by determining the amount of p-nitrophenol released when 1ml of 50mM PNP and 4ml 0.1M sodium acetate buffer, pH 5-6, was incubated with 100mg of fresh root tissue. The p-nitrophenol product was developed with 5ml 0.5M NaOH and determined spectrophotometrically in the same way as for rhizosphere phosphatase activity.

To obtain the pH range in which optimum enzyme activity occurred a series of buffers of the following composition and pH values were used: 3.8-5.8, sodium acetate; 5.7-6.8, sodium maleate; 6.5-8.0, tris/maleate; 7.5-9.5, tris/HCl (all 0.1M). All assays were replicated six times.

8.3. RESULTS AND DISCUSSION

Figures 8.1A and 8.1B show the effect of pH on the phosphatase activity in wheat roots infected by *Glomus mosseae* (Fig. 8.1A) and in the total rhizosphere (Fig. 8.1B). In Figure 8.1A it is apparent that the root has a range of activity of pH 5-7, with a peak at pH 5.5-6.0. In the rhizosphere, however, there is a more clearly defined peak at pH 5.0-5.5 with reduced activity above pH 6.0. This indicates that acid phosphatase activity predominates in the rhizosphere whilst there may also be alkaline phosphatase activity in the roots. A similar pattern was observed for plants infected with the other two VAM fungi tested. Gianinazzi *et al.* (1979) used histochemical studies to localise phosphatase activity within VAM-infected roots at an ultrastructural level. Acid phosphatase (pH<6) activity was noted in the cytoplasm of the plant root cells as well as at the growing tips of mycorrhizal hyphae. Alkaline phosphatase (pH>8) activity, however, was weak in root cells and was unaffected by VAM infection, but was conspicuous within the vacuoles of hyphae of the VAM fungus. McLachlan (1980a) found that the optimum pH for root phosphatase activity lay in the range pH 5-6 when he tested buckwheat, rye, subterranean clover and wheat, but found no evidence of alkaline phosphatase activity. On this evidence, it would appear that the phosphatase activity detected in the rhizosphere originated from root cells rather than from the associated fungus.

Levels of rhizosphere and root acid phosphatase activity are shown in Table 8.1 for each host in Experiment 1. Results for onion are from the 98 day harvest, those from rape are from the 72 day harvest, whilst those of wheat from the 53 day harvest. At these times adequate plant growth had occurred (Photos 63-65). As expected, rape did not become infected with VAM fungi and there was no significant difference between samples at any of the harvests in terms of shoot dry weights or enzyme activity. Onion was more difficult to analyse due to its slow

Photos 63-65 Comparative growth of experimental plants inoculated with different *Glomus* spp. during Experiment 1 of phosphatase assay investigation.

Photo 64 Onion (98 days after sowing)

Photo 63 Rape (72 days after sowing)

Photo 65 Wheat (53 days after sowing)


63


64
CONTROL
G. MONOSPORUM
G.GEOSPORUM
G. MOSSEAE


65
CONTROL
G. MOSSEAE
G.GEOSPORUM
G. MONOSPORUM

Figure 8.1A - Effect of pH on phosphatase activity of 25 day old wheat roots infected with G. mosseae. Activity is expressed in umol p-nitrophenol released (100mg fresh root tissue^{-1}) hour^{-1}.

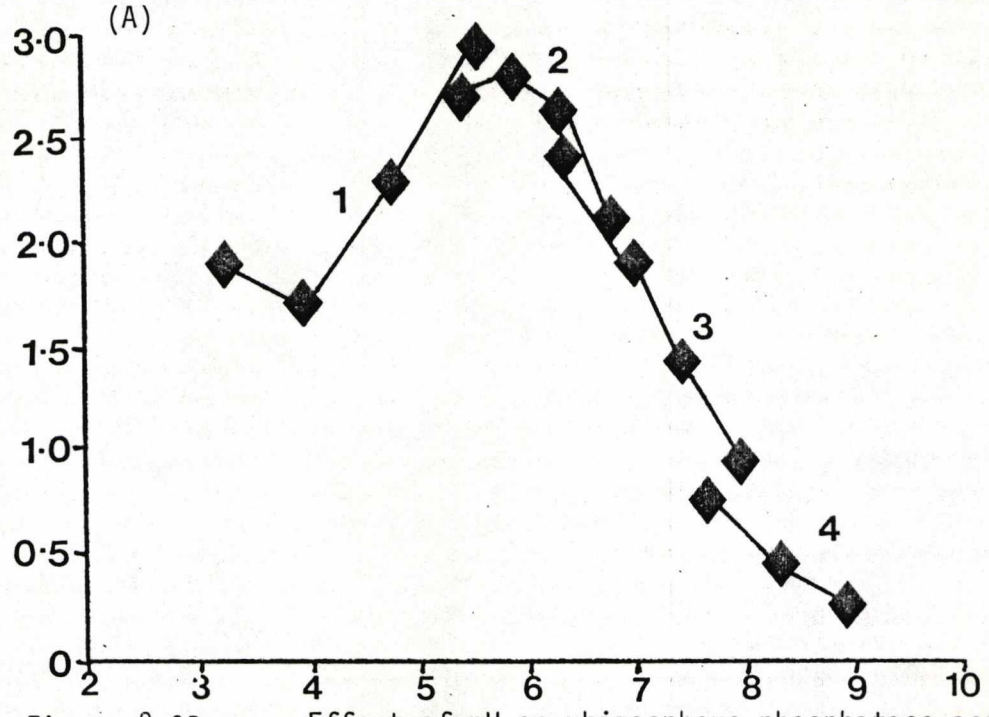
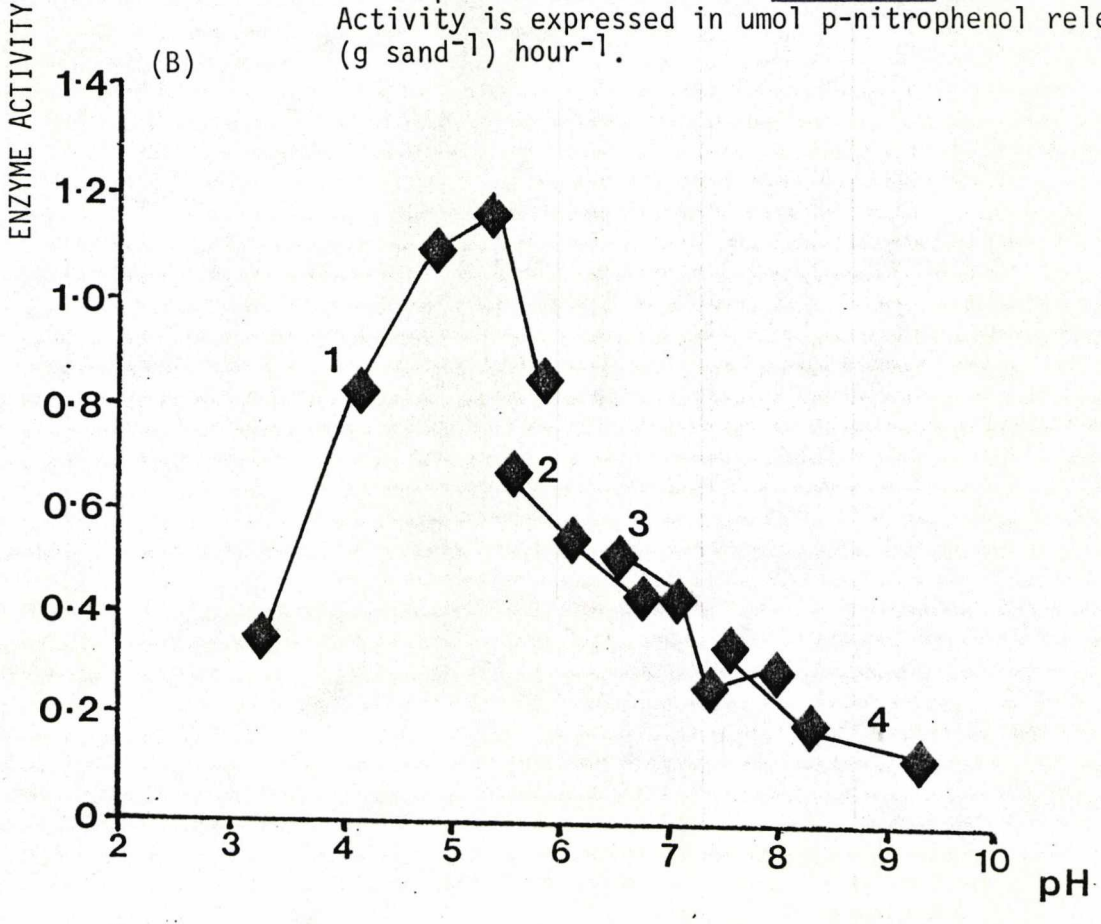


Figure 8.2B - Effect of pH on rhizosphere phosphatase activity of wheat plants infected with G. mosseae after 53 days. Activity is expressed in umol p-nitrophenol released (g sand^{-1}) hour^{-1}.



1 - sodium acetate buffer
2 - sodium maleate buffer
3 - Tris/maleate buffer
4 - Tris.HCl buffer

PLANT	TREATMENT	% VAM INFECTION	SHOOT D.W. (g) PER POT	ENZYME ACTIVITY		
				ROOT(+)	INNER(*) RHIZOSPHERE	OUTER(*) RHIZOSPHERE
RAPE	CONTROL	0	2.47	10.82	8.39	2.84
	G. MOSSEAE	0	2.18	9.28	8.99	2.20
	G. GEOSPORUM	0	2.45	11.38	9.18	2.89
	G. MONOSPORUM	0	2.25	10.58	8.60	2.65
	LSD, P=0.05	NT	NS	0.82	1.09	0.34
WHEAT	CONTROL	7	0.56	3.85	0.86	0.23
	G. MOSSEAE	66	0.99	4.56	1.19	0.38
	G. GEOSPORUM	57	0.51	4.17	1.07	0.25
	G. MONOSPORUM	59	0.58	4.01	0.92	0.28
	LSD, P=0.05	NT	0.30	0.34	0.15	0.03
					TOTAL RHIZOSPHERE	
ONION	CONTROL	10	0.03	4.10	0.48	
	G. MOSSEAE	65	0.56	5.23	0.57	
	G. GEOSPORUM	70	0.08	4.99	0.60	
	G. MONOSPORUM	80	0.10	4.25	0.49	
	LSD, P=0.05	NT	0.05	0.65	0.05	

Table 8.1 Acid phosphatase activities in Experiment 1 after 53 (wheat), 72 (rape) or 98 (onion) days.

NS - not significant; NT - not tested; ND - no data;
+ - μmol p-nitrophenol (100 mg fresh root tissue)$^{-1}$ hour^{-1}; * - μmol p-nitrophenol (g sand)$^{-1}$ hour^{-1}

TREATMENT	% VAM INFECTION	SHOOT D.W. (g) PER POT	% P(D.W.) CONCENTRATION IN SHOOTS	ENZYME ACTIVITY		
				ROOT(+)	INNER(*) RHIZOSPHERE	OUTER(*) RHIZOSPHERE
25 DAYS:						
CONTROL	0	0.20	ND	2.34	1.13	0.61
G. MOSSEAE	3	0.17		2.82	1.11	0.58
G. GEOSPORUM	7	0.26		2.81	0.98	0.59
G. MONOSPORUM	0	0.23		1.94	0.99	0.60
LSD, P=0.05	NT	NS		0.32	0.15	0.09
51 DAYS:						
CONTROL	0	0.32	0.14	5.70	1.04	0.54
G. MOSSEAE	60	0.42	0.19	6.32	1.41	0.79
G. GEOSPORUM	15	0.38	0.22	6.17	1.34	0.72
G. MONOSPORUM	28	0.53	0.20	5.43	1.01	0.58
LSD, P=0.05	NT	0.09	0.03	0.36	0.30	0.14
74 DAYS:						
CONTROL	1	0.24	0.09	5.02	1.21	0.66
G. MOSSEAE	76	0.45	0.16	7.81	1.82	0.78
G. GEOSPORUM	74	0.54	0.15	7.46	2.02	0.84
G. MONOSPORUM	68	0.55	0.14	4.93	1.19	0.65
LSD, P=0.05	NT	0.13	0.02	0.53	0.22	0.16

Table 8.2 Growth responses and acid phosphatase activities at three successive harvests of winter wheat plants in Experiment 2.

rate of growth and paucity of root material produced, and therefore the rhizosphere was not divided into inner and outer zones (even after 98 days). Rhizosphere levels of phosphatases were higher for plants infected with *Glomus mosseae* and *Glomus geosporum* compared with the control plants. Infection by *Glomus monosporum*, however, did not produce this increase in enzyme activity, but nevertheless a three-fold increase in shoot dry weight was noted compared with the uninfected controls. The reason for this anomaly is unclear. Wheat plants infected with *Glomus mosseae* grew better than the control, and had significantly higher rhizosphere levels of phosphatases. Control plants had become contaminated by extraneous or residual VAM infection by 53 days so further readings were not taken. The comparatively high levels of phosphatases produced by the non-mycorrhizal rape compared to either the mycorrhizal onions or mycorrhizal wheat should also be noted.

In Experiment 2 the wheat response to VAM infection was examined in more detail with respect to age of plant. Yields, phosphorus concentration in shoot material, and the effect on phosphatase levels in the root and rhizosphere were monitored. Results in Table 8.2 show that the level of acid phosphatase activity had increased relative to that of the control in both inner and outer rhizosphere zones and roots for plants infected with either *Glomus geosporum* or *Glomus mosseae* after 51 days and 74 days (but not by day 25). In contrast, the levels of activity associated with control plants or with those infected by *Glomus monosporum* do not increase. Significantly higher internal shoot P levels at days 51 and 74 were again noted following infection in all three endophyte treatments. Plants infected with *Glomus monosporum* showed no differences in root or rhizosphere phosphatase activity from that of the control (Table 8.2), but had significantly increased shoot dry weights and almost double the phosphorus concentration within the plant foliage compared with the control.

In both these experiments, infection of host plants by some VAM fungi has been shown to influence activities of root and rhizosphere phosphatases. In the second experiment, for example, infection of wheat by two out of the three VAM isolates tested resulted in a significant increase in phosphatase activity in the rhizosphere and root, coinciding with increased growth and higher internal P concentrations. This does not corroborate the results obtained by Azcon *et al.* (1982) with lavender. In Experiment 1, however, results indicated that only infection of wheat by *Glomus mosseae* increased root and rhizosphere levels of phosphatases compared with controls. One possibility for this difference may have been the slightly lower pH of the coarse sand used in the second experiment which was 0.5 units below that of the sand used in the first experiment, or may reflect other subtle differences in the experiment e.g. rates of spread of infection or early establishment of infection. Recent work by Hayman and Tavares (1985) showed that different endophytes respond better, with respect to root infection and stimulation of host growth response, at different soil pH's. This response was also shown to vary in different soils maintained at the same pH. Hedley *et al.* (1983) showed that rape grown in a sandy loam soil (pH 6.5), in pots, with a low P status acidified its rhizosphere (pH 4.3) and depleted acid-soluble forms of soil P. Plants thus absorbed twice the amount of P which could be desorbed from the control soil (non-acidified). It was also noted that an increase in rhizosphere phosphatase activity (a factor of ten higher than control soil levels) with increasing severity of P-deficiency appeared to be a response to increasing root density and decreasing concentration of soluble inorganic P in the soil. In these results rape produced higher levels of extracellular phosphatases compared with onion and wheat (Table 8.1). It may be that phosphatase production could be inversely related to mycorrhizal dependency of a crop species, since root phosphatase activity and the VAM symbiosis could be

regarded as alternative mechanisms for P uptake by plants growing in low soil-P conditions (see also Azcon *et al.*, 1982). There appears to a simple process of diffusion of these enzymes from the high activity of the root surface (rhizoplane) into the sand medium. It would appear therefore that the increased levels of phosphatases noted in the rhizosphere is not a result of direct secretion from external hyphae but occurs due to diffusion from the mycorrhizal root surface. This does not, however, explain the anomaly of *Glomus monosporum*, which may increase the rate of P uptake by more efficient uptake or translocation of phosphate, or may have a particularly efficient phosphate transfer system involving less overall production of internal phosphatase activity. Cooper and Tinker (1981) suggested that translocation, rather than uptake of P, is the rate limiting step in total transfer of P into the host plant. It is possible therefore that *Glomus monosporum* can translocate P at a faster rate than either of the other two isolates and, as result, an increase in phosphatase levels is not necessary and thus release is not observed. Hedley *et al.* (1983) using rape suggested that acidification of the rhizosphere led to dissolution of an acid-soluble form of inorganic P (Pi) which was not exchangeable with the ^{32}P used in their isotope-exchange technique for estimating P taken up from the labile pool. Alternatively, it may be hypothesised that if *G. monosporum* could 'tap' other sources of Pi unavailable to the non-mycorrhizal plants or even those infected with *G. geosporum* or *G. mosseae* and therefore not deplete the Pi in the labile pool the plant would not be severely P-deficient and no such increase in phosphatase activity would occur. The *G. geosporum* and *G. mosseae* symbioses may show increased root phosphatase activity as a result of depleting the available Pi supply. It is noteworthy that these experiments were carried out in coarse sands with little bicarbonate-soluble P and the source of the increased shoot P concentrations observed in Experiment 2 in VAM-infected plants is unclear and

deserves further study since such observations have been made before (Dodd *et al.*, 1983). It appears that models for P uptake based on *in vitro* measurements of physicochemical parameters controlling phosphate diffusion in soil apparently work well for plants supplied with sufficient P, but underestimate P uptake by P deficient plants grown in soils in which soluble P levels are sensitive to pH fluctuations (Hedley *et al.*, 1983). My *G. monosporum* isolate has consistently been the most efficient of the three isolates in stimulating wheat growth and P uptake (see chapter 6) in greenhouse experiments. It should also be noted that *Glomus monosporum* was originally isolated from a low P soil with a high clay content (see chapter 3) whereas the other two endophytes were isolated from a soil with a higher nutrient regime and with a lower clay component.

It would appear, therefore, that rhizosphere phosphatase activity is unrelated to the VAM external network and is a response to increased root phosphatase (from infected or non-infected root cells) activity diffusing into the rhizosphere. The role of phosphatases in the soil would obviously depend on the concentrations and chemical state of soil phosphorus as well as the chemical structure of the soil itself. It is of note, however, that Alexander and Hardy (1981) showed that Sitka spruce ectomycorrhizas increased activity of root surface phosphatases but trees grew poorly due to an apparent deficiency of inorganic P. They suggested that, in their investigations, high phosphatase activity could not compensate for an adequate supply of labile inorganic P.

CHAPTER 9

GENERAL DISCUSSION

Extensive early development of VAM infection in the roots of winter cereals has not been noted before and this novel observation may imply that they potentially have a more important role in seedling establishment and early nutrient uptake than previously acknowledged. Although this phenomenon occured chiefly in the Sussex fields, which had high clay contents and low bicarbonate-soluble phosphorus levels, these soil types are not unusual in being used for commercial cereal growth. The role of the indigenous VAM population in stimulating P uptake, however, remains unclear but from this study it appears that they are relatively inefficient when compared with single species inoculants of coarse endophytes introduced into partially sterile field soil eight months after methyl bromide fumigation (see Chapter 7). The application of a large quantity of chicken manure to the G-2 field resulted in reduced infection levels and higher shoot P concentrations in wheat in 1984/5 compared with the crops grown at the same site in 1982/3. These facts indicate that although high VAM infection levels can occur in winter cereals at the Sussex site they are not essential and the crop can still respond to added phosphorus. Buwalda *et al* (1985a,b) reached a similar conclusion in their investigations. A major component of the total infection recorded at both Hamill and Sussex field sites consisted of the fine endophyte. This was particularly noticeable in the winter and early spring period and later in the growing season. Fine endophytes have frequently been noted to be relatively inefficient in stimulating growth and P uptake in pot studies in the greenhouse. Rabatin (1979) studied graminaceous root systems at three field sites and suggested that these fungi may have adapted to a rapid infection strategy in periods when P availability in the soil is low and root attrition high (e.g. low moisture in the spring). This strategy may benefit both fine

endophytes and the perennial graminaceous roots of the hosts over short periods of time but this balanced association may be disturbed in cereal crops which are grown on a yearly cycle, and under higher soil nutrient conditions. The ecological significance of infections due to fine endophytes in cereal crops would provide a interesting project for future work. Wilson and Trinick (1983) noted different initial infection and infection spread characteristics of fine and coarse endophytes and this may suggest that each has adapted to forming a VAM association at a particular stage of the development of the host, governed by environmental factors such as soil moisture, temperature and root growth (see chapter 3, part 2). It should also be noted that added superphosphate reduced VAM infection in Experiment 1 of chapter 6 in my study, significantly reduced the weight of ear yields, yet increased straw yields. It may be concluded that the balance between a neutral symbiosis in wheat, as suggested in this and other studies (e.g. Buwalda *et al.* 1985a,b), and a pathogenic relationship may be small.

I have also shown that the use of spore wall morphological characteristics, determined by light microscopy, can lead to confusion and possible misidentification in taxonomic studies when VAM fungal spores of similar appearence are examined, e.g. *G. monosporum* and *G. mosseae*. I have confirmed that the use of PAGE and specific enzyme stains (Sen and Hepper, 1986) can be extremely useful in ascertaining the taxonomic status of VAM endophytes at a species level within *Glomus*. The future use of such a system in identifying individual VAM infections within root systems and estimation of relative levels within mixed infections (Hepper, pers.comm.) could prove to be a major advance in mycorrhizal studies.

The use of pesticides at equivalent field rates in this study has indicated that certain fungicides can inhibit the spread of VAM infection within wheat roots and

also spore germination *in vitro*, whilst other fungicides have no effect on the VAM symbiosis and the P uptake by the wheat crop. It is, however, noteworthy that specific active moities were consistent in their effects on the VAM association even though they were present in different agrochemical products. The use of such chemicals may be useful in the future for investigating the metabolic processes involved in VAM fungal systems either via a direct effect on the fungus in the plant and soil, or indirectly by altering host metabolism (e.g. photosynthesis inhibiting-herbicides).

In this thesis differences were noted concerning the relative efficiencies of the three VAM fungi in stimulating growth and P uptake of wheat grown in pots in sterile durite under low P conditions. These differences were not reflected by differences in root and rhizosphere phosphatase activity. Acid phosphatase activity was not apparently released from the external mycelial network but from the root surface. The increased levels of enzyme activity noted for plants infected with *G. mosseae* and *G. geosporum* compared with those infected with *G. monosporum* may reflect the original soil environment from which the fungi were obtained. *G. mosseae* and *G. geosporum* were isolated from the field at Hamill (with a relatively high soil P status) and *G. monosporum* from the site at Sussex (lower soil P levels). The differences in soil phosphatase levels may therefore be due to decreased P availability when all three fungi are investigated under low soil P conditions, and may reflect the ability of the *G. monosporum* to obtain P and translocate it more efficiently that the other two endophytes.

REFERENCES

ABBOTT, L.K., (1982) Comparative anatomy of vesicular-arbuscular mycorrhizas formed on subterranean clover. *Australian Journal of Botany*, vol. 30, pp. 485-499.

ABBOTT, L.K. and ROBSON, A.D., (1981) Infectivity and effectiveness of vesicular-arbuscular mycorrhizal fungi: Effect of inoculum type., *Australian Journal of Agricultural Research*, vol. 32, pp. 631-639.

ABBOTT, L.K. and ROBSON, A.D., (1982) The role of vesicular-arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation., *Australian Journal of Agricultural Research*, vol. 33, pp. 389-408.

ABBOTT, L.K. and , A.D. ROBSON, (1985) Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi., *New Phytologist*, vol. 99, pp. 245-255.

ALDWELL, F.E.B., HALL, I.R., and SMITH, J.M.B., (1985) Enzyme-linked immunosorbent assay as an aid to taxonomy of the Endogonaceae., *Transactions of the British Mycological Society*, vol. 84, pp. 399-402.

ALEXANDER, I.J. and HARDY, K., (1981) Surface phosphatase activity of Sitka spruce mycorrhizas from a serpentine site., *Soil Biology and Biochemistry*, vol. 13, pp. 301-305.

ALLEN, E.B. and ALLEN, M.F., (1980) Natural re-establishment of vesicular-arbuscular mycorrhizae following stripmine reclamation in Wyoming., *Journal of Applied Ecology*, vol. 17, pp. 139-147.

ALLEN, M.F., (1982) Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis*., *New Phytologist*, vol. 91, pp. 191-196.

ALLEN, M.F. and BOOSALIS, M.G., (1983) Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat., *New Phytologist*, vol. 93, pp. 67-76.

ALLEN, M.F., MOORE, T.S., and CHRISTENSON, M., (1980) Phytohormone changes in *Bouteloua gracilis* infected with vesicular-arbuscular mycorrhizae. I. Cytokinin increases in the host plant., *Canadian Journal of Botany*, vol. 58, pp. 371-374.

ALVEY, N.G. and others,, (1977) GENSTAT, A General Statistical Program., *Rothamsted Experimental Station*.

AMES, R.N., REID, C.P.P., PORTER, L.K., and CAMBARDELLA, C., (1983) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus., *New Phytologist*, vol. 95, pp. 381-396.

AMIJEE, F., STRIBLEY, D.P., and TINKER, P.B., (1986) The development of endomycorrhizal root systems. VI. The relationship between development of infection, and intensity of infection in young leek roots., *New Phytologist*, vol. 102, pp. 293-301.

ANON., (1984) Use of fungicides and insecticides on cereals., in *ADAS booklet 2257(84)*.

ANTIBUS, R.K., CROXDALE, J.G., MILLER, O.K., and LINKINS, A.E., (1981) Ectomycorrhizal fungi of *Salix rotundifiora* III. Resynthesized mycorrhizal complexes and their surface phosphatase activities., *Canadian Journal of Botany*, vol. 59, pp. 2458-2465.

ASAI, T., (1934) Uber das Vorkommen und die Bedeutung der Wurzelpilze in den Landpflanzen., *Japanese Journal of Botany*, vol. 7, pp. 107-150.

AZCON, R., BORIE, F., and BAREA, J.M., (1982) Exocellular acid phosphatase activity of lavender and wheat roots as affected by phytate and mycorrhizal inoculation., in *Les Mycorrhizes: biologie et utilization*, ed. S. GIANINAZZI, V. GIANINAZZI-PEARSON and A. TROUVELOT, INRA., vol. 13, pp. 83-85, Les Colloques de l'INRA.

AZCON, R. and OCAMPO, J.A., (1981) Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars., *New Phytologist*, vol. 87, pp. 677-685.

AZCON, R. and OCAMPO, J.A., (1984) Effect of root exudation on VA mycorrhizal infection at early stages of plant growth., *Plant and Soil*, vol. 82, pp. 133-138.

BACKHOUSE, D., WILLETTS, H.J., and ADAM, P., (1984) Electrophoretic studies of *Botrytis* species., *Transactions of the British Mycological Society.*, vol. 82, pp. 625-630.

BAILEY, J.E. and SAFIR, G.R., (1978) Effect of benomyl on soybean endomycorrhizae., *Phytopathology*, vol. 68, pp. 1810-1812.

BAKER, C.K., GALLAGHER, J.N., and MONTEITH, J.L., (1980) Daylength change and leaf appearence in winter wheat., *Plant Cell and Environment*, vol. 3, pp. 285-287.

BAREA, J.M. and AZCON-AGUILAR, C., (1982a) Interactions between mycorrhizal fungi and soil microorganisms., in *Les Mycorrhizes: Biologie et Utilization*, ed. S. GIANINAZZI, V. GIANINAZZI-PEARSON and A. TROUVELOT, pp. 181-193, INRA, Paris.

BAREA, J. M. and AZCON-AGUILAR, C., (1982b) Production of plant growth-regulating substances by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*., *Applied and Environmental Microbiology*, vol. 43, pp. 810-813.

BARRACLOUGH, P.B. and LEIGH, R.A., (1984) The growth and activity of winter wheat roots in the field: The effect of sowing date and soil type on root growth of high yielding crops., *Journal of Agricultural Science, Cambridge*, vol. 103, pp. 59-74.

BARTLETT, E.M. and LEWIS, D.H., (1973) Surface phosphatase activity of mycorrhizal roots of beech., *Soil Biology and Biochemistry*, vol. 5, pp. 249-257.

BATCH, J.J., (1981) Recent developments in growth regulators for cereal crops., *Outlook in Agriculture*, vol. 10, pp. 371-378.

BATEMAN, G.L. and NICHOLLS, P.H., (1982) Experiments on soil drenching with fungicides against take-all in wheat., *Annals of Applied Biology*, vol. 100, pp. 297-303.

BAYLIS, G.T.S., (1975) The magnolioid mycorrhiza and mycotrophy in root systems derived from it., in *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 373-389, Academic Press, New York and London.

BENJAMIN, R.K., (1979) Zygomycetes and their spores., in *The Whole Fungus*, ed. W.B. KENDRICK, vol. 2, pp. 573-621, National Museums of Canada, Ottawa.

BERTOLDI, M. DE, GIOVANETTI, M., GRISELLI, M., and RAMBELLI, A., (1977) Effects of soil applications of benomyl and captan on the growth of onions and the occurrence of endophytic mycorrhizas and rhizosphere microbes., *Annals of Applied Biology*, vol. 86, pp. 111-115.

BETHLENFALVAY, G.J., BROWN, M.S., and PACOVSKY, R.S., (1982) Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: Development

of the host plant. , *Phytopathology*, vol. 72, pp. 889-893.

BEVEGE, D.I., BOWEN, G.D., and SKINNER, M.E., (1975) Comparative carbohydrate physiology of ecto and endomycorrhizas. , In *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 152-174, Academic Press, New York and London.

BHAT, K.K.S., NYE, P.H., and BALDWIN, J.P., (1976) Diffusion of phosphate to plant roots in soil. IV. The concentration distance profile in the rhizosphere of roots with root hairs in low P soil. , *Plant and Soil*, vol. 44, pp. 63-72.

BIELESKI, R.L., (1973) Phosphate pools, phosphate transport and phosphate availability. , *Annual Review of Plant Physiology*, vol. 24, pp. 225-252.

BLACK, R.L.B. and TINKER, P.B., (1979) The development of endomycorrhizal root systems. II. Effect of agronomic factors and soil conditions on the development of vesicular- arbuscular mycorrhizal infection in barley and on the endophyte spore density. , *New Phytologist*, vol. 83, pp. 401-413.

BOATMAN, N., PAGET, D., HAYMAN, D.S., and MOSSE, B., (1978) Effects of systemic fungicides on vesicular-arbuscular mycorrhizal infections and plant phosphate uptake. , *Transactions of the British Mycological Society*, vol. 70, pp. 443-450.

BOLAN, N., ROBSON, A.D., BARROW, N.J., and AYLMORE, L.A.G., (1984) Specific activity of phosphorus in mycorrhizal and non-mycorrhizal plants in relation to the availability of phosphorus to plants. , *Soil Biology and Biochemistry*, vol. 16, pp. 299-304.

BONFANTE-FASOLO, P. and VIAN, B., (1984) Wall texture in the spore of a vesicular-arbuscular mycorrhizal fungus. , *Protoplasma*, vol. 120, pp. 51-60.

BOWEN, G.D., (1980) Misconceptions, concepts and approaches in rhizosphere biology., in *Contemporary Microbial Ecology*, ed. D.C. ELLWOOD, J.N. HEDGER, M.J. LATHAM, J.H. SLATER and J.M. LYNCH, pp. 283-304. Academic Press, New York and London.

BOWEN, G.D., , D.I. BEVEGE, and , B. MOSSE, (1975) Phosphate physiology of vesicular-arbuscular mycorrhizas., in *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 241-260, Academic Press, New York and London.

BOWMAN, R.A. and OLSEN, S.R., (1985) Assessment of phosphate-buffering capacity: 1. Laboratory methods., *Soil Science*, vol. 140, pp. 287-291.

BROOKS, D.H., (1972) Observations on the effects of mildew, *Erysiphe graminis*, on the growth of spring and winter barley., *Annals of Applied Biology*, vol. 70, pp. 149-156.

BRUIN, G.C.A. and EDGINGTON, L.V., (1984) The chemical control of diseases caused by zoosporic fungi., in *Zoosporic Plant Pathogens*, ed. S.T. BUCZACKI, pp. 193-232, Academic Press, New York.

BUWALDA, J., (1983) The development of vesicular-arbuscular mycorrhizal root systems and their role in the growth of cereals., in *Ph.D. Thesis.*, University of London.

BUWALDA, J., STRIBLEY, D.P., and TINKER, P.B., (1985a) Effects of vesicular-arbuscular infection in first, second and third cereal crops., *Journal of Agricultural Science, Cambridge*, vol. 105, pp. 631-647.

BUWALDA, J., STRIBLEY, D.P., and TINKER, P.B., (1985b) Vesicular-arbuscular mycorrhizae of winter and spring cereals., *Journal of Agricultural Science, Cambridge*, vol. 105, pp. 649-657.

CALLOW, J.A., CAPACCIO, L.C.M., PARISH, G., and TINKER, P.B., (1978) Detection and estimation of polyphosphate in vesicular-arbuscular mycorrhizas., *New Phytologist*, vol. 80, pp. 125-134.

CAPACCIO, L.C.M. and CALLOW, J.A., (1982) The enzymes of polyphosphate metabolism in vesicular- arbuscular mycorrhizas., *New Phytologist*, vol. 91, pp. 81-91.

CARR, G.R. and HINKLEY, M.A., (1985) Germination and hyphal growth of *Glomus caledonicum* on water agar containing benomyl., *Soil Biology and Biochemistry*, vol. 17, pp. 313-316.

CARR, G.R., HINKLEY, M.A., TACON, F. LE, HEPPER, C.M., JONES, M.G.K., and THOMAS, E., (1985) Improved hyphal growth of two species of vesicular-arbuscular mycorrhizal fungi in the presence of suspension-cultured plant cells., *New Phytologist*, vol. 101, pp. 417-426.

CHILVERS, M.T. and DAFT, J.F., (1982) Effects of low temperature on development of the vesicular- arbuscular mycorrhizal association between *Glomus caledonium* and *Allium cepa*., *Transactions of the British Mycological Society*, vol. 79, pp. 153-157.

CLARKE, C. and MOSSE, B., (1981) Plant growth responses to vesicular-arbuscular mycorrhiza. XII. Field inoculation responses of barley at two soil P levels., *New Phytologist*, vol. 87, pp. 695-703.

COOPER, K.M., (1975) Growth responses to the formation of endotrophic mycorrhizas in *Solanum*, *Leptospermum*, and New Zealand ferns., in *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 291-307, Academic Press, New York and London.

COOPER, K.M. and TINKER, P.B., (1978) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. II. Uptake and translocation of phosphorus, zinc and sulphur., *New Phytologist*, vol. 81, pp. 43-52.

COOPER, K.M. and TINKER, P.B., (1981) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. IV. Effect of environmental variables on movement of phosphorus., *New Phytologist*, vol. 88, pp. 327-339.

COX, G.C., SANDERS, F.E.T., TINKER, P.B., and WILD, J., (1975) Ultrastructural evidence relating to host-endophyte transfer in a vesicular-arbuscular mycorrhiza., in *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, p. pp297, Academic Press, New York and London.

COX, G., MORAN, K.J., SANDERS, F., NOCKOLDS, C., and TINKER, P.B., (1980) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. III. Polyphosphate granules and phosphorus translocation., *New Phytologist*, vol. 84, pp. 649-659.

COX, G. and TINKER, P.B., (1976) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. I. A quantitative ultrastructural study., *New Phytologist*, vol. 77, pp. 371-378.

CRESS, W.A., THRONEBERRY, G.O., and LINDSEY, D.L., (1979) Kinetics of phosphorus absorption by mycorrhizal and non-mycorrhizal tomato roots., *Plant Physiology*, vol. 64, pp. 484-487.

CRUSH, J.R., (1973) Significance of endomycorrhizas in tussock grassland in Otago, New Zealand., *New Zealand Journal of Botany*, vol. 11, pp. 645-660.

CRUSH, J.R., (1976) Endomycorrhizas and legume growth in some soils of the Mackenzie Basin, Canterbury, New Zealand., *New Zealand Journal of Agricultural Research*, vol. 19, pp. 473-476.

DAFT, M.J. and GIAHMI, A.A. EL. (1975) Effects of *Glomus* infection on three legumes., in *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 581-592, Academic Press, New York and London.

DANIELS, B.A. and DUFF, D.M., (1978) Variation in germination and spore morphology among four islates of *Glomus mosseae*., *Mycologia*, vol. 70, pp. 1261-1267.

DANIELS, B.A. and TRAPPE, J.M., (1980) Factors affecting spore germination of the vesicular- arbuscular mycorrhizal fungus *Glomus epigaeum*., *Mycologia*, vol. 72, pp. 457-471.

DANIELS, B.A and GRAHAM, S.O., (1976) Effects of nutrition and soil extracts on germination of *Glomus mosseae* spores., *Mycologia*, vol. 68, pp. 108-116.

DANIELS, B.A and MENGE, J.A., (1980) Hyperparasitisation of vesicular-arbuscular mycorrhizal fungi., *Phytopathology*, vol. 70, pp. 584-588.

DANIELS-HETRICK, B.A. and BLOOM, J., (1983) Vesicular-arbuscular mycorrhizal fungi associated with native tall grass prairie and cultivated winter wheat., *Canadian Journal of Botany*, vol. 61, pp. 2140-2146.

DANIELS-HETRICK, B.A., BOCKUS, W.W., and BLOOM, J., (1984) The role of vesicular-arbuscular mycorrhizal fungi in the growth of Kansas winter wheat., *Canadian Journal of Botany*, vol. 62, pp. 735-740.

DAVIDSE, L.C. and WAARD, M.A. de, (1984) Systemic fungicides, *Advances in Plant Pathology*, vol. 2, pp. 191-257.

DAVIS, E.A. and YOUNG, J.L., (1985) Endomycorrhizal colonisation of glasshouse grown wheat as influenced by fertilizer salts when banded or soil-mixed., *Canadian Journal of Botany*, vol. 63, pp. 1196-1203.

DIGHTON, J., (1983) Phosphatase production by mycorrhizal fungi., *Plant and Soil*, vol. 71, pp. 455-462.

DODD, J.C. and JEFFRIES, P., (1986) Early development of vesicular-arbuscular mycorrhizas in autumn-sown cereals., *Soil Biology and Biochemistry*, vol. 18, pp. 149-154.

DODD, J.C. and KRIKUN, J., (1984) Observations on endogonaceous spores in the Negev desert (Israel)., *Transactions of the British Mycological Society*, vol. 82, pp. 536-540.

DODD, J.C., KRIKUN, J., and HAAS, J., (1983) Relative effectiveness of indigenous populations of vesicular-arbuscular mycorrhizal fungi from four sites in the Negev., *Israeli Journal of Botany*, vol. 32, pp. 10-21.

DOMSCH, K.H., (1964) Soil Fungicides, *Annual Review of Phytopathology*, vol. 2, pp. 293-320.

ELMES, R.P., HEPPER, D.M., HAYMAN, D.S., and O'SHEA, J., (1983) The use of vesicular-arbuscular mycorrhizal roots grown by the nutrient film technique as inoculum for for field sites., *Ann. App. Biol.*, vol. 104, pp. 437-441.

ERWIN, D.C., (1973) Systemic fungicides: Disease control, translocation and mode of action., *Annual Review of Phytopathology*, vol. 11, pp. 389-422.

FANG, YU-CHENG, MCGRAW, A.-C., MODJO, HAKAM, and HENDRIX, J.W., (1983) A procedure for isolation of single-spore cultures of certain endomycorrhizal fungi., *New Phytologist*, vol. 93, pp. 107-114.

FITTER, A.H., (1985) Functioning of vesicular-arbuscular mycorrhizas under field conditions., *New Phytologist*, vol. 99, pp. 257-265.

GALLAGHER, J.N., BISCOE, P.V., and WALLACE, J.S., (1979) Field studies of cereal leaf growth. IV. Winter wheat leaf extension in relation to temperature and

leaf water status. , *Journal of Experimental Botany.* , vol. 30, pp. 657-668.

GERDEMANN, J.W., (1975) Vesicular-Arbuscular Mycorrhizae. , In *The Development and Function of Roots*, ed. J.E. TORREY and D.T. CLARKSONS, pp. 575-591, Academic Press, London.

GERDEMANN, J.W. and TRAPPE, J.M., (1974) Endogonaceae of the Pacific Northwest. , *Mycologia Memoir*, vol. 5, pp. 1-76.

GIANINAZZI, S., GIANINAZZI-PEARSON, V., and DEXHEIMER, J., (1979) Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III - Ultrastructural localisation of acid and alkaline phosphatase in onion roots infected by *Glomus mosseae* (Nicol & Gerd) , , *New Phytologist*, vol. 82, pp. 127-132.

GIANINAZZI-PEARSON, V. and GIANINAZZI, S., (1978) Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. II. Soluble alkaline phosphatase specific to mycorrhizal infection in onion roots. , *Physiological Plant Pathology*, vol. 12, pp. 45-53.

GIANINAZZI-PEARSON, V. and GIANINAZZI, S., (1981) The role of endomycorrhizal fungi in phosphorus cycling in the soil. , In *The Fungal Community, its organisation and role in the ecosystem.* , ed. D.T. WICKLOW and G.C. CALLOW, pp. 637-652, Marcel Dekker, New York.

GILDON, A. and TINKER, P.B., (1981) A heavy metal tolerant strain of a mycorrhizal fungus. , *Transactions of the British Mycological Society*, vol. 77, pp. 648-649.

GIOVANETTI, M. and MOSSE, B., (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. , *New Phytologist*, vol. 84, pp. 489-500.

GIOVANNETTI, M., (1985) Seasonal variations of vesicular-arbuscular mycorrhizas and Endogonaceous spores in a maritime sand dune. , *Transactions of the British*

Mycological Society, vol. 84, pp. 679-684.

GIOVANNETTI, M. and NICOLSON, T.H., (1983) Vesicular-arbuscular mycorrhizas in Italian sand dunes., *Transactions of the British Mycological Society*, vol. 80, pp. 552-557.

GRAHAM, J.H., (1982) Effect of citrus root exudates on germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeum*., *Mycologia*, vol. 74, pp. 831-835.

GRAHAM, J.H., LINDERMANN, R.G., and MENGE, J.A., (1982) Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of Troyer citrange., *New Phytologist*, vol. 91, pp. 183-189.

GRAHAM, J.H. and MENGE, J.A., (1982) Influence of vesicular-arbuscular mycorrhizae and soil phosphorus on take-all disease of wheat., *Phytopathology*, vol. 72, pp. 95-98.

GREEN, N.E., S.O.GRAHAM,, and SCHENCK, N.C., (1976) The influence of pH on the germination of vesicular-arbuscular mycorrhizal spores., *Mycologia*, vol. 68, pp. 929-934.

GREGORY, P.J., McGOWAN, M., BISCOE, P.V., and HUNTER, B., (1978) Water relations of winter wheat.I. Growth of the root system., *Journal of Agricultural Science, Cambridge*, vol. 103, pp. 91-102.

GROSSBARD, E., (1976) Effects on the soil microflora., in *Herbicides - Physiology, Biochemistry, Ecology*., ed. L.J. AUDUS, vol. 2, pp. 99-148, Academic Press.

GROTH, D.E. and MARTINSON, C.A., (1983) Increased endomycorrhizal infection of maize and soybeans after soil treatment with metalaxyl., *Plant Disease*, vol. 67, pp. 1377-1378.

HALE, K.A. and SANDERS, F.E., (1982) Effects of benomyl on vesicular-arbuscular mycorrhizal infection of red clover (*Trifolium pratense* L.) and consequences for phosphorus inflow., *Journal of Plant Nutrition*, vol. 5, pp. 1355-1367.

HALL, I.R., (1977) Species and mycorrhizal infections of New Zealand Endogonaceae., *Transactions of the British Mycological Society*, vol. 68, pp. 341-356.

HALL, I.R., (1984) Taxonomy of VA Mycorrhizal Fungi, in *VA Mycorrhiza*, ed. C.LI. POWELL and D.J. BAGYARAJ, pp. 57-94, CRC Press, Florida.

HALL, I.R. and FISH, B.J., (1979) A key to the Endogonaceae, *Transactions of the British Mycological Society*, vol. 73, pp. 261-270.

HALL, I.R., SCOTT, R.S., and JOHNSTONE, P.D., (1977) Effect of vesicular-arbuscular mycorrhizas on response of 'Grasslands Hula' and 'Tamar' white clovers to phosphorus., *New Zealand Journal of Agricultural Research*, vol. 20, pp. 349-355.

HARDIE, K., (1984) Germination of *Glomus mosseae* spores isolated from stock pots of different ages., *Transactions of the British Mycological Society*, vol. 83, pp. 693-696.

HARLEY, J.L., (1975) Problems of mycotrophy., in *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 1-24, Academic Press, New York and London.

HATTINGH, M.J., GRAY, L.E., and GERDEMANN, J.W., (1973) Uptake and translocation of ^{32}P-labelled phosphate to onion roots by endomycorrhizal fungi., *Soil Science*, vol. 116, pp. 383-387.

HAY, R.K.M. and TUNNICLIFFE-WILSON, G., (1982) Leaf appearance and extension in field grown winter wheat plants : the importance of soil temperature during

vegetative growth.. *Journal of Agricultural Science, Cambridge.*. vol. 99. pp. 403-410.

HAYMAM. D.S.. (1983) The physiology of vesicular-arbuscular endomycorrhizal symbiosis.. *Canadian Journal of Botany.* vol. 61. pp. 944-963.

HAYMAN. D.S.. (1970) *Endogone* spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment.. *Transactions of the British Mycological Society.* vol. 54. pp. 53-63.

HAYMAN. D.S.. (1974) Plant growth responses to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature.. *New Phytologist.* vol. 73. pp. 71-80.

HAYMAN. D.S.. (1978a) Mycorrhizal populations of sown pastures and native vegetation in Otago. New Zealand.. *New Zealand Journal of Agricultural Research.* vol. 21. pp. 271-276.

HAYMAN. D.S.. (1978b) Endomycorrhizas. in *Interactions between non-pathogenic soil microoorganisms and plants.*. ed. Y.R. DOMMERGUES and S.V. KRUPA. pp. 401-442. Elsevier. Amsterdam.

HAYMAN. D.S.. (1982) Practical aspects of vesicular-arbuscular mycorrhiza.. in *Advances in Agricultural Microbiology.* ed. N.S. SUBBA RAO. pp. 325-373. Oxford and IBH Publ. Co.. New Dehli.

HAYMAN. D.S.. (1984) Methods for evaluating and manipulating vesicular-arbuscular mycorrhiza.. in *Microbiological Methods for Environmental Biotechnology.* ed. J.M. GRAINGER and J.M. LYNCH. pp. 95-117. Academic Press. London.

HAYMAN. D.S. and MOSSE. B.. (1979) Improved growth of white clover in hill grasslands by mycorrhizal inoculation.. *Annals of Applied Biology.* vol. 93. pp. 141-148.

HAYMAN, D.S. and STOVOLD, G.E., (1979) Spore populations and infectivity of vesicular-arbuscular mycorrhizal fungi in New South Wales., *Australian Journal of Botany*, vol. 27, pp. 227-233.

HAYMAN, D.S. and TAVARES, M., (1985) Plant gowth responses to vesicular-arbuscular mycorrhiza.XV. Influence of soil p.H. on the symbiotic efficiency of different endophytes., *New Phytologist*, vol. 100, pp. 367-377.

HEDLEY, M.J., NYE, P.H., and WHITE, R.E., (1983) Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. IV. The effect of rhizosphere phosphorus status on the pH, phosphatase activity and depletion of soil phosphorus fractions in the rhizosphere and on the cation-anion balance in the plants., *New Phytologist*, vol. 95, pp. 69-82.

HEPPER, C.M., (1977) A colorimetric method for estimating vesicular-arbuscular mycorrhizal infection in roots., *Soil Biology and Biochemistry*, vol. 9, pp. 15-18.

HEPPER, C.M., (1979) Germination and growth of *Glomus caledonius* spores : The effects of inhibitors and nutrients., *Soil Biology and Biochemistry*, vol. 11, pp. 269-277.

HEPPER, C.M., (1985) Influence of age of roots on the pattern of vesicular-arbuscular mycorrhizal infection in leek and clover., *New Phytologist*, vol. 101, pp. 685-693.

HEPPER, C.M. and JAKOBSEN, I., (1983) Hyphal growth from spores of the mycorrhizal fungus *Glomus caledonicum*: Effect of amino acids., *Soil Biology and Biochemistry*, vol. 15, pp. 55-58.

HEPPER, C.M. and SMITH, G.A., (1976) Observations on the germination of *Endogone* spores., *Transactions of thr British Mycological Society*, vol. 66, pp. 189-194.

HO, I. and TRAPPE, J.M., (1973) Translocation of ^{14}C from *Festuca* plants to their endomycorrhizal fungi., *Nature*, vol. 244, pp. 30-31.

HO, I. and ZAK, B., (1979) Acid phosphatase activity of six ectomycorrhizal fungi., *Canadian Journal of Botany*, vol. 57, pp. 1203-1205.

HOWARD, R.J. and AIST, J.R., (1980) Cytoplasmic microtubules and fungal morphogenesis: ultrastructural effects of methyl benzimidazole-2-ylcarbamate determined by freeze-substitution of hyphal tip cells., *Journal of Cell Biology*, vol. 87, pp. 55-64.

HOWELER, R.H., CADAVID, L.F., and BURCKHARDT, E., (1982) Response of cassava to VA mycorrhizal inoculation and phosphorus application in greenhouse and field experiments., *Plant and Soil*, vol. 69, pp. 327-339.

JABAJI-HARE, S.H. and KENDRICK, W.B., (1985) Effects of fosetyl-Al on root exudation and on composition of extracts of mycorrhizal and nonmycorrhizal leek roots., *Canadian Journal of Plant Pathology*, vol. 7, pp. 118-126.

JAKOBSEN, I., (1983) Vesicular-arbuscular mycorrhizas in field grown crops. II. Effect of inoculation on growth and nutrient uptake in barley at 2 phosphorus levels in fumigated soil., *New Phytologist*, vol. 94, pp. 595-604.

JAKOBSEN, I. and NIELSON, N.E., (1983) Vesicular-arbuscular mycorrhiza in field grown crops. I. Mycorrhizal infection in cereals and peas at various times and soil depths., *New Phytologist*, vol. 93, pp. 401-413.

JALALI, B.L. and DOMSCH, K.H., (1975) Effect of systemic fungitoxicants on the development of endotrophic mycorrhiza., in *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 619-626, Academic Press, New York and London.

JARVIS, M.G., ALLEN, R.H., FORDHAM, S.J., HAZELDEN, J., MOFFAT, A.J., and STURDY, R.G., (1984) Soils and their use in South East England. in *Soil Survey of England and Wales Bulletin No. 15*. ed. R.D. Green and E.M. Thomson. Harpenden.

JENSEN, A., (1983) The effect of indigenous vesicular-arbuscular mycorrhizal fungi on nutrient uptake and growth of barley in two Danish soils., *Plant and Soil*, vol. 70, pp. 155-163.

KHAN, A.G., (1975) The effect of vesicular-arbuscular mycorrhizal associations on the growth of cereals. II. Effect on wheat growth., *Annals of Applied Biology*, vol. 80, pp. 27-36.

KIERNAN, J.M., HENDRIX, J.W., and MARONEK, D.M., (1983) Fertilizer-induced pathogenicity of mycorrhizal fungi to sweetgum seedlings., *Soil Biology and Biochemistry*, vol. 15, pp. 257-262.

KOIDE, R., (1985) The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection., *New Phytologist*, vol. 99, pp. 449-462.

KOSKE, R.E., (1981) *Gigaspora gigantea*: Observations on spore germination of a VA-mycorrhizal fungus., *Mycologia*, vol. 73, pp. 288-300.

KOUGH, J., MALAJCZUK, N., and LINDERMAN, R.G., (1983) Use of the indirect immunofluorescent technique to study the vesicular-arbuscular fungus *Glomus epigaeum* and other *Glomus* species., *New Phytologist*, vol. 94, pp. 57-62.

KRIKUN, J., SPIEGEL, Y., HAAS, J., DODD, J., and LIVESCU, L., (1982) The influence of vesicular-arbuscular mycorrhiza on phosphorus nutrition of pepper seedlings in a loessial soil., *Hassadeh (In Hebrew with English summary)*, vol. 62, pp. 989-991.

KRUCKELMANN, H.W., (1975) Effects of fertilizers, soils, soil tillage, and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infection in arable soils., In *Endomycorrhizas*, ed. F.E. SANDERS, B. MOSSE and P.B. TINKER, pp. 511-526, Academic Press, New York and London.

LESTER, E., (1971) Where do we stand with mildew?, *Arable Farmer*, p. 32.

LEVY, Y. and KRIKUN, J., (1980) Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations., *New Phytologist*, vol. 85, pp. 25-31.

LEWIS, D.H., (1973) Concepts in fungal nutrition and the origin of biotrophy., *Biological Reviews*, vol. 48, pp. 261-278.

LIVOLANT, F., GIRAUD, M.M., and BOULIGAND, Y., (1978) A goniometric effect observed in sections of twisted fibrous materials., *Biol. Cellulaire*, vol. 31, pp. 159-168.

MACDONALD, R.M. and LEWIS, M., (1978) The occurrence of some acid phosphatases and dehydrogenases in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*., *New Phytologist*, vol. 80, pp. 135-141.

MACKO, V., (1981) Inhibitors and stimulants of spore germination and infection structure formation in fungi., in *The Fungal Spore: Morphogenic controls*., ed. G. TURIAN and H.R. HOHL, pp. 565-584, Academic Press, London.

MANJUNATH, A. and BAGYARAJ, D.J., (1981) Components of VA mycorrhizal inoculum and their effects on the growth of onion., *New Phytologist*, vol. 87, pp. 355-361.

MARX, C., DEXHEIMER, J., GIANINAZZI-PEARSON, V., and GIANINAZZI, S., (1982) Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas. IV. Ultracytoenzymological evidence (ATPase) for active transfer processes in the host-arbuscle interface., *New Phytologist*, vol. 90, pp. 37-43.

McLACHLAN, K.D., (1976) Comparative phosphorus responses in plants to a range of available phosphorus situations., *Australian Journal of Agricultural Research*, vol. 27, pp. 323-341.

McLACHLAN, K.D., (1980a) Acid phosphatase activity of intact roots and phosphorus nutrition in the plants. I. Assay conditions and phosphatase activity., *Australian Journal of Agricultural Research*, vol. 31, pp. 429-440.

McLACHLAN, K.D., (1980b) Acid phosphatase activity of intact roots and phosphorus nutrition in plants. II. Variations among wheat roots., *Australian Journal of Agricultural Research*, vol. 31, pp. 441-448.

MENGE, J.A., (1982) Effects of soil fumigants and fungicides on vesicular-arbuscular fungi., *Phytopathology*, vol. 72, pp. 1125-1132.

MENGE, J.A., JOHNSON, E.L.V., and MINASSIAN, V., (1979) Effect of heat treatment and three pesticides upon the growth and production of the mycorrhizal fungus *Glomus fasciculatus*., *New Phytologist*, vol. 82, pp. 473-480.

MENGE, J.A., STEIRLE, D., BAGYARAJ, D.J., JOHNSON, E.L.V., and LEONARD, R.T, (1978) Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection., *New Phytologist*, vol. 80, pp. 575-578.

MILLER, R.M., , T.B. MOORMAN, and SCHMIDT, S.K., (1983) Interspecific plant association effects on vesicular-arbuscular mycorrhiza occurrence in *Atriplex confertifolia*., *New Phytologist*, vol. 95, pp. 241-246.

MORTON, J.B., (1985) Variation in mycorrhizal and spore morphology of *Glomus occultum* and *Glomus diaphanum* as influenced by plant host and soil environment., *Mycologia*, vol. 77, pp. 192-204.

MOSSE, B., (1959) The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular

mycorrhiza. . *Transactions of the British Mycological Society*, vol. 42, pp. 273-286.

MOSSE, B., (1970) Honey-coloured, sessile *Endogone* spores. III. Wall structure., *Archiv. Fur Mikrobiologie*, vol. 74, pp. 146-159.

MOSSE, B., (1972) The influence of soil type and *Endogone* strain on the growth of mycorrhizal plants in phosphate deficient soils. , *Rev. Ecol. Biol. Sol.*, vol. 9, pp. 529-537.

MOSSE, B., (1973) Advances in the study of vesicular-arbuscular mycorrhiza., *Annual Review of Phytopathology*, vol. 11, pp. 171-196.

MOSSE, B. and HAYMAN, D.S., (1971) Plant growth responses to vesicular-arbuscular mycorrhiza. II. In unsterilised field soils., *New Phytologist*, vol. 70, pp. 29-34.

MOSSE, B., HAYMAN, D.S., and ARNOLD, D.J., (1973) Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with ^{32}P., *New Phytologist*, vol. 72, pp. 809-815.

MOSSE, B. and HEPPER, C.M., (1975) Vesicular- arbuscular mycorrhizas in root organ cultures., *Physiological Plant Pathology*, vol. 5, pp. 212-223.

MOSSE, B., STRIBLEY, D.P., and LETACON, F., (1981) Ecology of mycorrhizae and mycorrhizal fungi., *Advances in Microbial Ecology*, vol. 5, pp. 137-210.

MOSSE, B., WARNER, A., and CLARKE, C.A., (1982) Plant growth responses to vesicular-arbuscular mycorrhiza XIII. Spread of an introduced VA endophyte in the field and residual growth effects of inoculation in the second year., *New Phytologist*, vol. 90, pp. 521-528.

NELSEN, C.E. and SAFIR, G.R., (1982) Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition., *Planta*, vol. 154, pp.

25-51.

NEMEC, S., (1980) Effects of 11 fungicides on endomycorrhizal development in sour orange., *Canadian Journal of Botany*, vol. 58, pp. 522-526.

NEMEC, S., (1985) Influence of selected pesticides on *Glomus* species and their infection in citrus., *Plant and Soil*, vol. 84, pp. 133-137.

NEMEC, S., MENGE, J.A., PLATT, R.G., and JOHNSON, E.L.V., (1981) Vesicular-arbuscular mycorrhizal fungi associated with citrus in Florida and California and notes on their distribution and ecology., *Mycologia*, vol. 23, pp. 112-127.

NEVILLE, A.C., (1975) in *Biology of the Arthropod Cuticle*, pp. 1-448, Springer, Berlin-Heidelberg-New York.

NICHOLLS, P.H. and BUXTON, B.S.M., (1982) Influence of weather on herbicide behaviour in soils., *Proceedings of 1982 British Crop Protection Conference: Weeds.*, pp. 161-170.

NIELSEN, J.D. and JENSEN, A., (1983) Influence of vesicular-arbuscular mycorrhizal fungi on growth and uptake of various nutrients as well as uptake ratio of fertilizer P for lucerne (*Medicago sativa*)., *Plant and Soil*, vol. 70, pp. 165-172.

NILSSON, H.E., (1973) Influence of the herbicide Mecoprop on*Gaeumannomyces graminis* and take-all disease of spring wheat. , *Swedish Journal of Agricultural Research* , vol. 3, pp. 105-113.

NYE, P.H., (1977) The rate-limiting step in plant nutrient absorption from soil., *Soil Science*, vol. 123, pp. 292-297.

NYE, P.H. and TINKER, P.B., (1977) in *Solute movement in the soil-root system.*, Blackwell Scientific, Oxford.

OCAMPO, J.A. and BAREA, J.M., (1982) Depressed metabolic activity of VA mycorrhizal fungi by photosynthesis inhibitor herbicides., *Colloq. Inst. Natl. Rech. Agron.*, vol. 13, pp. 267-270.

OCAMPO, J.A. and BAREA, J.M., (1985) Effect of carbamate herbicides on VA mycorrhizal infection and plant growth., *Plant and Soil*, vol. 85, pp. 375-383.

OCAMPO, J.A. and HAYMAN, D.S., (1980) Effects of pesticides on mycorrhiza in field-grown barley, maize and potatoes., *Transactions of the British Mycological Society*, vol. 74, pp. 413-416.

OLSEN, S.R., COLE, C.V., WATANABE, F.S., and DEAN, L.A., (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate., *Circular of the United States Department of Agriculture*, p. p.939.

OLSEN, S.R., WATANABE, F.S., and DANIELSON, R.E., (1961) Phosphorus absorption by corn roots as affected by moisture and phosphorus concentration., *Soil Science Society of America Proceedings*, vol. 25, pp. 289-294.

OWUSU-BENNOAH, E. and MOSSE, B., (1979) Plant growth responses to vesicular-arbuscular mycorrhiza. XI. Field inoculation responses in barley, lucerne and onion., *New Phytologist*, vol. 83, pp. 671-679.

OWUSU-BENNOAH, E. and WILD, A., (1979) Autoradiography of the depletion zone of phosphate around onion roots in the presence of vesicular-arbuscular mycorrhiza., *New Phytologist*, vol. 82, pp. 133-140.

PANG, P.C. and PAUL, E.A., (1980) Effects of vesicular-arbuscular mycorrhiza on ^{14}C and ^{15}N distribution in nodulated fababeans., *Canadian Journal of Soil Science*, vol. 60, pp. 241-250.

PARVATHI, K., VENKATESWARLU, K., and RAO, A.S., (1985) Effects of pesticides on development of *Glomus mosseae* in groundnut., *Transactions of the British*

Mycological Society. vol. 84. pp. 29-33.

PEARLMUTTER. N.L. and LEMBI. C.A.. (1978) Localisation of chitin in algal and fungal cell walls by light and electron microscopy.. *Journal of Histochemistry and Cytochemistry*. vol. 26. pp. 782-791.

PEARSON. V. and TINKER. P.B.. (1975) Measurement of phosphorus fluxes in the external hyphae of endomycorrhizas.. In *Endomycorrhizas*. ed. F.E. SANDERS. B. MOSSE AND P.B. TINKER. pp. 277-287. Academic Press. London.

PHILLIPS. J.M. and . D.S. HAYMAN. (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection.. *Transactions of the British Mycological Society*. vol. 55. pp. 158-161.

PLENCHETTE. C.. FORTIN. J.A.. and FURLAN. V.. (1983a) Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions.. *Plant and Soil*. vol. 70. pp. 199-209.

PLENCHETTE. C.. FORTIN. J.A.. and FURLAN. V.. (1983b) Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. II. Soil fumigation induced stunting of plants corrected by reintroduction of the wild endomycorrhizal flora.. *Plant and Soil*. vol. 70. pp. 211-217.

POPE. P.E.. CHANEY. W.R.. RHODES. J.D.. and WOODHEAD. S.H.. (1983) The mycorrhizal dependency of four hardwood tree species.. *Canadian Journal of Botany*. vol. 61. pp. 412-417.

POPE. P.E. and HOLT. H.A.. (1981) Paraquat influences development and efficacy of the mycorrhizal *Glomus fasciculatus*.. *Canadian Journal of Botany*. vol. 59. pp. 518-521.

PORTER, W. M., (1979) The 'most probable number' method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil., *Australian Journal of Soil Research*, vol. 17, pp. 515-519.

POWELL, C. LI., (1976) Development of mycorrhizal infections from *Endogone* spores and infected root segments., *Transactions of the British Mycologoical Society*, vol. 66, pp. 439-445.

POWELL, C. LI., (1979a) Spread of mycorrhizal fungi through soil., *New Phytologist*, vol. 83, pp. 671-679.

POWELL, C. LI., (1979b) Inoculation of white clover and ryegrass seed with mycorrhizal fungi in unsterile hill soils., *New Zealand Journal of Agricultural Research*, vol. 22, pp. 335-339.

POWELL, C. LI., (1981) Inoculation of barley with efficient mycorrhizal fungi stimulates seed yield., *Plant and Soil*, vol. 59, pp. 487-491.

PRIESTLEY, R. H. and BAYLES, R. A., (1982) Effect of fungicide treatment on yield of winter wheat and spring barley cultivars., *Plant Pathology*, vol. 31, pp. 31-37.

RABATIN, S., (1979) Seasonal and edaphic variation in vesicular-arbuscular mycorrhizal infection of grasses by *Glomus tenuis*., *New Phytologist*, vol. 83, pp. 95-102.

RATHMELL, W. G. and SKIDMORE, A. M., (1982) Recent advances in the chemical control of cereal rust diseases., *Outlook on Agriculture*, vol. 11, pp. 37-43.

RATNAYAKE, M., LEONARD, R. T., and MENGE, J. A., (1978) Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhiza formation., *New Phytologist*, vol. 81, pp. 543-552.

READ, D. J., KOUCHEKI, H. K., and HODGSON, J., (1976) Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection., *New*

Phytologist, vol. 77, pp. 641-653.

REDHEAD, J.F., (1977) Endotrophic mycorrhizas in Nigeria: species of the Endogonaceae and their distribution., *Transactions of the British Mycological Society*, vol. 69, pp. 275-280.

REID, C.P.P. and BOWEN, G.D., (1979) Effects of soil moisture on VA mycorrhiza formation and root development in *Medicago* ., In *The Soil-Root Interface*, ed. J.I. HARLEY and R. SCOTT-RUSSELL, pp. 211-219, Academic Press, London.

RHODES, L.H. and GERDEMANN, J.W., (1978a) Translocation of calcium and phosphate by external hyphae of vesicular-arbuscular mycorrhizae., *Soil Science*, vol. 126, pp. 125-126.

RHODES, L.H. and GERDEMANN, J.W., (1978b) Hyphal translocation and uptake of sulphur by vesicular-arbuscular mycorrhizae of onion., *Soil Biology and Biochemistry*, vol. 10, pp. 355-360.

RHODES, L.H. and GERDEMANN, J.W., (1980) Nutrient translocation in vesicular-arbuscular mycorrhizae., In *Cellular Interactions in Symbiosis and Parasitism*, ed. C.B. COOK, P.W. CAPPAS and E.D. RANDOLPH, pp. 173-195, Ohio State University Press, Columbia, Ohio.

RIDGE, E.H. and ROVIRA, A.D., (1971) Phosphatase activity of intact young wheat roots under sterile and non-sterile conditions., *New Phytologist*, vol. 70, pp. 1017-1026.

ROBERTSON, G.W., (1968) A biometeorological time scale for a cereal crop involving day and night temperatures and photoperiod., *Biometeorology*, vol. 12, pp. 191-223.

ROLAND, J-C., REIS, D., VIAN, B., and SATIAT-JEUNEMAITRE, B., (1983) Tradultion du temps en espace dans les parois des cellules vegetales., *Annales*

des Sciences Naturelles, Botanique, Paris. vol. 5 . pp. 173-192.

ROLDAN-FAJARDO, B.E., BAREA, J.M., OCAMPO, J.A. and AZCON-AGUILAR, C., (1982) The effect of season on VA mycorrhiza of the almond tree and of phosphate fertilisation and species of endophyte on its mycorrhizal dependency. . *Plant and Soil*, vol. 68, pp. 361-367.

ROVIRA, A.D., (1976) Studies on soil fumigation. I. Effects on ammonium nitrate and phosphate in soil and on the growth, nutrition and yield of wheat., *Soil Biology and Biochemistry*, vol. 8, pp. 241-247.

ROVIRA, A.D. and SIMON, A., (1985) Growth, nutrition and yield of wheat in calcareous sandy loams of South Australia: Effects of soil fumigation, fungicide, nematacide and nitrogen fertilizers., *Soil Biology and Biochemistry*, vol. 17, pp. 279-284.

SAIF, S.R., (1981) The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizae. I. Effect of soil oxygen on the growth and mineral uptake of *Eupatorium odoratum* .L. inoculated with *Glomus macrocarpum* . , *New Phytologist*, vol. 88, pp. 649-659.

SAIF, S.R., (1983) The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizas. II. Effect of soil oxygen on growth and mineral uptake in *Eupatorium odoratum* .L., *Sorghum bicolor* (.L.) Moench and *Guizotia abyssinica* (L.f.) Cass. inoculated with vesicular-arbuscular mycorrhizal fungi., *New Phytologist*, vol. 95, pp. 405-417.

SAIF, S.R. and KHAN, A.G., (1975) The influence of season and stage of development of plant on *Endogone* mycorrhiza of field-grown wheat., *Canadian Journal of Microbiology*, vol. 21, pp. 1020-1024.

SAIF, S.R. and KHAN, A.G., (1977) The effect of vesicular-arbuscular mycorrhizal associations. III. Effects on barley growth., *Plant and Soil*, vol. 47, pp. 17-26.

SAME, B.I., ROBSON, A.D., and ABBOTT, L.K., (1983) Phosphorus, soluble carbohydrates and endomycorrhizal infection., *Soil Biology and Biochemistry*, vol. 15, pp. 593-597.

SANDERS, F.E. and TINKER, P.B., (1971) Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas., *Nature*, vol. 233, pp. 278-279.

SANDERS, F.E., TINKER, P.B., BLACK, R.L.B., and PALMERLEY, S.M., (1977) The development of endomycorrhizal root systems. I. Spread of infection and growth-promoting effects with four species of vesicular- arbuscular endophyte., *New Phytologist*, vol. 78, pp. 257-268.

SANTAKUMARI, M. and DAS, V.S.R., (1979) Photosynthetic carbon fixation and changes in the levels of carbohydrate under the influence of diuron or atrazine in some crop and weed species., *Indian Journal of Plant Physiology*, vol. 22, pp. 263-268.

SCHENCK, N.C., GRAHAM, S.O., and GREEN, N.W., (1975) Temperature and light effects on contamination and spore germination of vesicular-arbuscular mycorrhizal fungi., *Mycologia*, vol. 67, pp. 1189-1192.

SCHENCK, N.C. and SMITH, G., (1982) Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida., *Mycologia*, vol. 74, pp. 77-92.

SCHONBECK, F., (1979) Endomycorrhizas in relation to plant diseases., in *Soil-Borne Plant Pathogens*, ed. B. SCHIPPERS and W. GAMS, pp. 271-280, Academic Press, London.

SCHREVEN, D.A. VAN, (1958) Some factors affecting the uptake of nitrogen by legumes., in *Nutrition of the Legumes*, ed. E.G. HALLSWORTH, pp. 137-163,

Butterworths. London.

SCHWAB, S.M., JOHNSON, E.L.V., and MENGE, J.A., (1982) Influence of simazine on formation of vesicular-arbuscular mycorrhizae in *Chenopodium quinona* Willd., *Plant and Soil*, vol. 64, pp. 283-287.

SEN, R. and HEPPER, C.M., (1986) Characterisation of vesicular-arbuscular mycorrhizal fungi (*Glomus* spp.) by selective enzyme staining following polyacrylamide gel electrophoresis., *Soil Biology and Biochemistry*, vol. 18, pp. 29-34.

SEVIOUR, R.J., PETHICA, L.M., SODDELL, J.A., and PITT, D.E., (1985) Electrophoretic patterns of sporangiophore proteins of *Rhizopus* isolates as taxonomic characters., *Transactions of the British Mycological Society*, vol. 84, pp. 701-708.

SIEVERDING, E., (1981) Influence of soil water regimes on VA mycorrhiza. I. Effect on plant growth, water utilization and development of mycorrhiza., *Journal of Agronomy and Crop Science*, vol. 150, pp. 400-411.

SMITH, S.E., (1980) Mycorrhizas of autotrophic higher plants., *Biological Reviews*, vol. 55, pp. 475-510.

SMITH, S.E. and DAFT, M.J., (1977) Interactions between growth, phosphate content and N_2 fixation in mycorrhizal and non-mycorrhizal *Medicago sativa*, *Australian Journal of Plant Physiology*, vol. 4, pp. 403-413.

SMITH, T.F., NOACK, A.J., and COSH, S.M., (1981) The effect of some herbicides on vesicular-arbuscular endophyte abundance in the soil and on infection of host roots., *Pesticide Science*, vol. 12, pp. 91-97.

SNELLGROVE, R.C., SPLITTSTOESSER, W.E., STRIBLEY, D.P., and TINKER, P.B., (1982) The distribution of carbon and the demand of the fungal symbiont in leek

plants with vesicular-arbuscular mycorrhizas.. *New Phytologist*. vol. 92. pp. 75-87.

SPEIR. T.W. and ROSS. D.J.. (1978) Soil phosphatase and sulphatase.. in *Soil Enzymes*. ed. R.G. BURNS. pp. 197-250. Academic Press.

SPIERTZ. J.H.J. and VOS. J.. (1985) Grain growth of wheat and its limitation by carbohydrate and nitrogen supply.. in *Wheat Growth and Modelling*. ed. W. DAY and R.K. ATKIN. pp. 129-141. Plenum Press. New York and London.

SPOKES. J.R.. MACDONALD. R.M.. and HAYMAN. D.S.. (1981) Effects of plant protection chemicals on vesicular- arbuscular mycorrhizas.. *Pesticide Science*. vol. 12. pp. 346-350.

SPURR. A.R.. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy.. *Journal of Ultrastructural Research*. vol. 26. pp. 31-43.

ST.JOHN. T.V.. (1980) Root size. root hairs and mycorrhizal infection: A re-examination of Baylis's hypothesis with tropical trees.. *New Phytologist*. vol. 84. pp. 483-487.

STEEL. R.G.D. and TORRIE. J.H.. (1960) in *Principles and Procedures of Statistics*. McGraw-Hill. New York.

STRIBLEY. D.P.. TINKER. P.B.. and RAYNER. J.H.. (1980) Relation of internal phosphorus concentration and plant weight in plants infected by vesicular-arbuscular mycorrhizas.. *New Phytologist*. vol. 86. pp. 261-266.

SWARD. R.J.. HALLAM. N.D.. and HOLLAND. A.A.. (1978) *Endogone* spores in a heathland area of south-eastern Australia.. *Australian Journal of Botany*. vol. 26. pp. 29-43.

SYLVIA. D.M. and SCHENCK. N.C.. (1983) Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-

arbuscular mycorrhizal fungi. , *New Phytologist*, vol. 95, pp. 655-661.

SYLVIA, D.M. and SCHENCK, N.C., (1983) Germination of chlamydospores of three *Glomus* species as affected by soil matric potential and fungal contamination., *Mycologia*, vol. 75, pp. 30-35.

TABATABAI, M.A. and BREMNER, J.M., (1969) Use of p-nitrophenol phosphate for assay of soil phosphatase activity., *Soil Biology Biochemistry*, vol. 1, pp. 301-307.

TARIQ, V.N., GUTTERIDGE, C.S., and JEFFRIES, P., (1985) Comparative studies of cultural and biochemical characteristics used for distinguishing species within *Sclerotinia*., *Transactions of the British Mycological Society*, vol. 84, pp. 381-397.

TEWARI, J.P., SKOROPAD, W.P., MUKERJI, K.G., and MISHRA, S., (1982) Surface morphology of *Glomus macrocarpum* chlamydospores. , *Transactions of the British Mycological Society*, vol. 79, pp. 364-366.

TIMMER, L.W. and LEYDEN, R.F., (1980) The relationship of mycorrhizal infection to phosphorus-induced copper deficiency in sour orange seedlings., *New Phytologist*, vol. 85, pp. 15-23.

TINKER, P.B., (1978) Effects of vesicular-arbuscular mycorrhizas on plant nutrition and plant growth., *Physiologie Vegetale*, vol. 16, pp. 741-761.

TINKER, P.B., (1980) Role of rhizosphere microorganisms in phosphorus uptake by plants., In *The Role of Phosphorus in Agriculture*, ed. F.E. KWASAHNEH, E.C. SAMPLE and E.J. KAMPRATH, pp. 617-654, American Society of Agronomy, Madison, Wisconsin.

TOMMERUP, I.C., (1983a) Spore dormancy in vesicular-arbuscular mycorrhizal fungi., *Transactions of the British Mycological Society*, vol. 81, pp. 37-45.

TOMMERUP, I.C., (1983b) Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizal fungi in soil., *Transactions of the British Mycological Society*, vol. 81, pp. 381-387.

TOMMERUP, I.C., (1985) Inhibition of spore germination of vesicular-arbuscular mycorrhizal fungi in soil., *Transactions of the British Mycological Society*, vol. 85, pp. 267-278.

TOMMERUP, I.C. and ABBOTT, L.K., (1981) Prolonged survival and viability of VA mycorrhizal hyphae after root death., *Soil Biology and Biochemistry*, vol. 13, pp. 431-433.

TOMMERUP, I.C. and BRIGGS, G.G., (1981) Influence of agricultural chemicals on germination of vesicular-arbuscular endophyte spores., *Transactions of the British Mycological Society*, vol. 76, pp. 326-328.

TRAPPE, J.M., (1982) Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi., *Phytopathology*, vol. 72, pp. 1102-1108.

TRAPPE, J.M., MOLINA, R., and CASTELLANO, M., (1984) Reaction of mycorrhizal fungi and mycorrhizal formation to pesticides., *Annual Review of Phytopathology*, vol. 22, pp. 331-359.

TRAPPE, J.M. and SCHENCK, N.C., (1982) Taxonomy of the fungi forming endomycorrhizae., in *Methods and principles of mycorrhizal research*, ed. N.C. SCHENCK, pp. 1-9, American Phytopathological Society, St. Paul, Minnesota.

TRINICK, M.J., (1977) Vesicular-arbuscular infection and soil phosphorus utilization in *Lupinus* spp., *New Phytologist*, vol. 78, pp. 297-304.

VERKADE, S.D. and HAMILTON, D.F., (1983) Effects of benomyl on growth of *Liriodendron tulipifera* L. seedlings inoculated with the vesicular-arbuscular fungus, *Glomus fasciculatus*., *Scientia Horticulturae*, vol. 21, pp. 253-260.

WALKER, C., (1982) Species in the Endogonaceae: A new species (*Glomus occultum*) and a new combination (*Glomus geosporum*)., *Mycotaxon*, vol. 15, pp. 49-61.

WALKER, C., (1983) Taxonomic concepts in the Endogonaceae: Spore wall characteristics in species descriptions., *Mycotaxon*, vol. 18, pp. 443-455.

WALKER, C., MIZE, C.W., and MCNABB, H.S., (1982) Populations of Endogonaceous fungi at two locations in central Iowa., *Canadian Journal of Botany*, vol. 60, pp. 2518-2529.

WALKER, C. and TRAPPE, J.M., (1981) *Acaulospora spinosa* sp.nov. with a key to the species of *Acaulospora*., *Mycotaxon*, vol. 2, pp. 515-521.

WANG, S.R., (1984) Effect of nitrogen and other factors on plant growth responses to vesicular-arbuscular mycorrhiza., *Ph. D. Thesis, University of London*.

WARNER, A. and MOSSE, B., (1980) Independent spread of vesicular-arbuscular mycorrhizal fungi in soil., *Transactions of the British Mycological Society*, vol. 74, pp. 407-410.

WARNOCK, A.J., FITTER, A.H., and USHER, M.B., (1982) The influence of a springtail *Folsomia candida* (Insecta, Collembola) on the mycorrhizal association of leek *Allium porrum* and the vesicular-arbuscular mycorrhizal endophyte *Glomus fasciculatum*., *New Phytologist*, vol. 90, pp. 285-292.

WELBANK, P.J., GIBB, M.J., TAYLOR, P.J., and WILLIAMS, E.D., (1974) Root growth of cereal crops., *Rothamsted Experimental Station Report part 2*, pp. 26-66.

WILSON, J.M., (1984) Inhibition of germination of spores of a *Gigaspora* species in sterilised soils., *Soil Biology and Biochemistry*, vol. 16, pp. 433-435.

WILSON, J.M. and TRINICK, M.J., (1983) Infection development and interactions between vesicular- arbuscular mycorrhizal fungi., *New Phytologist*, vol. 93, pp. 543-553.

YOST, R.S. and FOX, R.L., (1979) Contribution of mycorrhizae to P nutrition of crops growing on an oxisol., *Agronomy Journal*, vol. 71, pp. 903-908.

ZUBER, M. and MANIBHUSHANRAO, K., (1982) Studies on comparative patterns of proteins and enzymes from isolates of *Rhizoctonia solani* causing sheath blight disease in rice., *Canadian Journal of Botany*, vol. 28, pp. 762-771.

Additional References

ABBOTT, L.K. and ROBSON, A.D. (1985) The effect of Soil pH on the formation of VA mycorrhizas by two species of Glomus. Australian Journal of Soil Research, vol. 23, pp. 253-261.

BATEMAN, G.L. and NICHOLLS, P.H. (1982) Experiments on Soil drenching with fungicides against take-all in wheat. Annals of Applied Biology, vol. 100, pp. 297-303.

BRUNDRETT, M.C., PICHE, Y. and PETERSON, R.L. (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Canadian Journal of Botany, vol. 62, pp. 2128-2134.

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EARLY DEVELOPMENT OF VESICULAR–ARBUSCULAR MYCORRHIZAS IN AUTUMN-SOWN CEREALS

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Summary—Fractional infection of roots of winter cereals (wheat, barley and rye) by vesicular–arbuscular mycorrhizal fungi was monitored in three fields in south-east England over three successive seasons. In most cases there was a peak in fractional infection (up to 60% root length infected) in the period from November to December. This extensive development of mycorrhizas during this period has not previously been noted in winter cereals, and has importance as a determinant of the growth response of annual crops. Differences in experimental technique may have led to this phenomenon being overlooked in earlier work. Fractional infection levels then either dropped sharply or continued to rise gradually until late spring in the three seasons studied. In all cases, a second peak in arbuscular development was noted just prior to ear formation, followed by a post-anthesis decline. These fluctuations in fractional infection have been compared with similar trends recorded for dry weight increases of roots over a winter cereal season, and may be influenced by date of sowing. The winter decrease in fractional infection by VAM noted in two out of the three seasons was apparently due to a relative reduction in the coarse endophytic component of the mycorrhizal association. In contrast, infection due to fine endophytes was more stable and persisted as the major proportion of the association throughout the winter and post-anthesis declines in fractional infection.

INTRODUCTION

It is well established that vesicular–arbuscular mycorrhizas (VAM) improve phosphorus uptake and growth in a wide range of plants (Hayman, 1983) and that VAM occur in many field crops under a range of environmental conditions (Mosse *et al.*, 1981), especially when the availability of phosphorus in the soil is limited. Phosphorus uptake in annual crops may be significantly affected only if the infection is well established shortly after seedling emergence (Jakobsen and Neilsen, 1983). Rapid development of a VAM infection could be a major determinant of response in annual crops. Sanders *et al.* (1977) have, for example, presented data showing that low percentage root infections by VAM (< 10%) can have a beneficial value to the host out of all proportion to their size, especially when the plants are very young and a well-developed external mycelium is present. We report, for the first time, high levels of infection by VAM in field-grown winter wheat (*Triticum aestivum* L.) during late autumn and early spring.

Most previous studies in temperate climates have suggested that infection of cereals by VAM does not reach appreciable levels (> 30%) root length) until late spring or even early summer. In the U.K., for example, infection levels of winter wheat have been reported as sparse in May, but increasing to a peak in September (Hayman, 1970). In Denmark, very low infection by VAM (< 5% root length) was noted in the period up to April (Jakobsen and Neilsen, 1983), whilst no VAM colonization was detected until May in comparable studies in Kansas (Daniels, Hetrick and Bloom, 1983). In contrast, more than 50% of root segments of winter wheat seedlings examined in west Pakistan were mycorrhizal a month after they were sown (Saif and Khan, 1975) although this may reflect a much higher temperature regime, and the fact that large numbers of spores of Endogonaceous fungi were present in the soil. Higher levels of infection in December (7–8% root length) and March (15–16% root length) have been recorded in experimental plots of winter wheat in the U.K. (J. G. Buwalda, Ph.D. thesis, University of London, 1983) but this followed soil inoculation with VAM fungi. Such investigations have led to a general view that infection by VAM is unlikely to significantly influence the growth of winter cereals in temperate climates, at least until a period after flowering when any effects on grain yields would be much reduced. During a three-year survey of cereal fields in south-east England, we have recorded data which contradict this view.

MATERIALS AND METHODS

Soil samples, including cereal roots, were taken from three field sites, Gofflands 1 (G-1), Gofflands 2 (G-2), near Billingshurst, west Sussex, and Hamill, near Ramsgate, east Kent, through three consecutive winter cereal seasons 1982–1985. G-1, a clay loam soil, had been under wheat for 4 yr prior to 1982. G-2, another clay loam soil, had been permanent pasture for 12 yr prior to being planted with winter wheat in 1982. Hamill field, a silty clay loam soil, had been under continuous winter wheat since 1977.

On each occasion eight samples were taken from the top 10 cm of soil at approx. 10 m intervals along a transect across the field. The line of transect was different at each time of sampling. In the laboratory the samples were bulked and eight subsamples consisting of several plants with seminal and nodal roots attached were obtained by washing them free of soil.

Table 1. Scale for assessing qualities of VAM infection

Score on scale	Amount of root cortex infected
0	Absence of VAM structure
1	0–15% of root cortex
2	15–45% of root cortex
3	45–75% of root cortex
4	75–100% of root cortex

The roots were then chopped into 1–1.5 cm segments and stained in Trypan blue in lactic acid solution (Phillips and Hayman, 1970). At least 80 cm of root length per bulked sample were examined under the low power of the compound microscope (× 100) and the percentage root infection calculated by noting the presence or absence of internal VAM structures in each field of view (approx. 1.5 mm) and dividing by the number of fields of view examined. At the same time the quality of infection was assessed by recording, for each field of view, the proportion of infection due to arbuscules compared with that due to mycelium and vesicles, using the scale given in Table 1. Qualitative observations were also made of the relative amount of infection due to fine endophytes (= *Glomus tenue*) in relation to that resulting from infection by hyphae of the coarse species.

None of the sites were experimental plots but were in fields on commercial farms and were subjected to agricultural practices normal for intensively-grown cereals in north-west Europe. In the 1982–3 season for example, G-1 and G-2 received 100 kg ha^{-1} of NPK fertilizer (0-22-22) prior to sowing and 50 kg ha^{-1} of "Nitram" (ICI Agrochemicals) at growth stage 7 (Feekes scale). The crop received a post-emergence herbicide application and was treated with a broad-spectrum fungicide in late spring. At Hamill the "Nitram" application was given as 50 kg ha^{-1} at growth stage 6 and 150 Kg ha^{-1} at stage 8. Three herbicides were applied between growth stages 3 and 6 and five fungicides were applied as three mixtures between growth stages 7 and 10. Soil pH and available bicarbonate-soluble phosphorus (Olsen *et al.*, 1954) were determined several times during the investigation and the results are given in Table 2. The dates of sowing and the respective crops and varieties sown at each field site during the three seasons are recorded in Table 3.

RESULTS

Figures 1 and 2 show the % fractional infection of the roots by VAM during the three growing seasons. Fractional infection is the fraction of the length of host root containing mycorrhizal infection (D. Stribley, personal communication). During 1982–3 and 1984–5, the total infection levels increased rapidly at G-1 and G-2, and more gradually at Hamill, for the two to three months following sowing. Fractional infection then decreased during the subsequent 2–3 months. Infection levels then rose to a peak in late spring. These trends were reflected by the relative proportions of arbuscular infection, which dropped to zero by July or August (Figs 1 and 2). In the 1983–4 season a different, yet consistent, pattern of root colonization was observed. there was no decrease in infection levels during the winter period and the mycorrhizal proportion of the root system increased steadily to a January (G-2) or June (G-1 and Hamill) peak. Infection levels at Hamill were consistently lower than those recorded at either of the Gofflands sites.

The microscopic appearance of the mycorrhizal infection also changed during the growing season and reflected changes in the relative proportion of infection due to endophytes with coarse hyphae relative to those with fine hyphae (= *G. tenue*). Both types of endophyte were present in roots from all three sites when infection levels were high, but the proportion of roots infected by coarse hyphae dropped appreciably after late November in 1982 and 1984. During periods when infection levels were lowest (December to April, 1982–3 and 1984–5) colonization was primarily due to the presence of the fine endophytes. Few coarse hyphae were observed during the overwintering period of these two growing seasons. Appressorial formation by hyphae of coarse endophytes was frequently noted, but the infection did not spread laterally within the root cortex. The rise in infection levels noted during the spring periods was due to extensive mycelial and arbuscular growth of both types of endophyte within the root cortex. The observations during the 1983–4 season differed in that the

Table 3. Crop variety and sowing date at each field, 1982–1984

Site	Season	Crop–Variety	Sowing date
G-1	1982–3	Wheat—Avalon	2 October 82
	1983–4	Wheat—Rapier	2 October 83
	1984–5	Barley	30 September 84
G-2	1982–3	Wheat—Avalon	5 November 82
	1983–4	Wheat—Rapier	15 October 83
	1984–5	Wheat—Rapier	21 October 84
	1984–5	Rye	14 October 84
Hamill	1982–3	Wheat—Avalon	2 October 82
	1983–4	Wheat—Rapier	30 September 83
	1984–5	Oil seed rape	—

Table 2. Soil characteristics at each sample site

Hamill			Goffsland-1			Goffsland-2		
Sample date	Soil P[a]	Soil pH	Sample date	Soil P	Soil pH	Sample date	Soil P	Soil pH
September 1981	76	7.0	October 1982	16	6.9	October 1982	4[b]	6.4
November 1982	59	7.6	February 1983	13	7.0	February 1983	14	6.6
August 1983	39	7.6	November 1984	14	6.5	November 1984	30[d]	6.5
June 1984	35	7.5	March 1985	13	6.8	November 1984	40[d]	6.9
November 1984	62[c]	7.9				March 1985	76[d]	6.6

[a]Bicarbonate soluble P (Olsen) in mg P l^{-1}.
[b]Pasture soil prior to fertilization.
[c]Under oil seed rape.
[d]Chicken manure added at 10 t ha^{-1} prior to sowing.

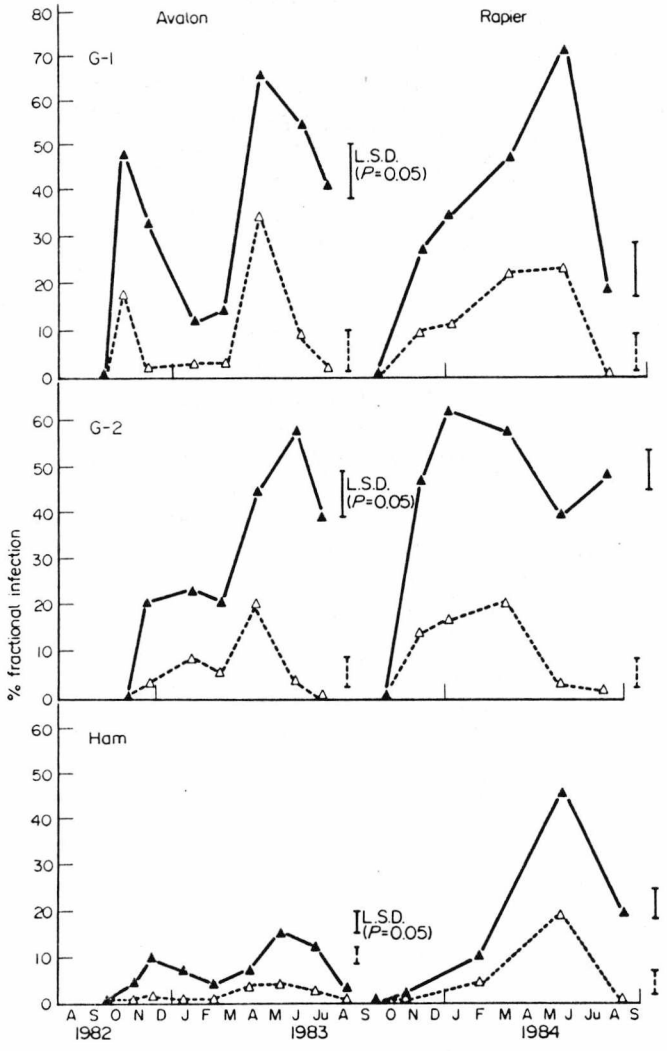

Fig. 1. Percentage fractional infection of VAM of winter wheat varieties Avalon and Rapier at 3 sites (G-1, G-2 and Hamill) during the period 1982–1984. ▲ = total infection (vesicles, intercellular mycelium and arbuscules), △ = infection due to arbuscules alone.

levels of infection due to coarse endophytes persisted overwinter.

Our results describe much more extensive colonization of winter cereal roots in the pre-winter period than has previously been noted. We suspect that this early peak in infection may have been overlooked before for two main reasons. Firstly, several reports do not present data for the late autumn–early winter period, but give observations only from late winter onwards. Secondly, different techniques used for estimation of infection of stained field material may give rise to conflicting results. In particular, the use of the grid-line intersect method for assessment of early season field material under a low power dissecting microscope may result in both young infection sites and the presence of fine endophytes being overlooked. This would result in underestimation of fractional infection. In contrast, the cortex of roots collected later in the season is often colonized by other fungi which stain using the Phillips and Hayman method (e.g. *Olpidium*, *Polymyxa* and *Rhizoctonia*). These are sometimes difficult to distinguish from dense VAM infection under a low power dissecting microscope and hence their presence may lead to overestimation of fractional infection by VAM if this method of assessment is used. In addition, some staining of cortical cells of the root occurs regularly which may also appear similar to a dense arbuscular infection if a low resolution microscope is employed. We have used a compound microscope ($\times 100$ magnification) for estimation of infection in field material throughout these investigations in order to overcome these problems.

Early development of VAM infection in autumn-sown cereals is consistent with the view that arbuscular colonization by VAM is favoured in the young, active regions of the root system. In our results the most extensive arbuscular phases in 1982–3 and 1984–5 occurred during periods when new root growth was likely to be most prolific,

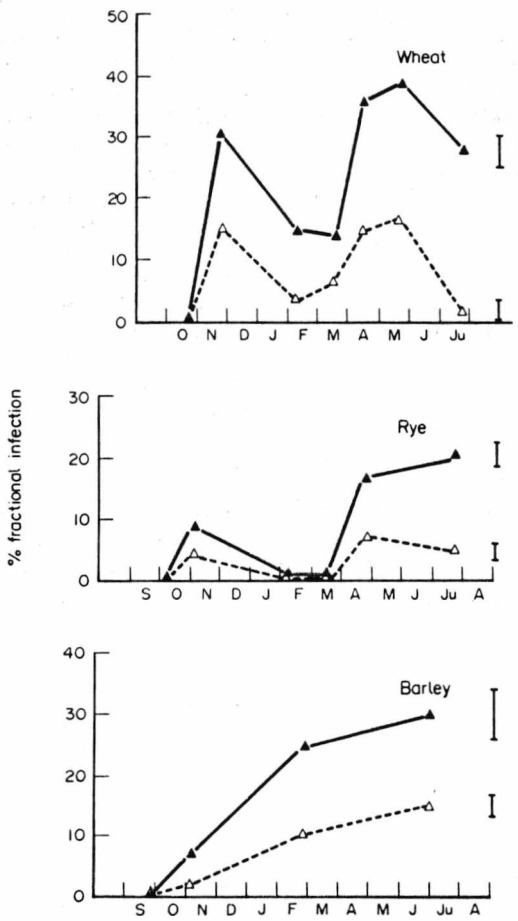

Fig. 2. Percentage fractional infection of VAM in the roots of winter wheat var. Rapier and winter rye at G-2, and winter barley at G-2, during the 1984–1985 season. ▲ = total infection; △ = infection due to arbuscules alone. Bars indicate the least significant differences at the 5% probability level.

particularly during late October and March to May, prior to ear emergence (Barraclough and Leigh, 1984). In contrast, much of the infection recorded during summer (June to August) consisted of vesicles and intercellular mycelium with little of the arbuscular component remaining. Total fractional infection also decreased during the period after anthesis and it may be significant that Welbank *et al.* (1974) reported a decline in dry weight of cereal roots after this growth stage. Buwalda (*loc. cit.*), however, did not record this decline in fractional infection but noted instead an end of season plateau in the amount of infection present. This difference in observations may reflect differences in microscopic technique as discussed above.

In 1983–4 the two peaks in arbuscular activity were masked by a much higher carry-over of infection from the autumn "flush" into the spring. One of the difficulties in interpreting these trends in infection, particularly in relation to the time-course of development, is that % VAM infection is a *ratio*. Changes in fractional infection thus result from two separate processes, root growth and growth of the fungus. In this respect, Barraclough and Leigh (1984) showed that early-sown (September) crops made substantial root growth by December and continued to grow throughout the winter at a faster rate than later-sown crops. The roots of October-sown crops, on the other hand, were slow to develop and their dry weight increased at a much slower rate overwinter. These two differing patterns of root dry weight increase correspond well with our two trends of VAM development through a winter cereal season. For example in 1984–5, early-sown (30 September 84; Table 2) barley showed a steady increase in VAM infection, whereas late-sown (14 October 84) rye and wheat (sown 21 October 84) had the overwinter decline of VAM infection. Thus the date of sowing may be of crucial importance to VAM development in autumn-sown cereals; an early sowing date increasing the likelihood of a longer period of environmental conditions favourable to the growth of roots and VAM fungi. It follows that that any unusual fluctuations in environmental conditions (e.g. in soil temperatures or soil water status) may have an overriding effect on the link between sowing date and patterns of development described here.

Where soil temperatures vary considerably with the season, as in temperate zones, it has been suggested that temperature may be the major rate-limiting factor in infection spread (Buwalda, *loc. cit.*). However, the average local daily temperatures at both locations were similar in all three seasons, and cumulative temperature data differ little over the winter periods. Thus we cannot explain our results solely by low temperature inhibition of infection spread. We have consulted with colleagues at Rothamsted Experimental Station and intend to carry out a more thorough analysis of temperature data, particularly with respect to thermal time.

Plants at both sites (approximately 250 km apart) exhibited the same trends of infection in each season, so local factors were apparently of minor importance in establishing these trends. The difference between the results in 1982–3 and 1983–4 might have been due to the change of winter wheat cultivar, especially since different spring wheat cultivars are known to vary in susceptibility to VAM infection (Azcon and Ocampo, 1981). On this basis, though, the results for 1984–5 (Fig. 2) would have resembled those of 1983–4 and not those of 1982–3. In addition, the two wheat cultivars (Avalon, Rapier) used in our studies have performed similarly with respect to percentage fractional infection in pot experiments using pure inoculum of VAM fungi (personal observations).

It is generally believed that the degree of mycorrhiza formation is inversely correlated with available soil phosphorus levels (Mosse *et al.*, 1981), and the lower infection levels recorded in Hamill soil are presumed to reflect the higher levels of bicarbonate-soluble phosphorus available at this site. It is notable, however, that over 40% of the root length was infected in May 1984, a value far higher than would be expected for a soil (Table 2) containing over 40 mg $P\,l^{-1}$ (ADAS rating 3). This peak in infection levels corresponded with a period of low rainfall, and it is likely that the drought conditions depressed the soil moisture content with a corresponding decrease in P mobility. The mobility of P and other mineral ele-

ments in the soil is lowered when soil water potentials decrease (Olsen *et al.*, 1961). This is known to lead to P-deficient plants during drought stress even though the soil P levels are adequate for greater growth under non-stress conditions (Nelsen and Safir, 1982). Interestingly, infection by VAM tends to be stimulated in plants growing in conditions of drought-stress (Sieverding, 1981). We obtained our highest infection levels in a soil with comparatively low P availability and it would be unreasonable to compare these results with those obtained for a highly fertile soil. Several studies, however, which report negligible infection levels in the late winter period have been carried out on soils with similar low levels of P (e.g. Jakobsen and Neilsen, 1983; Daniels Hetrick *et al.*, 1984). Such soils are not necessarily marginal with respect to cereal cultivation, and large areas of winter wheat are grown each year under these conditions.

We would now maintain that mycorrhizal colonization of autumn-sown crops can occur soon after seed germination, enabling appreciable levels of infection to build up prior to overwintering. The potential influence of the mycorrhizas on plant growth and vigour during the winter months could thus be extremely important, but must remain speculative until the appropriate physiological investigations have been carried out. The inflow of P to roots of winter wheat has a large peak in *mid-winter* and a smaller peak in spring (Gregory *et al.*, 1978), thus infection by VAM during late autumn and early winter could be extremely influential in optimizing nutrient uptake in these plants in agricultural soils. Recent research (Buwalda, *loc.cit.*) has made it clear that the dynamics of uptake of P in winter-sown cereals are such that VAM infection present in the roots at early stages of their growth may have a disproportionally large influence on subsequent growth. It must also be borne in mind that the mycorrhizal relationship may become imbalanced at low temperatures. A cut-off temperature (about 7°C) is believed to exist relating to infections due to coarse endophytes below which the fungus cannot take up P (Hayman, 1983). In such circumstances, a reappraisal is necessary of the influence of VAM on cereal growth.

Our observations draw attention to the frequent occurrence of infections due to fine endophytes in temperate agricultural soils, particularly during the winter months, and later towards the end of the cereal growth season, when these species make up the bulk of the mycorrhizal infection. Other workers have also noted a predominance of fine endophyte infection in crops during the winter months (Jakobsen and Neilsen, 1983). It has been suggested that the fine endophytes are less aggressive than the coarse with respect to infection ability from the growth medium (Wilson and Trinick, 1983), but once infection has occurred the mycelium of a fine endophyte is capable of producing many more secondary infection loci than that of a coarse endophyte. Furthermore, the fine endophyte was apparently slow to infect germinating seeds, but once established it was capable of a more rapid spread from plant to plant than other fungi investigated (Powell, 1979a). These results are reflected by our own observations, and the recolonization by coarse endophytes presumably relates to their more aggressive nature, and the restricted environmental range under which they are able to spread from plant to plant via the root system. Fine endophytes are often the most abundant mycorrhizal types in natural soils (Fitter, 1985). These fungi rarely produce beneficial growth responses, even in infertile soils (Powell, 1979b), and we have noted that they produce little external mycelium in the field. It may be that these two facts are related as Sanders *et al.* (1977) reported that of four VAM isolates introduced into onion roots, it was the one that produced least external mycelium that was the least efficient in terms of stimulating growth of the host.

A strategy for the manipulation of mycorrhizal infections for the future might be to introduce isolates of coarse endophytes that are able to rapidly infect autumn-sown cereals and maintain high infection levels throughout the winter period. Alternatively, cultural practices could be modified such that efficient indigenous fungi are favoured, an approach which would be particularly compatible with the current interest in developing sustainable systems of agriculture.

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REFERENCES

Azcon R. and Ocampo J. A. (1981) Factors affecting the vesicular–arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. *New Phytologist* **87,** 677–685.

Barraclough P. B. and Leigh R. A. (1984) The growth and activity of winter wheat roots in the field: the effect of sowing date and soil type on root growth of high yielding crops. *Journal of Agricultural Science, Cambridge* **103,** 59–74.

Daniels Hetrick B. A. and Bloom J. (1983) Vesicular–arbuscular mycorrhizal fungi associated with native tall grass prairie and cultivated winter wheat. *Canadian Journal of Botany* **61,** 2140–2146.

Daniels Hetrick B. A., Bockus W. W. and Bloom J. (1984). The role of vesicular–arbuscular mycorrhizal fungi in the growth of Kansas winter wheat. *Canadian Journal of Botany* **62,** 735–740.

Fitter A. H. (1985) Functioning of vesicular–arbuscular mycorrhizas under field conditions. *New Phytologist* **99,** 257–265.

Gregory P. J., McGowan M., Biscoe P. V. and Hunter B. (1978) Water relations of winter wheat. 1. Growth of the root system. *Journal of Agricultural Science, Cambridge* **103,** 91–102.

Hayman D. S. (1970) *Endogone* spore numbers in soil and vesicular–arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Transactions of the British Mycological Society* **54,** 53–63.

Hayman D. S. (1983) The physiology of vesicular–arbuscular endomycorrhizal symbiosis. *Canadian Journal of Botany* **61,** 944–963.

Jakobsen I. and Nielsen N. E. (1983) Vesicular–arbuscular mycorrhiza in field-grown crops. 1. Mycorrhizal infection in cereals and peas at various times and soil depths. *New Phytologist* **93,** 401–413.

Mosse B., Stribley D. P. and LeTacon F. (1981) Ecology of mycorrhizae and mycorrhizal fungi. *Advances in Microbial Ecology* **5,** 137–210.

Nelsen C. E. and Safir G. R. (1982) Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. *Planta* **154,** 25–51.

Olsen S. R., Cole C. V., Watanabe F. S. and Dean L. A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *Circular of the United States Department of Agriculture*, p. 939.

Olsen S. R., Watanabe F. S. and Danielson R. E. (1961) Phosphorus absorption by corn roots as affected by moisture and phosphorus concentration. *Soil Science Society of America Proceedings* **25,** 289–294.

Phillips J. M. and Hayman D. S. (1970) Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55,** 158–161.

Powell C. Ll. (1979a) Spread of mycorrhizal fungi through soil. *New Phytologist* **83,** 671–679.

Powell C. Ll. (1979b) Inoculation of white clover and ryegrass seed with mycorrhizal fungi in unsterile hill soils. *New Zealand Journal of Agricultural Research* **22,** 335–339.

Saif S. R. and Khan A. G. (1975) The influence of season and stage of development of plant on *Endogone* mycorrhiza of field-grown wheat. *Canadian Journal of Microbiology* **21,** 1020–1024.

Sanders F. E., Tinker P. B., Black R. L. B. and Palmerley S. M. (1977) The development of endomycorrhizal root systems: 1. Spread of infection and growth-promoting effects with four species of vesicular–arbuscular endophyte. *New Phytologist* **78,** 257–268.

Sieverding E. (1981) Influence of soil water regimes on VA mycorrhiza. 1. Effect on plant growth, water utilisation and development of mycorrhiza. *Journal of Agronomy and Crop Science* **150,** 400–411.

Welbank P. J., Gibb M. J., Taylor P. J. and Williams E. D. (1974) Root growth of cereal crops. *Rothamsted Experimental Station Report Part* 2, pp. 26–66.

Wilson J. M. and Trinick M. J. (1983) Infection development and interactions between vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **93,** 543–553.