The Structure of Chalk Grassland Communities and the Role of Arbuscular Mycorrhizal Fungi

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Abstract

Semi-natural chalk grassland is an internationally important habitat characterised by high species richness at a fine scale. In both the United Kingdom and other European Countries however, significant areas of chalk grassland have been lost to intensive agriculture practices. In the United Kingdom, conservation and expansion of existing chalk grassland sites has become a high priority. Research that leads to a better understanding of the processes that structure chalk grassland communities may aid these objectives.

A number of field trials have been conducted to examine the role of grazing in structuring grassland plant communities, but the role of arbuscular mycorrhizal fungi (AMF)/plant symbiosis has received less attention. In this research project the structure (presence and abundance of species) of chalk grassland communities growing on the South Downs in the United Kingdom is defined. This is achieved by detailed analysis of extensive plant survey data collected in 1991. Analysis revealed strong patterns relating to species presence and abundance in the chalk grassland communities. In particular evidence of „nestedness" and a frequency abundance relationship was found. From these patterns it was deduced that AMF/ plant symbiosis may have a significant role in structuring chalk grassland communities.

In the experimental component of this research project two separate, but connected, trials were conducted. In the first trial chalk grassland turf was sampled for study in the laboratory under controlled environmental conditions. The second trial was conducted on chalk grassland turf, in situ, at the same site from which the laboratory turf had been collected. In both trials the method adopted was to weaken AMF/plant symbiosis by apply increasing doses of the fungicide Iprodione. In the laboratory trial this was conducted over one growing season and in the field trial over two seasons.

There was a good level of consistency between the two trials. At low levels of fungicide there was no discernible change in community structure. But at the higher fungicide dose rates ($2gm^{-2}$ and $4gm^{-2}$), changes to community structure (presence and percentage cover were observed). The results from the field trial suggest that approximately 50% of plants

in the community benefited from the presence of AMF, around 25% were unaffected, whilst the remaining 25% benefited from the absence of AMF. The largest changes in cover were in the mycorrhizal grass *Brachypodium pinnatum* which declined in percentage cover with increased fungicide dose rates. This was associated with a corresponding increase in the cover of the non-mycorrhizal grass *Bromus erectus*. Examination of the roots of selected forbs and grasses suggests that in species that benefit from AMF/plant symbiosis the levels of root infection in individual plant species may be related to percentage cover in the community.

The research suggests that restoration of species rich chalk grassland communities might be better achieved by a process involving several stages. Initially this would involve creating a community consisting of the most abundant grasses and forbs (core species). These would be established in the presence of a full compliment of mycorrhizal fungi species native to chalk grassland. When this matrix community is established less abundant including scarce species would be added in a sequential process.

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Acknowledgements

Having completed my degree in Environmental Science at Brighton University in 2004, and enjoyed the ethos of the department and the company of fellow students and staff, it seemed like a natural progression to carry on and study for a PhD, with little appreciation at the time of the amount of work and effort this would involve.

As a keen gardener from an early age a project involving the ecology of plants was appealing. My third year degree dissertation concerned chalk grassland plants at which time I also became aware of AMF/plant symbiosis. Plants on chalk grassland grow in close proximity and the role of AMF/plant symbiosis, where cooperation as well as competition can occur, in structuring these communities, seemed a potentially useful area for research.

Dr Niall Burnside had been my dissertation supervisor for my first degree and I was delighted when he agreed to be the lead supervisor for my PhD project. Niall was joined by Dr Chris Joyce and Dr Steve Waite as my other two supervisors, each bringing his own areas of strength and expertise to the project. I would like to formally acknowledge the input of all three of my supervisors to this project, which without their help and guidance could never have been completed. In particular I would like to thank Niall for his unswerving enthusiasm, positivity and encouragement over the nearly seven years it has taken to complete this project.

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Last but not least I want to acknowledge the contribution of my wife Jan to the completion of this project. While pursuing her own academic studies she has provided unwavering support (including numerous cups of tea while at the computer) throughout the duration of the project. Without her support it would not have been completed.

Declaration

I declare that the research contained in this thesis, unless formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed ……

Dated ……

Chapter 1 - Introduction

1.1 General Introduction

Calcareous grassland growing on limestone is widespread globally and throughout Europe. However grassland growing on a chalk substrate is limited in extent and confined to north-western Europe, where its scarcity and high diversity of flora and fauna identify it as a habitat of international importance (Biodiversity Action Group, 2000; Willems, 2002). Chalk grassland throughout Europe is characterised by being species rich with many plants co-existing in a small space (Mitchley and Grubb, 1986). This gives it great conservation value. This research project aims to develop a better understanding of how the communities are maintained in a species rich state. The aims of the study were fulfilled by studying community structure through patterns of association, nestedness and frequency/abundance relationships and how these are influenced by arbuscular mycorrhizal fungi. This knowledge will be useful for conservation management and restoration of chalk grassland. The study sites used occurred on chalk grassland growing on the South Downs in the United Kingdom, but the findings will be applicable to chalk grassland in general.

The South Downs are a nationally and internationally important area of chalk grassland on a line of hills approximately 100km long running parallel to the English Channel, starting at Winchester in the west and finishing at Beachy Head in the east. The South Downs are typically 200m - 250m high with flat tops, a gentle south facing dip slope (Figure 1.1) and a steeper north facing scarp slope and intersected by steep, often wooded valleys. Typical soils of chalk grassland on the South Downs are shallow in depth, have a high pH, and are low in the major nutrients nitrogen (N), phosphorous (P) and potassium (K) (Grubb, 1986; Bonis *et al*. 1997). The chalk grassland sward is maintained in a state of plagioclimax (Tansley and Adamson, 1925; Hope-Simpson, 1940), the main arresting factor being moderate levels of grazing (Bacon, 1990; Denyer *et al*. 2009) which reduces the effectiveness of dominant grasses (Hillier, 1990) and holds scrub encroachment in check (Ward, 1990).

Figure 1.1 - View of flat top and south face of Westmeston (west) situated close to the city of Brighton and Hove (Holm, 2006)

1.2 Research Rationale

In Europe the area of calcareous grassland has been declining over the last 50 years (Green, 1990; Fischer and Stocklin, 1997; Poschlod and WallisDeViries, 2002) and the loss on the South Downs follows this general pattern (Burnside *et al.* 2002). On the South Downs estimates suggest that between 2500ha and 4000ha of chalk grassland remains, covering some 3-5% of the original area (Morris, 1990; Burnside *et al*. 2003;). Much of the remnant areas of chalk grassland are highly fragmented (Keymer and Leach, 1990) with individual sites ranging in area from 5 – 130ha (Steven and Muggeridge, 1992; Steven, 1992).

Over the last decade the need to reverse the trends of area loss and increasing fragmentation of chalk grassland on the South Downs has been recognised (Joint Nature Conservation Committee. 1998; Biodiversity Action Group, 2000) and efforts have been made to identify suitable sites for restoration to species-rich chalk grassland (Burnside *et al*. 2002). Even at suitable sites, restoration of species-poor to species-rich chalk grassland can be a slow process (Hutchings and Booth, 1996a; Hutchings and Booth, 1996b).

Research that leads to a better understanding of the factors and mechanisms that determine or influence chalk grassland establishment and development has the potential to aid the conservation of existing species rich chalk grassland. A thorough understanding of the factors which determine the structure and diversity of chalk grassland is essential to facilitate the restoration of species poor, to species rich, chalk grassland.

1.3 Research Aim

Semi-natural chalk grassland is a habitat with high species richness and diversity where species co-exist in complex plant communities. These plant communities are under threat from loss of area and fragmentation.

The aim of this research was to gain a better understanding of two of the critical factors which have the potential to influence species rich chalk grassland plant communities. These factors are the community structure at fragmented semi-natural chalk grassland sites and the potential for the biotic mechanism of the arbuscular mycorrhizal fungi (AMF)/plant symbiosis to influence the structure of chalk grassland communities at a micro scale. There were three experimental strands to this study. A desk based analysis of existing data sets, laboratory studies and manipulative field experiments.

The objectives were to;

Determine the composition of chalk grassland communities, the regional species pool, the functional types and individual species present and their relative abundance in the community. Examine the structure of chalk grassland communities by testing interclass frequency associations and measuring nestedness and abundance-frequency relationships, through the analysis of detailed survey data collected from sites on the South Downs in Sussex

Examine in detail the role of AMF/plant symbiosis in structuring and maintaining chalk grassland plant communities, by creating semi-natural microcosms in the laboratory using turf taken from the site selected for field studies. To treat the turf with graduated doses of fungicide and measure changes in species presence and abundance. Examine the roots of selected species for the presence of AMF.

Determine the role of AMF/plant symbiosis in structuring chalk grassland communities under field conditions using the same fungicide regime as in the laboratory trials. Examine the roots from selected species for the presence of AMF and relate the results to changes in species abundance.

1.4 Thesis Structure

The eight chapters that comprise this thesis are summarised.

1.4.1 Chapter 1 - Introduction

This chapter provides the general background to the thesis and the context for the area in which the research was conducted. The research rationale presented describes briefly the extent of the problem of fragmentation and species loss in calcareous grasslands in Europe and chalk grassland on the South Downs in particular. The research aim and three objectives designed to achieve it are presented together with an outline of the thesis structure and chapter contents.

1.4.2 Chapter 2 - Literature Review

The review begins with a historical perceptive of calcareous grasslands, its distribution and importance with particular reference to chalk grassland on the South Downs. Chalk grassland is maintained in a state of plagioclimax by grazing. The processes of succession are defined and described.

Chalk grassland communities are shaped by a number of abiotic factors, e.g. soil depth, pH, nutrient levels, rainfall and insolation and biotic factors e.g. grazing and plant/AMF symbiosis. These factors are considered in detail and their influence on community composition considered.

The definition of a plant community is considered. Species richness, diversity and grain structure are important terms used for describing the structure of plants growing together in groups. These terms are defined and discussed.

Many mechanisms have been advanced to explain how groups of plant come together to form communities. Those considered to be most relevant to the current research on chalk grassland are presented and evaluated

The second half of the literature review deals with AMF/plant symbiosis. Mycorrhizal fungi have been present in the soil for hundreds of millions of years. Today ~ 80% of vascular plants have been shown to form some type mycorrhizal symbiosis. This is considered along with the different types of mycorrhizal fungi.

This is followed by a description of the components of AMF and why plants might choose to form a symbiotic relationship with AMF. The relationship between plants and AMF is complex and different forms of the symbiosis are considered.

Next the R* concept (of limited resource) for plant species in competition is described and how AMF might alter the balance between plants competing for the same resources. This leads on to consideration of the idea that the relationship between plants and different species of AMF is not symmetric and a spectrum of relationships from the beneficial to the antagonistic can exist. The concept of positive and negative feedback in the symbiotic relationship offers a mechanism by which subordinate species might be able to compete with more dominant species in a plant community and this is discussed.

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The relative merits of research into the potential role of AMF/plant symbiosis in structuring plant communities using a reductionist or a natural community approach are considered within the context of this research project and thesis.

In the final section, research papers describing studies using microcosm and field trials are reviewed and the findings summarised. Three areas considered important to this research project and thesis are reviewed. These are the effect of AMF on seedling establishment, the role of AMF in intra and interspecific plant competition and the role of AMF in determining the composition and structure of complex plant communities.

1.4.3 Chapter 3 - The Research Site

The location of Newmarket Hill, at which the field trials were conducted and from which turf was selected for the laboratory trials, is described. Abiotic and biotic characteristics of the site and site management are presented. The NVC classification (Rodwell, 1990) for Sites 1 and 2 at Newmarket Hill where the field trials took place are given. A review of previous studies at the site is conducted which provides background and context for the present study.

1.4.4 Chapter 4 - The Structure of Chalk Grassland Communities

The aim of the work presented in this chapter was to determine the structure of chalk grassland communities growing on the South Downs in Sussex by detailed analysis of a comprehensive body of survey data. This involved attempting to establish regional species pool composition from species listing and site occurrence. Once the potential regional species pool was established it was used to identify the range of functional plant groups present. Placing the plants into frequency classes and examining interclass relationships allowed interclass patterns to be determined. By calculating the extent of nestedness in the functional group strong calcicoles chalk grassland plants in Sussex were shown to be strongly nested. Examining the survey data showed a relationship between frequency of occurrence (at sites) and abundance.

1.4.5 Chapter 5 - Influence of AMF on Chalk Grassland Community Structure: Laboratory Trials

In this chapter the effect on community structure of weakening AMF/plant symbiosis by the application of fungicide is described. Three trials, a pilot and two full trials were carried out between 2006 and 2008. The methods of collection, stabilisation, and care of the turf used in the laboratory based studies are presented and the doses and procedures for the application of fungicide are given. The survey methods used to obtain a quantitative measure of species presence and abundance throughout the trial are described.

The results of the surveys assessing changes in species presence and abundance with time and treatment are presented in graphical and tabular form and subjected to appropriate statistical analysis. The results of the examination of the roots of selected species for the

presence of AMF are described. The results are discussed in terms of the treatments received; the mycorrhizal affinity of the species involved and compared and contrasted with previously reported studies of chalk grassland species.

1.4.6 Chapter 6 - Influence of AMF on Chalk Grassland Community Structure: Field Trials

This chapter describes the changes to chalk grassland communities following the application of fungicide under field conditions. The survey methods adopted are described. The spraying procedures for the fungicide, dose rates and frequency of application are given. The survey results, collected over four seasons are presented. The two main variables collected were species presence and percentage cover. The data is subjected to analysis with changes in mean percentage cover associated with the different treatments considered in detail.

Changes in rank order of the thirty five most abundant species is considered for the period of the trial at which higher doses of fungicide were applied. A principal component analysis for changes in mean percentage cover resulting from the higher doses of fungicide is performed. The results of root examination of selected species for the presence of AMF and how this relates to changes in mean percentage cover are described. The overall changes observed in plant community structure reslting from the application of fungicide under field conditions are discussed.

1.4.7 Chapter 7 – Discussion

At the start of this chapter chalk grassland at the landscape scale and the importance of the species pool is discussed. The concept of „core and satellite" species and their role in determining community structure is considered. Nestedness and its relationship to species entering and leaving the community are discussed. Nestedness in chalk grassland is compared to nestedness in other habitats.

An aim of the research project was to investigate the role of AMF/plant symbiosis in structuring chalk grassland communities. The two methods adopted, the laboratory turf trials and the field trials are compared and the merits and disadvantages of the two

methods discussed. Differences and commonality in the results from the two methods are highlighted and reasons for the differences advanced.

The results from the laboratory and field trials are compared with those from other relevant research. Firstly the current results are compared to those carried out in artificial microcosms and secondly with results from other field trials.

This is followed by a section that interprets the results of the laboratory and field trials within the context of current ideas on the mechanisms that may be operating within the complex networks of AMF that interconnect plant species in the below ground structure of plant communities.

1.4.8 Chapter 8 – Conclusions

In this final chapter the completion of the aims set out in Chapter 1 are assessed. The main findings from each of the three analysis chapters are summarised. The limitations of the research are considered. Finally recommendations for future research are made. These are for a re-survey of representative sites of chalk grassland sites in Sussex to evaluate changes in species abundance and rank order since the last survey 20 years ago. To examine in greater detail „mycorrhizal interactions" between the dominant grasses *Brachypodium pinnatum*, *Bromus erectus* and *Festuca ovina*. To conduct field studies in chalk grassland at the inter-plant scale, to look for patterns of nearest neighbours within the context of their mycorrhizal relationships. Finally within the area of chalk grassland restoration, to conduct a small trial in which „core" species are allowed to become established and „intermediate" and „scarce" are introduced sequentially.

Chapter 2 - Literature Review

2.1 Introduction

The literature review is divided into three sections. The first section deals with the historical development of calcareous and in particular chalk grassland across Europe. The distribution and international importance of chalk grassland is discussed. In this section the abiotic and biotic parameters that combine to produce the conditions in which semi-natural chalk grassland communities can thrive are described.

In the second section the term „plant community" is defined and the terms used to describe the components and characteristics of plant communities are explained. Plant interactions and AMF are important factors that influence plant community structure. Concepts and mechanisms developed to explain plant community structure are described and discussed with particular emphasis on those most relevant to chalk grassland communities.

In the third section various aspects of AMF/plant symbiosis are considered in detail. This section begins by outlining the different types of mycorrhizal fungi and continues with the description of the anatomical components of arbuscular mycorrhizal fungi. Next the advantages and disadvantages of AMF/ plant symbiosis to the plants and fungi and the range of relationships that can be occurring are considered. The concept of R* in the context of competition for scarce nutrients and how AMF/plant symbiosis may influence the competitive outcome between plant species is discussed. This is followed by a discussion of community feedback between host plants and different species of AMF, where asymmetric relationships between AMF species and plant species may favour one plant species over another.

The third section continues by discussing the merits of detailed observations of plant behaviour within the context of artificial microcosms when compared to field trials, which have less precision but often greater ecological relevance. In the final sections other studies involving AMF/plant symbiosis are described and reviewed. These studies were in the form of both microcosms and field trials. The section starts with work on single or small numbers of plants growing in competition and builds to research where artificially constructed and natural communities were studied.

2.2 The History and Defining Characteristics of Calcareous / Chalk Grassland

2.2.1 Historical perspective

Calcareous grasslands are usually semi-natural habitats many of which have come into existence as a result human intervention. Calcareous and semi-natural chalk grassland is held in a state of arrested succession by intermediate levels of grazing and occasionally cutting. Despite being semi-natural habitats chalk grassland displays many of the properties of natural grassland such as Prairie (Green, 1990). Semi-natural chalk grassland is rich in plant species, associated invertebrates and supportive of higher fauna that are ecologically important (Green, 1990).

The climax community at many calcareous grassland sites would typically be woodland (Green, 1990). According to Poschlod and WallisDeViries (2002) the maximum spread of calcareous grassland in Europe took place between the 15th and 20th centuries when woodland was cleared for grazing, with large flocks of sheep maintained. The farming methods were extensive and in the absence of mineral fertiliser, three field rotation and transhumance were common practices (Poschlod and WallisDeViries, 2002). Studies of calcareous grassland have shown that former land use and age of habitat has a strong influence on recent patterns of species presence (Karlik and Poschlod, 2009; Reitalu *et al*. 2009; Heubes *et al*. 2011)

Low intensity agriculture was still the norm in the United Kingdom in the first half of the 20th century and a review by Hodgson *et al.* (2005) states that in 1940 two thirds of food was being imported. Between 1950 and the 1980‟s the goal of agriculture in the UK was to substantially increase food production (Hodgson *et al.*, 2005). This was achieved firstly by ploughing lowland grass sites and secondly via the increased use of mineral fertiliser and the „improvement‟ of semi- natural grassland by the addition of fertiliser (Poschlod *et al.* 2005). Poschlod *et al.* (2005) give these as the principal reasons for the large scale loss of semi-natural grassland. Poschlod *et al.* (2005) also suggest that more recently the economic pressures arising from the cheap imports of sheep into Europe, e.g. from New Zealand, led to the opposite effect of nutrient poor soil being abandoned for

grazing or converted to woodland. The overall result has been a dramatic loss of lowland calcareous/chalk grassland (Hodgson *et al.*, 2005).

2.2.2 Distribution and importance of calcareous/ chalk grassland

Calcareous grassland is widely distributed throughout Europe and is high in species diversity (Willems, 1990). A study of plant species and soils in Germany has shown the number of calcicoles (for example plants favouring alkaline soils with high pH) is disproportionately high when compared to the areas of alkaline soils present (Ewald, 2003). A similar effect was observed in north-western Europe (Scandinavia and the Baltic States) (Pärtel *et al.*, 2004). They found that in these countries, calcicoles were relatively well protected in reserves. They also found that many of the rarer species calcicoles occurred in a narrow pH band (strong calcicoles) and their presence was an indicator of the health of the larger species pool associated with calcareous grassland.

Calcareous soil and grassland overlying limestone is common throughout Europe and the world, however calcareous grassland growing on chalk (chalk grassland) is restricted to north-western Europe and the high diversity of flora and fauna makes it a habitat of international importance (Biodiversity Action Group, 2000). In the United Kingdom the presence of a chalk substrate close to the surface is restricted to lowland areas in southern England. One of the most important areas for chalk soils providing the right conditions for the creation and maintenance of chalk grassland are the South Downs (see Chapter 1, section 1.1 for a detailed description of the South Downs).

In Europe large areas of semi-natural chalk grassland have been lost and the United Kingdom conforms to this pattern. The loss of semi-natural chalk grassland has been particularly severe on the South Downs (Burnside, 2000; Burnside *et al.*, 2002; Burnside *et al.* 2003). In a series of studies of chalk grassland on the South Downs in Sussex Burnside *et al.*, (2002 and 2003) used aerial photographs of the South Downs taken over the period 1971-1991 to study changes in the areas of semi-natural chalk grassland. Over the 20 year period Burnside *et al.* (2003) found that the area had decreased by ~ 60% and the number of patches by ~ 75%, and the mean distance between sites had increased, i.e. the landscape had become highly fragmented, an observation also made by Keymer and Leach (1990). The problems associated with habitat fragmentation is recognised in the

current white paper published by the Department for Environment, Food and Rural Affairs (Depart. for Environment, 2011)

Currently the area of unimproved grassland on the South Downs is estimated at less than 5% of that potentially available (Burnside *et al.*, 2002). In an attempt to reverse this decline Burnside *et al.* (2002) conducted a GIS based study to identify areas on the South Downs whose characteristics, i.e. slope, aspect, soil, current status and proximity to existing sites of semi-natural grassland, identified them as potential sites for restoration. A north facing aspect with steep slopes and at low- to med- elevation and thin rendzina soils were identified as the most suitable sites for restoration although some south facing sites were also identified (Burnside *et al.*, 2002).

The importance of conserving and restoring unimproved chalk grassland is not confined to the flora involved, but also for the fauna that relies on them, in particular invertebrates (Thomas, 1990; Morris *et al.*, 1997). The Adonis Blue butterfly only breeds on the chalk grassland plant species *Hippocrepis comosa* (Thomas, 1990). The Sussex Wildlife Trust identify the Wartbiter Cricket (*Decticus verrucivorus*), the Chalkhill Blue (*Polyommatus coridon*), Adonis Blue (*Polyommatus bellargus*) and Marbled White (*Malannargia gallathea*) butterflies, the downland snail (*Hellicilia itala)* and the Skylark (*Alauda arvensis*) as species that would benefit from increased availability of semi-natural chalk grassland (Sussex Wildlife Trust., 1995).

Table 2.1 lists species identified in the National Biodiversity Action Plan as being on the short and medium list and as important species needing protection and found in chalk grassland in Sussex (Habitat Action Plan for Sussex, 2000). The status of threatened species in Sussex is monitored and the results published annually by the Sussex Biodiversity Record Centre (ADASTRA, 2010).

Table 2.1 Short and middle list species for chalk grassland in Sussex. (Habitat Action Plan for Sussex, 2000)

Species	Status
Skylark (*Alauda arvensis*)	SL
Glaucus beard moss* (*Barbula glauca*)	SL
Wart-biter cricket* (*Decticus verrucivorus*)	ML
Early gentian* (*Gentianella anglica*)	SL
Silver spotted skipper* (*Hesperia comma*)	SL
Juniper (*Juniperus communis*)	ML
Brown hare (*Lepus europaeus*)	SL
Adonis Blue* (*Lysandra bellagus*)	ML
Corn bunting (*Miliaria calandra*)	ML
Grey Partridge (*Perdix perdix*)	SL

Key. * Largely restricted to chalk grassland. SL- on short list. ML- on medium list. Both of these lists comprise about 400 species taken from a long list of about 1250 species and targeted for conservation.

In addition to its ecological value, a well managed and attractive landscape is an important visual and recreational resource, to be enjoyed by the wider public (Haines-Young *et al.*, 2006).

2.2.3 Abiotic conditions that affect chalk grassland communities

Semi-natural chalk grassland is valued for its high biodiversity which results from the abiotic (and biotic) factors that combine to produce a particular set of properties conducive to the establishment, stability and regeneration of the chalk grassland species that make up the community. In this section the abiotic conditions present in chalk grassland are reviewed and discussed.

2.2.3.1 Soil pH

The soil under chalk grassland is generally alkaline and the South Downs are typical with the soil at some sites having pH values ≥ 7.5 (see Chapter 3). This means that they are suitable for plants that are strong calcicoles (Ellenberg *et al.* 1991) and this functional

group are an important component of the plant community. A high pH also discourages species favouring more neutral or acid soils (Chytry *et al*. 2003).

2.2.3.2 Nutrient levels

The nutrient levels in soils under semi-natural chalk grassland tend to be low, particularly in P and N. High levels of nutrients in particular N encourage the growth of dominant grasses and a subsequent reduction in forbs (Jacquemyn *et al.*, 2003). Jacquemyn *et al.* (2003) report the results of a trial in which increasing levels of nitrate were applied to chalk grassland in Belgium, while varying grazing and disturbance levels. They observed decreasing species richness with increasing nitrogen levels and reduced levels of light penetration. A further effect of low levels of nutrients in semi-natural chalk grassland is severe miniaturisation of the species present (Grime, 1990) and according to Koide (1991) low phosphorous in particular will result in both slow growth and miniaturisation. Through miniaturisation there is also less competition for light among the forb species present (Grime, 1990).

2.2.3.3 Site characteristics: Soil depth, aspect, slope, insolation and rainfall

In chalk grassland generally and on the South Downs in particular, a whole spectrum of different abiotic conditions can occur, for example north facing hills with steep slopes and thin soils and south facing hills with shallow slopes and some areas of deeper soils and south facing hills with steep slopes and largely thinner soils (Burnside *et al.*, 2002). In a GIS study of plant communities growing on the South Downs Burnside *et al.* (2002) showed that the hills with the steeper slopes, thin soils, typically North facing, are favoured by calcareous (CG) plant communities (Rodwell, 1990 and 1991), while south facing slopes with gentler slopes and deeper soils had a heavier presence of more mesotrophic (MG) plant communities (Rodwell, 1991). However various studies (Sussex Wildlife Trust., 1995; Sussex Downs Conservation Board, 1996) have shown that steep south facing slopes can maintain a diverse mixture of plant species.

More recently in a comprehensive study of the landscape characteristics of chalk grassland Bennie *et al.* (2006) found that steep south facing slopes had shown the highest resistance to degradation and invasion by coarse grasses, when they revisited a study carried out some 50 years earlier (Perring, 1958a; Perring, 1958b; Perring, 1959). Bennie

et al. (2006) hypothesise that this resistance to change at sites with south facing, steep slopes results from phosphorous limitation in the shallow rendzina soils and the greater resistance of the plant communities to drought effects, resulting from rapid soil drainage and high insolation. These ideas are consistent with a study of abandoned grassland in the limestone dales of Derbyshire (Grime and Curtis, 1976) which found that under conditions of nutrient and drought stress on steep south facing slopes, *Festuca ovina* was able to resist the invasion of the more vigorous *Arrhenantherum elatius*. *F. ovina* is an important grass species present in semi-natural chalk grassland. Buckland *et al.* (1997); in a related study in the same area, measured the relative water content of plant species growing in different soil depths in a period of severe drought in 1995. They found that many species, which are also found in chalk grassland growing in shallow soil, for example *Sanguisorba minor, Carex flacca, Pimpinella saxifrage* and *Centaurea nigra* maintained the highest turgor. Buckland *et al.* (1997) also highlighted the lack of drought resistance of a range of dominant grasses. Low drought resistance in dominant grasses may be as important as the drought tolerance of subordinate species in maintaining calcareous grassland communities. Rainfall on the South Downs is generally higher in the autumn and winter months with the prospect for drought conditions being greater during the summer months when insolation levels are high. Evidence of differing drought resistance among species may be significant for chalk grassland communities growing on steep South facing slopes on the South Downs where the vigorous and dominant grasses, *Brachypodium pinnatum* and *Bromus erectus* have the potential to overtop other slower growing species.

A study in Finland by Pykälä *et al.* (2005) examined species richness in 162 grazed or abandoned patches of semi-natural mesic grassland growing on a clay soil. The results of the study showed the importance of high solar radiation in maintaining species richness and that shading by trees may be more detrimental to species richness than lack of grazing.

2.2.4 Biotic factors that affect chalk grassland communities

Two biotic factors that have the potential to influence plant community structure in semi-natural chalk grassland are grazing (Sankaran and McNaughton, 2005; Denyer *et al*. 2009) and AMF/plant symbiosis (Fitter, 2005; Karanika *et al.* 2008)

2.2.4.1 Grazing

Grazing of vegetation for nutrition is carried out by a wide range of herbivores ranging in size from tiny insects to large ungulates (Crawley, 1997b). In respect of herbivory, Crawley (1997b) lists four factors that will affect the performance of the plants being grazed. These are their phenology (the time in the plants life cycle at which it is attacked), which parts of the plant are grazed and the intensity and frequency at which the plant is attacked.

The effect of grazing on increasing or maintaining species diversity at the local scale will, according to Sankaran and McNaughton (2005), depend on the form of the herbivory. To maintain species diversity the herbivory must: (1) selectively consume competitive dominants, permitting subordinate species to establish or survive; (2) increase heterogeneity through soil disturbance; and (3) reduce plant size allowing greater plant packing within a given area. Sankaran and McNaughton (2005) go on to state that without grazing the dominants are able to grow very large and overwhelm the sub-dominants, thereby reducing community diversity. The scenario described by Sankaran and McNaughton (2005) is very applicable to the conditions pertaining to semi-natural chalk grassland.

A study by Bacon (1990) addressing grazing on calcareous grassland allows the factors identified by Crawley (1997b) to be evaluated. Bacon (1990) is of the view that where a site has been traditionally managed this should continue. He makes the point that in areas of low productivity, for example *Festuca ovina* grassland on shallow soil with south facing slopes, there is no need to graze other than between the months of October to March, which allows forbs the chance to set seed and reduces negative impacts on invertebrates which tend to be dormant during the winter. Potential disturbance and hoof marks in wetter winter conditions provide beneficial disturbance and breaks up the litter layer. A general guide to a sustainable grazing intensity is ~ 1 sheep per ha, or 0.2 -1.5 cattle ha/year depending on the aims of the grazing regime (Bacon, 1990). Bacon (1990) makes the point that a high stocking density for a short period will have a different effect on the plant communities than less intense stocking density over a longer period. If, as can be the case in semi-natural chalk grassland, productivity is low, plants may be less

resilient to grazing resulting in severe damage from short periods of heavy grazing (Bacon, 1990). The effect of heavy grazing on invertebrates is even more severe (Bacon, 1990). In a more recent study of grazing of semi-natural grassland in Sweden, Hessle *et al.* (2008) studied the foliage eaten by two breeds of cattle. Hessle *et al*. (2008) found that their diet consisted mainly of competitive grasses with some herbs and that their diet changed with the season. The cattle also grazed juvenile woody species keeping scrub in check (Hessle *et al.* 2008).

Sheep are the traditional grazing animal on chalk grassland and have been used in restoration studies (Gibson *et al*. 1987) where they were shown to increase species richness. The nibbling action of sheep can produce a very close sward (Bacon, 1990). Sheep however tend to be selective eaters which results in uneven grazing. Cattle are less selective grazers than sheep removing vegetation by wrapping their tongues around the vegetation and can produce quite a low sward over a period of time. The hoof marks of cattle are a source of soil disturbance. Rabbits, if present in high numbers, can be significant grazers (Denyer, 2005; Denyer *et al*. 2009) and their scrapes provide further potential sites for plant regeneration.

The literature contains several studies of the effects of grazing and abandonment on species diversity in communities and for individual plant population growing in different soils and under different abiotic conditions. Pykälä (2004) studied the effect of grazing and abandonment on species richness of mesic semi-natural grassland growing on clay soils in southern Finland. Pykälä, (2004) observed that regularly grazed grassland had the highest species richness and abandoned grassland the lowest. Grassland, where grazing had been resumed after a period of abandonment, had intermediate levels of species richness. In a study of steppe-like grassland in Romania Enyedi *et al*. (2008) found that grassland where grazing had been abandoned had lower species diversity compared to grazed areas, but ungrazed grassland still contained rare and threatened species.

Grazing of upland grassland by sheep in northern England was evaluated by Smith and Rushton (1994). Over a four year period traditional hay meadows were subjected to different cutting and grazing regimes. Species richness was highest in plots cut and grazed in the autumn and lowest in plots cut and not grazed (Smith and Rushton, 1994) The effect of different grazing regimes on both beetle diversity and population numbers

(and associated plant communities) in calcareous grassland on Salisbury Plain, England has also been studied (Woodcock *et al.*, 2005). One of their findings was that long term grazing by sheep produced a sward richer in herbaceous species and that this increased the number of phytophagous beetle species present (Woodcock *et al.*, 2005). The effect of grazing regimes and abandonment on the populations of the plant species *Primula veris* in calcareous grassland in eastern Belgium has been studied (Brys *et al.*, 2004). Brys *et al* (2004) found that lack of disturbance as produced by grazing or mowing was detrimental to the survival of *Primula veris*. From a diverse range of grazing studies similar findings emerge, which indicates that regularly grazed grassland has higher species richness than is found in abandoned grassland. However the studies caution that over or too intense grazing reduces species richness.

The study conducted by Pykälä, (2004) on cl;ay soil and grassland showed that grazing increased the richness of hemicryptophytes (herbaceous perennial with over wintering parts close to the ground) and chamaephytes (perennial woody plant with survival buds at or just above the ground) but a reduction in geophytes (perennial herbaceous plant with dormant parts below ground) that is that grazing affected community structure.

Overall the research shows that the biotic component of grazing can and does have a strong influence on the structure of calcareous grassland communities including chalk grassland communities.

2.2.4.2 Arbuscular mycorrhizal fungi/plant symbiosis

A second biotic component with the potential to affect the species composition and abundance of plants in semi-natural chalk grassland communities is the symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and the plant species present. In this symbiosis the AMF transfer nutrients from the soil via hyphae into the plant roots in exchange for carbon. This relationship is not discussed here but will be explored in depth in the third section of this literature review.

2.3 Plant Community Structure

2.3.1 Definition of plant communities

Plant communities can be considered in an abstract form or with more relevance to this study as practical plant communities consisting of „concrete stands of vegetation" or as defined by Westhoff and van de Maarel (1978) as a „phytocoenase". The early practical attempts to define large scale communities were by Clements (1916) and Gleason (1926) and are summarised in van der Maarel (2005). The conclusions however of the studies by Clements (1916) and Gleason (1926) were in sharp contrast. Clements (1916) compared plant communities to an „organism", and Gleason (1926) proposed that the individualistic behaviour of plants precluded cooperative behaviour as a community. More recent research suggests that the reality probably lies in between these two extremes (van der Maarel, 2005).

A more recent definition of a phytocoenase has been provided by (van der Maarel, 2005), „*a phytocoenosis is a piece of vegetation in a uniform environment with a relatively uniform floristic composition and structure that is distinct from the surrounding vegetation*". The insertion of the words „relatively uniform" is important within the context of chalk grassland communities on the South Downs. Although the „core species" (that is, those present with a high frequency and abundance) will be easily located within a site, scarce species may be difficult to locate or be absent from individual sites. However collectively all sites will come under the umbrella of semi-natural chalk grassland. This difference in the species present or absent at sites containing the same chalk grassland communities is at least partly due to „nestedness" resulting from fragmentation of the landscape (see Chapter 4).

2.3.2 Species richness and diversity

The species richness of a community is the number of plant species in that community and this number will be dependent on the area of the community being considered (van der Maarel, 2005), for example a $1m^2$ quadrat or a 20ha site. The richness will also depend on which plant species are included (Leps, 2005), for example forbs only, forbs and grasses, forbs and grasses and lower plants. For a complete stand or landscape of defined vegetation such as semi-natural chalk grassland on the South Downs the number of species present equates to the „species pool" (Zobel *et al.*, 1998). Species richness will provide information on the number and identity of species forming a community but

incomplete information on the structure of the community. Further information on community structure is provided by species diversity.

Species diversity has two components as defined by Leps (2005). Species richness describes the number of species present, and equability or evenness expresses how evenly the species are represented in the community. A typical graphical representation of diversity is provided by diversity dominance curves for the abundance of each species present, where abundance of the most dominant species is calculated as a percentage of the whole, followed in rank order by the remaining species. Where a small number of species produce most of the abundance the curve is very steep and corresponding diversity low. If there is greater evenness between the biomass of the species the curve is shallower and the corresponding diversity higher (Leps, 2005).

In many plant communities, including those present in semi-natural chalk grassland, the community structure consists of a small number of dominant species with high abundance (e.g. biomass or percentage cover), a medium number of subordinate species with medium abundance and in communities with high species richness, for example high quality semi-natural chalk grassland has a large number of rare species with low abundance (Leps, 2005). While the species with low abundance will have minimum impact on biological processes such as biomass production and nutrient recycling, they may have a more subtle role in the general community ecosystem as food for rare insects (Leps, 2005) or as symbiotic partners for AMF species (Fitter, 2005) and are often of conservation value for their rarity.

An alternative approach to diversity other than the measurement of evenness is the range of functional types present (Leps, 2005), for example a community consisting of four annuals is less diverse than one containing of two annuals and two perennials. According to Leps (2005) community structure can be described as a hierarchy consisting of functional groups and species diversity within functional groups. Possible functional groups are Raunkiaer life forms (Raunikiaer, 1934), clonal versus non-clonal species, in grassland communities graminoids, legumes and forbs (McLaren and Turkington, 2011) and in chalk grassland, the strong calcicoles.

2.3.3 Grain structure

Within a community species diversity may indicate which species are present, their evenness, and which are dominants, subordinates or rare, but does not describe how individual species are distributed through the community, for example clumped or as individuals. Species that are clumped together will have their own species as nearest neighbour and be subject to intraspecific competition, while isolated individuals will have different species as neighbours and experience interspecific competition (Crawley, 1997a) or possibly co-operation. Plant communities with individual species in clumps are described as coarse grained and where the individual plants of the species present are spread throughout the community as fine grained. Examination of high quality semi-natural chalk grassland at the $1m^2$ scale and smaller shows individual species to be distributed throughout the community, that is it is fine grained (Mitchley and Grubb, 1986).

2.3.4 Mechanisms contributing to plant community structure

The mechanisms that determine plant community structure can operate at different scales. This may involve external abiotic inputs to the plant community for example high temperatures, drought and atmospheric pollution. Biotic factors may also be operating, for example the phenology of individual plants and their ability to set and disperse seed. Biotic interactions might involve intra and interspecific plant competition for resources or alternative AMF facilitation. In this section on mechanisms some of the ecological models, hypotheses and concepts are reviewed within the context of chalk grassland communities.

2.3.4.1 The competitive exclusion principle

The competitive exclusion principle states that; when two (or more) species are in competition one will have an advantage over the other(s) in the prevailing conditions. In time this species will eliminate the other(s), producing a community consisting of one species (Crawley, 1997a). This is a somewhat simplistic model and in practice the environments that plants inhabit are not uniform and have spatial heterogeneity. For example the environment is not temporally or spatially constant; it is affected by seasonal variations, patchy nutrient distribution and different responses to biotic variables such as grazing, pathogens, pollinators and dispersal agents (Crawley, 1997a). Thus in natural

plant communities monocultures are rare and species richness and diversity can be very high, for example chalk grassland.

2.3.4.2 The niche concept

A further mechanism or model that has relevance to plant community structure is the concept of the ecological niche. An early definition of the niche was provided by Hutchingson (1957). He described the niche as a "*multidimensional description of a species needs, habitat requirements and environmental tolerances*". According to Crawley (1997a) the niche concept serves as "*a shorthand summary of a species complex suite of ecological attributes including its abiotic tolerances, its maximum relative growth rate, its phenology, its susceptibility to various enemies and its relative competitive abilities with other plant species*".

For plant species growing in chalk grassland the important abiotic factors defining the generalised niche are insolation, shallow soils, good drainage, high pH and low nutrient levels and in a plant community as species rich as chalk grassland, the ability of plant species to be able to compete with their neighbours (Burnside *et al.*, 2002; Hutchings and Booth, 1996b; Hutchings and Booth, 1996a). Tilman (1997) describes a scenario where competing plant species have requirements for several abiotic inputs, for example water, pH and nutrients. Where there are gradients of these variables within the area being considered, different niches may be created allowing competing species to coexist (Tilman, 1997).

The idea of interspecific competition is a fundamental concept of the niche, and leads to the ideas of a species having a „fundamental niche" and a „realised niche". Crawley (1997a) describes the fundamental niche as the optimum conditions for maximum growth and survival that a plant species exhibits when growing alone. In the realised niche the conditions are those existing when the same plant species is growing in competition with other species.

In a series of trials lasting seven years Mitchley and Grubb (1986) studied the competitive ability of a number of chalk grassland perennial dicotyledons. The abundance of these species ranged from high (dominants) to low (subordinates). They

found that the more abundant species were the most aggressive as measured by their above ground dry weight and they had greater interference ability. They also considered that below ground competition might be as important as above ground competition (Mitchley and Grubb, 1986). The most abundant species were found to be long lived, reproducing primarily through lateral spread of persistent clones, whilst the less abundant species were short lived and reproduced sexually through their seeds.

2.3.4.3 The regeneration niche

An expanded model of the niche concept is that of the regeneration niche which was proposed by Grubb (1977). The concept of „regeneration niche" is applicable to chalk grassland and is one of several niches described by Grubb, (1986) in his paper on sparse and patchily distributed species in species rich chalk grassland communities. Grubb (1986) stresses that it is not only the physical characteristics of the regeneration niche that are important, the dispersal of seeds in space and time, seed germination, establishment and onward growth are also important. These will in turn be affected by weather, disturbance, gap size, grazing and pests and diseases. He emphasises that the regeneration niche is concerned with regeneration of existing species, not succession.

In chalk grassland most species are perennials, but annuals and biennials are also represented. The perennials of chalk grassland vary in form, but many are hemicryptophytes (with growth points close to the ground) forming small tussocks, rosettes and creeping sub-shrubs. Collectively they form a matrix into the interstices of which the short lived plants come and go. The model of a matrix of long lived plants in which shorter lived plants can set seed is also addressed in a paper by Gay *et al.*, (1982) on turf compatible and turf incompatible species. These are species that can regenerate directly into closed turf and those that require disturbance to produce gaps in the turf for the seed to germinate. More recently Zobel *et al*. (2010) discuss the community balance between matrix forming clonal plants of low mobility and mobile inter-matrix species regenerating from seed.

Grubb (1986) also identified a number of characteristics associated with scarce species found in chalk grassland. His study showed that scarce species are less affected by close grazing, and in fact low grazing is necessary for them to survive. He demonstrated that

short lived dicotyledons are better at establishing from seed than more abundant species and they are more likely to be in competition with abundant (matrix) species than each other. Many scarce species are annual or biennials, and flowering and seed production will vary from year to year. Scarce interstial pauciennials e.g. *Daucus carrota* and *Picris hieracoides* take a relatively long time to flower (2-4 years) have low seed production and are subject to drought, predation and shading.

2.3.4.4 The carousel model

The „carousel model" is an adaptation or possibly a successor to the regeneration niche (Grubb, 1986). The „carousel model" was proposed by van der Maarel and Sykes (1993) and was based on their observations of small scale plots $0.01m^2$ to $0.001m^2$ in limestone grassland in Sweden. They observed that at the $0.01m^2$ scale plant species might be present in one year, be absent in the following year, and then re-appear in later years. As one species disappeared another one took its place in the plot. However over the 6 years of the study the species present in an area of $2.5m^2$, encompassing the small scale plots, remained essentially unchanged. For this model to work, the plant species involved require high dispersion rates, which allows them to colonise sites where competition for their particular requirements are low. This is a dynamic model that requires extinction and colonisation at the small scale producing stable plant communities at the larger scale. This model places the emphasis on dynamic dispersal process as opposed to the need for niche separation for a species to survive (van Andel, 2005).

2.3.4.5 Mechanisms of plant competition with relevance to chalk grassland

A review of mechanisms and traits for chalk grassland species commonly found at Newmarket Hill (the research site) was carried out by Grime (1990). Grime (1990) highlights that species rich calcareous grassland will typically experience chronic deficiency in the essential nutrients nitrogen and phosphorous. The plants that are able to survive these conditions are described as „stress tolerant" and have the attributes of small stature, low growth and low turn over of their vegetative parts (Grime, 1990).

The low stature of many chalk grassland plants, that form tussocks and rosettes with short rootstocks, makes for efficient recycling of the limited nutrients. The small nature of many of the perennial plants may be an adaptation to close grazing by sheep and rabbits

over many generations. Grime (1990) considers that it is a combination of nutrient stress and grazing, which results in plant miniaturisation. The slow growth rates and turn over of plant material may also have a beneficial effect on species generation from seed in micro sites. Slow turnover allows the sites to remain unexploited for longer periods and colonisation and seedling establishment to occur.

Another factor which affects competition and allows greater species richness is temporal difference in species phenology. This enables species to access essential nutrients at times of maximum requirement at different times during the growing season (Grime, 1990).

The last mechanism suggested by Grime (1990) to explain the close packing of chalk grassland species, involves the transfer of nutrients between plants whereby dominant plants supply assimilates to subordinate plants via interconnected AMF networks. These findings were based on the results from a trial in which chalk grassland plants were grown in microcosms in the presence and absence of AMF (Grime *et al.*, 1987). The idea of transfer of nutrients between plants is controversial and an alternative explaination is that transfer of material, for example carbon along networks, is for the benefit of the fungi (Fitter *et al.*, 1998).

2.4 The Role of Arbuscular Mycorrhizal Fungi (AMF)/Plant Symbiosis in Structuring Plant Communities

2.4.1 Introduction

Fossil records show that arbuscular mycorrhizal fungi (AMF)/plant symbiosis was occurring some 400 million years ago at a period of time when plants first began to colonise terrestrial habitats (Karandashov *et al.*, 2004). Today around 80% of vascular plants are thought to be in some form of relationship with AMF (Karandashov *et al.*, 2004) and although many of these relationships are mutually beneficial a range of relationships including some that are antagonistic can occur (Read, 1999; Johnson *et al*. 1997; Klironomos *et al.*, 2000).

Studies of mycorrhizal fungi have been carried out for more than a hundred years and although vast numbers of plant species have been identified as mycorrhizal only around 130 (Read, 2002) to 150 (Karandashov *et al.*, 2004) species of AMF have been

identified. Within these AMF species however there is evidence of high genetic variability (Koch *et al.*, 2004). A recent study of DNA from AMF collected from calcareous grassland in the north of England identified 70 species of AMF in an area of approximately 7m^2 (Dumbrell *et al*. 2011). This was twice the number of AMF species previously identified at this site and suggests that there are more species of AMF to be discovered. The main symbiotic relationship was considered to be the transfer of phosphates from the fungus to the roots of the plant species in exchange for carbon (Jakobsen, 1999) but more recent studies suggest that the relationship is far more complex (Fitter, 2005; Helgason and Fitter, 2009; Feddermann *et al*. 2010)

2.4.2 The types of mycorrhizal fungi

There are two main types of mycorrhizal fungi (Read, 2002). The first of these sheath the roots with a mycorrhizal fungi (sheathing) and are allocated to three sub- divisions defined as monotropoid, arbutoid and ecto. The second group of mycorrhizal fungi are those that penetrate the roots of the plants (endo) and are also divided into three sub groups; orchid, ericoid and arbuscular. Many of the arbuscular forms of fungi also contain vesicles. Figure 2.1 shows the families containing the species of AMF.

Figure 2.1 The families comprising the phylum Glomeromycota. INVAM (2008)

It can be seen in Figure 2.1 that the phylum *Glomeromycota* has two suborders *Glomineae* and *Gigasporineae*. The main difference between the two orders is that

Glomineae produce vesicles within the roots of the plants whereas *Gigasporineae* do not produce vesicles, but produce storage cells outside of the root.

2.4.3 The components and functions of AMF

AMF have five main components (Smith and Read, 1997b): spores, hyphae, arbuscules, mycelium and vesicles.

2.4.3.1 Spores

Spores present in the soil are a means of survival for the fungus during stressful periods, when little photosynthesis is occurring, (for example in winter) and provide a means for the fungus to regenerate, along with infected root fragments and hyphae (Smith and Read, 1997b). Spores were considered to be the most reliable method of identifying individual AMF (Smith and Read, 1997c) but studies of AMF DNA may be more reliable in some circumstances (Dumbrell *et al*. 2011).

2.4.3.2 Hyphae

Hyphae are the internal highways of the fungus within the roots of the host plant, along which for example, nutrients are transferred to the plant and carbohydrate to the fungus. Hyphae can take many forms, including tight coils within plant cell and longitudinal growth along cells. Two main morphological forms of hyphal growth have been identified (Karandashov *et al.*, 2004); *Arum* – type mycorrhizas in which the hyphae spread between the cortical cells of the host plant with arbuscules forming in the cells and *Paris* – type in which thick coiled hyphae form within the plant cortical cells.

2.4.3.3 Arbuscules

Arbuscules are multi–branched membranes, usually within cells, attached to the internal hyphae (Smith, and Read, 1997c). Arbuscules have a very high surface area and act as interfaces between the fungus and the plant across which ions and molecules can be transferred.

2.4.3.4 Mycelium

External hyphae or mycelium only start to grow after internal infection of the plant root has taken place. The mycelium are typically 2 – 20µm in diameter (Smith and Read, 1997a), and are able to extract nutrients from the soil. During nutrient transfer the mycelium provide the route along which the nutrients are transported to the plant roots. The length and hence surface area provided by the mycelium is extensive with up to 25m per gram of soil being quoted (van der Heijden, 2002).

2.4.3.5 Vesicles

According to Smith and Read (1997b), about 80% of arbuscular mycorrhizal species produce vesicles. Vesicles are the storage sacks or containers, typically spherical in form and located within the plant roots, in which the fungus stores excess carbohydrate, surplus to its immediate requirements. Carbohydrate stored within vesicles is likely to be used in spore production, which has a high carbon demand (Smith and Read, 1997b).

2.4.4 The basis of the symbiotic relationship

Most plants involved in a relationship with mycorrhizas can survive in the absence of the fungi, provided sufficient nutrients are available. AMF however are ecologically obligate symbionts (Smith and Read, 1997b) and cannot survive in the absence of plant hosts. Thus the relationship is essential for the AMF. The value of the symbiosis is not as obvious from the standpoint of the plant. However one reason proposed is the desirability for the plant to minimise energy expenditure while maximising root function (Fitter, 1997).

Growing roots results in the expenditure of energy/resources by the plant (Fitter, 1997). The energy expended on the growth of a root is proportional to its volume and in nutrient poor soils in particular, long thin roots appear to be advantageous in absorbing nutrients (Fitter, 1997). However fine roots can be short lived and prone to predation, while thicker roots are longer lived, resistant to herbivory and help to anchor the plant. Thicker roots operating in symbiosis with AMF appear to offer an energetic advantage. Whereas fine plant roots may have diameters in the range 100 -1500µm, the diameters of mycelium are in the range 2-20µm, (Smith and Read, 1997a). Thus the cross-section of mycelium are about two order of magnitude smaller than that of roots and the energy cost to the plant of

acquiring phosphate symbiotically through mycelium will be lower (Helgason and Fitter, 2009)

Phosphate ions have low mobility in soils, readily form insoluble compounds with the ions of $Al^{3+,}$ $Ca^{2+,}$ and Fe^{3+} (Fitter, 2005) and will rapidly become exhausted in the zone close to the roots. This effect is greater in soils with a high pH where micronutrients such as iron can be difficult to access and conditions favour calcicoles (Zohlen and Tyler, 2000). The increased volume of soil accessed by the more extensive, but small diameter mycelium, makes the symbiosis energetically attractive to the plant (Smith and Read, 1997a). There is some evidence that the amount of phosphate supplied by the fungi is dependent on the amount of carbohydrate delivered by the plant (Bucking and Shachar-Hill, 2005).

In field conditions some plant species can become completely reliant upon mycorrhizal fungi for their supply of nutrients, for example *Hyancinthoides non-scripta*. In a greenhouse trial conducted by Merryweather and Fitter (1995b) *H. non-scripta* was shown to be unable to assimilate sufficient phosphorous to maintain an adequate P content in plant tissue in the absence of mycorrhizal fungi, despite being fed with a nutrient solution (Merryweather and Fitter, 1995b). Moreover in a study of *H. non-scripta* in their natural habitat it was observed that over time, the bulbs descended in the soil to depths where P levels were low, but that this was mitigated by increased levels of mycorrhizal infection (Merryweather and Fitter, 1995a). In addition to nutrient acquisition different types of mycorrhizal fungi may offer additional benefits to the plant (Fitter, 2005) which are described in the next sub-section.

2.4.5 Variable characteristics of AMF/plant symbiosis

Early studies of the AMF/plant relationship concentrated on the movement of phosphorous from the soil via fungal mycelium to the plant in exchange for carbohydrate. The number of AMF species studied was low and tended to be the more common ones, which were not specific to the plant species they colonised. More recent studies reviewed by Fitter (2005) have shown the relationship to be far more complex than first believed, with many AMF species performing a range of symbiotic functions. Molecular techniques used to analyse fungal hyphae in roots from plants growing in natural

communities have shown up to 20 fungal species present in the roots of one plant, with many of the fungal taxa unknown in culture collections (Fitter, 2005). Thus in addition to the well known generalist species there are a number of specialist species.

Even within the more common fungal species there is high genetic variation (Koch *et al.*, 2004). Different plants within a community will have different combinations of mycorrhizal fungi, thus in a community of a 50 plants there is likely to be a similar number of fungal species, the properties of which may be modified by environmental conditions, for example pH (Fitter, 2005). Environmental variations and the biotic conditions within the roots of different plants are likely to result in abiotic and biotic niche differentiation by fungal species (Fitter, 2005). In summary the properties and function of what initially appeared to be a comparatively small number mycorrhizal species is far more complex than first thought, with their own diversity and community ecology.

In addition to supplying P from mineral sources (Jakobsen, 1999) and organic sources (Kiode and Kabir, 2000), mycorrhizal fungi may be able to transfer N from organic sources (Barrett *et al.* 2011). AMF also transfer water from the soil to the plant (Azcon et al. 2003;Marulanda *et al*. 2003), along with micronutrients e.g. Zn, Cu, Fe and Mn (Liu *et al.* 2000; van der Heijden, 2002; Azcon *et al*. 2003). Furthermore, studies have shown that plants with fine roots may be less dependent on AMF for nutrient supply, but may gain protection from soil pathogens through a mycorrhizal symbiosis (van der Heijden, 2002; Sikes *et al.* 2009). Specialist AMF growing close to the root may be capable of binding soil particles together (Piotrowski *et al*. 2004) and to maintain water close to the root and give protection against drought (Subramanian and Charest, 1999; Fitter, 2005). AMF may also be capable of altering the palatability of leaves eaten by herbivorous insects and the production and properties of flowers and seeds (Gange *et al.*, 2005). AMF have also been found to change the time of flowering and number of seeds produced in desert ephemerals (Sun *et al*. 2008). In a trial involving the plant *Leucanthemum vulgare* the performance of a leaf mining insect was altered by the associated AMF species, thought to be due to fungal induced changes in plant nitrogen content (Gange *et al.*, 2005). In a study involving growing the perennial forb *Chamerion augustifolium* in the presence and absence of two AMF species it was found that plants with the AMF species present had more visits by pollinators and percentage seed set was

twice that of non-mycorrhizal plants (Wolfe *et al.*, 2005). Gange and Smith (2005) also found that the presence of AMF influenced the rate at which pollinating insects visited plants. Three plants were studied *Centaurea cyanus*, *Tagetes erecta* and *Tagetes patula* and although the presence of AMF increased the rates at which pollinating insects visited, the mechanisms involved were different. These mechanisms an increase in flower numbers, the size of individual inflorescence and the availability of nectar (Gange and Smith, 2005).

2.4.6 Competition for resources in conditions of limited supply

Different plant species growing together, for example in a community, will experience competition, in particular for nutrients (Tilman, 1997). Plants need some 15 inorganic nutrients with the major ones being nitrogen (N), carbon (C), potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) (Tilman, 1997). It is often the case that the growth and survival of a species in a habitat under a particular set of abiotic conditions will be determined by one resource (for example nutrient) at its lowest availability relative to the species requirement for all resources (Tilman, 1997). This will be the limiting resource for that species in that habitat. The growth of individual plants will be reduced if its neighbours are consuming its limiting resource, this being the basic mechanism of resource competition (Tilman, 1997). The concentration of a resource in the soil is dependent on the balance between the rate at which the resource is consumed and the rate at which it is re-supplied in the habitat. For the nutrient P, because of its low solubility and mobility, (see section 2.4.4) local concentrations can rapidly become depleted.

In a study of resource competition Tilman (1997) used the symbol R* to depict the resource (nutrient) concentration in the soil required for the growth of a specie to exactly balance its various sources of loss. A species will only be able to survive in a habitat if the resource concentration is at least R*. In effect if the resource concentration in the habitat is less than R* the species will not be able to grow and will be eliminated from the community (Tilman, 1997). If several plant species compete for the same resource then the species with the lowest R* should displace the other species from the habitat. So one must question how can species rich communities such as those found on chalk grassland co-exist?

One potential mechanism that could promote plant species co-existence is AMF/plant symbiosis, with particular focus on AMF species whose primary function is nutrient acquisition and transfer. In Figure 2.2 it is shown how in a situation where two plant species are competing for the same limited resources e.g. N and P, the presence of mycorrhizal fungi could in principle alter the balance of competition.

Figure 2.2 - Without AMF being present species A out competes species B. With AMF present species A and B are able to coexist. (van der Heijden, 2002)

In the left hand section of the diagram shown in Figure 2.2, plant species A has the lowest R* values and is able to out compete species B due to the absence of AMF. In the right hand section of the diagram, plant species B has formed a symbiotic relationship with an AMF and its R* value for phosphorus is now below that of plant species A allowing both species to co-exist. This resource model provides a good representation of the co-existence of two species resulting from the presence of AMF; however it does not adequately explain how multiple species might co-exist.

2.4.7 Community feedback in AMF/plant symbiosis

In an attempt to explain the complexities of multiple species communities the concept of community feedback involving the interaction of AMF species was developed (Bever *et al.*, 1997; Bever, 2002; Bever *et al.*, 2002). In plant communities where there are a number of different plant and AMF species, there is scope for a whole range of

relationships between the plants and the AMF species present (Bever, 2002; Bever *et al.*, 2002). The basis of the model is one of positive and negative feedback between individual plant and AMF species (Bever *et al.*, 1997; Bever, 2002; Bever *et al.*, 2002).

If the symbiotic relationship between a plant species and a particular mycorrhizal fungus is completely beneficial to both species (that is the plant receives the maximum quantity of P in exchange for carbohydrate), both the plant and AMF will grow stronger and may eliminate weaker plant species from the community. Thus positive feedback is occurring. However not all symbiotic relationships are equal and a plant species may be colonised by different AMF species that give less P for the same quantity of carbohydrate, described as negative feedback (Bever, 2002). If a dominant plant species experiences negative feedback and a subordinate species positive feedback while being linked by common mycelia, a mechanism for co-existence can be proposed. Given that plant species might be colonised by several AMF species which may react differently with different plants, there is scope for a range of relationships between the plants and the AMF species present (Bever, 2002; Bever *et al.*, 2002). Current ideas on negative and positive feedback between AMF and plants within a plant community have been reviewed by Bever *et al.* (2010).

2.4.8 The development of research into AMF/plant symbiosis

A major achievement of the first one hundred years of research into mycorrhizal symbiosis has been to demonstrate that symbiosis is present in all natural plant communities (Read, 2002). There are currently two basic approaches to research into AMF/plant symbiosis and its effects on plant competition and community structure. The first reductionist method is to study AMF/plant interactions in artificial microcosms where complex relationships can be simplified to aid interpretation. The second method is to study natural communities in the field, where the results are less precise and the interpretation more tentative (Read, 2002).

The reductionist approach has tended to concentrate on the study of the transport of P to the plant, while the broader range of symbiotic relationships have received less attention (Read, 2002). Many insights into the processes occurring in AMF/plant symbiosis have been gained by taking this reductionist approach, but there are difficulties in applying

these results to natural communities. At best the result from studies using microcosms provide a simplification of the processes occurring in natural communities and a number of researchers have called for more studies of natural communities (Vierheilig *et al.*, 1998; Read, 2002; Klironomos and Rillig 2008). In a recent evaluation of the role of mycorrhizal fungi Klironomos *et al.* (2011) call for an increased quantification of the extent to which mycorrhizal symbiosis structures plant communities, where field trials are accompanied by relevant laboratory experiments to validate the results. It can be seen from Figure 2.3 that studies of natural communities have high ecological relevance but a lower level of precision than those carried out on a smaller scale in microcosms.

Figure 2.3 - The balance between precision of studies at the small scale compared to increased relevance at a coarser scale. (Read, 2002)

Figure 2.3 illustrates the advantages of greater precision in the data obtained from studies of plants at the small scale, compared to the greater ecological relevance of studies conducted at the community level.

The advantages and disadvantages of field studies on natural communities have been summarised by Read (2002). Read (2002) states that advantages of field based studies are that they are a true representation of the conditions in which the symbiosis is occurring, with climate, soil, physical and chemical heterogeneity represented. Naturally selected communities of both the plants and mycorrhizal fungi will be present and the community is likely to contain different functional types of both plant and AMF species. There is the potential for long term studies to cover the complete lifecycles of the species present.

The disadvantages of field based trials (Read, 2002) include difficulties in studying the below ground phenomena without disturbing the overall balance of the underground ecosystem. Field experiments may be susceptible to natural disasters, (for example excessive disturbance, herbivore and pathogen attack). Perhaps most importantly there are difficulties in identifying single factor effects where the manipulation involved will have the potential to affect the physical and biological components of the underlying systems.

2.4.9 Pot trials, microcosm research and field trials

There has been a wealth of research into AMF/plant symbiosis and its effects on plant interactions and community structure. In this section, papers, considered to be relevant to plant interactions and plant community structure in chalk grassland are reviewed. Three areas of research have been identified as of particular relevance: 1) the role of AMF in community regeneration by promoting seedling establishment. 2) the mitigation or reinforcement of intra or interspecific competition between plants of the same species, pairs of species and small groups of species 3) the role of AMF in the determination of the structure of plant communities.

2.4.9.1 Seedling establishment

While many plant species in chalk grassland communities are perennial and able to propagate clonally (Mitchley and Grubb, 1986), many species including some subordinates are comparatively short lived and regenerate through seeds. These species are typically found in closed turf or in areas of minor disturbance (Gay *et al.*, 1982). Therefore factors that promote seedling establishment in mature natural communities will be important in promoting species richness and diversity within the community thereby maintaining community stability. Studies by van der Heijden (2004) suggest that AMF play a major role in seedling establishment. Earlier studies by Gay *et al.* (1982) noted that the roots of seedlings, in a natural chalk grassland community, became infected with mycorrhizal fungi within 7 -10 days, with arbuscules recorded as present after 2 weeks and vesicles within 3 weeks (Gay *et al.*, 1982). In a review of some 60 experiments van der Heijden and Horton (2009) found that that in 48% of cases mycorrhizal networks promoted seedling growth.

2.4.9.2 Experiments on AMF/seedling establishment

Using microcosms of 3 litres of calcareous sand, with controlled nutrition levels Grime *et al.* (1987) were able to create artificial calcareous grassland. In these microcosms, the grass *Festuca ovina* was the dominant species and less dominant grasses and forbs commonly found in calcareous grassland were planted in various random configurations within the *Festuca ovina* matrix. In this research the major influence on seedling establishment, as measured by their biomass, was the presence of AMF when compared to the results from plants grown in conditions where AMF were absent (Grime *et al.*, 1987). It was also found that trimming the grasses to simulate grazing also encouraged the establishment of subordinates (Grime *et al.*, 1987).

An example of the benefits of AMF in establishing subordinate forbs is given by Grime *et al.* (1987) using *Centaurium erythraea*. The seedlings that were established in the presence of AMF grew successfully, whereas in the absence of AMF they remained small and eventually died (Grime *et al.*, 1987). Based on this observation Grime *et al.* (1987) proposed that the establishment and growth of *C. erythraea* seedlings was a result of the movement of assimilate from dominant to subdominant species through a common mycorrhizal network (Grime *et al.*, 1987). This hypothesis was supported by experimental results involving the observation of the movement of C^{14} between plants (Grime *et al.*, 1987). However this proposition and the experimental results are contentious, (for example Fitter *et al.*, 1998; Robinson and Fitter, 1999) as other workers claim to show that assimilate and/or carbohydrate remains within the mycorrhizal network and are used exclusively for the benefit of the AMF species community. In a study by Zabinski *et al.* (2002) the forb *Centaurea maculosa* was grown in conjunction with a range of native grasses. The study showed no transfer of carbon between plants and any advantages gained by the forb resulted from higher P delivery when AMF species were present, but only when its nearest neighbour was a grass species (Zabinski *et al.*, 2002).

A more recent study into seedling establishment carried out by van der Heijden (2004) involved trials of forty eight microcosms. The microcosms consisted of a 2:1 mixture of autoclaved calcareous soil from Switzerland and autoclaved quartz sand. The microcosms were injected separately with four individual *Glomus* species of AMF native to

Switzerland and with the four AMF species combined, plus controls with no AMF. Seventy plants from eleven plant species native to calcareous grassland were planted in each microcosm, the number of plants from each species reflecting their relative abundance in the natural plant community. The microcosms were allowed to settle for 1 year after which the seeds from four species, two grasses *B. pinnatum* and *B. erectus* and two forbs *Prunella vulgaris* and *Trifolium pratense*, were added to each microcosm with ten seeds at two locations for each of the four species. The main variable measured was above ground biomass. The results for the adult plant showed that *B. pinnatum*, *P. vulgaris* and *T. pratense* all benefited from the presence of AMF with different species of AMF producing different levels of benefit. The results for *B. erectus* showed no benefit from the presence of AMF with the plants from the control have a slightly increased biomass compared to those treated with AMF. The seedlings of all four species benefited from the presence of AMF. *T. pratense* showed the greatest benefit from the presence of AMF where the average biomass across treatments was about twenty five times greater than that in the controls. The variation in benefit across treatments was again apparent being particular strong for *T. pratense*. Measurements of the P content of seedlings showed it to be higher in seedlings grown in the presence of AMF. The results of the van der Heijden (2004) trial on seedling establishment is broadly in line with that of Grime *et al.*, (1987) and confirms that mycorrhizal fungi are important in seedling establishment, although the mechanisms involved are not firmly established. In fact van der Heijden, (2004) suggests that the situation may be more complex and protection against pathogens may also be involved.

There is also evidence that AMF/plant symbiosis may affect the plant life cycle by influencing the nutrient content of the seeds produced and the subsequent vigour of the seedlings (Lewis and Koide, 1990). In a greenhouse trial, Lewis and Koide (1990) studied the effect of AMF infection on the seeds produced by the plants *Sinapis alba* and *Abutilon theothrasti*. The adult plants were grown under varying conditions including high and low P nutrition and with and without AMF being present. No large effects on germination of the seeds produced was found, but the seeds produced under mycorrhizal conditions had increased seedling vigour, even though some non-mycorrhizal seeds contained similar levels of P. It was postulated that variations of phosphate fractions within the seeds may be important in imparting vigour (Lewis and Koide, 1990). In a field trial using the forb *Achillea millefolium* Allison (2002) studied four abiotic and four

biotic variables and found that *A. millefolium* grew more vigorously when AMF infection was reduced by the application of fungicide, suggesting a non beneficial (for the plant) symbiosis. The largest plants produced the most seed and the highest germination rates; there were no data on subsequent seedling vigour (Allison, 2002).

During the life cycle of a plant the need to reproduce and at the same time to maintain its own vigour, may result in competition for nutrients between the plant and its seedlings. This potential conflict between adult plants and seedlings of the same species has been tested in a greenhouse experiment on the forb *Gnaphalium norvegicum* (Pietikainen and Kytoviita, 2007). In the trial, seedlings were grown in the vicinity and remote from adult plants, in the presence and absence of AMF. Pietikainen and Kytoviita (2007) found that AMF increased below ground competitive intensity and that when seedlings were close to adult plants AMF benefit for seedlings was low. However if the adult plant was defoliated by removing 50% or 75% of leaf area the benefit to the seedlings increased with increasing defoliation of the adult plant, defoliation presumably reducing the intensity of the symbiotic relationship for the adult plant. This result has important implications for regeneration of plant communities in the field by seedling establishment, as it suggests that grazing not only increases access to light but may also act as a trigger for seedlings to become established. However there is evidence that the relationship between foliage removal by artificial or natural grazing and the presence of AMF is complex and may vary between plant species. In a field trials involving the study of three lowland grasses, *Anthroxanthum oderatum*, *Holcus lanatus* and *Agrostis tenuis,* Wearn and Gange (2007) found that compared to ungrazed plots, the levels of AMF in these species from plots moderately grazed by rabbits was up to 1.6 times higher

In summary, the results from the glasshouse and field trials outlined above have shown how intimately AMF are involved in the life cycles of plants. In the majority of cases they have a positive influence upon seedling fitness and their ability to grow vigorously through the seedling stage (Grime *et al.*, 1987; van der Heijden, 2004; Lewis and Koide, 1990). The mechanisms involved are only poorly understood and are likely to vary according to the environmental circumstances and the plant and AMF species involved. However it does appear that AMF tolerate a short term loss of benefit to increase their long term benefit by facilitating seedlings to progress to the adult stage. In a recent review of the importance of AMF networks as facilitators in natural ecosystems a major

conclusion of van der Heijden and Horton (2009) was that AMF networks have a key role in influencing and facilitating seedling establishment in plant communities.

2.4.9.3 Intra and Interspecific Plant Competition

In plant communities nearest neighbours may be plants of the same species leading to intraspecific competition, or plants from different species, leading to interspecific competition. It is generally accepted that AMF will play a role in plant competition particularly between species that have different degrees of mycorrhizal dependence (Scheublin *et al.*, 2007). A number of studies on the effects of AMF/plant symbiosis on plant populations and intraspecific competition have been performed and these have been reviewed by Koide and Dickie (2002). One of the findings of Koide and Dickie (2002) was that a positive response in growth to AMF colonisation may be inversely related to population density. It is argued that at high population densities AMF infection of roots may be less effective in supplying nutrients, the greater number of roots leading to lower percentage root infection (Koide and Dickie, 2002), and this may in turn lead to stabilisation of plant populations over time. Another possible effect of mycorrhizal colonisation was to produce greater size inequality, for example some plants growing very large while others remain small. If the underlying cause of size differences is genetic, the effect of more vigorous plants setting seed could have genetic implications for subsequent generations (Koide and Dickie, 2002).

A study of the role mycorrhizal fungi in intraspecific competition in populations of the forb *Plantago lanceolata* grown under field and glasshouse conditions has been undertaken by Ayres *et al.* (2006). Some of the findings were consistent with those of Koide and Dickie (2002) but there were also differences. The study by Ayres *et al.*, (2006) showed the importance of the starting condition of the plants and that small variation in environmental conditions can result in differences in plant population dynamics. In this trial 14 day old seedlings grown in compost were transplanted either as singles or in a grid arrangement into the field or the glasshouse, where the same soil as that in field trial was used. The most interesting set of results were for *P. lanceolata* grown in the presence of AMF under non-competing conditions, which produced contrasting results for field and glasshouse conditions (Ayres *et al.* 2006). In the field the effect of AMF was to produce larger plants, but plant size inequality was low, but under

glasshouse conditions, although the levels of AMF infection were high, the plants were reduced in size, (i.e. the AMF were antagonistic). Larger plants in the glasshouse were shown to have lower levels of infection, and in contrast to the field trials, size inequality was high (Ayres *et al.*, 2006). The differences between the behaviour in *P. lanceolata* under field and glasshouse conditions is difficult to explain, particularly as the presence of AMF is generally thought to be beneficial (Ayres *et al.*, 2006). A possible explanation for the observed behaviour of size equality in the field trial was that the seedlings were all the same size at the start of the trial, that is variable germination was eliminated. In the glasshouse trial although the seedlings were also the same size, the soil was taken from an adjacent area to the field trial and, different species of AMF might have been present (Ayres *et al.*, 2006).

In a study by Hartnett *et al.* (1993) two co-occurring American Prairie grasses, *Andropogon gerardii* (strongly mycorrhizal) and *Elymus Canadensis* (weakly mycorrhizal) were subjected to intra and interspecific competition trials and the following effects were observed. In the case of *A. gerardii* its competitive dominance disappeared in the absence of mycorrhizas and was also reduced when competing intra-specifically in crowded conditions in the presence of AMF. However the competitive effectiveness of *E. canadensis* was less affected by the presence of AMF and it competed more strongly with *A. gerardii* in the absence of AMF. Overall the study showed that AMF/plant symbiosis had a strong influence both on intra and interspecific competition.

In a glasshouse experiment two grasses *Holcus lanatus* and *Dactylis glomerata*, were assessed for the effects of intra and interspecific competition in the presence of AMF (West, 1996). The effects, although measurable, appeared to be weaker than those found in the Prairie grasses studied by Hartnett *et al.* (1993). This may be because both are regarded as weakly mycorrhizal with only around 10% of roots infected in both species. This relative balance between the species manifests itself in the fact that, at high plant densities, *H. lanatus* was more aggressive when AMF were present, but at low densities this was reversed with *D. glomerata* being more competitive (West, 1996).

A further study has been conducted in China, where the separate and combined effects of AMF root infection and subsequent P concentrations in the leaves of three plants with different mycorrhizal dependence was examined (Chen *et al.*, 2005). The species

Digitaria cilaris (weakly mycorrhizal), *Ixeris denticulate* (moderately mycorrhizal) and *Kummerowia stratia* (highly mycorrhizal) were planted in separate pots. The degree of root infection reflected their stated AMF dependence, but the level of infection was not correlated with the level of P in the leaves which was lowest in the species with the highest root infection (Chen *et al.*, 2005). In three way plant mixtures the performance of the most mycorrhizal species was unaffected, but the mycorrhizal root infection and P concentration of the least mycorrhizal species was increased, while that of the moderately mycorrhizal species was decreased (Chen *et al.*, 2005). These results illustrate that different plant species, grown in combination, can behave differently compared to when grown separately and that the level of root infection does not necessarily translate into nutrient benefit to the leaves.

The relationship between grasses and forbs in the presence of AMF has important implications for the structuring of grassland communities. A trial by Zabinski *et al.*, (2002) involving an invasive forb *Centaurea maculosa* paired with five plant species native to USA mountain grassland, three grasses and two forbs produced interesting results. Zabinski *et al.* (2002) showed that *C. masculosa* was able to exploit AMF symbiosis when growing with a grass as a neighbour, but this exploitation was lower when the neighbour was a forb. There was no evidence of carbon transfer from the native grasses to *C. masculosa* via hyphal linkage. An alternative explanation was that C. *masculosa* was able to access higher levels of P in the presence of AMF but only when competing with the grasses (Zabinski *et al.*, 2002) .

A recent study by Scheublin et al. (2007) on a legume (*Lotus corniculatus*), a grass (*F. ovina*) and a forb (*P. lanceolata*) grown separately and as pairs in the presence of four different AMF species showed that the legume and grass both benefited from the presence of AMF when grown alone. However the findings also showed that when grown together, the legume suppressed the growth of the grass. The extent to which the grass was suppressed depended on the species of AMF with which it was grown, but this effect was not seen in competition between the legume and forb. The forb was generally unresponsive to AMF in both monoculture and in competition (Scheublin *et al.*, 2007).

Many of the plants in chalk grassland regenerate clonally and a study by Streitwolf-Engel *et al.* (1997) of two clonal plant species, *P. vulgaris* and *Prunella grandiflora* grown in

conjunction with three species of AMF present in calcareous grassland and a non mycorrhizal control, found that both *Prunella* species were highly mycorrhizal. The addition of P to the study did not increase the growth of *P. vulgaris* in mycorrhizal or non-mycorrhizal conditions. The effect of the individual AMF isolates was strong, with different AMF producing different growth characteristics in the two *Prunella* species. Perhaps more significantly the different AMF species had different effects on the formation of stolon and stolon branching on both species. The effect of the AMF species on stolons was independent of the percentage root colonisation and, in *P.vulgaris,* the AMF that gave the highest growth rates was not the one that produced the highest stolon branching. Streitwolf-Engel *et al.* (1997) demonstrated that AMF fungi species could affect different plant species in different ways and that biotic factors as well as abiotic factors could influence plant foraging characteristics. Streitwolf-Engel *et al.* (1997) conclude that the diversity of the AMF species present has the potential to structure plant populations in ecosystems.

In summary, this series of papers on intra and interspecific plant competition in the presence of AMF has shown that high density and overcrowding can reduce or alter mycorrhizal benefit. Species grown in isolation will behave differently to those grown in combination. When plant species were grown in combination, each species had the potential to alter the mycorrhizal behaviour and growth characteristics of the other species present. The research has also shown that the extent of AMF root infection is not necessarily a good guide to the effects of the symbiosis on plant function. Different AMF can have different effects on the plant when grown alone or when the plant is growing in competition.

2.4.9.4 The study of community structure using microcosms

In the previous section the interaction of pairs and three way combinations of plants was considered. In this final section the level of complexity is increased to consider the effect of AMF on the functioning and structure of plant communities and how diversity in plant communities is affected. The studies fall into two groups: sophisticated glasshouse studies using microcosms and medium to long term field trials.

In the much referenced study by Grime *et al.* (1987) microcosms were set up with *F. ovina* as the dominant matrix species into which various combinations of subordinate species of both grasses and forbs were introduced. This was done with both AMF present and absent, and with variable levels of simulated grazing and soil heterogeneity. The study showed greater plant diversity in the microcosms in which AMF was present, and this was at least partially the result of higher seedling survival (Grime *et al.*, 1987). The effect of AMF was to reduce the yield of *F. ovina* and increase the yields of the forbs, such as. *Scabiosa columbaria, H. pilosella* and *P. lanceolata,* all of which were found to have roots with high levels of mycorrhizal infection present (Grime *et al.*, (1987).

At the conclusion of the microcosm trial Grime *et al.* (1987) performed an experimental procedure in which ^{14}C (introduced as $^{14}CO_2$) was tracked between plants. Based on the results of this procedure Grime *et al.*, (1987) proposed a mechanism of assimilate transfer from dominant to subordinate species via hyphal networks. However the experimental bases of these findings has since been disputed (Fitter *et al.*, 1998; Robinson and Fitter, 1999). More recent studies of inter–species competition and the concept of positive and negative feedback (Bever, 2002; Bever *et al.*, 2002), suggest that the mechanisms that allow subordinate species to survive in plant communities, are likely to be more complex than a single mechanism of the transfer of carbon assimilates between dominant and subordinate species. However a study by Wilson *et al*. (2006) has demonstrated that AMF facilitated the movement of P between two tall Prairie grass species, although it was not possible to demonstrate conclusively that the transfer took place via a common mycelial network.

A further study of note was that of van der Heijden *et al.* (1998) in which microcosms were assembled to represent two plant community types, calcareous grassland and North American old field. In the study of calcareous grassland the species *B. erectus, F. ovina, C. flacca, B. pinnatum, H. pilosella, P. vulgaris, L. corniculatus, T. pratense, Sanguisorba officinalis, P. grandifolia* and *C. erythraea* were grown in microcosms in the presence of four AMF species. The the plants were grown in the presence of the four AMF species separately, with the four species combined and with AMF absent. It was found that with the exceptions of *B. erectus, F. ovina* and *C. flacca,* other species were so strongly mycorrhizal as to be almost totally dependent on the symbiotic relationship for their survival (van der Heijden *et al.*, 1998). The biomass of *F. ovina* and *C. flacca*

was greater under non–AMF conditions, whereas *B. erectus* appeared to be unaffected by the presence or absence of AMF. Those species that were mycorrhizal produced differing levels of biomass in the presence of different AMF species. Individual plant species produced maximum biomass when different AMF species were present. Although individual plant species produced variable levels of biomass with different AMF, the overall biomass of the species present was independent of the AMF species and was the same under non–mycorrhizal conditions. However it was observed that total biomass increased with an increasing number of mycorrhizal plant species present, as did the total P in the plant tissue. The increase in P present in the foliage was accompanied by lower P levels in the soil and increases in the length of mycelium present. It was suggested that the increased P in the foliage was the result of more efficient exploitation of the P resource available in the soil (van der Heijden *et al.*, 1998).

In a trial by Klironomos *et al.* (2000) two species of AMF (*Glomus etunicatum* and *Glomus intraradices*) and up to 15 plant species, chosen at random from a pool of 35 species were grown in microcosms. It was observed that one of the AMF species, *G. etunicatum,* produced double the plant biomass compared to the other AMF species. The study also found that with both AMF species total plant biomass appeared to be approaching a maximum level of productivity with 15 plant species present. The difference in productivity between the two AMF species was considered to be due to the spectrum of symbiotic – parasitic relationships that can exist between plants and AMF (Johnson *et al*. 1997; Klironomos *et al.*, 2000). These findings might also be considered as evidence of positive and negative feedback between plants and AMF as proposed by Bever (2002) and Bever *et al.* (2002).

2.4.9.5 The study of community structure using field trials

A field trial in Argentina carried out on a „community" of only two salt marsh plants, *Spartina densiflora* and *Spartina alterniflora* comes closest to creating a „microcosm experiment" under field conditions (Daleo *et al*. 2008). *S. densiflora* is a high marsh species which dominates under the relatively benign conditions that exist here and is colonised by AMF. *S. alterniflora* grows in the more hostile environment of the lower salt marsh, and is a stress tolerant species that does not respond to AMF.

The trial consisted of applying the fungicide Benomyl and nutrients separately, and in combination, and measuring the species response (Daleo *et al*., 2008). At low nutrient levels root colonisation increased the growth of *S. densiflora* but at high nutrient levels growth was reduced. If fungicide and nutrient were applied separately, S. *alterniflora* replaced *S. densiflora* in the high marsh. However if fungicide and nutrient were applied in combination *S. densiflora* was able to retain its dominance in the high marsh (Daleo *et al*., 2008). Daleo *et al.* (2008) conclude that the trial demonstrated that AMF can determine the outcome of plant competition. However changes to the environment, e.g. eutrophication, can also change the competitive balance between plant species and alter community structure.

In the UK field trials were initiated by Gange *et al.* (1990 and 1993) to study the effects of mycorrhizal fungi on plants in early succession In these field trial two areas of 450m^2 of sandy soil (pH 4.5) were cleared of existing vegetation and allowed to regenerate naturally. The trials were conducted in 2.5 x 2.5m quadrats separated by 1.5m walkways. Quadrats were either treated on a regular basis with the contact fungicide Iprodione or as untreated controls. The effect of the fungicide was to reduce the percentage root infection in the plant species. For example, root infection in the forb *Veronica persica* was reduced from approximately 50% to 25% (Gange *et al.*, 1993).

For the plot started in 1988 the difference between quadrats treated with Iprodione and untreated quadrats was pronounced by 1990, with an average of 42 species in untreated quadrats and 33 in the treated quadrats. The equivalent values for the plot started in 1990 were 31 species in untreated and 21 species in treated quadrats (Gange *et al.*, 1993). In each study 13 species were found only in the untreated quadrats. Perennial forbs were the functional group most affected by the application of fungicide with 6 species being absent from the 1988 plot and 7 from the 1990 plot (Gange *et al.*, 1993). It was also observed that seedling emergence in fungicide treated quadrats was lower than in the untreated quadrats, which was evidence of the role of AMF in seedling establishment.

A field trial carried out on semiarid herbland in Australia was on emerging annuals (O' Connor *et al.* 2002). The field trial was supported by parallel glasshouse experiments. In the glasshouse experiments the AMF /plant association of six plants that made up greater than 90% of the biomass of the communities was assessed. Three species, the dominant

species *Medicago minima* and two subordinates *Vittadinia gracilis* and *Velleia arguta*, were strongly mycorrhizal responsive. *Salvia verbenaca* became colonised by AMF but showed no growth response and *Carrichtera annua,* the second most abundant species, remained uncolonised by AMF. In the field trial the effect of fungicide (Benomyl) application did not change species richness but diversity was increased as a result of greater evenness. The major change was that *M. minima* was greatly reduced in biomass. *C. annua* increased biomass slightly and *S. verbenaca* by a factor of x10 from a low base value. The biomass of the subordinate mycorrhizal species *V. gracilis* and *V. arguta* was largely unchanged by the application of fungicide. O' Connor *et al*. (2002) concluded that the mycorrhizal response of species grown separately in the glasshouse was not a good predictor of that species response in field conditions where interspecific competition was occurring. That the two mycorrhizal subordinate species *V. gracilis* and *V. arguta* were not greatly reduced by the application of fungicide might have been due to competitive release from the dominant *M. minima* (O' Connor *et al*. 2002).

A study of a predominantly lichen/moss community growing on sandy soil in Suffolk UK has shown that community structure was also affected by AMF (Newsham *et al.*, 1995). The application of the fungicide Benomyl over a three year period resulted in the elimination of the lichen *Cladonia rangiformis* and a large increase in the abundance of the moss *Ceratodon pupureus*, while the abundance of mycorrhizal forbs decreased and non-mycorrhizal forbs and grasses increased. Two species that increased in abundance were *F. ovina* and *P. lanceolata*, a further example of *F. ovina* apparently preferring non-AMF conditions. These observations are further evidence of the dual behaviour of *P. lanceolata*, which appears to exhibit both mycorrhizal and non-mycorrhizal behaviour in different environmental conditions (see Grime *et al.*, 1987; Ayres *et al.*, 2006).

A study by Hartnett and Wilson (1999), focusing on a tall Prairie grass community, has shown that reducing mycorrhizal infection by the application of fungicide can increase species diversity in a community. This reverse effect on species diversity appears to occur if the dominant species are strongly mycorrhizal and is a possible example of positive feedback in the dominant species (Bever *et al.*, 2002). The fungicide Benomyl was applied during the growing season over a five year period (Hartnett and Wilson, 1999) and the result was to reduce the abundance of the dominant warm season matrix grasses *Andropogon gerardi*, *Andropogon scoparius* and *Sorghastrum nutans*, and

increase the abundance of the subordinate grasses *Poa pratensis* and *Carex* species. There was also an increase in the abundance of forbs, such as *Aster ericoides*, *Aster oblongifolius* and *Salvia azura*. Two important observations were that it took several years for the effects of fungicide application to show and that the overall biomass of the new community was similar to that of the original community (Hartnett and Wilson, 1999) .

A field study on a traditionally managed hay meadow in the Czech Republic was carried out by Smilauer and Smilauerova (2000). The trial took place between 1994 and 1998 on an unimproved meadow, cut annually in June and which had been managed in this way for at least 120years. There were two treatments applied over a five year period, the addition of phosphate and the fungicide Benomyl, applied separately and in combination (Smilauer and Smilauerova, 2000). The effect of the fungicide was to reduce the biomass of the forbs present with the exception of *Achillea millefolium* which increased in biomass. The biomass of the grasses and sedges present was not affected by the application of fungicide (Smilauer and Smilauerova, 2000). The response of *A millefolium* to the reduction of AMF is similar to that found by Allison (2002) (see page 38).

A further study of a grassland community in Greece was undertaken by Karanika *et al*. (2008a). The montane grassland was nutrient poor, with low levels of P and other nutrients in the soil. The two dominant species in the community were a grass (*Agrostis capillaris*) and a forb (*Galium lucidium*) both of which are non mycorrhizal and subordinate forbs which are mycorrhizal. The fungicide Benomyl was applied to the community during the growing season of April to July. The non-mycorrhizal *A. capillaris* and *G. lucidium* were not affected by the application of the fungicide. Many of the mycorrhizal forbs were reduced in biomass by the application of fungicide, some being eliminated from the community. An unexpected observation was the increase in biomass of the mycorrhizal legume *Doricnium herbaceum* (Karanika *et al*. 2008a). The increase in biomass occurred despite a parallel study showing that the legumes in the community, including *D. herbaceum,* had higher levels of root infection than the grasses and forbs present (Karanika *et al*. 2008b)

In summary, the studies involving microcosms and field trials have shown that the presence of AMF has a clear role in determining species richness and diversity in plant communities. However plant communities growing in different soils at various locations will interact with AMF in different ways and environmental factors such as nutrient levels can also affect the outcome of interspecific competition. Individual AMF species can affect the plant species present in different ways and increasing the number of AMF species present facilitates greater complexity in community structure and allows more species to compete through more efficient exploitation of available resources. Removal of AMF through the application of fungicide has the ability to remove some plant species from the community and retard or prevent them from regeneration. The evidence suggests that the application of fungicide in most cases lowers the level of root infection but does not eliminate the fungus from the roots. There appears to be evidence that the application of fungicide to a plant community does not change the overall biomass produced but leads to redistribution amongst the remaining species.

2.5 Synthesis

In this review factors and mechanisms which contribute to the characteristics and stability of community structure in chalk grassland have been described and discussed, in particular the role of AMF/plant symbiosis.

Chalk grassland has been identified as a habitat of international importance under threat from fragmentation. The abiotic factors pH, slope and aspect have been identified as important factors in structuring chalk grassland communities, as has the resistance of calcareous grassland to drought (Grime and Curtis, 1976). The biotic factor grazing has also been demonstrated to affect community structure (Pykälä, 2004) where moderate grazing increase species diversity. The concept of the fundamental and realised niche was shown to be important, particularly within the context of AMF/plant behaviour. Studies involving plant species growing in isolation or in competition with AMF present have shown that there is a fundamental and realised symbiotic niche. The regeneration niche (Grubb, 1977) and the carousel model (van der Maarel and Sykes, 1993) have been identified as key mechanisms in the dynamic regeneration and stability of plant communities. Positive and negative feedback relationships between AMF and plants (Bever, 2002; Bever *et al.*, 2002) have been recognised as a potential mechanism for

subordinate species survival in tightly packed communities such as chalk grassland. The desirability of conducting field trials for their increased ecological relevance (Read, 2002) has been discussed against the background of only small numbers of field trials being reported in the literature.

The role of AMF in seedling establishment has been reviewed. A significant finding was that „grazing" adult plants facilitates AMF benefit in competing seedlings (Pietikainen and Kytoviita, 2007). A review of intra and interspecific competition found that over a wide range of plant species mycorrhizal behaviour was affected by packing density in single species populations and by the identity of nearest neighbours in interspecific competition. In the context of chalk grassland communities the paper by Zabinski *et al.*, (2002) that suggested that a forb in the presence of AMF could only obtain more P if its nearest neighbour was a grass species is of particular interest in the context of chalk grassland communities. From microcosm research the finding by van der Heijden *et al.* (1998) that *B. erectus, F. ovina* and *C. flacca* did not benefit from an association with AMF was important and aids the interpretation of the research in this thesis. Two of the main conclusions from the field trials reviewed were that the application of fungicide to reduce AMF/plant symbiosis did change community structure and that these changes took several seasons to become apparent. This suggests that application of fungicide to natural chalk grassland will change community structure, but in may take several seasons for the changes to be become apparent.

Chapter 3 - The Research Site

3.1 General Introduction

The research site for the field trial was situated within the recently designated South Down National Park at a at a location about 8km east of the city of Brighton and Hove (Figure 3.1)

Figure 3.1 - The research site was located on the South Downs about 8km east of the city of Brighton and Hove, United Kingdom

The field trials were conducted at Newmarket Hill within the Castle Hill Nature Reserve a designated Site of Special Scientific Interest (S.S.S.I.) and a Special Area of Conservation (S.A.C.) (Figure 3.2). The overall Castle Hill Site contains several downland areas including Bullock Hill, Castle Hill and Newmarket Hill extending to an overall area of 115ha, and is managed by Natural England (formerly English Nature). More specifically Newmarket Hill has an area of approximately 27ha of which 13ha are

designated as chalk grassland and 4ha as mesotrophic grassland (Steven and Muggeridge, 1992).

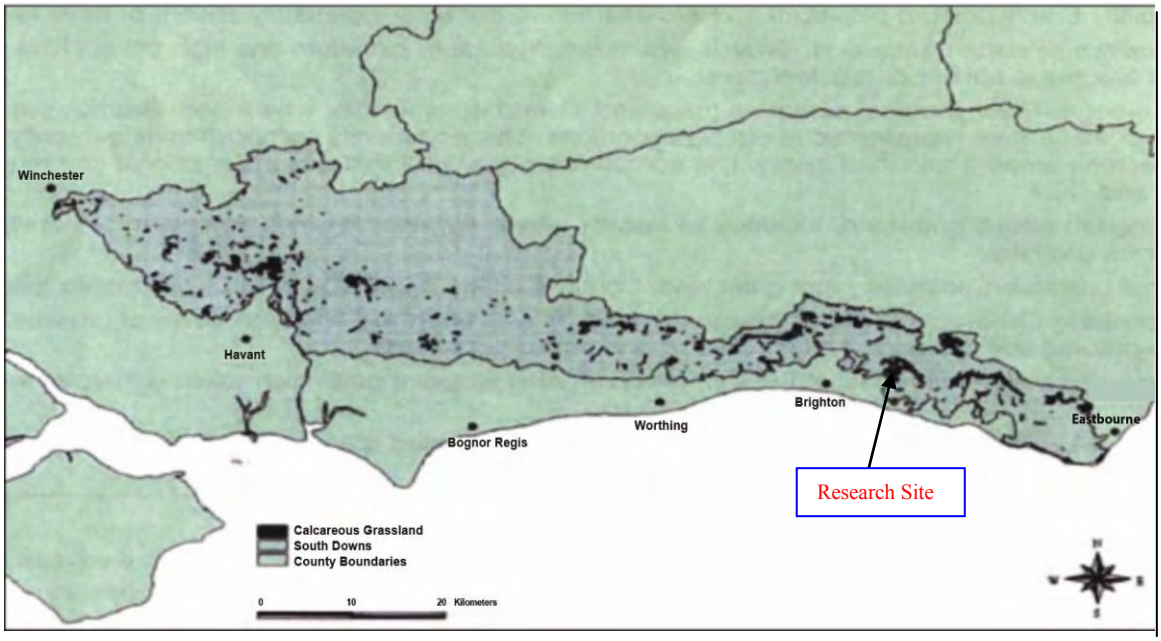


Figure 3.2 - The general location of the Castle Hill SSSI

The overall site is described as semi-natural grassland and scrub facies on calcareous substrates (Natural England, 2008). The chalk grassland is made up of a mosaic of CG2 *Festuca ovina - Avenula pratensis* grassland, CG3 *Bromus erectus* grassland and CG4 *Brachypodium pinnatum* grassland (Rodwell, 1990). Details of the communities present are given in Figure 3.5. The soil is described as basic, nutrient poor and the geology as sedimentary (Natural England, 2008). .

The site contains a substantial number of rare species including *Ophrys sphegodes* (Early Spider Orchid)(Hutchings, 1987), *Orchis ustulata* (Burnt Orchid) and *Gentianella anglica* (Early Gentian.). The site is considered to be vulnerable, without regular grazing by sheep and cattle high species diversity and in particular rare chalk grassland species would be at risk (Natural England, 2008). The proximity of agricultural land adjacent to unimproved areas makes them vulnerable to chemical drift.

3.2 Location of Field Experiments

The area chosen for the field trials was on the South facing slope of Newmarket Hill. The area was inspected in April 2006 and two sites were chosen for the field experiments. The two sites chosen were at locations where there was evidence of high species diversity and

regular grazing. Site 1 was situated towards the bottom of the south facing slope (TQE 36936 N06859) and Site 2 was situated in a more elevated position half way up the south facing slope (TQE 36861 N 06865) (Figure 3.3). Each site, approximately 12m x 6m, was fenced off to exclude domestic livestock. Detailed plans of the quadrat size, spacing and treatments applied are described in Chapter 6 section 6.4.3. In addition to the field trials, turf was also selected from Newmarket Hill for the purpose of laboratory trials. Turf was removed from within a rectangular area located between Sites 1 and 2 used for the field experiments.

Figure 3.3 - The approximate location of Sites 1 and 2 (Natural England, 2008)

3.3 Topography

The Newmarket Hill site varies in elevation between 106m and 150m above sea level (Burnside, pers. comm). Topographically Newmarket Hill varies in aspect with the more eastern section having a south eastern aspect and as the hill curves westward the aspect becomes more southerly. At Sites 1 and 2, the location of the field trials, the aspect is close to due south. The slope at Newmarket Hill typically varies between 0 – 30^{o} (Burnside, pers. comm). The slope at Site 1 is estimated at approximately 20^{o} with Site 2 being marginally steeper than Site 1. The importance of south facing steep slopes in controlling coarse grasses by encouraging drought conditions in the summer months is

described by Grime and Curtis, (1976) and confirmed by a study of British chalk grassland by Bennie *et al*. (2006).

3.4 Soil pH

The soil on the South facing slope of Newmarket Hill is thin with a high pH. In the period 1979 to 1980, Gay *et al.* (1982) were conducting field trials at Newmarket Hill and described the soil as being approximately 30cm down to bedrock, with the top 10cm being mostly free of stones and the top 3cm being alkaline with a pH in the range 7.4 – 7.6. More recently, soil samples taken in 2006 from areas around Sites 1 and 2 yielded pH values in the range 7.7 – 8.1. Independent analysis by the Macauley Soil Institute (Owen, 2008) of soil collected from within Sites 1 and 2 in October 2007 (Figure 3.4) gave pH values for Site 1 as being in the range 7.64 – 7.86 (Table 1) and for Site 2 as 7.77 – 7.84 (Table 2). Thus the pH levels are conducive to plants with a preference for conditions of high alkalinity that is strong calcicoles.

3.5 Nutrient status, Nitrate Levels and Organic Content

The level of nutrients in the soil of chalk grassland is considered to be important in maintaining high species diversity, in particular the levels of available phosphorous (Bennie *et al.*, 2006). In order to have independently produced values of nitrate and the major nutrients at Sites 1 and 2, soil samples were collected in October 2007 and sent to The Macaulay Soil Institute for analysis (Owen, 2008).

Figure 3.4 is a schematic diagram showing the positions at which soil sample were taken. In order not to disturb the vegetation within quadrats, soil samples were taken from within the buffer zones between the $1m^2$ quadrats. The ten samples were taken to a depth of about 12cm with an auger and from an area about 10cm in diameter. The upper layer of vegetation was not included in the soil sample. The results of the analysis, (Owen, 2008) are set out in tables 3.1 and 3.2.

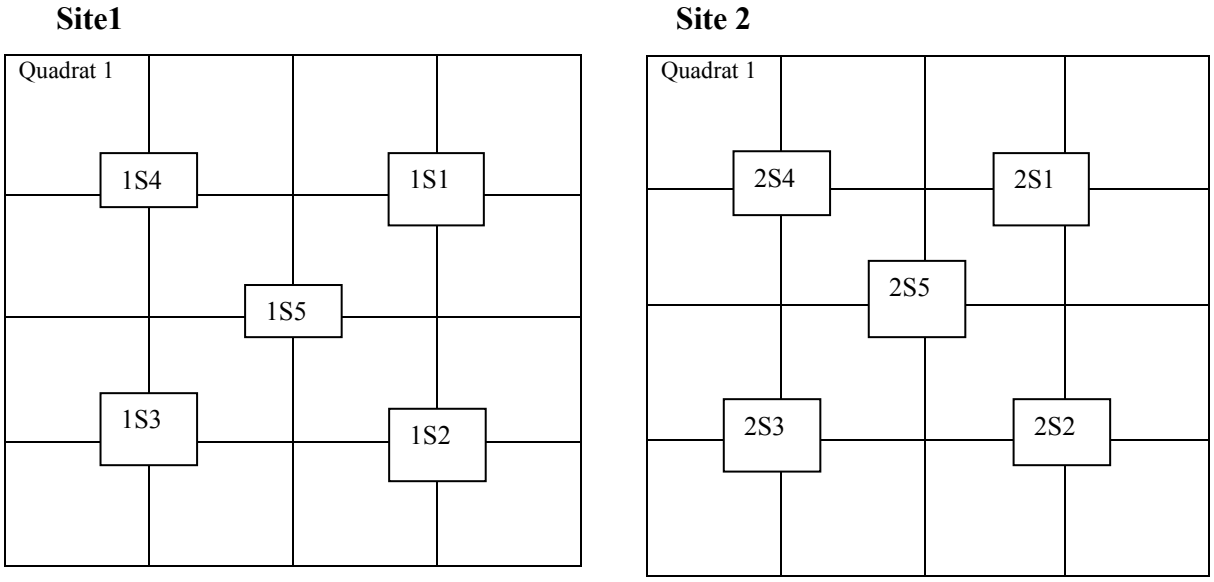


Figure 3.4 - Schematic diagram showing the locations from which soil samples were taken in October 2007 from the two field trial sites.

Table 3.1 - Results of soil analysis for Site 1 (Owen, 2008)

Sample ID	Nitrate (mg/kg)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Organic Content %	pH
1S1	388.5	35.21	238.8	708.1	17	7.64
1S2	223.8	31.98	232.4	683.4	18	7.73
1S3	<0.07	26.43	188.8	638.5	18	7.78
1S4	17.3	27.69	165.2	649.7	17	7.86
1S5	5.41	31.95	174	714.8	17	7.83
Mean	127	30.65	199.84	679.04	17.4	7.77

Table 3.2 - Results of soil analysis for Site 2 (Owen, 2008)

Sample ID	Nitrate (mg/kg)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Organic Content %	pH
2S1	11.65	31.95	174	714.5	20	7.77
2S2	<0.07	24.4	172.8	527.3	20	7.83
2S3	84.05	25.69	346.7	599.8	20	7.81
2S4	<0.07	26.75	246.5	613.1	23	7.84
2S5	<0.07	27.75	276.4	639.4	22	7.8
Mean	47.84	27.31	243.2	618.8	21.8	7.81

Examination of the soil analysis data in Tables 3.1 and 3.2 shows the values for the nutrients at each site to be similar although there was a wide variation in the levels of nitrate present at both Sites. The amount of available phosphorous in the soil is critical to plant survival. The mean values of P at Sites 1 and 2 were 30.65 mg/kg and 27.31 mg/kg respectively and P was evenly distributed throughout Sites 1 and 2. It was expected that the values of P at Newmarket Hill would be low given the management strategy of no artificial nutrient input practised over many years. The values obtained from the soil analysis were at the boundary between low and medium according to Owen (2008).

The level of P in the soil and available for plant nutrition is likely to be a critical factor in determining plant community structure, i.e. which plant species can survive and grow under the prevailing conditions. According to Koide (1991) if P levels are high mycorrhizal fungi are not needed in plant P acquisition and conversely if P levels are very low, mycorrhizal networks are unable to supply sufficient phosphorus to be of benefit.

The level of nitrate in the individual soil samples was very variable with some samples having < 0.07mg/kg present and the maximum value recorded of ~ 390mg/kg (Table 3.1). The high local levels of nitrogen do not appear to be consistent with the management of the site (that is only rabbits had access to research Sites 1 and 2 in the 18 months prior to collection of the soil samples). A possible explanation is provided by the work of Hurst and John, (1999) who found that nitrate tended to build up under the grass *Brachypodium*

pinnatum and was thought to have been accumulated from adjacent soil. Both Sites 1 and 2 contain substantial amounts of *B. pinnatum*. Several papers have been written on the effect of high nutrient levels on chalk grassland, in particular the effect of high nitrogen levels resulting from atmospheric deposition. Two of them based on field trials (Bobbink, 1991; Willems *et al*. 1993) have found that adding nitrogen to the soil at an annual rate of $10gm^{-2}$ reduce species diversity and promote the growth of *B. pinnatum*, while a trial carried out in microcosms (Wilson *et al*. 1995) found that high nitrogen levels did not enhance the growth of *B. pinnatum.*

The slightly higher levels of soil organic content at Site 2, higher up the slope, may result from the generally drier conditions than those at Site 1. Lower water levels may slow the soil processes that are water dependent and this could also account for the slightly lower levels of available P at site 2.

3.6 Rainfall and Insolation

The average rainfall calculated from meteorological data for the surrounding area in the period 2006 to 2009 was 834mm within the range 795 – 877 mm (Meeching, 2010). The value of 834mm is close to the value quoted by Gay *et al.* (1982) who give the annual rainfall at Newmarket Hill as 850mm. An interesting statistic was the rainfall for the May, June and July in 2007, totalled 322mm, more than twice the amount for the same months in 2006, 2008 and 2009 (Meeching, 2010). In the field trials (Chapter 6) there was a large increase in the percentage cover of *B. pinnatum* between early May and October 2007 (Figure 6.22). The south coast of England is the sunniest place in the United Kingdom (Met Office, 2008) with average sunshine levels of ~ 1750 hours per annum, and maximum daily levels occurring in May and June. Thus with a south facing aspect and steep slope Sites 1 and 2 will receive high levels of insolation and the top levels of soil containing most of the roots will become very dry (drought stressed) during periods of low rainfall in the summer months.

3.7 Grazing and Disturbance

Grassland systems in the United Kingdom and in particular chalk grassland are maintained by grazing and moderate disturbance. Bacon (1990) states that grazing needs to take place at the correct time of year, with the appropriate livestock and at appropriate

intensity. For south facing slopes and shallow soils winter grazing between October and March is sufficient to keep the grasses in check (Bacon, 1990) and ensure flowering forbs complete their lifecycle and release seed. Cattle are ideal as, unlike sheep which are selective grazers, they will eat long rank vegetation and stocking densities of up to 1.5 cattle per $ha^{-1}year^{-1}$ can be accommodated. Rabbits can also be an important source of grazing. (Denyer, 2005; Denyer *et al.* 2009).

Until recently grazing at Newmarket Hill was performed by cattle during the autumn and winter months but in 2009 Exmoor ponies were used. There was ample evidence of rabbit activity through the presence of scrapes and droppings. There was no evidence that molluscs had a significant grazing impact and none were detected during the surveys. However in a study of the impact of mollusc grazing in grassland Hanley *et al*. (1995) found grazing by mollusc could effect seedling recruitment and survival in some plant species. In their study at Newmarket Hill, Gay *et al.* (1982) report winter grazing of the site by cattle. Thus this grazing regime has been operating for at least 30 years.

In addition to controlling the dominant grasses, the cattle and rabbits are an important source of disturbance. This disturbance and the creation of areas of bare earth are considered to be important in the regeneration of plant species that are „turf incompatible", that is they cannot regenerate in „closed turf" (Grubb, 1976; Gay *et al.*, 1982)

3.8 Grassland Plant Communities Present at Newmarket Hill

The most recent detailed information on the plant communities comes from the surveys of Steven and Muggeridge (1992). This is illustrated in a GIS map of communities present (Figure 3.5) (Burnside, 2005). The NVC plant communities shown in Figure 3.5 are a representation of the communities present in 1991, however it should be noted that there may have been some changes since. Indeed the scrub encroachment shown higher up the slope (in black) appears to have been cut back, in compliance with current management targets. With minimal changes in the management history of Newmarket Hill (that is winter grazing by cattle and no artificial nutrient or nitrate input), initial site inspection in 2006 suggested little change in the overall community structure had occurred (but see Table 6.9).

Figure 3.5 - National Vegetation Classification of communities present at Newmarket Hill in 1991 taken from Steven and Muggeridge (1992), GIS representation by Burnside (2005)

Key (at the side of Figure 3.5); Brachy – *Brachypodium pinnatum*, CG – Calcicolous Grassland, MG – Mesotrophic Grassland.

Figure 3.5 shows that the south slopes of Newmarket Hill represent the largest continuous area of unimproved chalk grassland on the whole of the Castle Hill complex. Three communities are present 1) CG4a *Brachypodium pinnatum – Avenula pratensis - Thymus praecox* sub community, described by Steven and Muggeridge (1992) as the most strongly calcicolous sub – community with many of the forb species found in CG2 being present. 2) CG3b – *Bromus erectus – Centaurea nigra* sub - community and 3) CG3b/CG4b - *Brachypodium pinnatum – Centaurea nigra – Leontodon hispidus* sub-community The initial survey in 2006 of the two research sites (Site 1 and Site 2 shown in Figure 3.5) shows that both sites have more *B. pinnatum* than *B. erectus* and the presence of strong calcicoles (see Chapter 4) and high species richness suggests that they are closer to CG4a than CG4b.

3.9 Research History at Newmarket Hill

The south and south- eastern facing slopes of Newmarket Hill have historically provided high quality chalk grassland with high species diversity. These characteristics have made Newmarket Hill a favoured location for previous trials and studies into a variety of aspects of chalk grassland ecology. The current study reported in this thesis seeks to

contribute to and build on these earlier studies. Three studies that have particular relevance to the current study are those of Gay *et al.*, (1982) and those of Mitchley and Grubb (Mitchley and Grubb, 1986b; Mitchley, 1988a; Mitchley, 1988b) and the comprehensive surveys of species and communities carried out by Steven and Muggeridge (1992).

The research by Gay *et al.* (1982) looked at turf compatible species (those species able to regenerate in closed turf) and turf incompatible species (those needing bare earth to regenerate). Variations in the seasonal concentrations of the nutrients N, P and K in the vegetation were studied and comparisons made. Studies were also conducted into the presence and percentage infection of the roots of the plants with mycorrhizal fungi. An important observation was that the roots of newly germinated plants were infected with mycorrhizal fungi within two weeks. The research by Mitchley and Grubb (1986b), Mitchley (1988a) and Mitchley (1988b) was primarily concerned with the relative abundance of perennial dicotyledons in chalk grassland, the rank order in which they occurred and if this order changed with time and factors which might affect rank order. The survey data collected by Steven and Muggeridge (1992) was not directly aimed at research, and was part of a regional survey of the chalk grassland resource in the South East of England, but the data collected for Newmarket Hill allows comparisons with data collected in this current research.

3.10 Summary

Newmarket Hill situated within the Castle Hill SSSI; is an ideal location to perform research into the factors which influence the plant community structures found in high quality semi-natural chalk grassland. The remoteness of the site reduces the probability of the trials being affected by anthropogenic inputs. The steep slope, south facing aspect, high pH, grazing management and low to medium phosphorus levels combine to produce grassland with high species richness. This high species and diversity creates conditions where changes in community structure following the applications of fungicide can be detected and measured. The quality of the chalk grassland present increases confidence that results of the trials conducted at this site have a more general applicability. The availability of the findings of previous research conducted at this site makes it an ideal

location for the current research and presents an opportunity to add to the understanding of chalk grassland communities.

Chapter 4 - The Structure of Chalk Grassland Communities

4.1 General Introduction

The purpose of this chapter is to describe and define the structure of chalk grassland communities by analysing survey data collected by Steven and Muggeridge (1992) and Steven (1992) at 136 chalk grassland sites on the South Downs in Sussex, United Kingdom. Defining the structure of chalk grassland communities on the South Downs will be a useful contribution to the understanding of how the communities are constructed and will provide a base line for the laboratory and field trials, where community structure will be altered by the application of fungicide. The analysis addresses four areas; the species pool and its composition, the relationship between the frequency classes of the species present, nestedness, and the relationship between local abundance and frequency of presence at the sites surveyed.

One of the definitive characteristics of plant communities growing in a particular habitat within a designated area, for example chalk grassland growing on the South Downs, is the species pool (Zobel *et al*. 1998; Partel and Zobel, 1999; Tofts and Silvertown, 2000). This is the total inventory of plants found growing within that habitat. The species pool is a collection of plant species which can be subdivided into functional groups for example shrubs, forbs, grasses and mosses and lichens and by environmental preferences, for example strong calcicoles, which are plants preferring dry, nutrient poor and strongly alkaline soils (Ellenberg *et al.* 1991).

Examination of the distribution of the plant species present in the species pool, at different scales for example at the larger site and smaller $1m^2$ quadrat scale, reveals some species to have a high level of presence (frequency) at both the site and quadrat scale while others may occur infrequently. This difference in frequency of occurrence for individual species allows them to be arbitrarily categorized into frequency of occurrence classes (Hanski, 1982; Gibson *et al*. 1999; Partel *et al*. 2001) and relationships between these classes can then be tested.

Earlier studies of species distributions at many sites over large areas have found that those species that are widely distributed tended to be the ones that have a high level of abundance at the small scale (Tansley and Adamson, 1926; Gaston *et al*. 1997; Gaston *et*

al. 2000; Pärtel *et al,* 2001; Holt *et al*. 2002), that is species that are locally abundant are likely to be widely distributed.

Chalk grassland on the South Downs has become highly fragmented into many individual sites of variable area. None of these sites is of sufficient area for the complete species pool to be present. This relates to the concept of „nestedness" where the plants found at small sites are found to be subsets of the plants found at sites with larger areas (Wright and Reeves, 1992; Atmar and Patterson, 1993; Patterson and Atmar, 2000;). Nestedness at fragmented sites is considered in Study 3 of this chapter.

4.2 Aim

The aim of this chapter is to describe and define the structure of chalk grassland communities. This will be achieved from an analysis of detailed survey data, in which four related components, the species pool, interclass frequency relationships, nestedness and frequency/abundance relationships are addressed. By describing and defining community structure a case will be made for the study of AMF/plant symbiosis as a potentially important mechanism in the structuring of natural chalk grassland communities.

The objectives:

Study 1. To list the plant species that make up the species pool and define the species pool in terms of the plant functional groups present.

Study 2. To place the species present in the pool into four frequency classes and examine inter class relationships.

Study 3. To determine the extent of nestedness in the functional group strong calcicoles.

Study 4. To examine the relationship between local abundance and species frequency of occurrence at sites.

4.3 Introduction to the Data Base

The data base was compiled from an extensive field survey of semi-natural chalk grassland on the South Downs in Sussex in 1991 (Steven, 1992; Steven and Muggeridge, 1992). In total 136 sites were identified and surveyed, corresponding to a total of 3400ha of grassland including calcareous and more mesotrophic communities. The individual sites varied from 5ha to 136ha with a mean of ~25ha. The mean number of species present at a site was 56 with a standard error of ± 19.

Chalk grassland communities were identified in the field survey as discrete patches in accordance with National Vegetation Classification (NVC) types CG1, CG2, CG3 and CG4 (Rodwell, 1991), based on species presence and abundance. The most frequent and abundant calcareous community recorded during the survey was the NVC-CG2 community (*Festuca ovina – Avenula pratensis*), which reflects the drier climate and free draining rendziniform soils overlying chalk in the southeast of England (Rodwell, 1991). Within the identified community patches, a total of normally 8 quadrats per site were placed at random, each quadrat being $1m^2$ (Steven, 1992; Steven and Muggeridge, 1992). Within each quadrat the species present and their abundance using the Domin scale (Dahl and Hadac, 1941) was recorded. The total number of species found at each site was determined by combining the species data from the 8 quadrats. At a small number of heterogeneous sites up to 15 quadrats were taken (Steven, 1992; Steven and Muggeridge, 1992).

4.4 Study 1 The Composition, Characteristics and Functional Groups of the Species Pool

4.4.1 Introduction

In this study three tables of data are assembled. The first one is the species pool. The term species pool has a number of interpretations (Zobel *et al*. 1998; Partel and Zobel 1999; Tofts and Silvertown, 2000), but within this research project it taken as the total number of different plant species recorded by Steven (1992) and Steven and Muggeridge (1992) in their survey of the 136 chalk grassland sites on the South Downs in Sussex. The second table assembled was for the functional group strong calcicoles from within the species pool. The presence of strong calcicoles in a calcareous grassland community can be an indicator of the general health of that community (Pärtel *et al*. 2004). The third

table lists the species found to be present at the research site, Newmarket Hill. This allows comparisons to be made with the regional species pool and the local community species composition at the research site. In addition to listing the species the tables also show the frequency of occurrence at which they occurred at the 136 sites and which frequency category they were placed in, that is core, intermediate, scarce or rare.

4.4.2 Methods

In 1991 a comprehensive survey was carried out of the plant species presence at identified chalk grassland sites on the South Downs in East and West Sussex (Steven and Muggeridge, 1992; Steven, 1992). The survey data was recorded on Excel spread sheets, but not subjected to detailed analysis. The species pool (Table 4.1) was assembled by examining the survey lists for each of the 136 sites surveyed and constructing a list of the species present. Having identified the species they could then be classified into plant functional types that is forbs, grasses and sedges, mosses and lichens and shrubs.

In northern Europe vascular plants that prefer soils with a high pH, calcicoles, have been recognised as an important functional group responsible for increased species richness, and whose presence is indicative of the health of a habitat (Ewald, 2003; Wohlgemuth and Gigon, 2003; Chytry *et al*. 2007). The functional group strong calcicoles were defined as species that prefer conditions of high pH, low nutrient levels in the soil and dry conditions. Applying the tables of Ellenberg indicators (Ellenberg *et al*. 1991) to the plant species present in the species pool for the South Downs in Sussex a table (Table 4.2) of strong calcicoles was assembled. The table (Table 4.3) for the species present at Newmarket Hill was taken from the survey data of Steven and Muggeridge (1992).

4.4.3 Results

4.4.3.1 The species pool

In Table 4.1 the number of different plant species identified from a survey of 136 chalk grassland sites in Sussex is listed.

Table 4.1 - The species present at chalk grassland sites on the South Downs in Sussex (Steven and Muggeridge, 1992; Steven, 1992)

Key. **Column 1** gives the Scientific name of the plant. *(F)* – Forb, *(G)* – Grass or Sedge, *(M)* – Moss, *(L)* – Lichen, and *(S)* – Shrub.
Column 2 gives the Common name.
Column 3 gives the percentage frequency of occurrence of the species over all sites surveyed.
Column 4 shows the frequency category into which the species was placed. (Based on column 3, with the categories defined in Table 4.4)

Scientific Name	Common Name	% of sites at which present	Class Category
Dactylis glomerata (G)	Cocksfoot	100	Core
Festuca ovina (G)	Sheep"s Fescue	100	Core
Lotus corniculatus (F)	Birds – Foot –Trefoil	100	Core
Plantago lanceolata (F)	Ribwort Plantain	100	Core
Carex flacca (G)	Glaucous Sedge	99	Core
Cirsium acaule (F)	Dwarf Thistle	97	Core
Sanguisorba minor (F)	Salad Burnet	95	Core
Trifolium pratense (F)	Red Clover	95	Core
Leontodon hispidus (F)	Rough Hawkbit	94	Core
Avenula pratensis (G)	Meadow Oat -Grass	92	Core
Pimpinella saxifraga (F)	Burnet-saxifrage	91	Core
Achillea millefolium (F)	Yarrow	90	Core
Linum catharticum (F)	Fairy Flax	90	Core
Centaurea nigra (F)	Black Knapweed	89	Core
Scabiosa columbaria (F)	Small Scabious	89	Core
Pseudoscleropodium purum (M)	Common Moss (chalklands)	88	Core
Thymus praecox (F) articus	Thyme	88	Core
Bromus erectus (G)	Upright Brome	86	Core
Prunella vulgaris (F)	Selfheal	86	Core
Viola hirta (F)	Hairy Violet	85	Core
Briza media (G)	Quaking Grass	83	Core
Holcus lanatus (G)	Yorkshire Fog	81	Core
Ranunculus bulbosus (F)	Bulbous Buttercup	78	Core

Hieracium pilosella group (F)	Hawkweeds	78	Core
Agrostis stolonifera (F)	Creeping Bent	77	Core
Medicago lupulina (F)	Black Medick	77	Core
Carex caryophyllea (G)	Spring Sedge	77	Core
Galium verum (F)	Ladies Bedstraw	76	Core
Koeleria macrantha (G)	Crested Hair Grass	76	Core
Senecio jacobaea(F)	Common Ragwort	76	Core
Trisetum flavens (G)	Yellow Oat grass	74	Core
Plantago media(F)	Hoary Plantain	73	Core
Galium mollugo (F)	Hedge Bedstraw	71	Core
Asperula cynanchica (F)	Squinancywort	70	Intermediate
Polygala vulgaris (F)	Common Milkwort	67	Intermediate
Avenula pubescens (G)	Downey Oat Grass	66	Intermediate
Succisa pratensis (F)	Devil‟s Bit Scabious	66	Intermediate
Phyteuma orbiculare (F)	Round Headed Rampion	63	Intermediate
Cerastium fontanum (F)	Common Mouse- ear	62	Intermediate
Filipendula vulgaris (F)	Dropwort	62	Intermediate
Primula veris (F)	Cowslip	57	Intermediate
Euphrasia officinalis (F)	Eyebright	53	Intermediate
Gentianella amarella (F)	Autumn Gentian	52	Intermediate
Hippocrepis comosa(F)	Horseshoe Vetch	52	Intermediate
Phyleum pratense bertolonii (F)	Small Cats Tail	51	Intermediate
Rumex acetosa(F)	Sorrel	50	Intermediate
Bellis perennis (F)	Daisy	49	Intermediate
Taraxacum seedlings (F)	Dandelion.	48	Intermediate
Calliergon cuspidatum (M)		47	Intermediate
Leontodon autumnalis (F)	Autumn Hawkbit	46	Intermediate
Leucanthemum vulgare (F)	Oxeye Daisy	46	Intermediate
Trifolium repens (F)	White Clover	46	Intermediate
Anthoxanthum odoratum (G)	Sweet Vernal Grass	45	Intermediate
Homalothecium lutescens (M)		45	Intermediate
Campanula rotundifolia (F)	Harebell	43	Intermediate
Fissendens sp (M)		43	Intermediate
Crataegus monogyna (S)	Hawthorn (seedlings)	40	Intermediate
Anthyllis vulneraria (F)	Kidney Vetch	39	Intermediate
Crepis capillaris (F)	Smooth Hawks Beard	39	Intermediate
Cynosurus cristatus (G)	Crested Dog‟s Tail	38	Intermediate
Leontodon taraxacoides(F)	Lesser Hawkbit	38	Intermediate
Luzula campestris (G)	Field Woodrush	38	Intermediate
Daucus carota (F)	Wild Carrot	36	Intermediate
Blackstonia perfoliata (F)	Yellow Wort	35	Intermediate
Festuca arundinacea (G)	Tall Fescue	35	Intermediate
Carlina vulgaris (F)	Carline Thistle	34	Intermediate
Brachypodium pinnatum (G)	Tor Grass	32	Intermediate
Helianthenum nummularium (F)	Rockrose	32	Intermediate
Picris hieracioides (F)	Hawkweed Oxtongue	32	Intermediate

Centaurium erythraea (F)	Common Centaury	30	Intermediate
Rhinanthus minor (F)	Yellow Rattle	30	Intermediate
Agrostis capillaris (G)	Common Bent	29	Intermediate
Danthonia decumbens (G)	Heath Grass	29	Intermediate
Viola riviniana (F)	Common Dog Violet	29	Intermediate
Festuca rubra (G)	Red Fescue	27	Intermediate
Poa pratensis (G)	Smooth Meadow Grass	27	Intermediate
Ctenidium molluscum (M)		26	Intermediate
Rhytidiadedelphus squarrosus (M)		26	Intermediate
Brachypodium sylvaticum (G)	False Brome	24	Intermediate
Clinopodium vulgare (F)	Wild Basil	24	Intermediate
Hypochoeris radicata (F)	Common Cat‟s Ear	24	Intermediate
Ranunculus acris (F)	Meadow buttercup	24	Intermediate
Rubus fruticosus agg (S)	Bramble	24	Intermediate
Lolium perenne (G)	Perennial Rye Grass	23	Intermediate
Veronica chamaedrys (F)	Germander Speedwell	23	Intermediate
Agrimonia eupatoria (F)	Agrimony	21	Intermediate
Arrhenatherum elatius (G)	False Oat Grass	21	Intermediate
Weissa sp (M)		21	Intermediate
Brachythecium rutabulium (M)	Common Moss	20	Intermediate
Cirsium vulgare (F)	Spear Thistle	20	Intermediate
Potentilla erecta (F)	Tormentil	20	Intermediate
Ononis repens (F)	Restharrow	19	Scarce
Dactylorhiza fuchsii (F)	Common Spotted Orchid	18	Scarce
Stachys officinalis (F)	Betony	17	Scarce
Hypnum cupressiforme (M)		15	Scarce
Origanum vulgare (F)	Marjoram	15	Scarce
Plagiomnium undulatum (M)		15	Scarce
Polygala calcarea(F)	Chalk Milkwort	15	Scarce
Hypericum perforatum (F)	Perforated St John‟s Wort	14	Scarce
Potentilla reptans (F)	Creeping Cinquefoil	14	Scarce
Vicia cracca(F)	Tufted Vetch	14	Scarce
Arenaria serpyllifolia (F)	Thyme-leaved Sandwort	13	Scarce
Festuca pratensis (G)	Meadow Fescue	13	Scarce
Rhytidiadelphus triquetus (M)		13	Scarce
Centaurea scabiosa (F)	Greater Knapweed	12	Scarce
Dicranum scoparium (M)		12	Scarce
Eurhynchium striatum (M)		10	Scarce
Necera crispa (M)		10	Scarce
Gymnadenia conopsea (F)	Fragrant Orchid	9	Scarce
Heracleum sphondylium (F)	Hogweed	9	Scarce
Knautia arvensis (F)	Field Scabious	9	Scarce
Tragopogon pratensis (F)	Goats Beard	9	Scarce
Anacamptis pyramidalis (F)	Pyramidal Orchid	8	Scarce
Bryophytes (M)		8	Scarce

Campanula glomerata (F)	Clustered Bellflower	8	Scarce
Cirsium arvense (F)	Creeping Thistle	8	Scarce
Deschampsia caespitosa (G)	Tufted Hair-grass	8	Scarce
Senecio erucifolius (F)	Hoary Ragwort	8	Scarce
Thymus pulegioides (F)	Large Thyme	8	Scarce
Veronica officinalis (F)	Heath Speedwell	8	Scarce
Bryum capillare (M)		7	Scarce
Cladonia rangiformis (L)		7	Scarce
Pastinaca sativa (F)	Wild Parsnip	7	Scarce
Plagiomnium affine (M)		7	Scarce
Ranunculus repens (F)	Creeping Buttercup	7	Scarce
Teucrium scorodonia (F)	Wood Sage	7	Scarce
Ulex europaeus (S)	Common Gorse	7	Scarce
Centaurium puchellum (F)	Lesser Centaury	6	Scarce
Fissidens cristatus (M)		6	Scarce
Rosa seedlings (S)	Wild Roses	6	Scarce
Sonchus asper (F)	Prickly Sow Thistle	6	Scarce
Thesium humifusum (F)	Bastard Toadflax	6	Scarce
Cirsium palustre (F)	Marsh Thistle	5	Rare
Glechoma hederacea (F)	Ground Ivy	5	Rare
Senecio intergrifolius (F)	Field Fleawort	5	Rare
Spiranthes spiralis (F)	Autumn Ladies-tresses	5	Rare
Trifolium dubium (F)	Lesser Trefoil	5	Rare
Bromus hordaeceus (G)	Soft Brome	4	Rare
Calluna vulgaris (F)	Ling	4	Rare
Clematis vitalba (S)	Travellers Joy	4	Rare
Coeloglossum viride (F)	Frog Orchid	4	Rare
Cruciata laevipes (F)	Crosswort	4	Rare
Erica cinerea (F)	Bell Heather	4	Rare
Eupatorium cannabinum (F)	Hemp Agrimony	4	Rare
Euhynchium praelongum (M)		4	Rare
Fragaria vesca (F)	Wild Strawberry	4	Rare
Geranium molle (F)	Dove‟s foot Cranesbill	4	Rare
Listera ovata (F)	Common Twayblade	4	Rare
Veronica arvensis (F)	Wall Speedwell	4	Rare
Arabis hirsuta (F)	Hairy Rock-cress	3	Rare
Cladonia fucata (L)		3	Rare
Cladonia squamules (L)		3	Rare
Convolvulus arvensis (F)	Field Bindweed	3	Rare
Cornus sanguinea (S)	Dogwood	3	Rare
Fissidens taxifolius(M)		3	Rare
Galium saxatile (F)	Heath Bedstraw	3	Rare
Hypericum hirsuthm (F)	Hairy St John‟s Wort	3	Rare
Hypericum pulchrum (F)	Slender St John‟s Wort	3	Rare
Lathyrus pratensis (F)	Meadow Vetchling	3	Rare
Plantago coronopus (F)	Buck‟s Horn Plantain	3	Rare
Rumex acetosella (F)	Sheep‟s Sorrel	3	Rare
Sedum acre (F)	Biting Stonecrop	3	Rare

Taraxacum sect erythroserma (F)	Lesser Dandelion	3	Rare
Valeriana officinalis (F)	Common Valerian	3	Rare
Ajuga repans (F)	Bugle	2	Rare
Cladonia pyxidata(L)		2	Rare
Filipendula ulmaria (F)	Meadowsweet	2	Rare
Hieracium vulgatum (F)	Hawkweed	2	Rare
Hyloomium spendens (M)		2	Rare
Inula conyza (F)	Ploughman‟s-spikenard	2	Rare
Onobrychis viciifolia (F)	Sainfoin	2	Rare
Orchis mascula (F)	Early Purple Orchid	2	Rare
Rhamnus catharticus (F)	Buckthorn	2	Rare
Thuidium tamariscum (M)		2	Rare
Viola reichenbachiana (F)	Early Dog Violet	2	Rare
Aquilegia vulgaris (F)	Columbine	1	Rare
Brachythecium rivulare (M)		1	Rare
Bryum pseudotriquetrum (M)		1	Rare
Campanula trachelium (F)	Nettle-leaved Bellflower	1	Rare
Campylium stellatum (M)		1	Rare
Campylium chrysophyllum (M)		1	Rare
Capsella bursa-pastoris (F)	Shepherd‟s Purse	1	Rare
Cerastium semidecandrum (F)	Little Mouse Ear	1	Rare
Cerastium arvense (F)	Field Mouse Ear	1	Rare
Cladonia impexa (L)		1	Rare
Cladonia verticillata (L)		1	Rare
Cynoglossom officinale (F)	Hound‟s Tongue	1	Rare
Desmazeria rigida (M)		1	Rare
Dicranium bonjeani (M)		1	Rare
Dipsacus fullonum	Teasel	1	Rare
Echium vulgare (F)	Vipers- bugloss	1	Rare
Elymus repens (G)	Common Couch	1	Rare
Elymus pycnathus(G)	Sea Couch	1	Rare
Epilobium parviflorum (F)	Hoary Willowherb	1	Rare
Equisetum arvense (M)		1	Rare
Erodium cicutarium (F)	Common Stork‟s-bill	1	Rare
Geranium dissectum (F)	Cut-leaved Crane‟s-bill	1	Rare
Galeopsis tetrahit (F)	Common Hemp-nettle	1	Rare
Galium uliginosum (F)	Fen Bedstraw	1	Rare
Hieracium accuminatum (F)	Hawkweed	1	Rare
Hypericum humifusum (F)	Trailing St John‟s Wort	1	Rare
Linaria vulgaris (F)	Common Toadflax	1	Rare
Melilotus officinalis (F)	Ribbed Meliot	1	Rare
Mentha aquatica (F)	Water Mint	1	Rare
Odonitites verna (F)	Red Bartsia	1	Rare
Porella arbores-vitae (M)		1	Rare
Parapholis strigosa(M)		1	Rare

Peltigera collina (L)		1	Rare
Potentilla sterilis (F)	Barren Strawberry	1	Rare
Potentilla tabernaemontani (F)	Spring Cinquefoil	1	Rare
Rapistrum rogosum (F)	Bastard Cabbage	1	Rare
Resedea lutea (F)	Wild Mignonette	1	Rare
Reseda luteola (F)	Weld	1	Rare
Rosa pimpinellifolia (F)	Burnet Rose	1	Rare
Salvia verbenaca (F)	Wild Clary	1	Rare
Scrophularia nodosa (F)	Common Figwort	1	Rare
Sherardia arvensis (F)	Field Madder	1	Rare
Serratula tinctoria (F)	Saw-Wort	1	Rare
Sonchus oleraceus (F)	Smooth Sow Thistle	1	Rare
Spergularia marina (F)	Lesser Sea Spray	1	Rare
Stellaria graminae (F)	Lesser Stichwort	1	Rare
Stellaria media (F)	Common Chickweed	1	Rare
Torilis japonica (F)	Upright Hedge Parsley	1	Rare
Tussilago farfara (F)	Colts-foot	1	Rare
Veronica filiformis (F)	Slender Speedwell	1	Rare
Vicia sepium (F)	Bush Vetch	1	Rare

Inspection of Table 4.1 shows that there were 226 plant species in the species pool. Of these 154(68%) were forbs, 28 (13%) were grasses or sedges, 38 (17%) were mosses or lichens and 6 (2%) were shrubs.

4.4.3.2 Strong calcicoles.

In Table 4.2 the 32 strong calcicole species from within the species pool (Table 4.1) are identified.

Table 4.2 - The species within the species pool identified as strong calcicoles

(Strong Calcicoles; Ellenberg Indicator values. F (Moisture) ≤ 4, R (pH) ≥ 7, N (Nitrogen) ≤ 4.)

Key. **Column 1** gives the Scientific name of the plant. *(F)* – Forb, *(G)* – Grass or Sedge, *(M)* – Moss, *(L)* – Lichen, and *(S)* – Shrub.
Column 2 gives the Common name.
Column 3 gives the percentage frequency of occurrence of the species over all sites surveyed.
Column 4 shows the frequency category into which the species was placed.

Latin Name	Common Name	% of sites at which present	Class Category
Cirsium acaule (F)	Dwarf Thistle	97	Core
Sanguisorba minor (F)	Salad Burnet	95	Core
Pimpinella saxifraga (F)	Burnet-saxifrage	91	Core
Scabiosa columbaria (F)	Small Scabious	89	Core
Bromus erectus (G)	Upright Brome	86	Core
Viola hirta (F)	Hairy Violet	85	Core
Ranunculus bulbosus (F)	Bulbous Buttercup	78	Core
Carex caryophyllea (G)	Spring Sedge	77	Core
Koeleria macrantha (G)	Crested Hair Grass	76	Core
Plantago media (F)	Hoary Plantain	73	Core
Galium mollugo (F)	Hedge Bedstraw	71	Core
Asperula cynanchica (F)	Squinancywort	70	Intermediate
Phyteuma orbiculare (F)	Round Headed Rampion	63	Intermediate
Filipendula vulgaris (F)	Dropwort	62	Intermediate
Primula veris (F)	Cowslip	57	Intermediate
Hippocrepis comosa (F)	Horseshoe Vetch	52	Intermediate
Anthyllis vulneraria (F)	Kidney vetch	39	Intermediate
Daucus carota (F)	Wild Carrot	36	Intermediate
Carlina vulgaris (F)	Carline Thistle	34	Intermediate
Brachypodium pinnatum (G)	Tor Grass	32	Intermediate
Helianthenum nummularium (F)	Rockrose	32	Intermediate
Picris hieracioides (F)	Hawkweed Oxtongue	32	Intermediate
Polygala calcarea (F)	Chalk Milkwort	15	Scarce
Centaurea scabiosa (F)	Greater knapweed	12	Scarce
Anacamptis pyramidalis (F)	Pyramidal Orchid	8	Scarce

Campanula glomerata (F)	Clustered Bellflower	8	Scarce
Thymus pulegioides (F)	Large Thyme	8	Scarce
Thesium humifusum (F)	Bastard Toadflax	6	Scarce
Sedum acre (F)	Biting Stonecrop	3	Rare
Inula conyza (F)	Ploughman"s-spikenard	2	Rare
Onobrychis viciifolia (F)	Sainfoin	2	Rare
Rosa pimpinellifo1ia (F)	Burnet Rose	1	Rare

Inspection of Table 4.2 shows that there are 32 species within the functional group strong calcicoles, of which 28 (88%) were forbs, 4 (12%) were grasses or sedges, with 14% of the species pool being strong calcicoles. No data were found as to whether there were mosses or lichens that were strong calcicoles.

4.4.3.3 Newmarket Hill

The species found by Steven and Muggeridge (1992) at Newmarket Hill in their 1991 survey are shown in Table 4.3.

Table 4.3 - The species present at Newmarket Hill in 1991 (Steven and Muggeridge, 1992)

Key. **Column 1** gives the Scientific name of the plant. *(F)* – Forb, *(G)* – Grass or Sedge, *(M)* – Moss, *(L)* – Lichen, and *(S)* – Shrub.
Column 2 gives the Common name.
Column 3 gives the percentage frequency of occurrence of the species over all sites surveyed.
Column 4 shows the frequency category into which the species was placed.
[C] – strong calcicoles.

Latin Name	Common Name	% of sites at which presen	Class Category
Dactylis glomerata (G)	Cocksfoot	100	Core
Festuca ovina (G)	Sheep"s Fescue	100	Core
Lotus corniculatus (F)	Birds – Foot –Trefoil	100	Core
Plantago lanceolata (F)	Ribwort Plantain	100	Core
Carex flacca (G)	Glaucous Sedge	99	Core
Cirsium acaule (F)	Dwarf Thistle	97	Core [C]
Sanguisorba minor (F)	Salad Burnet	95	Core [C]
Trifolium pratense (F)	Red Clover	95	Core
Leontodon hispidus (F)	Rough Hawkbit	94	Core
Avenula pratensis (G)	Meadow Oat -Grass	92	Core
Pimpinella saxifraga (F)	Burnet-saxifrage	91	Core [C]
Achillea millefolium (F)	Yarrow	90	Core
Linum catharticum (F)	Fairy Flax	90	Core
Centaurea nigra (F)	Black Knapweed	89	Core
Scabiosa columbaria (F)	Small Scabious	89	Core [C]

Pseudoscleropodium purum (M)	Common Moss (chalklands)	88	Core
Thymus praecox (F)	Thyme	88	Core
Bromus erectus (G)	Upright Brome	86	Core [C]
Prunella vulgaris (F)	Selfheal	86	Core
Viola hirta (F)	Hairy Violet	85	Core [C]
Briza media (G)	Quaking Grass	83	Core
Ranunculus bulbosus (F)	Bulbous Buttercup	78	Core [C]
Hieracium pilosella group (F)	Hawkweeds	78	Core
Agrostis stolonifera (F)	Creeping Bent	77	Core
Medicago lupulina (F)	Black Medick	77	Core
Carex caryophyllea (G)	Spring Sedge	77	Core [C]
Galium verum (F)	Ladies Bedstraw	76	Core
Koeleria macrantha (G)	Crested Hair Grass	76	Core [C]
Senecio jacobaea (F)	Common Ragwort	76	Core
Plantago media(F)	Hoary Plantain	73	Core [C]
Galium mollugo (F)	Hedge Bedstraw	71	Core [C]
Asperula cynanchica (F)	Squinancywort	70	Intermediate [C]
Polygala vulgaris (F)	Common Milkwort	67	Intermediate
Avenula pubescens (G)	Downey Oat Grass	66	Intermediate
Succisa pratensis (F)	Devil's Bit Scabious	66	Intermediate
Phyteuma orbiculare (F)	Round Headed Rampion	63	Intermediate [C]
Cerastium fontanum (F)	Common Mouse- ear	62	Intermediate
Filipendula vulgaris (F)	Dropwort	62	Intermediate [C]
Primula veris (F)	Cowslip	57	Intermediate [C]
Euphrasia officinalis (F)	Eyebright	53	Intermediate
Gentianella amarella (F)	Autumn Gentian	52	Intermediate
Hippocrepis comosa(F)	Horseshoe Vetch	52	Intermediate [C]
Bellis perennis (F)	Daisy	49	Intermediate
Calliergon cuspidatum (M)		47	Intermediate
Leucanthemum vulgare (F)	Oxeye Daisy	46	Intermediate
Homalothecium lutescens (M)		45	Intermediate
Campanula rotundifolia (F)	Harebell	43	Intermediate
Fissendens sp (M)		43	Intermediate
Crataegus monogyna (S)	Hawthorn (seedlings)	40	Intermediate
Anthyllis vulneraria (F)	Kidney vetch	39	Intermediate [C]
Leontodon taraxacoides(F)	Lesser Hawkbit	38	Intermediate
Blackstonia perfoliata (F)	Yellow Wort	35	Intermediate
Carlina vulgaris (F)	Carline Thistle	34	Intermediate [C]
Brachypodium pinnatum (G)	Tor Grass	32	Intermediate [C]
Picris hieracioides (F)	Hawkweed Oxtongue	32	Intermediate [C]
Centaurium erythraea (F)	Common Centaury	30	Intermediate
Rhinanthus minor (F)	Yellow Rattle	30	Intermediate
Viola riviniana (F)	Common Dog Violet	29	Intermediate
Ctenidium molluscum (M)		26	Intermediate
Clinopodium vulgare (F)	Wild Basil	24	Intermediate
Ranunculus acris (F)	Meadow buttercup	24	Intermediate
Weissa sp (M)		21	Intermediate

Stachys officinalis (F)	Betony	17	Scarce
Hypnum cupressiforme (M)		15	Scarce
Plagiomnium undulatum (M)		15	Scarce
Polygala calcarea (F)	Chalk Milkwort	15	Scarce [C]
Hypericum perforatum (F)	Perforated St John‟s Wort	14	Scarce
Necera crispa (M)		10	Scarce
Gymnadenia conopsea (F)	Fragrant Orchid	9	Scarce
Bryum capillare (M)		7	Scarce
Cladonia rangiformis (L)		7	Scarce
Centaurium puchellum (F)	Lesser Centaury	6	Scarce
Senecio integrifolius (F)	Field Fleawort	5	Rare
Orchis mascula (F)	Early Purple Orchid	2	Rare
Viola reichenbachiana (F)	Early Dog Violet	2	Rare

Inspection of Table 4.3 shows that in 1991 the survey by Steven and Muggeridge, (1992) identified 75 plant species, of which 21 belonged to the functional group strong calcicoles. Fifty three (71%) were forbs, 10 (13%) were grasses or sedges, 11 (15%) were mosses or lichens and 1 (1%) was a shrub.

4.4.4 Discussion

The regional species pool consisted of 226 species of which 154 (68%) were forbs, 28 (13%) were grasses and 38 (17%) were mosses or lichens. No direct comparison with earlier surveys could be made, however data was available in tabular form from a paper by Tansley and Adamson (1926). In this paper they report on survey data from chalk grassland on the South Downs, mainly in Sussex. In this survey carried out in 1921 and 1923, Tansley and Adamson (1926) visited 41 sites and recorded a total of 151 different plant species. Of these 108 (71%) were forbs, 18 (12%) were grasses or sedges and 25 (17%) were mosses or lichens. Despite a time interval of about 70 years the percentage presence of forbs, grasses and sedges and mosses and lichens in the surveys by Tansley and Adamson 1926) and Steven and Muggeridge (1992) and Steven (1992) are remarkably similar. The total number of species 151 recorded by Tansley and Adamson (1926) is lower than the 226 recorded by Steven and Muggeridge (1992) and Steven (1992) and probable reflects the number of sites visited, 41 compared to 136. The data also shows that despite the fragmentation of chalk grassland between 1921 and 1991 the proportion of forbs, grasses and sedges and mosses and lichen has been maintained. However the abundance of individual species may have changed. Comparing the frequency of occurrence of two dominant grasses *Bromus erectus* and *Brachypodium*

pinnatum and the forb *Senecio jacobaea* in the 1921 survey by Tansley and Adamson (1926) and the 1991 survey of Steven and Muggeridge, (1992) and Steven, (1992) all three have increased in frequency of occurrence. *B. erectus* has increased in frequency of presence from 62% to 86% of sites and *B. pinnatum* from 20% to 32% of sites, the whilst the presence of *S. jacobaea* has more than doubled from 37% to 76% of sites. The increase in the presence of *S. jacobaea* can at least be partially explained by it being unpalatable to grazers (Bossuyt *et al*. 2005)

The number of strong calcicoles (32) is comparative small compared to the total species pool (226). This may result from conditions at chalk grassland sites favouring other species, for example lower pH, wetter soils and deeper more nutrient rich soils. Some generalist species may be adapted to conditions favoured by calcicoles. In addition many scarce and rare species recorded may be marginal species in chalk grassland habitat but be more abundant (Pärtel *et al*. 2001) or dominants (Grime, 1998) in other habitats.

The area of the site at Newmarket Hill (17ha) is lower than the mean value for the 136 sites surveyed of 25ha; this and the high number of species recorded as present (76) compared to the mean site value of 56, identifies it as a site of high quality as does the high number, 21 (27%) of calcicoles present (Pärtel *et al*. 2004). The ratios of forbs (71%) to grasses and sedges (13%) to mosses and lichens (15%), is very close to the values for the species pool as a whole with a slightly increased value for forbs. Thus the characteristics of the chalk grassland present at Newmarket Hill are typical of species rich semi-natural chalk grassland.

4.5 Study 2 Analyses of Frequency Classes

4.5.1 Introduction

One method that can be used to describe the structure of a plant community is to analyze frequency classes, that is the frequency (as a percentage occurrence) of a species in for example the total number of sites or total number of quadrats surveyed at a site. These frequency data can then be grouped into different categories for descriptive or analytical purposes. Different researchers have taken slightly different approaches. The concept of core (common) and satellite (less common) has been used to describe community structure (Hanski, 1982; Collins and Glenn, 1997a; Pärtel *et al*. 2001) and the terms

dominant and subordinate have been used in respect of salt marsh communities by Olff and Bakker (1998), while the terms dominants, subordinates and transients have been used by Grime (1998) to describe the components of plant communities. However as described in Partel *et al.* (2001) the terms matrix, common species, fugitive, interstitial, redundant, scarce and rare have also been used.

In this analysis the terms core, intermediate, scarce and rare were used, although for statistical analysis scarce and rare were treated as one group. The divisions between frequency classes are arbitrary and different researchers have set different boundaries. Partel *et al*. (2001) defined core species as occurring at more than 75% of stands (sites) and satellite species at less than 25% of sites, while Collins and Glenn (1997a) defined core as occurring at 90% of sites and satellites at less than 10%.

4.5.2 Method: Assigning species to frequency categories

In this study having examined the percentage frequency of occurrence of the 226 species making up the regional species pool (Table 4.1), the boundaries were set as follows: core occurring at 71 to 100% of sites, intermediate at 21 to 70%, scarce at 6 to 20% and rare at 1 to 5% of sites. These categories and ranges are summarised in Table 4.4.

Table 4.4 - Frequency classes categories and ranges

Name of Range	Frequency (%)
Core	71 - 100
Intermediate	21 - 70
Scarce	6 – 20
Rare	1 - 5

4.5.3 Results

4.5.3.1 Assigning the species pool to frequency categories

Analysis of Table 4.1 shows that for the complete species pool there are 33 core species, 55 intermediate species, 44 scarce species and 94 rare species. These are summarised in terms of the percentage of species in each category in Table 4.5.

Table 4.5 - Percentage of species in each frequency class

Frequency Class	Percentage of Total
Core	15
Intermediate	24
Scarce	20
Rare	41

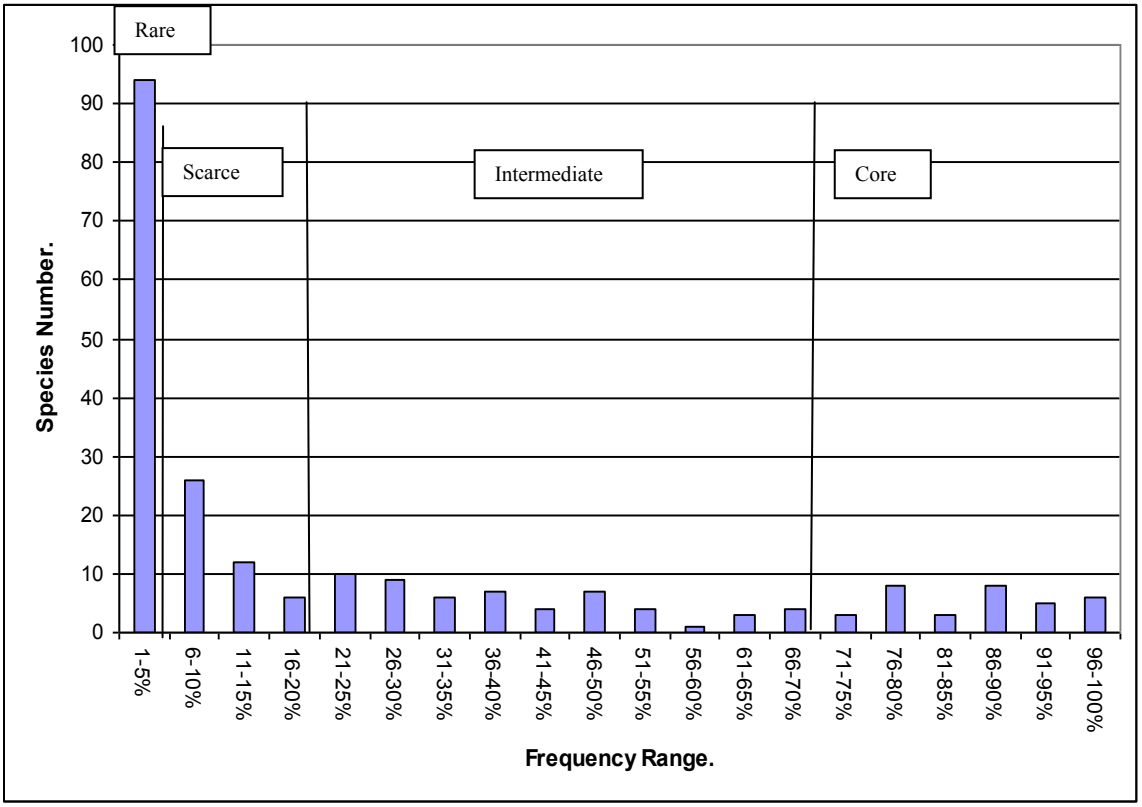


Figure 4.1 - The number of species observed in each frequency class for the 136 chalk grassland sites surveyed in Sussex

Figure 4.1 represents the frequency of each species across the total number of sites surveyed. Over 90 species from a total of 226 were recorded at less than 5% of sites and these species may be considered rare. The great majority of species occur at less than 50% of sites, with only 44 species being found at greater than 50% of sites.

4.5.3.2 The distribution of plant functional types within frequency categories for the species pool

The categorization of the plant species detailed in Table 4.1 into the functional groups forbs, grasses and sedges, mosses and lichen, and shrubs allows their distribution within the frequency classes to be ascertained. The results are shown in Table 4.6.

Table 4.6 - Number (and %) of species in functional groups present in the categories core, intermediate, scarce and rare

	Core	Intermediate	Scarce	Rare	Total
Forbs	22 (67%)	34 (62%)	28 (64%)	70 (74%)	**154 (68%)**
Grasses and Sedges	10 (30%)	13 (24%)	2 (5%)	3 (3%)	**28 (12%)**
Mosses and Lichens	1 (3%)	6 (11%)	12 (27%)	19 (20%)	**38 (17%)**
Shrubs	0 (0%)	2 (4%)	2 (4%)	2 (2%)	**6 (3%)**
Total	**33**	**55**	**44**	**94**	**226**

Table 4.6 shows that forbs were the major contributor to species richness at the site scale. Examination of the percentage presence in the frequency classes core, intermediate, scarce and rare shows that forbs were fairly evenly distributed between the four classes contributing between 62 to 74% of the total for each frequency class. Grasses and sedges had their highest presence (30%) in the core category where their presence was a major contributor to community structure. The presence of grasses and sedges declined through the categories intermediate, scarce and rare. Mosses and lichens showed a reverse trend to grasses and sedges having their maximum presence in the scarce (27%) and rare (20%) categories and a low presence in the core (3%) category.

4.5.3.3 Assigning the functional group strong calcicoles to frequency categories

Inspection of Table 4.2 shows that for strong calcicoles there were 11 core, 11 intermediate, 6 scarce and 4 rare species present. These are shown as percentages in Table 4.7.

Table 4.7 - Percentage of species in each frequency class for strong calcicoles

Frequency Class	Percentage of Total.
Core	34
Intermediate	34
Scarce	19
Rare	13

The results for strong calcicoles (Table 4.7) differed from those for the overall species pool. The percentage of rare species was much lower than that for the species pool (Table 4.5), with a resulting increase in the percentage of core and intermediate species present. The probable explanation is that the rare calcicoles were likely to be true members of the chalk grassland communities; where as many of the rare species in the species pool were likely to be vagrants from other habitats, for example. some were agricultural weeds.

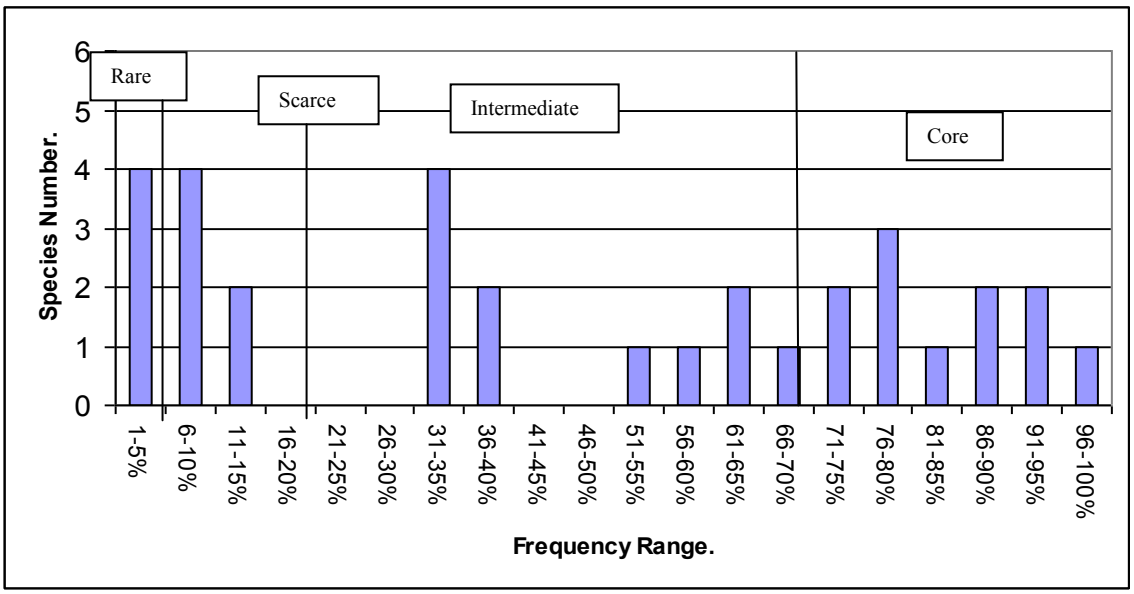


Figure 4.2 - The number of species in each frequency band for the functional group strong calcicoles

It can be seen from Figure 4.2 that the functional group strong calcicoles was spread throughout the frequency range with all four frequency classes represented. In the species pool the number of species in the categories rare and scarce was much higher than those present in the categories intermediate and core (Figure 4.1). In the functional group strong calcicoles the species in each frequency class were more even, with a maximum of 4 species in any one frequency range (Figure 4.2).

4.5.3.4 The distribution of plant functional types within frequency categories for strong calcicoles

The distribution of plant functional types within categories for strong calcicoles is shown in Table 4.8.

Table 4.8 - Number (and %) of species present in the categories core, intermediate, scarce and rare for the functional group strong calcicoles

	Core	Intermediate	Scarce	Rare	Total
Forbs	8 (73%)	10 (92%)	6 (100%)	4 (100%)	**28 (88%)**
Grasses and Sedges	3 (27%)	1 (8%)	0 (0%)	0 (0%)	**4 (12%)**
Total	**11**	**11**	**6**	**4**	**32**

Direct comparison with the results from the species pool can not be made as no mosses, lichens and shrubs were identified as strong calcicoles and the sample size was small. However the general trend was that forbs provide most species in all categories and that grasses were more prevalent in the core category.

4.5.3.5 Assigning the species present at Newmarket Hill to frequency categories

Inspection of Table 4.3 gives a count of 31 core species, 31 intermediate species, 10 scarce species and 3 rare species present at Newmarket Hill. These are summarised in terms of the percentage of species in each category in Table 4.9.

Table 4.9 - Percentage of species in each frequency class

Frequency Class	Percentage of Total
Core	43
Intermediate	41
Scarce	13
Rare	4

The percentage values for the frequency classes core and intermediate at Newmarket Hill (Table 4.9) were approximately equal showing a similar pattern to that for the functional group strong calcicoles (Table 4.7). Newmarket Hill is situated within a S.S.S.I. and is a

valued chalk grassland site and it can be seen in Figure 4.3 that it contains a very high number of core species, a high number of intermediate species, some scarce species and a few rare species.

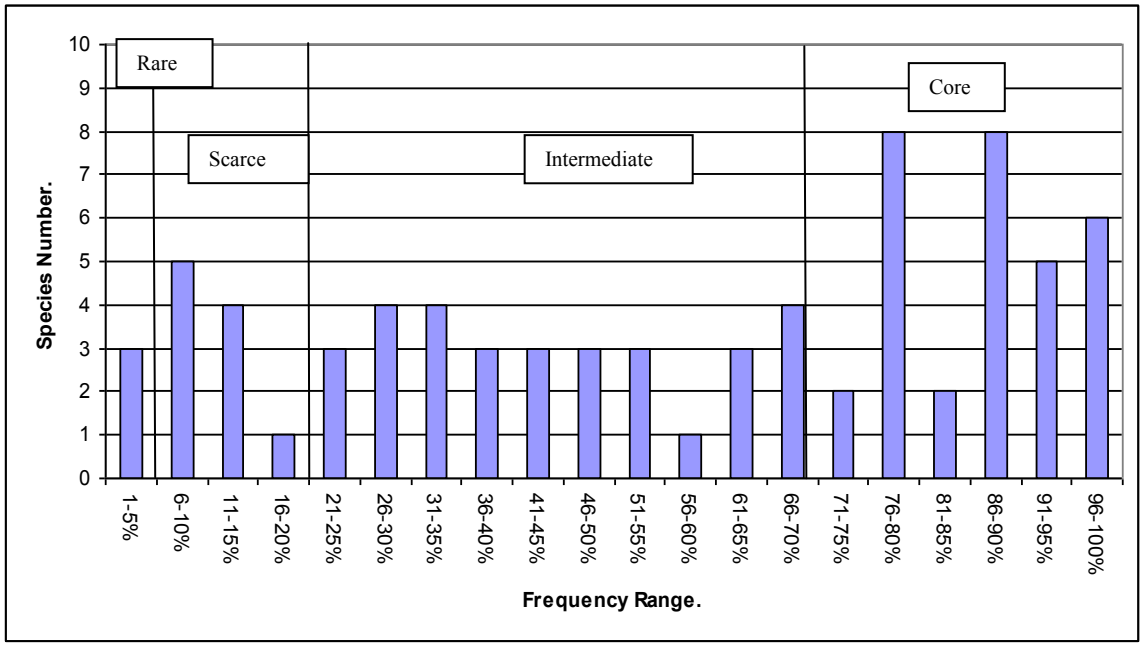


Figure 4.3 - The number of species in each frequency range at Newmarket Hill

If the species present in the frequency bands shown in Figure 4.3 for Newmarket Hill are compared with those in Figure 4.1 for the species pool, it can be seen that for the species occurring at greater than 50% of sites, there are very strong similarities. Thirty one of the 33 core species were present at Newmarket Hill and 37 of the 44 species that were present at greater than 50% of sites in the species pool were also present. This strong grouping of species within the categories core and intermediate with few of the expected species absent is indicative of nestedness (see Study 3) and of high species richness at this site. It can also be observed that the number of species in each frequency band was fairly even, which suggests that the structure of the chalk grassland community present at Newmarket Hill is not a random collection of species that have come together by chance but conforms to a pattern.

4.5.3.6 The distribution of plant functional types within frequency categories for the species present at Newmarket Hill

The distribution of plant functional types within categories for species found at Newmarket Hill is shown in Table 4.10 below.

Table 4.10 - Number (and %) of species present at Newmarket Hill in the categories core, intermediate, scarce and rare

	Core	Intermediate	Scarce	Rare	Total
Forbs	22 (71%)	23 (74)	5 (50%)	3 (100%)	**53 (71%)**
Grasses and Sedges	8 (26%)	2 (6%)	0 (0%)	0 (0%)	**10 (13%)**
Mosses and Lichens	1 (3%)	5 (16%)	5 (50%)	0 (0%)	**11 (15%)**
Shrubs	0 (0%)	1 (3%)	0 (0%)	0 (0%)	**1 (1%)**
Total	**31**	**31**	**10**	**3**	**75**

Examination of the data in Table 4.10 shows it to be consistent with the percentages in the species pool. Forbs were the most frequent members of the community with about 70% presence. The levels for grasses and mosses and lichens were similar at ~10% with grasses being more prevalent in the core category and declining in the other categories, with mosses and lichens having their highest percentage presence in the scarce category.

4.5.3.7 The relationship between frequency classes

In the first part of Study 2 the analysis has considered the distribution of species within frequency classes and the division of plant functional types within the categories core, intermediate, scarce and rare. This analysis was at the landscape scale for the regional species pool and the functional group strong calcicoles and at the site scale for Newmarket Hill. However the quadrat ($1m^2$) scale may also be important as it is at this small scale that above and below ground interactions take place. In the next section of this study the relationship between frequency classes is explored at both the quadrat ($1m^2$) and site scale.

4.5.3.7.1 Quadrat scale

To analyze frequency class data at the quadrat level, 15 quadrats were selected from the survey data for East Sussex (Steven and Muggeridge, 1992). Each quadrat was from a different community sub–group (Rodwell 1991): CG1e, CG2a, CG2a (Mossy), CG2a (Acid), CG2b, CG2c, CG3a, CG3a, CG3b, CG3c, CG4a, CG4b, CG4c, CG2a/CG2d and CG2a/MG5b. The quadrats ranged in species richness from low, 5 species per quadrat to very high 40 species per quadrat with a mean of 22. Data from sample quadrats were

placed into frequency classes‟ core, intermediate and scarce and subjected to regression analysis. For this analysis the species present in scarce and rare categories were combined under the category scarce.

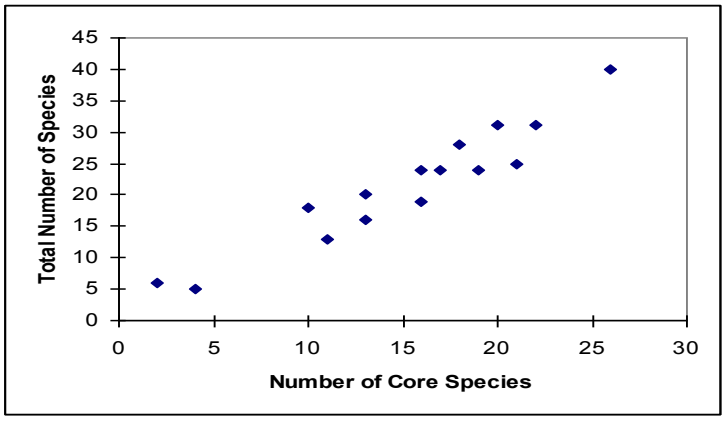


Figure 4.4 - Relationship between total number of species in a quadrat and number of core species in a quadrat. ($r^2 = 0.91$, $p < 0.01$)

It can be seen in Figure 4.4 that the the number of species in a quadrat was strongly related to the number of core species present ($r^2 = 0.91$, $p < 0.01$).

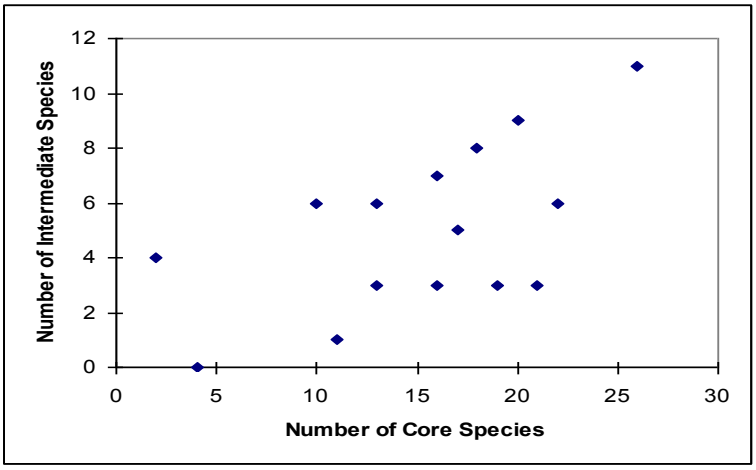


Figure 4.5 - Relationship between number of intermediate species in a quadrat and number of core species in a quadrat. ($r^2 = 0.35$, $p = 0.02$)

The number of intermediate species was also related to the number of core species present (Figure 4.5) ($r^2 = 0.35$, $p = 0.02$).

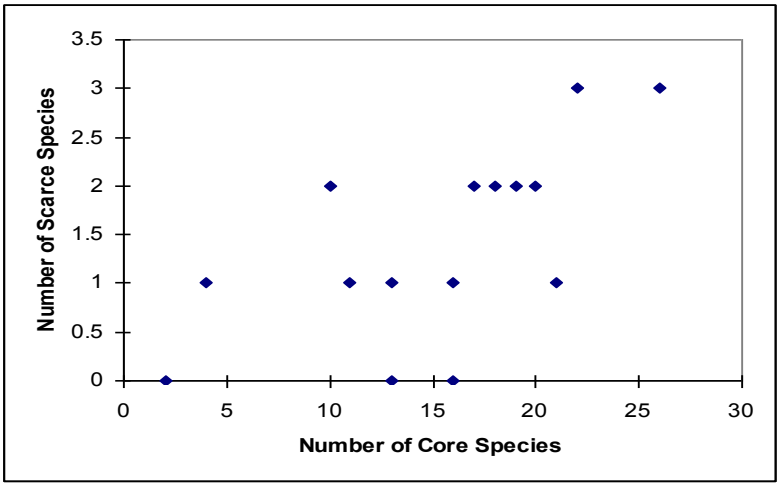


Figure 4.6 - Relationship between scarce species in a quadrat and number of core species in a quadrat. ($r^2 = 0.41$, $p = 0.01$)

In Figures 4.6 and 4.7 it can be observed that plotting core species against the number of scarce species and intermediate species against scarce species both showed strong relationships ($r^2 = 0.41$, $p = 0.01$) and ($r^2 = 0.32$, $p = 0.02$).

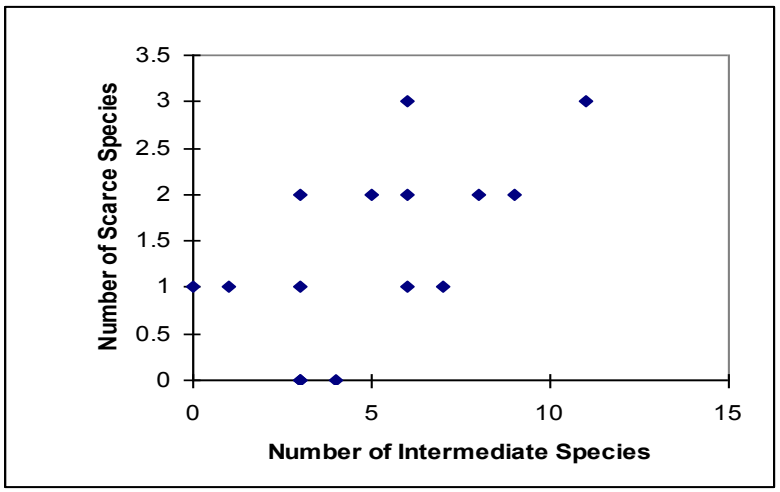


Figure 4.7 - Relationship between the number of scarce species in a quadrat and the number of intermediate species in a quadrat. ($r^2 = 0.32$, $p = 0.02$)

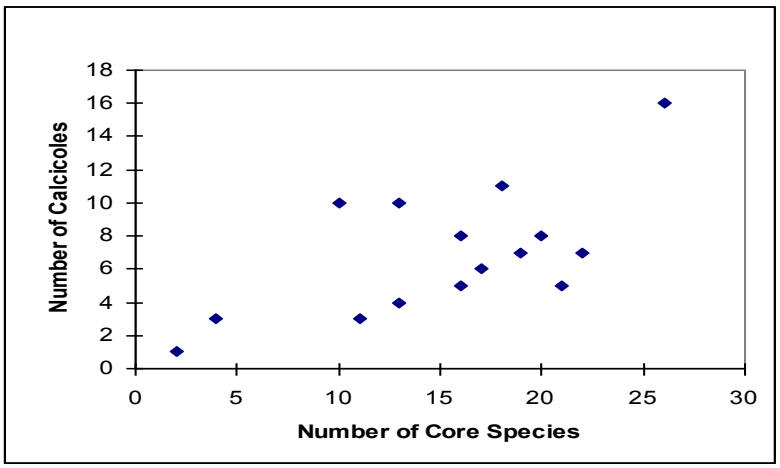


Figure 4.8 - Relationship between number of strong calcicoles in a quadrat and the number of core species in a quadrat. ($r^2 = 0.37$, $p < 0.01$)

It can be seen in Figure 4.8 that the number of strong calcicoles in a quadrat is related to the number of core species in the quadrat ($r^2 = 0.37$, $p < 0.01$).

If the data shown in Figures 4.4, 4.5, 4.6 and 4.7 are considered as a whole it can be deduced that the presence of core species is the key to species richness within the quadrat, that is the species richness of the local community. Without a substantial number of core species present there are few intermediate species present and an even smaller number of scarce species. Thus the presence of individual species in quadrats is not a random process and follows a predictable pattern.

4.5.3.7.2 Site scale

The relationships between frequency classes at the site level were explored by taking data from all of the 136 chalk grassland sites in Sussex surveyed by Steven (1992) and Steven and Muggeridge (1992) and subjecting it to regression analyses. For each site the number of core species was plotted against the total species, the number of intermediate species and the scarce species present and intermediate species against scarce species. The results are shown in Figures 4.9, 4.10 and 4.11 and 4.12.

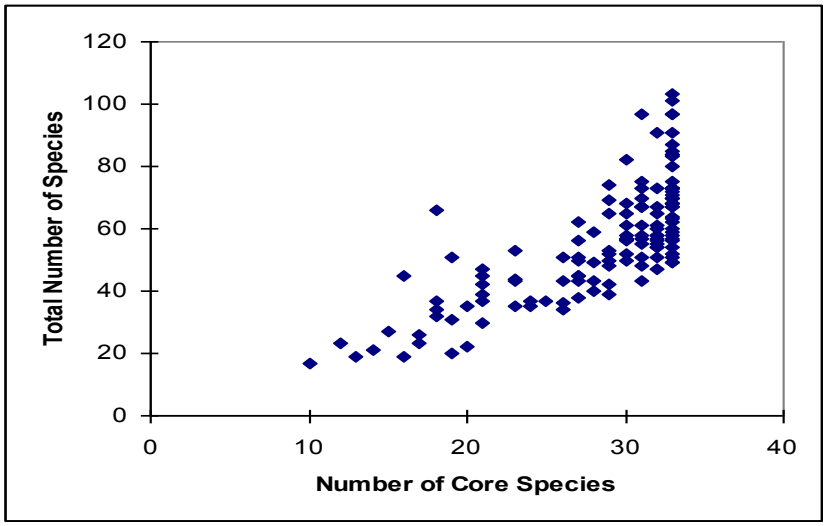


Figure 4.9 - The relationship between the total number of species at a site and number of core species at a site ($r^2 = 0.57$, $p < 0.01$)

It can be seen in Figure 4.9 that there is a strong relationship between the number of core species at a site and the total number of species at a site ($r^2 = 0.57$, $p < 0.01$).

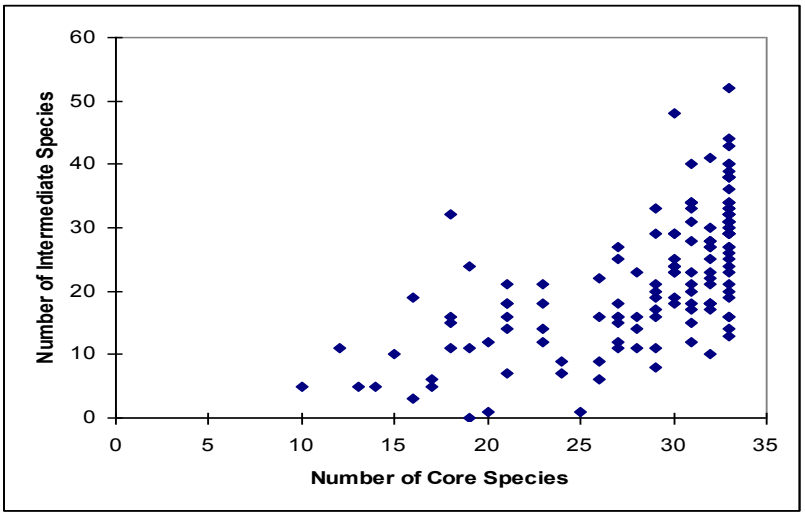


Figure 4.10 - The relationship between the number of intermediate species at a site and number of core species at a site ($r^2 = 0.39$, $p < 0.01$)

It can be seen in Figure 4.10 that there was a strong relationship between the number of core species at a site and the number of intermediate species at a site ($r^2 = 0.39$, $p < 0.01$).

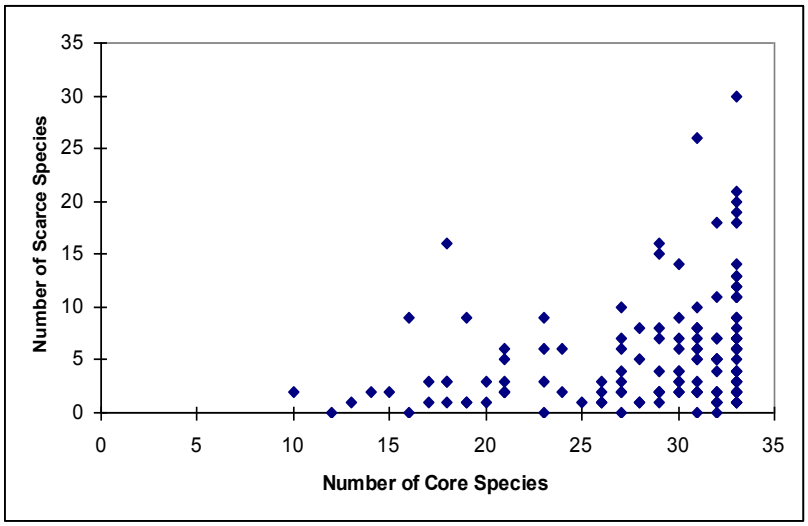


Figure 4.11 - Relationship between the number of scarce species at a site and the number of core species at a site ($r^2 = 0.08$, $p < 0.01$)

It can be observed in Figure 4.11 that the relationship between the number of core species at a site and the number of scarce species is quite weak ($r^2 = 0.08$, $p < 0.01$)

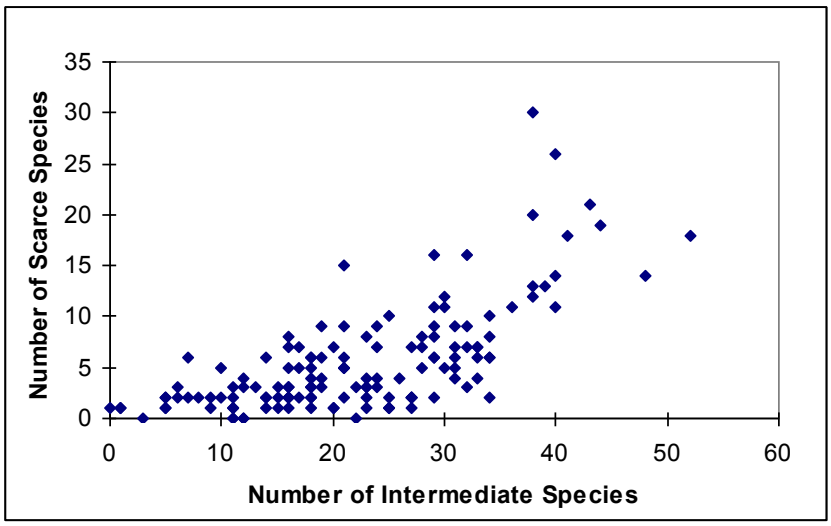


Figure 4.12 - Relationship between the number of scarce species at a site and the number of intermediate species at a site.($r^2 = 0.48$, $p < 0.01$).

It can be seen from Figure 4.12 that there was a stronger relationship between the number of intermediate species at a site and the number of scarce species at a site. The relationship is stronger than that between core species at a site and scarce species at a site (Figure 4.10). These results show that the association between scarce and core species at the quadrat scale is weaker at the site scale. However, comparatively strong site-scale

relationships were found between adjacent frequency classes, i.e. core-intermediate and intermediate-scarce.

4.5.4 Discussion

One of the first formalisation of the concept of core and satellite species being present in plant and other species communities was by Hanski (1982) in which a number examples of the U-shaped bi-modal distribution of core and satellite species is discussed. However as early as 1926 Tansley and Adamson (1926) were allocating chalk grassland species in Sussex to constancy bands based upon the frequency of occurrence at sites. Tansley and Adamson (1926) recorded 25 „œore‟ species occurring at > 70% of sites which compares quite closely with the 33 core species in the current study and the 34 recorded by Pärtel *et al*. (2001) in a study of 16 stands of Alvar calcareous grasslands in Estonia. Although the sample is quite small the data suggest that the number of dominant core species in grassland in a community is limited.

There is much discussion in the literature as to whether the relationship between core and satellite species is bimodal. There is evidence that both a bimodal and unimodal relationships can occur. In a study of four species types present in Prairie grassland in Kansas USA Collins and Glenn (1997b) found that the distribution of grasshoppers and small mammals was bimodal whereas the distribution of plants and birds was unimodal. In the current research the relationship between core and scarce Figure 4.1 was unimodal whereas as the results of Pärtel et al. (2004) for Alvar grassland was bimodal. Collins and Glenn (1997b) emphasise that scale will be important in determining the relationship between core and satellite species. Collins and Glenn (1997b) make the point that as the scale increases the number of scarce satellite species increases whereas the number of core species will be constant. It follows that as the scale increases at some point core and satellite species will balance giving a bimodal distribution. An example of the effect of scale on core and satellite species can be seen by comparing the relationship between core and scarce species at the quadrat ($1m^2$) scale Figure 4.6 and the site (25ha) scale Figure 4.10. At the quadrat scale there was a strong relationship between the number of core and scarce species present ($r^2 = 0.41$) whereas at the site scale the relationship was weak ($r^2 = 0.08$). A common observation between the results from the Alvar grasslands in Estonia (Pärtel *et al*. 2001) and chalk grasslands in Sussex was the very strong

relationship between the number of core species present and the total number of species present. Thus in both habitats the presence core species was a requirement for high species richness.

In modelling plant community behaviour the emphasis has been on the relationship between core and satellite species (Hanski, 1982; Collins and Glenn, 1997b; Pärtel *et al*. 2001). However the research presented in this thesis suggests that intermediate species have an equally important role in determining plant community structure. Examination of Figures 4.5, 4.7, 4.10 and 4.12 shows that intermediate species have a strong relationship with both of the adjacent frequency bands core and scarce. In fact the analysis suggests that without the presence of intermediate species there would be few scarce or satellite species. At the1m^2 quadrat scale plants with intermediate presence will be an important group in the mechanisms of competition and facilitation between plants that determine the overall structure of the community. The idea of interacting groups from adjacent frequency bands is taken up by Grime (1998) in his paper on filter and founder effect. In the paper by Grime (1998) the classes core, intermediate and scarce are represented by the categories dominants, subordinates and transients. In Grime (1998) dominants are characterised as relative large and coarse grained in their foraging characteristics, while subordinates are of smaller stature and occupy microhabitats dictated by the structure and phenology of the dominants. Transients tend to be a diverse collection of plant species of low abundance, often being juveniles of species having a dominant or subordinate status in another habitat. The idea of scarce or satellite species being vagrants from other habitats is taken up by Pärtel *et al*. (2001) in their paper on Alvar grassland In this paper Pärtel *et al*. (2001) suggest that satellite species may be more abundant in surrounding areas of high fertility.

4.6 Study 3 Nestedness

4.6.1 Introduction

In Study 2 concerning frequency classes it was demonstrated that by allocating plant species to frequency classes based upon their frequency of occurrence both at the quadrat and site scale and exploring the relation ships between frequency classes, information regarding the structure of chalk grassland communities could be ascertained. The phenomenon of „nestedness" provides an alternative approach to studying community

structure, particularly in highly fragmented landscapes as is the case with chalk grassland communities growing on the South Downs in Sussex (Burnside *et al*. 2002; Burnside *et al*. 2003).

Nestedness occurs when species found at depauperate sites are subsets of more species-rich sites within the same habitat (Patterson and Atmar, 1986). An alternative description is that scarce or rare species occur at sites with many species (Silvertown and Wilson, 1994), but tend to be absent at sites with few species. The concept of nested subsets was first applied to island species (Patterson and Atmar, 1986), but is equally applicable to fragmented landscapes, where the individual fragments were once part of a larger area of continuous habitat.

The early interpretation of nestedness was based on linking fragment (site) area and the processes of extinction and colonization. As fragments reduce in area the most vulnerable species, i.e. those prone to extinction because of small populations and low abundance are progressively lost. Thus, where a fragmented landscape exhibits nestedness, species richness should be highest in the largest surviving fragment and decrease with site area (Wright and Reeves 1992). In practice nestedness tends to be imperfect (Fischer and Lindenmayer, 2002 and 2005), that is some species that might be expected to be present are absent.

Since the early studies of Patterson and Atmar (1986) species nestedness has been demonstrated for a wide range of habitats and communities, including desert perennials (Silvertown and Wilson, 1994), plants of improved and unimproved meadows (Myklestad and Sætersdal, 2003), alpine flora (Bruun and Moen, 2003), sedge meadows (Mathews, 2004) Alvar grasslands (Pärtel *et al.* 2001), herbaceous plants in deciduous forests (Honnay *et al.* 1999), Oak-Hazel woodland (Hansson, 1998), birds and butterflies (Fleisman *et al*. 2002), land snails (Hylander *et al*. 2005), plant communities in the Sonoran Desert (Stiles and Scheiner, 2008), plant species in refugial mires in Bulgaria (Hajek *et al*. 2009), plants of herb-rich boreal forests in Finland (Hokkanen *et al*.2009) and insect assemblages in Bahamian Island (Murakami and Hirao, 2010).

4.6.2 Method

The plant species present in fragmented sites of the same habitat could in theory be a random collections of species drawn from the regional species pool. In practice as shown in Study 2, the examination of the relationship between frequency classes, in chalk grassland there are strong patterns of association between species. As site area is reduced species with small population tend to be lost from that site through extinction. If as sites reduce in area species are lost in strict order of their frequency of occurrence over all sites, the sites would be considered to be perfectly ordered. In practice this does not occur (Fischer and Lindenmayer, 2002 and 2005).

Atmar and Patterson (1993) published a computer programme for the calculation of nestedness, which they made available for other researchers. The programme, which was Windows based was updated (Atmar and Patterson, 1995) and guidance on the interpretation of results was provided by Patterson and Atmar (2000). The basis of the programme is that it compares the presence, absence and order of species at sites and compares this to randomly generated sets of the same species. The result is a value between 0^{oC} and 100^{oC} where an analogy is drawn with entropy, 0^{oC} is perfectly ordered and 100^{oC} completely random. Thus fragmented habitats where low values are calculated are regarded as being strongly nested.

Using the temperature calculator programme to evaluate nestedness over 136 sites using the whole species pool of 226 species was beyond the scope of this research project. However performing the calculation of nestedness for the 32 species in the functional group strong calcicoles was practical and Studies 1 and 2 has shown the frequency class distribution of this sub-group to have characteristics consistent with the species pool as a whole. The calculation was performed at two scales; the site and quadrat. In calculating nestedness at the site scale 136 sites were ordered with the site with most species at the top and the least at the bottom. For each site the species were arranged in sequence starting with the most frequent on the left. The matrix produced was then in a form that the temperature programme could be run. To examine nestedness at the quadrat scale three sites were selected which had a high number (> 65) of species present, but were different in area, and had different numerical ranges of species present in the quadrats. The data from eight ($1m^2$) quadrats taken at each of the sites was used for the analysis.

4.6.3 Results

4.6.3.1 Nestedness at site level

Calculation of the „nested temperature" for the 136 chalk grassland sites in Sussex using the functional group strong calcicoles gave a temperature of 17^{oC}. The range for nested temperatures is 0^{oC} to 100^{oC} and a value of 17^{oC} indicates that the functional group strong calcicoles were strongly nested. This result suggests that the whole of the species pool was likely to be strongly nested.

Two factors which other studies (Honnay *et al*. 1999)) and ((Hylander *et al*. 2005) have identified as promoting nestedness are site heterogeneity and quality. While quality is difficult to quantify it can be argued that site environmental heterogeneity is reflected in the number of different of NVC communities present at a site and site area has already been identified as an important factor. The grass area and communities present at each chalk grassland site in Sussex were recorded by Steven and Muggeridge (1992 and Steven (1992). These data can be used to analyze how the variables area and heterogeneity affect the presence of strong calcicoles. Analyzing these data gives a single regression for the presence of strong calcicoles with log10 area as $r^2 = 0.35$, $p < 0.01$ and strong calcicoles against log10 communities a value of $r^2 = 0.41$, $p < 0.01$. If grass area and community number are combined using multivariate regression the following expression is obtained: Total Strong Calcicoles Number = 5.01 + 8.16 log Community Number + 2.63 log Grass Area (ha), $r^2 = 0.44$, $p < 0$ .01. This suggests that the presence of strong calcicoles is enhanced by increasing area and site heterogeneity.

4.6.3.2 Nestedness at quadrat level

The results of running the temperature calculator programme for quadrats at Beachy Head, Castle Hill and Newmarket Hill are shown in Table 4.11.

Table 4.11 - Summary of data associated with nestedness temperature calculations at the quadrat level

Site	Total Grass Area (ha)	Total Species at Site	No. of Quadrats	Minimum Number of Species in Quadrat	Maximum number of Species in Quadrat	Maximum Strong Calcicoles in a Quadrat	Total Strong Calcicoles at Site	Nestedness Temperature Degrees	R^2 value Calcicoles Vs Total
Beachy Head	136	101	8*	13	24	12	18	**42.73°**	0.19
Castle Hill	35	67	8	18	32	15	21	**36.08°**	0.37
Newmarket Hill	17	75	8	20	40	16	21	**27.47°**	0.68

* 8 most species rich quadrats

The results from running the nested temperature calculator program show that at the Newmarket Hill site the strong calcicoles in the quadrats were more strongly nested (temperature = 27.47^{o}) than at the other two sites. This means that there were fewer of the expected calcicoles absent from these quadrats. Table 4.11 shows that the quadrats from Newmarket Hill have the highest minimum and maximum total species per quadrat. The maximum number of strong calcicoles per quadrat is also highest at Newmarket Hill. Thus, high levels of nestedness for strong calcicoles and high total species richness per quadrat are indicators of high strong calcicole numbers. The data also show that a comparatively small area (17ha) of chalk grassland with high species richness at the quadrat level can give high total species numbers at the site level.

4.6.4 Discussion

At the site scale the temperature of 17^{oC} obtained using the nested temperature calculator suggests that the chalk grassland sites in Sussex were nested. The most appropriate habitats to compare the results for chalk grassland with are other grassland or sedge habitats. In sedge meadow (containing at least 25% sedge species) wetland in Illinois, USA Mathews (2004) studied nestedness in 56 sites and using the temperatures calculator (Atmar and Patterson, 1995) reported a temperature of 16^{oC}. In a similar exercise a total of 68 un-fertilised and fertilised grassland meadows in Norway were examined for nestedness and a temperature of 22^{oC} was calculated (Myklestad and Sætersdal, 2004). In an examination of Alvar grassland in Estonia by Pärtel *et al*. (2001) it is claimed that the sites were nested although no temperature was quoted. Thus fragmented chalk grassland sites appear to conform to the pattern of nestedness in other fragmented grassland habitats.

The research on grasslands also considered the effect of area and site heterogeneity on nestedness. In sedge meadows Mathews (2004) found that large sites rather than small sites contained the species targeted for conservation, while smaller sites had random distributions of species of lesser conservation value and as sites decreased in size species richness decreased. In the study of Norwegian meadows by Myklestad and Sætersdal (2004) they found that traditional unfertilised meadows were at the top of the nestedness hierarchy and artificial fertilised meadows at the bottom. Myklestad and Sætersdal, (2004) also found that there was a significant effect of environmental heterogeneity on

nestedness but no effect of area, the areas of the sites were small (0.03 – 5.1ha). In the study of Alvar grassland (Pärtel *et al*. 2001) site area was between 1ha and 150ha but a strong effect of area was not found. In the current study on chalk grassland the r^2 values of 0.35 and 0.41 for community presence and area in respect of species presence suggests that these variables will affect nestedness in chalk grassland.

The evaluation of nestedness at the quadrat scale does not appear to have parallels in the research literature. At this $1m^2$ scale the effect of area is eliminated and the measurement of nestedness is within sites, rather that across sites. The results are of particular interest to this thesis as this is the scale at which plant to plant interactions will be taking place. Environmental heterogeneity across individual quadrats should be low and biotic factors such as AMF/plant symbiosis may be important. If the results from the three sites Beachy Head, Castle Hill and Newmarket Hill are considered together (Table 4,11) it can be seen that high species richness at the site scale does not necessarily translate to strong nestedness at the quadrat scale. It is apparent that quadrats (see data for Newmarket Hill) with high species richness have strong levels of nestedness. Silvertown and Wilson (1994) describe a characteristic of nestedness is that scarce species are only found at sites with many species. The results for fragmented chalk grassland in Sussex show this characteristic of nestedness to be present at both the site and quadrat scale.

4.7 Study 4 Abundance – Frequency Relationship

4.7.1 Introduction

In an analysis of the relationship between species frequency and abundance (Gaston *et al*. 1997; Gaston *et al*. 2000; Holt *et al*. 2002) attention was drawn to the links between range size of a species (that is frequency of occurrence of a species at different sites within a range) and its abundance at a local level. One generalized observation was that where species are abundant at a local level they tend to be widely distributed while species that were locally scarce have a restricted range (Gaston *et al*. 1997). Species becoming rarer over time also tended to show a decline in the range occupied and conversely species becoming more locally abundant extended their range (Gaston *et al*. 2000). In chalk grassland in Sussex the relationship between the frequency of occurrence

of a species at individual sites and local abundance was observed as early as 1921 (Tansley and Adamson, 1926).

4.7.2 Methods

Using the survey data of Steven and Muggeridge (1992) and Steven (1992) the relative abundance and frequency of occurrence of species present in 15 quadrats identified as typical of chalk grassland communities in Sussex (Steven and Muggeridge 1992; Steven 1992) were extracted . These were the same 15 quadrats used in the analysis of frequency classes in Study 2. The frequency of occurrence at the site scale (Table 4.1) of the species present in the 15 quadrats was available. Using these data, the relationship between species abundance and frequency of occurrence at the quadrat scale and species abundance at the quadrat scale and frequency of occurrence at the site scale was examined.

4.7.3 Results

The relationship between relative abundance and frequency of occurrence at the quadrat scale is shown in Figure 4.13.

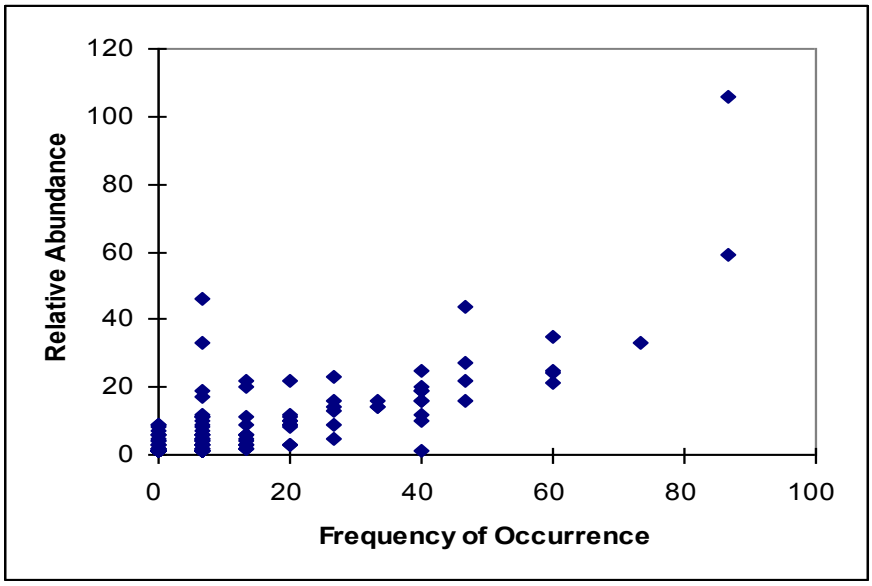


Figure 4.13 - Relative abundance (Domin scale) versus frequency of occurrence in sample of 15 quadrats typical of chalk grassland communities present in Sussex ($r^2 = 0.61$, $p < 0.01$)

It can be seen in Figure 4.13 that there is a strong relationship between the relative abundance of species in quadrats and the frequency at which that species was present in the quadrats.

In Figure 4.14 the frequency of occurrence at the quadrat scale is linked to frequency at the site scale.

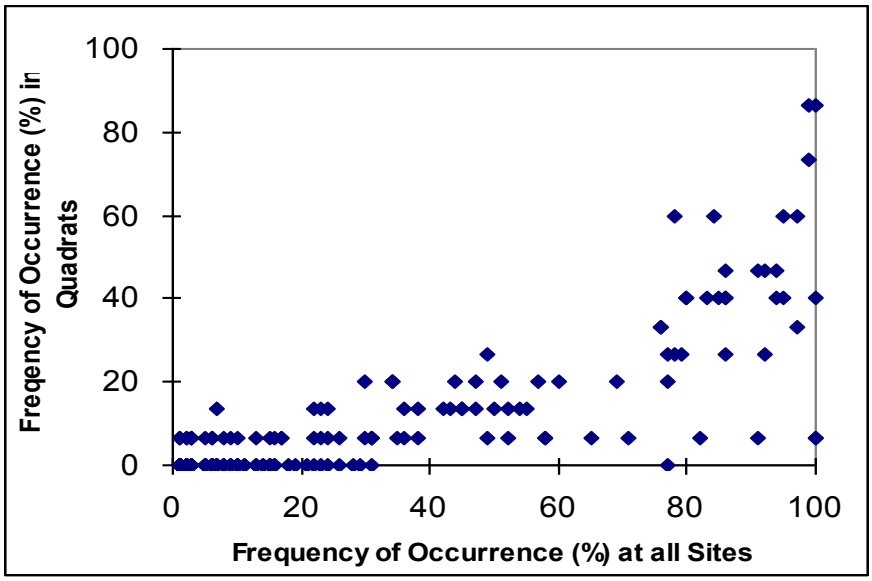


Figure 4.14 - Frequency of occurrence of species at the quadrat scale versus frequency of occurrence at the site scale for chalk grassland in Sussex. ($r^2 = 0.67$, $p < 0.01$)

Figure 4.14 shows that there is a strong relationship ($r^2 = 0.67$, $p < 0.01$) between frequency of occurrence of species at the quadrat ($1m^2$) scale and the site (25ha mean) scale. Thus there is a link between the quadrat scale at which plant species above ground and below ground interactions will take place and observations of species occurrence and abundance at the broader scale.

4.7.4 Discussion

Observations of abundance-frequency relationships are widespread and numerous examples are given in a review by Gaston, (1996) which included farmland birds, freshwater fish, grasshoppers, bumble bees and plant species from a variety of habitats. Thus the detection of an abundance-frequency relationship in chalk grassland in Sussex conforms to a generalised pattern. In fact abundance-frequency patterns in chalk

grassland in Sussex was observed as long ago as 1921 by Tansley and Adamson (1926). Although the levels of abundance and frequency for individual species appears to have changed between the 1921 survey of Tansley and Adamson (1926) and that of Steven and Muggeridge, (1992 and Steven, (1992) the pattern of abundance-frequency is maintained. This suggests underlying factors or mechanisms driving the process. Other grasslands where abundance-frequency relationships have been observed are Alvar grassland (Pärtel *et al.* 2001) and Prairie grassland (Collins and Glenn, 1990). In a paper on Prairie grassland Collins and Glenn (1990), which links the abundance-frequency relationship to the core-satellite hypothesis, the point is made that large scale structure is composed of numerous small-scale units of similar structure. These observation by Collins and Glenn (1990) are consistent with the observations in the present study that abundance-frequency at the quadrat level extend upwards in scale to the site scale.

In an appraisal of mechanisms which might underlie the observed abundance-frequency relationship observed in classes of species Gaston *et al*. (1997) identified a number of potential candidates. These included „range position", „breadth of resource" „resource availability" and „habitat selection". However Gaston *et al*. (1997) concluded that no single mechanism has unequivocal support. In their review Gaston *et al*. (1997) did not identify biotic mechanisms as potential candidates for the underlying processes driving abundance-frequency relationships. However the relative immobility of plant communities and the observation of an abundance-frequency relationship at the quadrat scale make biotic mechanisms potential candidates for this group of species. The potential for biotic process to be important is reinforced by the observation of patterns of plant association at the small scale in the study of nestedness (Study 3) and frequency classes (Study 2). A strong candidate for consideration is the symbiotic relationship between mycorrhizal fungi and plant. It has already been established in microcosm based research that mycorrhizal fungi can aid seedling establishment (Grime *et al*. 1987; van der Heijden, 2004) and have the potential to affect the structure of plant communities (van der Heijden *et al*. 1998). Thus the potential of AMF/plant symbiosis to affect community structure in natural chalk grassland is a mechanism worthy of study.

4.7.5 Conclusions

The species composition of chalk grassland communities growing on the South Downs in Sussex in 1991 (Steven and Muggeridge 1992; Steven 1992) has been evaluated. Two hundred and twenty six plant species were found to comprise the species pool, of these 68% were found to be forbs, 11% grasses or sedges and 17% mosses or lichens. Thirty two species were identified as strong calcicoles whose presence can be an indicator of the overall „health" of the community (Pärtel *et al*. 2004). Comparisons between the 1921 survey of chalk grassland in Sussex (Tansley and Adamson, 1926) and a survey of the same area in 1991 (Steven and Muggeridge, 1992; Steven, 1992) has shown the balance between forbs, grasses and sedges and mosses and lichens to be largely unchanged. However there was evidence that the presence of individual species at sites had changed, both *B. pinnatum* and *B. erectus* had increased their presence. Examination of the species present at the research site Newmarket Hill showed it to be a species rich site with the ratios of forbs, grasses and sedges and mosses and lichens present to be typical of chalk grassland communities.

In an examination of the relationship between the frequency classes core, intermediate and scarce at the quadrat scale this analysis showed the total number of species present to be strongly related to the number of core species, a result similar to that observed by (Partel *et al*. 2001) in calcareous Alvar grassland. The number of strong calcicoles in a quadrat was also strongly related to the number of core species present. At the site scale the same relationships were found as at the quadrat scale but the relationships tended to be weaker. The literature (Hanski, 1982; Collins and Glenn, 1997b) tends to concentrate on the relationship between „core" and „satellite" species but the current research suggests that intermediate species may be equally important in the context of mechanisms that underpin community structure.

Using the functional group strong calcicoles as representative of the species pool as a whole, it has been demonstrated that chalk grassland species are strongly nested at both the site and quadrat scale. The finding of strong nestedness at the quadrat scale suggests that colonisation and extinction process are operating at this scale. It is shown that strong nestedness at the quadrat scale, that is few expected species were missing, was related to high species richness. Examination of abundance-frequency relationships at the quadrat

and site scales shows a strong abundance-frequency relationship in chalk grassland. It is demonstrated that chalk grassland exhibits similar behaviour to other grassland habitats, for example Alvar grassland (Partel *et al*. 2001) and Prairie grassland (Collins and Glenn, 1990).

The separate studies of the relationships between frequency classes‟ core, intermediate and scarce, of nestedness and an abundance-frequency relationship have all shown strong patterns of association between the species present in chalk grassland communities. These strong patterns of association were present at the site scale but more particularly at the quadrat scale. Thus chalk grassland communities have been shown not to be random collections of species, but have an order of assembly determined by underlying mechanism and rules. That chalk grassland communities are structured at the small scale, where plant interactions take place, strengthens the validity of research into the role of AMF/plant symbiosis on chalk grassland community structure.

Chapter 5 - Influence of AMF on Chalk Grassland Community Structure: Laboratory Trials

5.1 Introduction

A feature of European chalk grassland is the high level of species richness (Hillier, 1990; Willems, 1990; Willems, 2002) with many forb species present. The turf is usually fine grained (Mitchley, 1990) with many plants of small stature growing in close proximity. Several theories and mechanisms have been proposed in attempts to explain how closely packed grasses and forbs are able to coexist. The reduction in vigour of dominant grasses by grazing is important and has been the subject of a number of studies (Bacon, 1990; Myklestad and Sætersdal, 2004; Denyer *et al*. 2009). Tilman (1997)) has suggested that niche differentiation along small scale nutrient gradients may modify competition. Grime (1990) has proposed two mechanisms, the first being species miniaturization as an adaptation to low nutrient availability and secondly temporal variation in plant phenology reducing peaks in nutrient demand. A further factor that has the potential to modify interspecies competition is the relationship between plants and mycorrhizal fungi. The relationship between AMF and plant can exist in different forms with the effect on plant fitness ranging from beneficial to neutral to antagonistic (Johnson *et al*. 1997). In a species rich and mature plant community, such as chalk grassland, many species of AMF are present (Fitter, 2005; Dumbrell *et al.* 2011). The plants and AMF present in chalk grassland have the potential to form many complex relationships (Bever *et al*. 1997; Bever *et al*. 2002; Bever *et al*. 2010), which may produce a positive or negative outcome for the fitness of different plant species thereby altering their competitiveness.

As reviewed in Chapter 2 extensive research has been conducted into the relationship between AMF and plant species common to calcareous grassland. Much of this research has been carried out in artificially constructed microcosms (Grime *et al*. 1987; van der Heijden *et al*. 1998b; van der Heijden *et al*. 1998a; van der Heijden 2004), has shown that individual plant species react differently to AMF infection in general and to different species of AMF and that these different relationships have the potential to affect community structure. Despite the potential role of AMF in structuring plant communities few studies have been conducted at the community level and even fewer on natural or semi-natural communities. In a recent paper presented at a conference in Krakow on

Plant-Microbial Interactions, Klironomos and Rillig (2008) identified the current trend for studies to focus at a fine scale towards the genetic and molecular level but highlighted the need for more studies on natural communities (Klironomos and Rillig, 2008; Read, 2002; Vierheilig *et al.* 1998; Rillig and Mummey, 2006; Hartnett and Wilson, 2002).

The research described in this chapter on semi-natural chalk grassland turf studied under laboratory conditions aims to bridge the gap between research in artificial microcosms and full field trials. While the study of chalk grassland turf in the laboratory lacks the precision of the artificial microcosm approach, it has the considerable merit that it encompasses the full diversity and complexity of the plants, AMF and soil fauna system found in chalk grassland. Thus the results will therefore have much ecological relevance in the context of restoration and conservation.

5.2 The Aim of Laboratory Trials

The aim of the laboratory trials was to examine whether AMF have a significant role in structuring semi-natural chalk grassland communities.

This was achieved through the following objectives.

1. To successfully grow, treat and examine turf under laboratory conditions for a minimum of one season.

2. To weaken AMF/plant symbiosis with graduated increases in the dose level of the fungicide Iprodione applied to the experimental sward turf and to assess the impact of fungicide application by a) recording forb species presence, size and abundance at the start and finish of the trial and b) changes in grass and forb species presence and abundance throughout the trial using point samples.

3. To examine the roots of selected grasses and forbs at the conclusion of a trial and measure the percentage length of roots infected with AMF.

4. To relate the percentage length of roots infected to the fungicide dose received and changes in species percentage cover

5.3 Methods

5.3.1 Introduction

The laboratory turf experiments comprised three separate trials, namely a pilot study carried out in 2006 and the two separate trials studies carried out in 2007 and 2008.

5.3.2 Collection of turf

The turf for the pilot study was collected from Newmarket Hill (see Chapter 3) in December 2005. The turf was taken adjacent to Site 1 and Site 2 (see Chapter 3) and a location midway between the two sites. The turf was taken to a laboratory at Brighton University and allowed to stabilise until the start of the trial in January 2006. The turf for the two main trials were collected from Newmarket Hill in December 2006 and 2007 and allowed to stabilise until January 2007 and 2008 respectively. The location from which turf was collected for the main trials is shown in Figure 5.1.

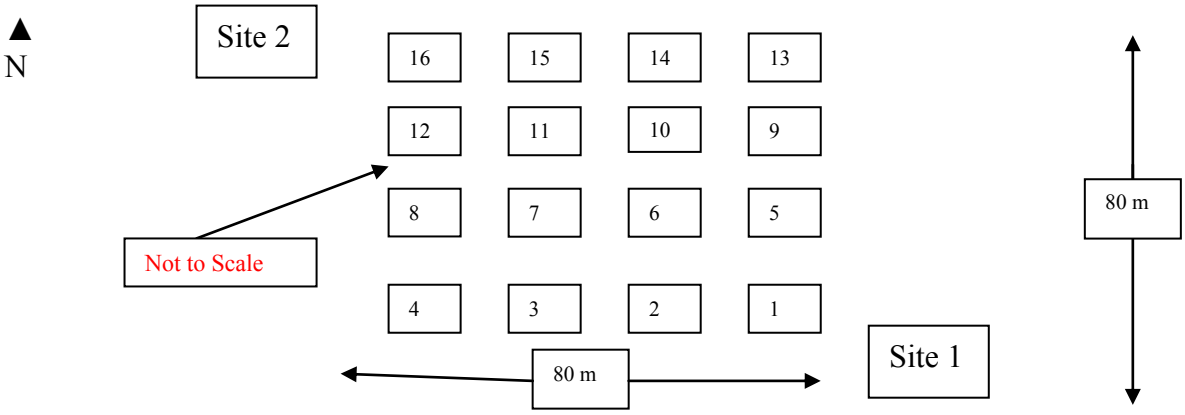


Figure 5.1 - Schematic of locations from which turf was removed for the trials.

In the schematic diagram Figure 5.1 it can be seen that the turf was collected from sixteen locations. Turf collection was spaced out at approximately 20m along the slope (at the same elevation) and up the slope at 20m intervals. Thus there were four turf samples at each of the four elevations. The area from which turf was taken was between the two sites (Site 1 and Site 2) used for the field trials (Chapter 6). In the 2007 trial turf was taken at locations 1, 2, 3 etc and in the 2008 trials 1m to the left of the 2007 locations at 1A, 2A 3A, etc. The shallow soils present a Newmarket Hill made standard seed trays (36cm x 22cm x 8cm) the preferred containment vessel for the turf samples. The turf was removed

by cutting around the outline of the seed tray with a sharp knife. A spade was used to dig down to a depth of 10cm and the turf was extracted labelled and placed in the seed tray prior to removal to the laboratory.

5.3.3 Allocation of trays to treatments

Two rules were applied in assigning trays to a particular treatment. The first was that trays assigned to each treatment should be from different locations both along the slope and up the slope (see Figure 5.1). Examination of Table 5.1 shows that the number of forbs in a tray varied from a minimum of 20 to a maximum of 55. The second rule was that trays for a particular treatment would have low, medium and high numbers of forbs present (Table 5.1).

Table 5.1 - The designation of trays to treatments for each trial

Notes. 1) 1.0g Ip – Tray treated with 1.0gm^{-2} Iprodione etc. 2) Values in ( ) are the number of forbs in each tray at start of study. 3. Tray 6A was a spare treated with 4.0gm^{-2} IP in the 2008 trial.

Trial 1 (Pilot Trial) 2006		**Trial 2 (Main Trial) 2007**				**Trial 3 (Main Trial) 2008**		
Control	**3.0 g Ip**	**Control**	**0.5 g Ip**	**1.0 g Ip**	**2.0 g Ip**	**Control**	**2.0 g Ip**	**4.0 g Ip**
Tray 2	Tray 1	Tray 1 (36)	Tray 4 (27)	Tray 3 (55)	Tray 2(33)	Tray 2A (44)	Tray 3A (34)	Tray 1A (30)
Tray 4	Tray 3	Tray 6 (28)	Tray 5 (35)	Tray 8 (39)	Tray 7 (29)	Tray 7A (31)	Tray 5a (38)	Tray 4A (27)
Tray 6	Tray 5	Tray 11 (37)	Tray 10 (34)	Tray 9 (20)	Tray 12 (32)	Tray 9A (20)	Tray 8A (28)	Tray 11A (44)
Tray 8	Tray 6	Tray 16 (30)	Tray 15 (34)	Tray 14 (42)	Tray 13 (50)	Tray 12A (40)	Tray 10A (51)	Tray 13A (51)
						Tray 15A (32)	Tray 14A (37)	Tray 16 A (38)
		Mean (32.75)	**Mean (32.5**	**Mean (39**	**Mean (36)**	**Mean (33.4)**	**Mean (37.6)**	**Mean (38)**

In 2006 and 2007 there were four trays for each treatment and in 2008 5 trays for each treatment. In Table 5.1 it can be seen that for the 2007 and 2008 trials the mean number of forbs in trays for each treatment ranged from 32.5 to 39.

5.3.4 Maintenance and treatment of turf samples

5.3.4.1 Stabilisation of turf

Following the collection of turf in December 2005, 2006 and 2007 it was allowed to stabilise for approximately 3 weeks prior to commencement of the treatments in the

following January. Prior to starting the trial the vegetation was trimmed to a height of 2.5cm.

5.3.4.2 Watering

The trays were situated in south facing window sills and subject to full sun. Each tray received a minimum of 1litre of distilled water per week. During the summer months this was increased to 2 litres per week. Although the turf could become dry excessive wilting was avoided. The position of the trays was randomised every two weeks to reduce the impact difference of light levels and temperatures.

5.3.4.3 Trimming

To simulate grazing and prevent excessive overtopping of forbs by the dominant grasses, both grasses and forbs were trimmed to a height of 2.5cm three times during the growing season (Table 5.2 and 5.3). The clippings from individual trays were dried for 48 hours at 35^{oC} and weighed.

Table 5.2 - Details of spraying, surveying and photographing dates for the 2007 trial

* All foliage trimmed to ~2.5 cm on 30/05/07. Plant matter collected, dried and weighed.

** All foliage trimmed to ~2.5 cm on 20/07/07. Plant matter collected, dried and weighed.

*** All foliage trimmed to ~2.5 cm on 11/09/07. Plant matter collected, dried and weighed.

Spraying Date	Photographing	25 point Survey	Individual Plant
12/01/07	4/01/07	9/01/07	5/01/07
7/02/07	13/02/07	12/02/07	
7/03/07	13/03/07	12/03/07	
12/04/07		17/04/07	
11/05/07	30/05/07	22-24/05/07	
15/06/07	20/07/07	16/07/07	
23/07/07	11/09/07	28/08/07	9/10/07
20/09/07		6/11/07	

Table 5.3 - Details of spraying, surveying and photographing dates for the 2008 trial

* All foliage trimmed to ~2.5 cm on 13/05/08. Plant matter collected, dried and weighed.

* *All foliage trimmed to ~2.5 cm on 14/07/08. Plant matter collected, dried and weighed.

* * *All foliage trimmed to ~2.5 cm on 4/09/08. Plant matter collected, dried and weighed.

Spraying Date	Photographing	25 point Survey	Individual Plant
14/01/08	2/01/ 08	7-8/01/08	2-4/01/08
11/02/08	30/01/08	4-5/02/08	
11/03/08	26/02/08	28-29/02/08	
8/04/08	2/04/08	2/04/08	
15/05/08	12/9/08	12-13/05/08	
12/06/08	20/11/08	10/06/08	
21/07/08		15/07/08	
8/09/08		1 /09/08	
16/10/08		5/11/08	8/11/08

Photographic records of the trays were taken at regular intervals (Table 5.2 and Table 5.3)

5.3.5 Choice of fungicide and spraying details

The fungicide chosen for the trials was Iprodione, which had been used to successfully lower AMF activity in an earlier field trial (Gange *et al*. 1993). Iprodione is a complex organic compound (FRAC 2; dicaroboximide) whose function is as a contact fungicide (Bayer Environmental Science, 2009). It is sold under a number of trade names including „Chipco Roval Green". Iprodione is claimed to have both preventative and curative properties including inhibition of the germination of fungal spores and the growth of mycelium. The level of toxicity to a range of organisms from soil biota to mammals is claimed to be very low. The recommended dose for commercial use is up to 1.2gm^{-2} dissolved in 1.5 litres of water.

To increase accuracy of the applied doses of the fungicide it was initially diluted by a factor of 10. Solutions were then made up according to the dose to be applied. All

solutions were again diluted to give an application rate equivalent to 1.5l of solution per m^2 of turf. This equated to 130ml of solution per tray. Control trays were sprayed with 130ml of distilled water. Trays were sprayed at approximately monthly intervals. Details of the precise spraying dates for the 2007 and 2008 trials are given in Tables 5.2 and 5.3.

5.3.6 Surveying the species in individual trays

Two methods were adopted for surveying the trays.

5.3.6.1 Recording position and number of forbs present

Although the primary method adopted for surveying the trays was a 25 point random survey conducted at regular intervals, further information regarding changes in species presence and cover was obtained by recording the identity and position of individual forbs in all of the trays at the start and conclusion of the trial

5.3.6.2 Twenty five point random surveys

Surveys were performed at 25 randomly generated points in each tray. The positions of the 25 random points were generated using the Excel programme „random between". Pairs of coordinates were generated from adjacent columns of random numbers, where the first column represented the length and the second column the width of the tray. The same 25 coordinates were used for all trays on a particular date, with new sets of coordinates generated for each of the survey dates. The trays were surveyed at an approximately monthly interval (Tables 5.2 and 5.3)

The method adopted was to have a fixed rule along the length of the tray and moving a second rule along the width. Starting at the identified front end of the tray, with the length rule always on the left, the moving rule progressed along the tray until a point coordinate was reached. At this point, a slim pencil (5mm cross-section) was inserted through the foliage down to the soil; all forb and grass species in contact with the body of the pencil were recorded. This procedure was repeated for all 25 random points. The data for each tray was collated to give the number of contacts (relative abundance) for each forb and grass species.

5.3.7 Microscopic examination of roots for the presence of AMF

The techniques for detecting AMF in plant roots have been refined over the last 40 years (Phillips *et al*. 1970; Brundett *et al*. 1984; Koske *et al*. 1989). Different combinations of chemicals and microscope techniques have been used, and these were reviewed and assessed by Gange *et al*. (1999). The method chosen in this research for clearing and staining roots was that attributed to Vierheilig *et al*. (1998) using chemicals with minimum toxicity. The cross hair technique (McGonigle *et al*. 1990) currently used by other workers (Ayres *et al*. 2006) for the microscopic evaluation of the presence and extent of AMF components in roots was adopted.

5.3.7.1 Procedures for preparation, staining and microscopic examination of roots

Although the basic clearing and staining technique used was that attributed to Vierheilig *et al*. (1998) it was necessary to optimize it for the range of forb and grass roots to be examined. Most of the experimentation in this study was on the roots of *Brachypodium pinnatum*, which proved difficult to clear and stain. At the conclusion of this experimentation a standard procedure for clearing, staining and de-staining roots was adopted for both the grasses and forbs to be examined.

At the conclusion of the 2008 trials the turf from individual trays was soaked in water to remove the soil and loosen the roots. The roots of individual plants were gently separated by shaking in water and teasing apart. The plants from each of the different treatments were separated firstly into grasses and forbs and then into individual species. Thus pooled bags of individual species for a particular treatment were collected, e.g. *Brachypodium pinnatum* that had been treated with $4.0gm^{-2}$ Iprodione.

Grasses and forbs from the three treatments (controls, $2.0gm^{-2}$ and $4.0gm^{-2}$) that were present in sufficient numbers (minimum of 5) to be representative were selected for examination. From the pooled bags five plants of each species were selected and the roots separated from the foliage. The pooled roots from each individual species and treatment were placed in a boiling solution of 10% potassium hydroxide for 10 minutes, removed and thoroughly washed in water. The roots were then placed in a boiling solution of 5% black (Parker Quink) ink in vinegar for 3 minutes. The roots were washed in water and

left to de-stain in 5% vinegar in water for 16 hours in accordance with the method of Vierheilig *et al*. (1998).

The roots were placed in clean water and chopped into 5 – 30 mm lengths and stirred to randomise. Individual pieces of roots representing a range of root thicknesses were removed from the beaker. These were then mounted in parallel lines on a 30mm x 40mm glass slides. For most species there was sufficient material to produce two slides each with ~ 150mm of roots.

The roots were examined using a Nikon microscope at a magnification of x 100. Placing a crosshair at 90^{o} to the root, the stage was advanced at 1mm intervals. Where the crosswire intersected the root the presence or absence of mycorrhizal infection and vesicles was recorded, that is using the method attributed to McGonigle *et al*. (1990).

5.3.8 Statistical analysis of data

The main data collected in this trial was individual species presence; that is which species were present in trays and their abundance, which was the number of point contacts for each of the species present. The data for each tray from a particular treatment, that is controls and fungicide treated trays, were combined to give a mean value and standard error. These variables were tested first for normality and then for difference between treatments using a one-way analysis of variance (ANOVA) with the significance level set at 0.05. This was followed by a Tukey *post-hoc* test. The relationship between the length of plant root infected and the presence of vesicles was tested using regression analysis.

5.4 Results

5.4.1 Trial 1 (Pilot Study 2006)

The pilot study was carried out at the start of the research and although Iprodione had been used to reduce AMF activity in a natural community in early succession (Gange *et al*. 1993) there was uncertainty on the effect of Iprodione on semi-natural chalk grassland communities. Thus the pilot study was a simple experiment in which Iprodione was applied at a dose rate of $3gm^{-2}$ at 3 week intervals. The trial was conducted in a growth cabinet set for 12 hours daylight at a temperature of 20^{oC} and ran for a period of 100 days.

The main finding was that Iprodione applied at a dose rate of $3gm^{-2}$ affected the relative abundance of the species present. The grasses and sedges increased and the forbs decreased in abundance compared to the controls (Figures 5.2 and 5.3).

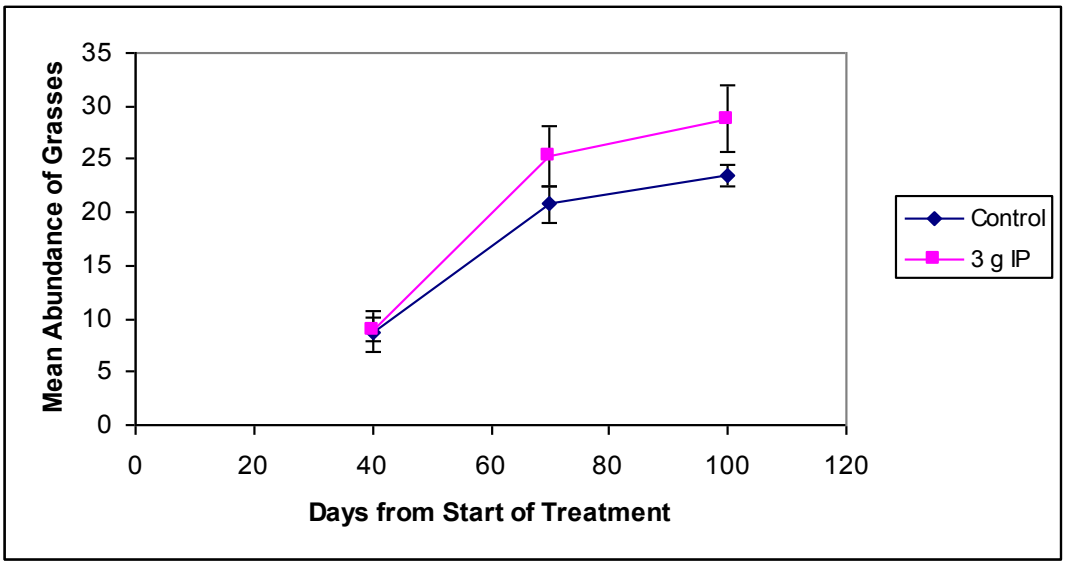


Figure 5.2 - The change in mean (n = 4. ± 1 S.E) abundance of grasses and sedges in control and $3gm^{-2}$ treatments in pilot study

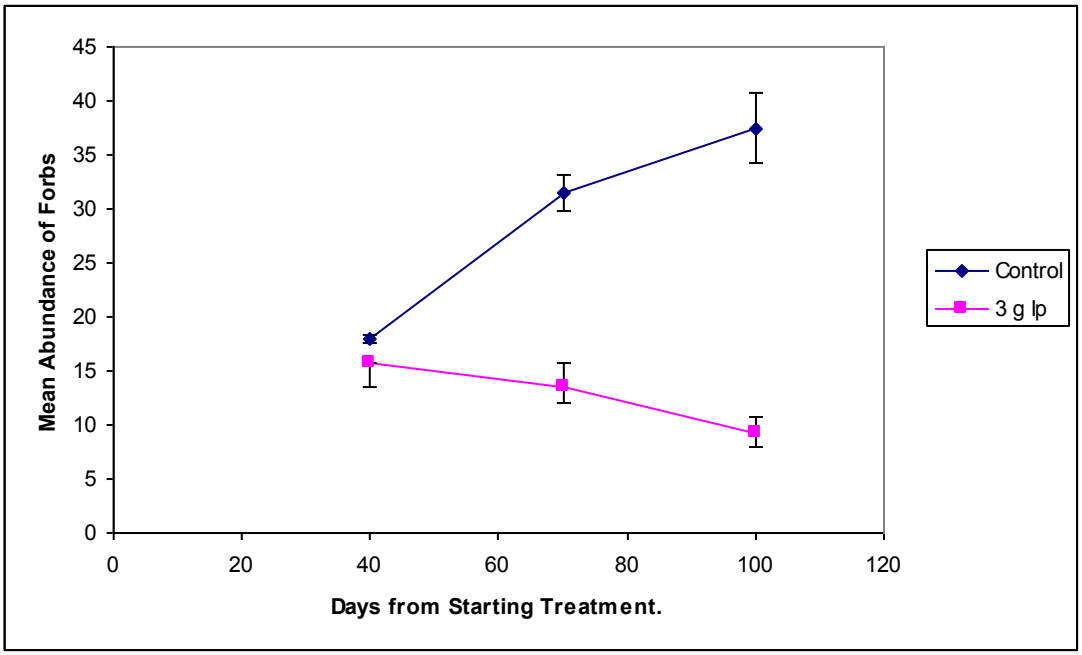


Figure 5.3 - The change in mean (n = 4. ± 1 S.E) abundance of forbs in control and $3gm^{-2}$ treatments in pilot study

It can be seen in Figure 5.2 that up to 40 days from when the fungicide was first applied there was little difference in the mean abundance of grasses and sedges in the controls

and treated trays. At about 70 days the means start to diverge and at 100 days the abundance of grasses and sedges in the fungicide treated trays was about 20% higher than that in the control. In Figure 5.3 the means for forb abundance is similar in control and fungicide treated trays after 40 days. After 70 days the means have diverged and at 100 days there was about a 70% reduction in the mean abundance of forbs in trays treated with fungicide compared to the controls. It should also be noted that the abundance of forbs and grasses in the control trays were also changing during the trial.

In Trial 1 (Pilot Trial) the application of the fungicide Iprodione to chalk grassland communities at a dose rate of $3gm^{-2}$ has resulted in changes to community structure on a short (100 day) timescale. The results of this trial were used as a guide for setting dose rates for Trials 2 and 3 and the field trials (Chapter 6).

5.4.2 Trial 2 (2007)

5.4.2.1 The identity and number of individual forb species present at the start and conclusion of the trial.

At the start of the trial in January the forbs in all trays were small of stature. At the conclusion of the trial (October) the forbs in the control trays and those treated with $0.5gm^{-2}$ and $1.0gm^{-2}$ Iprodione had increased in stature but were uniform in size. In contrast the forbs in the trays treated with $2.0gm^{-2}$ were fewer in number but were of larger stature. One species *Ranunculus bulbosus* did not adapt to laboratory conditions and the 68 plants spread across all treatments had died within 2 months, although 2 plants of *R. bulbosus* were present in the October survey.

The data on the number of plant from each species present in the trays at the start and end of the trial are summarised in Tables 5.4, 5.5, 5.6 and 5.7. Table 5.4 shows the data for the control, Table 5.5 data for trays treated with $0.5gm^{-2}$ Iprodione, Table 5.6 data for trays treated with 1.0 gm^{-2} Iprodione and Table 5.7 data for trays treated with 2.0 gm^{-2} Iprodione.

Table 5.4 - The number of plants of each species present in control trays at start and end of trial

Key. A.C – *Asperula cynanchica*. C.N – *Centaurea nigra*. C.E – *Centaurium erythraea*. C.S. – *Cirsium species*. F.V – *Filipendula vulgaris*. G.M – *Galium mollugo*. H.P – *Hieracium pilosella*. H.C – *Hippocrepis comosa*. H.Pe – *Hypericum perforatum* L.H – *Leontodon hispidus*. L.V – *Leucanthemum vulgare*. L.C - *Lotus corniculatus*. P.O – *Phyteuma orbiculare*. P.L – *Plantago lanceolata*. P.M – *Plantago media.* P.C – *Polygala calcarea*. Pr.V – *Prunella vulgaris*. R.B – *Ranunculus bulbosus*. S.M – *Sanguisorba minor*. S.S – *Scabiosa columbaria*. S.P – *Succisa pratensis*. T.P – *Thymus praecox*. Tr.P – *Trifolium pratense*. V.S – *Viola species.*

January 2007	A.C	C.N	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LH	L.V	LC	P.O	P.L	P M	P.C	Pr.V	R.B	S.M	S.C	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 1	2	0	0	2	0	2	1	3	0	2	0	0	0	0	3	2	0	4	7	0	0	1	0	3	**32**	**12**
Tray 6	3	0	1	0	8	4	0	0	0	1	0	0	0	1	0	0	0	7	1	0	0	1	0	2	**29**	**10**
Tray 11	3	0	0	2	1	8	3	0	0	5	3	0	0	0	3	0	0	4	2	0	0	0	0	1	**35**	**11**
Tray 16	5	0	0	0	9	0	5	0	0	1	0	0	0	0	1	0	0	0	5	0	0	2	0	1	**29**	**8**
Total	**13**	**0**	**1**	**4**	**18**	**14**	**9**	**3**	**0**	**9**	**3**	**0**	**0**	**1**	**7**	**2**	**0**	**15**	**15**	**0**	**0**	**4**	**0**	**7**	**125**	**41**
																						Mean ± 1S.E.			**31.25 ± 1.44**	**10.25 ± 0.85**

October 2007	A.C	C.N	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LH	L.V	LC	P.O	P.L	P.M	P.C	Pr.V	R.B	S.M	S.C	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 1	3	0	0	1	1	2	4	2	0	0	0	0	0	0	3	1	0	0	3	0	0	1	1	4	**26**	**12**
Tray 6	2	0	0	0	4	0	0	0	0	1	0	1	0	0	1	0	0	0	2	0	1	3	1	4	**20**	**10**
Tray 11	8	1	2	2	2	2	2	0	1	5	2	2	0	0	2	0	0	1	1	1	1	1	0	2	**39**	**18**
Tray 16	3	0	1	1	6	0	5	0	0	1	0	5	1	0	0	0	0	0	1	5	5	1	0	3	**34**	**12**
Total	**16**	**1**	**3**	**4**	**13**	**4**	**11**	**2**	**1**	**7**	**2**	**8**	**1**	**0**	**6**	**1**	**0**	**1**	**7**	**6**	**7**	**6**	**2**	**15**	**119**	**52**
																						Mean ± 1 S.E.			**29.25 ± 4.21**	**13 ± 1.73**

Table 5.5 - The number of plants of each species present in trays treated with 0.5gm^{-2} Iprodione at start and end of trial

Key. A.C – *Asperula cynanchica*. . C.E – *Centaurium erythraea*. C.S. – *Cirsium species*. F.V – *Filipendula vulgaris*. G.M – *Galium mollugo*. H.P – *Hieracium pilosella*. H.C – *Hippocrepis comosa.* L.H – *Leontodon hispidus*. L.V - *Leucanthemum vulgare*. L.C - *Lotus corniculatus*. O.R – *Ononis repens*. P.O – *Phyteuma orbiculare*. P.S – *Pimpinella saxifrage*. P.L – *Plantago lanceolata*. P.M – *Plantago media.* P.C – *Polygala calcarea*. Pr.V – *Prunella vulgaris*. R.B – *Ranunculus bulbosus*. S.M – *Sanguisorba minor*. S.P – *Succisa pratensis*. T.P – *Thymus praecox*. Tr.P – *Trifolium pratense*. V.S. *Viola species.*

January 2007	A.C	C.E	C.S	F.V	G.M	H.P	H.C	LH	L.V	LC	P.O	P.S	P.L	P M	P.C	Pr.V	R.B	S.M	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 4	0	0	1	7	2	1	2	0	0	0	0	0	0	0	0	0	5	2	0	5	1	2	**28**	**10**
Tray 5	4	0	0	2	0	3	1	4	0	0	0	4	2	0	0	0	7	2	0	3	0	3	**35**	**11**
Tray 10	3	0	1	3	6	4	1	1	3	0	0	0	3	0	0	0	3	5	0	0	0	1	**33**	**11**
Tray 15	5	0	0	3	4	7	0	2	0	2	0	0	0	0	1	0	4	6	0	2	0	0	**37**	**11**
Total	**12**	**0**	**2**	**13**	**12**	**15**	**4**	**7**	**3**	**2**	**0**	**4**	**5**	**0**	**1**	**0**	**19**	**15**	**0**	**10**	**1**	**6**	**133**	**43**
																				Mean ± 1 S.E.			**33.25 ± 1.93**	**10.75 ± 0.25**

October 2007	A.C	C.E	C.S	F.V	G.M	H.P	H.C	LH	L.V	LC	P.O	P.S	P.L	P.M	P.C	Pr.V	R.B	S.M	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 4	0	1	1	7	2	0	2	2	1	0	1	0	0	1	0	0	0	4	8	0	4	0	**34**	**12**
Tray 5	0	1	1	2	0	3	0	4	0	2	1	3	0	0	0	0	0	2	3	0	3	2	**28**	**12**
Tray 10	6	1	0	1	0	3	1	2	5	2	1	0	0	1	0	1	0	3	3	0	0	1	**34**	**14**
Tray 15	4	2	0	1	0	6	0	2	0	6	1	0	0	0	0	1	0	6	5	1	0	6	**38**	**12**
Total	**10**	**5**	**2**	**11**	**2**	**12**	**3**	**10**	**6**	**10**	**4**	**3**	**0**	**2**	**0**	**2**	**0**	**15**	**19**	**1**	**7**	**9**	**134**	**50**
																				Mean ± 1 S.E.			**33.5 ± 2.06**	**12.5 ± 0.5**

Table 5.6 - The number of plants of each species present in trays treated with 1.0gm^{-2} Iprodione at start and end of trial

Key. A.C – *Asperula cynanchica*. C.E – *Centaurium erythraea*. C.S. – *Cirsium species*. F.V – *Filipendula vulgaris*. G.M – *Galium mollugo*. H.P – *Hieracium pilosella*. H.C – *Hippocrepis comosa*. L.H – *Leontodon hispidus*. L.V – *Leucanthemum vulgare*. L.C - *Lotus corniculatus*.. P.O – *Phyteuma orbiculare*. P.S – *Pimpinella Saxifrage*. P.L – *Plantago lanceolata*. P.M – *Plantago media.* P.C – *Polygala calcarea*. P.V. *Polygala vulgaris*. Pr.V – *Prunella vulgaris*. R.B – *Ranunculus bulbosus*. S.M – *Sanguisorba minor*. S.J – *Senecio jacobaea*. S.P – *Succisa pratensis*. T.P – *Thymus praecox*. Tr.P – *Trifolium pratense*. V.S. – *Viola species*

January 2007	A.C	C.E	C.S	F.V	G.M	H.P	H.C	LH	L.V	LC	P.O	P.S	P.L	P M	P.C	P.V	Pr.V	R.B	S.M	S.J	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 3	5	0	2	6	3	1	4	3	1	0	0	0	0	1	1	0	0	8	9	0	0	0	0	7	**51**	**13**
Tray 8	5	0	0	0	2	1	2	1	1	0	0	0	1	1	7	0	0	3	11	0	0	0	0	0	**35**	**11**
Tray 9	1	0	2	0	1	0	0	2	0	0	0	0	4	0	0	0	0	2	3	5	0	0	0	0	**20**	**8**
Tray 14	1	0	3	6	4	10	1	6	0	1	0	0	0	0	0	1	0	2	6	0	0	1	0	1	**43**	**13**
Total	**12**	**0**	**7**	**12**	**10**	**12**	**7**	**12**	**2**	**1**	**0**	**0**	**5**	**2**	**8**	**1**	**0**	**15**	**26**	**5**	**0**	**1**	**0**	**8**	**149**	**45**
																						Mean ± 1 S.E.			**37.25 ± 6.61**	**11.25 ± 1.18**

October 2007	A.C	C.E	C.S	F.V	G.M	H.P	H.C	LH	L.V	LC	P.O	P.S	P.L	P M	P.C	P.V	Pr.V	R.B	S.M	S.J	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 3	0	0	4	10	1	1	1	1	5	4	2	1	0	2	0	0	5	0	3	0	8	5	0	2	**55**	**16**
Tray 8	6	0	1	0	6	2	1	1	1	0	3	0	0	2	7	0	1	1	6	0	2	1	0	2	**43**	**16**
Tray 9	0	0	2	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	1	8	0	0	0	2	**20**	**5**
Tray 14	1	1	3	5	1	5	0	3	3	2	0	0	0	0	0	0	2	0	4	0	4	1	0	1	**36**	**14**
Total	**7**	**1**	**10**	**15**	**8**	**8**	**2**	**12**	**9**	**6**	**7**	**1**	**0**	**4**	**7**	**0**	**8**	**1**	**14**	**8**	**14**	**7**	**0**	**7**	**154**	**51**
																						Mean ± 1 S.E.			**38.5 ± 7.31**	**12.75 ± 2.63**

Table 5.7 - The number of plants of each species present in trays treated with 2.0gm^{-2} Iprodione at start and end of trial

Key. A.C – *Asperula cynanchica*. C.E – *Centaurium erythraea*. C.S. – *Cirsium species*. F.V – *Filipendula vulgaris*. G.M – *Galium mollugo*. H.P – *Hieracium pilosella*. H.C – *Hippocrepis comosa*. L.H – *Leontodon hispidus*. L.V – *Leucanthemum vulgare*. L.C - *Lotus corniculatus*. O.R – *Ononis repens*. P.O – *Phyteuma orbiculare*. P.S – *Pimpinella Saxifrage*. P.L – *Plantago lanceolata*. P.M – *Plantago media.* P.C – *Polygala calcarea*. P.V. – *Polygala vulgaris*. Pr.V – *Prunella vulgaris*. R.B – *Ranunculus bulbosus*. S.M – *Sanguisorba minor*. S.P – *Succisa pratensis*. T.P – *Thymus praecox*. Tr.P – *Trifolium pratense*. V.S. – *Viola species.*

January 2007	A.C	C.E	C.S	F.V	G.M	H.P	H.C	LH	L.V	LC	P.O	P.S	P.L	P M	P.C	P.V	Pr.V	R.B	S.M	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 2	4	0	1	7	4	0	0	2	0	0	0	0	2	1	0	0	0	3	5	0	0	2	1	**33**	**12**
Tray 7	1	0	0	3	5	1	1	6	0	0	0	0	4	0	0	0	0	5	0	0	0	0	1	**27**	**9**
Tray 12	2	0	4	0	2	3	2	2	2	0	0	0	0	1	0	1	0	3	5	0	3	0	0	**30**	**12**
Tray 13	5	0	0	0	3	9	3	5	0	0	0	0	0	0	0	0	0	8	10	0	0	0	1	**44**	**8**
Total	**12**	**0**	**5**	**10**	**14**	**13**	**6**	**15**	**2**	**0**	**0**	**0**	**6**	**2**	**0**	**1**	**0**	**19**	**20**	**0**	**3**	**2**	**3**	**134**	**41**
																					Mean ± 1 S.E.			**33.5 ± 3.71**	**10.25 ± 1.03**

October 2007	A.C	C.E	C.S	F.V	G.M	H.P	H.C	LH	L.V	LC	P.O	P.S	P.L	P.M	P.C	P.V	Pr.V	R.B	S.M	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 2	0	0	0	5	5	1	0	2	0	0	0	0	0	1	0	0	0	0	5	1	3	0	2	**25**	**9**
Tray 7	0	1	1	7	5	0	0	4	1	3	0	0	0	0	0	0	0	0	1	0	0	0	3	**26**	**9**
Tray 12	0	2	1	0	1	2	0	1	3	5	0	0	0	1	0	0	0	0	3	2	3	0	1	**25**	**12**
Tray 13	1	0	0	4	0	5	0	0	0	1	0	0	0	1	0	0	5	0	1	1	1	0	1	**21**	**10**
Total	**1**	**3**	**2**	**16**	**11**	**8**	**0**	**7**	**4**	**9**	**0**	**0**	**0**	**3**	**0**	**0**	**5**	**0**	**11**	**4**	**7**	**0**	**7**	**97**	**40**
																					Mean ± 1 S.E.			**24.25 ± 1.11**	**10.0 ± 0.71**

Examination of Tables 5.4, 5.5, 5.6 and 5.7 shows that there were a total of 25 different species present in the trays. At the start of the trial a maximum of 51 and a minimum of 20 plants were present in individual trays. The maximum number of species in individual trays was 13 and the minimum 8. At the conclusion of the trial the maximum number of plants in a tray was 55 and the minimum 20, i.e. there was little change during the trial. At the conclusion of the trial there were a maximum of 18 and a minimum of 5 different species in trays. The mean values for total forbs and total species present in January and October for the control and three treatments (Tables 5.4, 5.5 and 5.6 and 5.7) were tested for significant difference using a one-way ANOVA. None were found to be significantly different, however the change between January and October in the means for total forbs present in the 2.0gm^{-2} treatment was close to being significantly reduced with $p = 0.054$.

5.4.2.2 The 25 random point surveys

The species that showed the highest response to the application of fungicide was *Brachypodium pinnatum* (Figure 5.4).

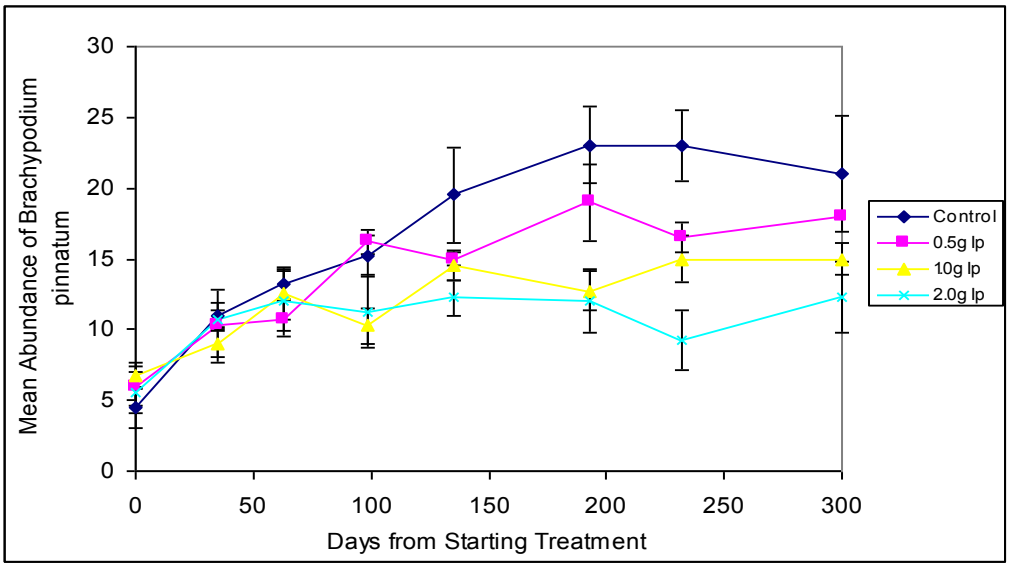


Figure 5.4 - Mean (n = 4 ± 1 S.E) abundance of *Brachypodium pinnatum* with different doses of Iprodione over a period of 300 days

At the start of the trial the relative abundance (point counts) were similar for the controls and the three fungicide treatments. They start to diverge after approximately 100 days and appear to reach a new equilibrium after about 200 days. It can be seen in Figure 5.4 that there is a trend for the mean abundance of *B. pinnatum* to decrease as the fungicide dose is

increased. Testing the means for significant difference using a one-way ANOVA gave a significant difference ($p < 0.01$) between the control and 2.0gm^{-2} treatment after 230 days.

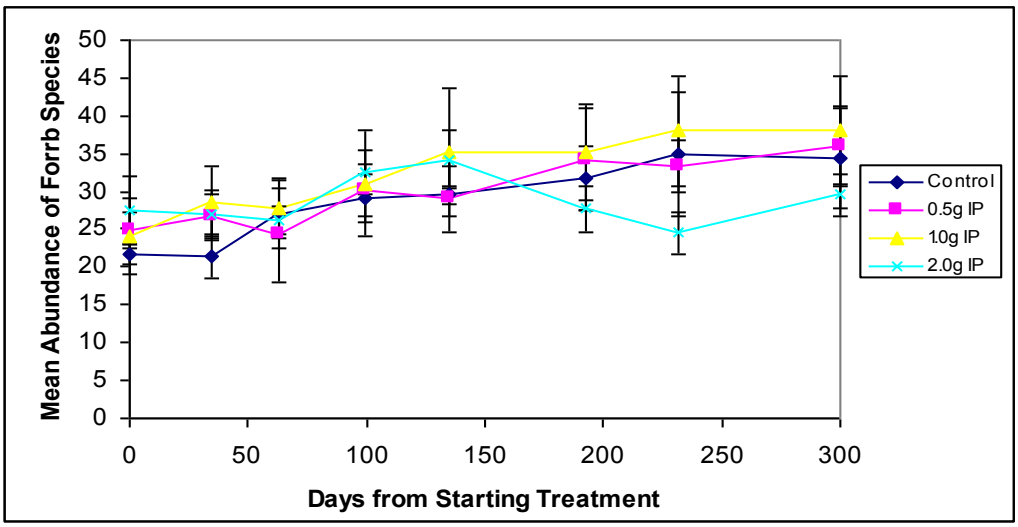


Figure 5.5 - Mean (n = 4 ± 1 S.E) abundance of forb species with different doses of Iprodione over a period of 300 days

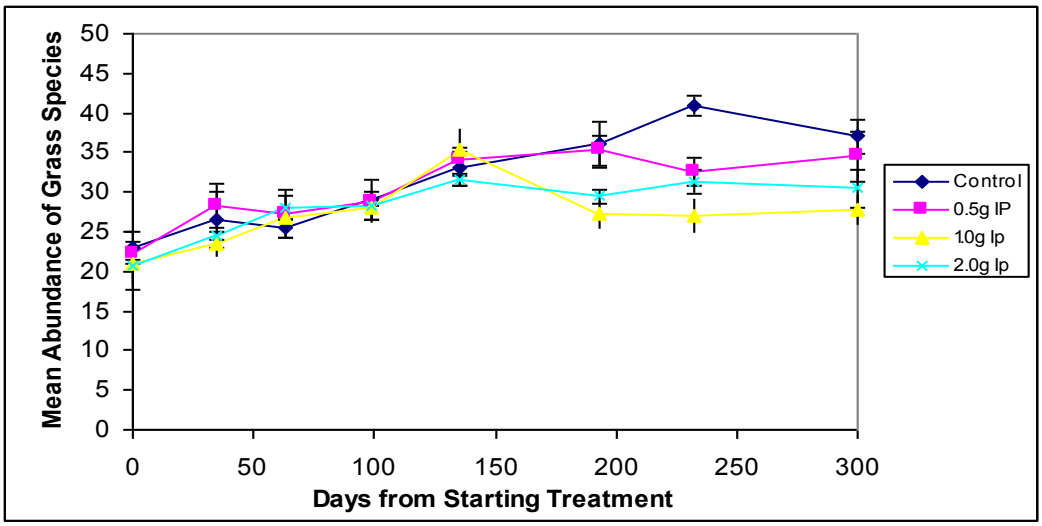


Figure 5.6 - Mean (n = 4 ± 1 S.E) abundance of grass species with different doses of Iprodione over a period of 300 days

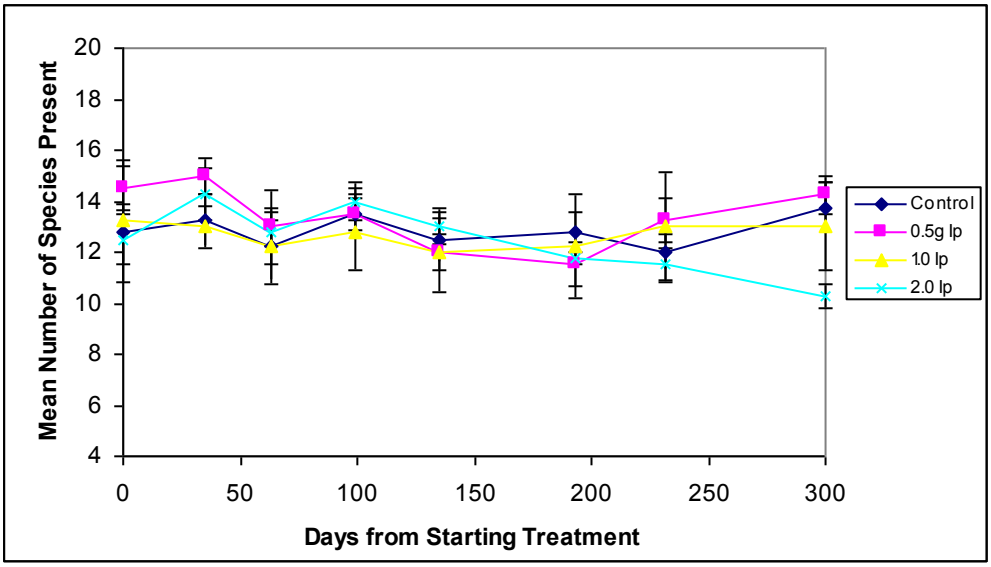


Figure 5.7 - Mean (n = 4 ± 1 S.E) number of species present with different doses of Iprodione over a period of 300 days

Figure 5.5 shows that up to about 150 days there was little difference in the mean abundance of forbs in the controls and the three fungicide treatments. After 150 days there was a decline in the mean abundance of forbs in trays treated with 2.0gm^{-2} fungicide. However testing for significance, using a one-way ANOVA showed no significant differences between the control and any of the three fungicide treatments.

The mean abundance of grass species is shown in Figure 5.6 where it can be seen that for the first 150 days abundance for controls and fungicide treated trays is rising. Between 150 and 300 days grass abundance in the control trays is generally rising while that in the treated trays levels off or declines. The greatest difference between controls and treated trays occurs at 230 days where testing with a one-way ANOVA shows significant differences for all three fungicide treatments ($p < 0.01$). There were also significant differences between the controls and the 1.0gm^{-2} treatments at 190 and 300 days ($p = < 0.05$).

Figure 5.7 shows the changes in the mean number of species (forbs + grasses) in the trays. There was little difference in the means from the controls and three fungicide treatments up to 230 days, although there was a gradual decline in trays treated with 2.0gm^{-2} fungicide from about 100 days. Between 230 and 300 days the mean for the 2.0gm^{-2} treatment

declines in comparison to the control and other fungicide treatments. Testing using a one-way ANOVA showed a significant difference between mean number of species present in controls and 2.0gm^{-2} treated trays after 300 days ($p < 0.05$)

5.4.2.3 Biomass

The weight data shown in Table 5.6 are a compilation of the total sward biomass (grasses and forbs) collected for each treatment after cutting in May, July and September. The weight of the material collected was strongly dependent on the abundance of grasses and sedges in the trays, with grasses and sedges accounting for about 80% of the material collected.

Table 5.8 - Summary of dry mass of material collected from turf in Trial 2 (2007)

Tray	Treatment	30/05/07 WT (g)	20/07/07 WT (g)	11/09/07WT (g)	Total WT (g)	Mean WT (g)	± 1S.E
1	Control	3.145	1.683	2.304	7.132	11.7	4.27
6	Control	7.330	9.17	7.989	24.489		
11	Control	1.934	0.992	4.047	6.973		
16	Control	1.793	2.146	4.286	8.225		
4	0.5g Ip	2.410	1.822	2.316	6.548	10.82	1.65
5	0.5g Ip	5.746	4.339	4.459	14.544		
10	0.5g Ip	4.803	1.76	4.021	10.584		
15	0.5g Ip	3.062	3.347	5.187	11.596		
3	1.0g Ip	4.574	1.630	2.368	8.572	12.42	1.94
8	1.0g Ip	3.214	3.061	5.067	11.342		
9	1.0g Ip	7.376	4.776	5.658	17.81		
14	1.0g Ip	4.916	2.305	4.734	11.955		
2	2.0g Ip	4.494	2.717	5.137	12.348	11.61	2.01
7	2.0g Ip	3.830	2.797	2.983	9.61		
12	2.0g Ip	2.532	1.475	3.584	7.591		
13	2.0g Ip	7.140	4.656	5.105	16.901		
	Total	**68.07**	**48.87**	**69.24**	**186.18**		

Inspection of Table 5.8 shows that the biomass collected from trays was quite variable with the maximum biomass collected from an individual tray being about 3 times that for the minimum. The total biomass collected in May, July and September showed some variation with that collected in May and September being similar at slightly less than 70g, whole the weight of biomass collected in July was about 30% lower. The mean total biomass for trays designated to controls, 0.5gm^{-2}, 1.0gm^{-2}, and 2.0gm^{-2} fungicide treatments were similar and testing with a one-way ANOVA showed no significant difference between treatments. However the biomass mean in control trays was increased by the contribution from a „grassy" tray 6 reflected in the high standard error.

5.4.3 Trial 3 (2008)

5.4.3.1 Species survival and distribution

Not all forb species, even those growing in the control trays, remained viable under laboratory conditions for the duration of the trial. As in the 2007 trial *R. bulbosus* did not thrive under laboratory conditions. In the 2008 trial, 65 *R. bulbosus* plants were present at the start. After about 3 weeks a white fungal growth appeared on the leaves accompanied by an aphid species. Ninety days after the start of the trial all of the *R. bulbosus* plants had died.

There was also a tendency for *Plantago lanceolata* and *Leontodon hispidus* to be lost from the trays of all treatments, although small specimens of both species re-appeared later in the trial. The reason may be a deeper root system making these species more prone to damage during translocation from the field.

It was also observed that the forbs present in the trays were not evenly dispersed but showed a tendency to form clusters of typically 4 – 7 plants within a matrix of grasses. The plants of an individual species did not clump together but were dispersed throughout the clusters. This configuration of forb species produces a pattern where nearest neighbours of individual plants are likely to be plants of several other species, i.e. the individual plant is experiencing interspecific competition as opposed to intraspecific competition.

In Figures 5.8, 5.9 and 5.10 photographs of trays that were either controls or treated with $2.0gm^{-2}$ or $4.0gm^{-2}$ fungicides are shown. It can be seen from the Figures that the control trays contained forbs and the trays treated with $4.0gm^{-2}$ fungicide contained less forbs and more grasses.

Tray 2A a control tray photographed in April 2008

Tray 2A a control tray photographed in November 2008

Figure 5.8 - A control tray photographed in April and November 2008

Tray 5A treated with 2.0gm^{-2} Iprodione photographed in April 2008

Tray 5A treated with 2.0gm^{-2} Iprodione photographed in November 2008

Figure 5.9 - A Tray treated with 2.0gm^{-2} Iprodione photographed in April and November 2008

Tray 16A. treated with 4.0gm^{-2} Iprodione photographed April 2008

Tray 16A. treated with 4.0gm^{-2} Iprodione photographed November 2008

Figure 5.10 – A Tray treated with 4.0gm^{-2} Iprodione photographed in April and November 2008

In Figure 5.8 the forbs in a control tray 2A photographed in April 2008 had increased in size from about 1cm in diameter at the start of the trial in January to be typically 2 – 4cm

in diameter. In November, 300 days from the start of the trial, the forbs were still of a similar size to that observed in April with many different species present.

In Figure 5.9 the forbs in tray 5A treated with 2.0gm^{-2} fungicide and photographed in April 2008 were typically 2 – 6cm in diameter. In November when photographed it was noticeable that the number of plants in the tray had decreased from April 2008 and tended to be larger at typically 4 – 8 cm in diameter.

In Figure 5.10 the forbs in tray 16A treated with 4.0gm^{-2} fungicide and photographed in April 2008 were typically 4 – 8 cm in diameter and about 90% of the forb plants present were from one species (*Filipendula vulgaris*). When the tray was photographed again in November 2008 *F. vulgaris* was no longer present in the tray which consisted almost exclusively of grasses, of which *Bromus erectus* was prominent, and sedges.

5.4.3.2 Species presence and abundance

The data obtained from recording the presence and number of each forb species present at the start of the trial in January 2008, November 2008 and when the turf in the trays was broken up for root sampling in January 2009 are given in Tables 5.9, 5.10 and 5.11. Table 5.9 contains the data for the trays designated as controls and Tables 5.10 and 5.11 data for trays treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide respectively. Examination of Tables 5.9, 5.10 and 5.11, shows that a total of 29 different forb species were observed over the period of the trial. At the start of the trial a maximum of 47 individual plants and a minimum of 18 were observed in individual trays. The maximum number of forb species present was 16 and the minimum 8. Details of the forbs found in individual trays can be found in the Tables.

Table 5.9 - The number of plants of each species present in control trays

Key. A.C – *Asperula cynanchica*. C.E – *Centaurium erythraea*. C.S. – *Cirsium species*. F.V – *Filipendula vulgaris*. G.M – *Galium mollugo*. H.P – *Hieracium pilosella*. H.C – *Hippocrepis csmosa*. . H.Pe – *Hypericum perforatum* L.H – *Leontodon hispidus*. L.V – *Leucanthemum vulgare*. L.C - *Lotus corniculatus*.. P.O – *Phyteuma orbiculare*. P.S – *Pimpinella Saxifrage*. P.L – *Plantago lanceolata*. P.M – *Plantago media.* P.C – *Polygala calcarea*. P.V. *Polygala vulgaris*. Pr.V – *Prunella vulgaris*. R.B – *Ranunculus bulbosus*. S.M – *Sanguisorba minor*. S.J – *Senecio jacobaea*. S.P – *Succisa pratensis*. T.P – *Thymus praecox*. Tr.P – *Trifolium pratense*. V.S. – *Viola species*

January 2008	A.C	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	L.S	L.V	L.C	O.R	P.O	P.L	P.M	P.C	P.V	Pr.V	R.B	S.M	S.C	S.J	S.O	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 2	2	0	0	10	1	4	4	0	1	3	0	0	0	1	0	0	1	0	1	2	0	0	0	2	5	2	2	**41**	**15**
Tray 7	0	2	0	6	1	2	1	0	2	3	0	0	0	3	0	0	0	3	5	0	0	0	0	0	1	1	0	**30**	**12**
Tray 9	0	0	3	5	0	0	0	0	2	1	0	0	0	0	2	2	0	0	0	2	0	1	0	0	0	0	0	**18**	**8**
Tray 12	3	0	1	3	0	8	0	0	2	0	0	0	0	2	0	0	2	0	8	2	0	0	0	2	0	1	2	**34**	**12**
Tray 15	10	1	1	1	0	4	2	0	1	1	8	0	0	0	0	5	0	0	1	3	0	0	0	0	2	0	0	**40**	**13**
Total	**15**	**3**	**5**	**25**	**2**	**16**	**7**	**0**	**8**	**8**	**8**	**0**	**0**	**6**	**2**	**7**	**3**	**3**	**14**	**9**	**0**	**1**	**0**	**4**	**8**	**4**	**4**	**163**	**60**
																										Mean		**32.6**	**12**
																										± 1 S.E		**4.17**	**1.14**

November 2008	A.C	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	L.S	L.V	L.C	O.R	P.O	P.L	P.M	P.C	P.V	Pr.V	R.B	S.M	S.C	S.J	S.O	S.P	T.P	Tr.P	V.S	Total Forbs	Total Species
Tray 2	0	1	1	9	12	5	2	0	0	8	1	0	0	0	0	0	1	0	1	6	0	0	1	1	7	1	0	**57**	**15**
Tray 7	1	7	0	8	10	1	0	0	0	4	4	1	0	1	0	0	0	0	0	3	0	2	1	1	3	2	1	**52**	**16**
Tray 9	0	9	0	7	2	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	**22**	**5**
Tray 12	0	4	0	3	0	22	0	0	0	0	1	0	2	0	0	0	0	1	0	3	0	0	1	0	2	0	11	**50**	**10**
Tray 15	5	3	1	1	0	5	1	0	1	6	4	0	0	0	0	1	0	0	0	2	0	0	1	1	0	0	4	**36**	**14**
Total	**6**	**24**	**2**	**28**	**24**	**33**	**3**	**0**	**1**	**21**	**10**	**1**	**2**	**1**	**0**	**1**	**1**	**2**	**1**	**14**	**0**	**2**	**4**	**3**	**12**	**3**	**16**	**217**	**60**
																										Mean		**43.4**	**12**
																										± 1 S.E		**6.38**	**2.02**

January 2009	A.C	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	L.S	L.V	L.C	O.R	P.O	P.L	P.M	P.C	P.V	Pr.V	R.B	S.M	S.C	S.J	S.O	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 2	0	3	0	16	2	4	0	0	0	10	0	0	0	0	0	0	0	0	0	5	0	0	0	5	3	0	1	**49**	**9**
Tray 7	0	12	0	10	4	2	0	0	0	4	5	0	0	1	0	0	0	0	0	4	0	1	0	0	6	3	3	**55**	**12**
Tray 9	0	3	0	7	0	0	0	1	0	2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	**15**	**6**
Tray 12	0	4	0	2	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	9	**36**	**5**
Tray 15	0	4	0	0	0	6	0	0	0	5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	**18**	**6**
Total	**0**	**26**	**0**	**35**	**6**	**29**	**0**	**1**	**0**	**21**	**5**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**1**	**0**	**5**	**10**	**3**	**14**	**173**	**38**
																										Mean		**34.6**	**7.6**
																										± 1 S.E		**8.02**	**1.29**

Table 5.10 - The number of plants of each species present in trays treated with 2.0gm^{-2} Iprodione

Key. A.C – *Asperula cynanchica*. C.N – *Centaurea nigra*. C.E – *Centaurium erythraea*. C.S. – *Cirsium species*. F.V – *Filipendula vulgaris*. G.M – *Galium mollugo*. H.P – *Hieracium pilosella*. H.C – *Hippocrepis comosa*. H.Pe – *Hypericum perforatum* L.H - *Leontodon hispidus*. L.V - *Leucanthemum vulgare*. L.C- *Lotus corniculatus*. O.R – *Ononis repens*. P.O – *Phyteuma orbiculare*. P.S – *Pimpinella saxifraga*. P.L – *Plantago lanceolata*. P.M – *Plantago media.* P.C – *Polygala calcarea*. P.V. *Polygala vulgaris* . R.B – *Ranunculus bulbosus*. S.M – *Sanguisorba minor*. S.S – *Scabiosa columbaria*. S.J – *Senecio jacobaea*. S.O - *Sonchus oleraceus*. S.P – *Succisa pratensis*. T.P – *Thymus praecox*. Tr.P – *Trifolium pratense*. V.S. *Viola species.*

January 2008	A.C	C.N	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LH	L.V	L.C	O.R	P.O	P. S	P.L	P.M	P.C	P.V	R.B	S.M	S.C	S.J	S.O	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 3	0	0	0	0	8	0	1	0	0	1	0	0	0	0	1	4	0	0	0	6	5	0	0	0	2	0	5	0	**33**	**9**
Tray 5	0	0	1	0	0	0	5	1	0	1	0	0	0	0	1	2	1	0	0	4	4	0	0	0	10	0	2	2	**34**	**12**
Tray 8	6	0	1	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	8	2	0	0	0	3	1	0	0	**25**	**8**
Tray 10	1	0	0	2	5	1	2	0	0	2	3	0	0	0	0	6	2	1	1	3	3	0	0	0	3	6	0	1	**42**	**16**
Tray 14	3	0	0	2	4	3	1	0	0	3	0	0	0	0	1	2	1	0	0	10	3	0	0	0	0	0	0	0	**33**	**11**
Total	**10**	**0**	**2**	**4**	**17**	**5**	**9**	**4**	**0**	**7**	**3**	**0**	**0**	**0**	**3**	**14**	**4**	**1**	**1**	**31**	**17**	**0**	**0**	**0**	**18**	**7**	**7**	**3**	**167**	**56**
																												Mean	**33.4**	**11.2**
																												± 1 S.E	**2.69**	**1.39**

November 2008	A.C	C.N	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LH	L.V	L.C	O.R	P.O	P.S	P.L	P.M	P.C	P.V	R.B	S.M	S.C	S.J	S.O	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 3	0	1	0	0	9	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0	0	0	7	0	0	2	**26**	**8**
Tray 5	1	0	0	0	1	5	4	0	0	0	0	0	0	2	0	2	0	0	0	0	2	0	0	0	10	0	0	1	**28**	**9**
Tray 8	0	2	0	0	0	12	1	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2	0	2	**24**	**8**
Tray 10	0	0	0	0	7	0	0	0	0	1	6	0	0	0	0	1	1	0	0	0	3	0	0	0	1	1	0	1	**22**	**9**
Tray 14	1	0	0	0	8	3	0	0	0	0	2	1	0	2	2	0	0	0	0	0	3	0	0	0	0	0	0	1	**23**	**9**
Total	**2**	**3**	**0**	**0**	**25**	**21**	**6**	**0**	**0**	**1**	**10**	**1**	**0**	**4**	**2**	**4**	**1**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**20**	**3**	**0**	**7**	**123**	**43**
																											Mean		**24.6**	**8.6**
																											± 1 S.E		**1.08**	**0.25**

January 2009	A. C	C. N	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LH	L.V	L.C	O.R	P.O	P.S	P.L	P.M	P.C	P.V	R.B	S.M	S.C	S.J	S.O	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 3	0	0	0	4	12	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0	0	6	0	0	0	**27**	**7**
Tray 5	0	0	4	0	2	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	12	0	0	0	**24**	**5**
Tray 8	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0	1	0	0	0	3	3	0	1	**14**	**7**
Tray 10	0	0	0	0	15	1	0	0	0	2	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**25**	**5**
Tray 14	0	0	1	2	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	**19**	**5**
Total	**0**	**0**	**5**	**6**	**43**	**1**	**4**	**0**	**0**	**2**	**9**	**1**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**3**	**0**	**1**	**0**	**24**	**3**	**0**	**1**	**109**	**29**
																											Mean		**21.8**	**5.8**
																											± 1 S.E		**2.35**	**0.49**

Table 5.11 - The number of plants of each species present in trays treated with 4.0gm^{-2} Iprodione

Key. A.C – *Asperula cynanchica*. C.E – *Centaurium erythraea*. C.S. – *Cirsium species*. F.V – *Filipendula vulgaris*. G.M – *Galium mollugo*. H.P – *Hieracium pilosella*. H.C – *Hippocrepis comosa*. H.Pe – *Hypericum perforatum* L.H - *Leontodon hispidus*. L.V - *Leucanthemum vulgare* L.C - *Lotus corniculatus*. O.R – *Ononis repens*. P.O – *Phyteuma orbiculare*. P.S – *Pimpinella saxifraga*. P.L – *Plantago lanceolata*. P.M – *Plantago media.* P.V - *Polygala vulgaris*. Pr.V – *Prunella vulgaris*. R.B – *Ranunculus bulbosus*. S.M – *Sanguisorba minor*. S.J – *Senecio jacobaea*. . S.P – *Succisa pratensis*. T.P – *Thymus praecox*. Tr.P – *Trifolium pratense*. V.S. *Viola species*

January 2008	A.C	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LH	L.V	L.C	O.R	P.O	P.S	P.L	P.M	P.V	Pr.V	R.B	S.M	S.J	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 1	1	0	0	2	0	3	2	0	2	1	0	0	0	0	2	0	1	0	1	6	0	2	3	0	0	**26**	**12**
Tray 4	0	1	0	3	0	0	6	0	0	2	0	0	0	0	0	1	0	0	6	2	0	4	4	0	1	**30**	**10**
Tray 11	8	0	0	5	1	2	0	0	4	0	1	0	0	1	1	1	0	0	7	2	0	3	3	0	1	**43**	**14**
Tray 13	1	1	1	4	0	12	0	0	2	0	0	0	0	0	1	1	1	0	5	16	1	0	1	0	0	**47**	**13**
Tray 16	5	1	0	8	0	1	4	0	5	0	0	0	0	0	2	0	0	1	1	2	0	1	5	1	0	**37**	**13**
Total	**15**	**3**	**1**	**22**	**1**	**16**	**10**	**0**	**13**	**3**	**1**	**0**	**0**	**1**	**6**	**3**	**2**	**1**	**20**	**28**	**1**	**10**	**16**	**1**	**2**	**183**	**62**
																									Mean	**36.6**	**12.2**
																									± 1 S.E	**3.91**	**0.68**

November 2008	A.C	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LS	L.V	L.C	O.R	P.O	P.S	PL	P.M	P.V	Pr.V	R.B	S.M	S.J	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 1	0	0	3	0	1	4	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	**14**	**6**
Tray 4	0	5	0	6	5	0	0	0	0	4	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	**22**	**6**
Tray 11	0	3	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	1	**13**	**6**
Tray 13	0	1	1	5	0	13	0	3	0	0	1	0	0	0	0	0	0	0	0	3	1	9	0	0	0	**37**	**9**
Tray 16	0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	5	0	0	0	**9**	**3**
Total	**0**	**9**	**4**	**19**	**6**	**17**	**0**	**3**	**0**	**6**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**8**	**2**	**17**	**0**	**0**	**1**	**95**	**30**
																									Mean	**19.0**	**6.0**
																									± 1 S.E	**4.97**	**0.95**

January 2009	A.C	C.E	C.S	F.V	G.M	H.P	H.C	H.Pe	LH	L.V	L.C	O.R	P.O	P.S	P.L	P.M	P.V	Pr.V	R.B	S.M	S.J	S.P	T.P	Tr.P	V.S	**Total Forbs**	**Total Species**
Tray 1	0	0	1	0	0	10	0	0	0	4	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	**18**	**6**
Tray 4	0	5	0	10	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**17**	**3**
Tray 11	0	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	**6**	**3**
Tray 13	0	1	1	6	0	17	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	4	0	0	0	**34**	**7**
Tray 16	0	1	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	**7**	**4**
Total	**0**	**9**	**2**	**21**	**0**	**27**	**0**	**3**	**0**	**7**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**2**	**2**	**7**	**1**	**0**	**0**	**82**	**23**
																									Mean	**16.4**	**4.6**
																									± 1 S.E	**5.05**	**0.82**

In Table 5.9 it can be seen that the mean number of forbs present in control trays at the start of the trial in January 2008 was 32.6 ± 4.17. In November 2008 this had risen to 43.4 ± 6.38 and by January 2009 had reduced to 34.6 ± 8.02. The mean number of forb species present in January 2008 was 12 ± 1.14 with a similar number 12 ± 2.02 recorded in November 2008 and in January 2009 mean species present had dropped to 7.6 ± 1.29. The mean forbs and mean species recorded at the three dates were tested for significant difference using a one-way ANOVA. The only significant difference was between forb species present in January 2008 and January 2009 ($p < 0.05$).

Reference to Table 5.10 shows that the mean number of forbs presents in trays treated with 2.0gm^{-2} Iprodione in January 2008 was 33.4 ± 2.69 and by November 2008 this had fallen to 24.6 ± 1.08 and in January 2009 to 21.8 ± 2.35. The mean number of forb species present in January 2008 was 11.2 ± 1.39 which by November 2008 had decreased to 8.6 ± 0.25 and by January 2009 to 5.8 ± 0.49. The mean forbs and mean species recorded at the three dates for the 2.0gm^{-2} treatment were tested for significant difference using a one-way ANOVA. The only significant difference was between mean forbs present in January 2008 and mean forbs present in November 2008 ($p < 0.05$)

In Table 5.11 it can be seen that the mean number of forbs presents in trays treated with 4.0gm^{-2} Iprodione in January 2008 was 36.6 ± 3.91 which fell to 19.0 ± 4.97 by November 2008 and to 16.4 ± 5.05 by January 2009. The mean number of forb species present in January 2008 was 12.2 ± 0.68 falling to 6.0 ± 0.95 in November 2008 and 4.6 ± 0.82 in January 2009. The mean forbs and mean species recorded at the three dates for the 4.0gm^{-2} treatment were tested for significant difference using a one-way ANOVA. Significant differences were found between both mean forbs and mean forb species present in January 2008 and mean forbs and mean forb species present in trays in November 2008 ($p < 0.05$).

5.4.3.3 The 25 random point surveys

The results from the 2008 laboratory trial show strong similarities to those of the 2007 trial, in particular the response of *B. pinnatum.* As in the 2007 trial there was a graduated reduction in the abundance as measured by the point count of *B. pinnatum* with increasing dose rate of Iprodione (Figure 5.11).

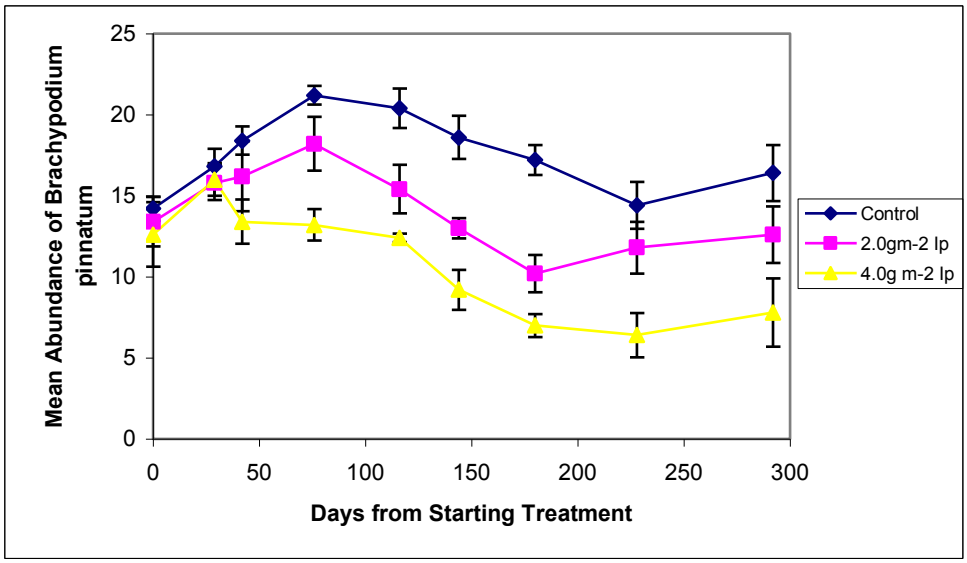


Figure 5.11 - Mean (n = 5 ± 1 S.E) abundance of *Brachypodium pinnatum* with different doses of Iprodione over a period of 290 days

It can be seen from Figure 5.11 that, as in the 2007 trial (Figure 5.4), the abundance of *B. pinnatum* flattens out after approximately 200 days. It can also be seen for the 2.0gm^{-2} fungicide treatments that after approximately 300 days the relative abundance (point count) was very similar for the 2007 and 2008 trials. The strong consistency between the two trials increases the confidence that the observations represent a genuine effect. Statistical testing using a one-way ANOVA showed significant difference in mean abundance between controls and the 2.0gm^{-2} and 4.0gm^{-2} treatments. Comparing the means of the controls and the 2.0gm^{-2} fungicide treatment showed a significant difference between 120 and 180 days from start of treatment ($p < 0.05$). Comparing the means of controls with those of the 4.0gm^{-2} fungicide treatment showed significant differences 80 days after the start of the treatment through to the end of the trial at 290 days ($p < 0.01$).

In the 2008 trial the reduction in relative abundance of *B. pinnatum* was accompanied by an increase in the relative abundance of *B. erectus*. The increase in *B. erectus* with increasing dose rate of Iprodione is shown in Figure 5.12.

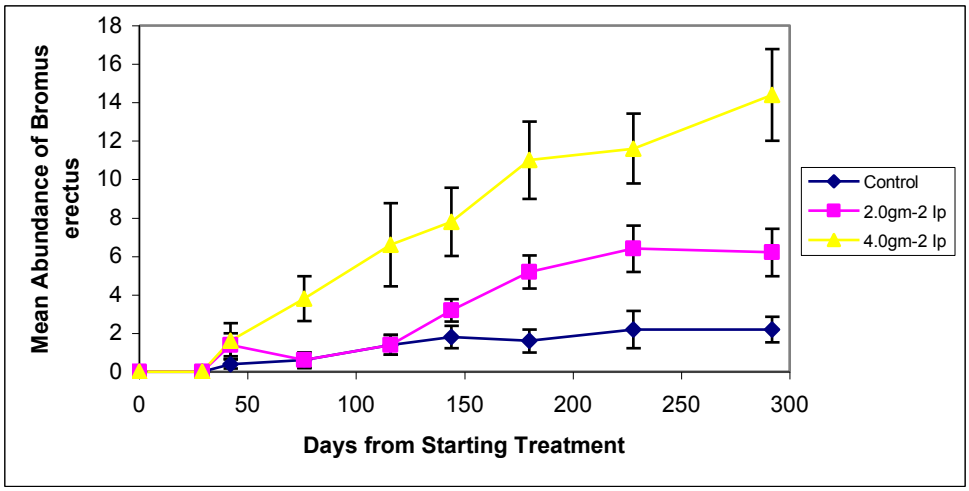


Figure 5.12 - Mean (n = 5 ± 1 S.E) abundance of *Bromus erectus* with different doses of Iprodione over a period of 290 days

It can be seen in Figure 5.12 that the trays treated with 2.0gm^{-2} show an increase in *B. erectus* after about 140 days from starting treatment and after 290 days mean abundance was a factor 3 greater than that in the controls. The trays treated with 4.0gm^{-2} Iprodione showed a stronger response than the 2.0gm^{-2} treatments with a clear separation from the control trays after 80 days and after 290 days mean abundance was 7 times higher than in the control trays. Significance testing using a one-way ANOVA showed significant difference between mean abundance in control trays and trays treated with 2.0gm^{-2} Iprodione after 180 days through to the conclusion of the trial at 290 days ($p < 0.05$). In the 4.0gm^{-2} treatment there was a significant difference in means from 80 days through to the conclusion of the trial ($p < 0.01$).

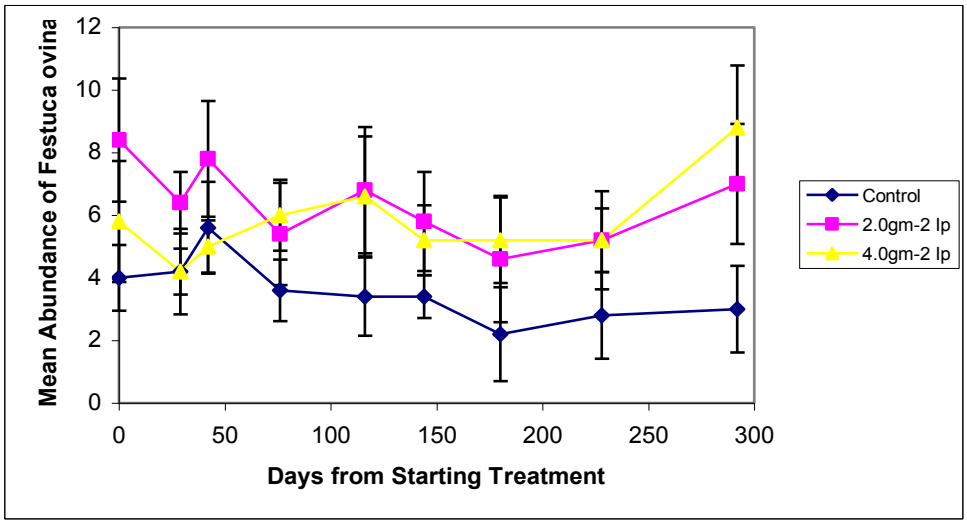


Figure 5.13 - Mean (n = 5 ± 1 S.E) abundance of *Festuca ovina* with different doses of Iprodione over a period of 290 days

Festuca ovina is the third dominant grass species found in chalk grassland and the effect of the application of Iprodione to trays in which *F. ovina* was present is shown in Figure 5.13. As was the case with *B. erectus, F. ovina* shows a positive response to the application of Iprodione at the 2.0gm^{-2} and 4.0gm^{-2} dose rates but to a much reduced extent. The means were tested for significant difference using a one-way ANOVA and the only significant difference was between the mean for control trays and those treated with 4.0gm^{-2} Iprodione after 290 days from start of treatment ($p < 0.05$).

Carex flacca is the most common sedge found in chalk grassland communities. In Figure 5.14 the mean abundance of *C. flacca* is shown for the trays designated to controls and the two fungicide treatments. It can be seen that in Figure 5.14 that there might have been a small increase after 290 days in the means of *C. flacca* in trays treated with 2.0gm^{-2} and 4.0gm^{-2} Iprodione, but the change was small. Testing the means using a one-way ANOVA showed no significant difference between the means from controls and those treated with 2.0gm^{-2} or 4.0gm^{-2} Iprodione.

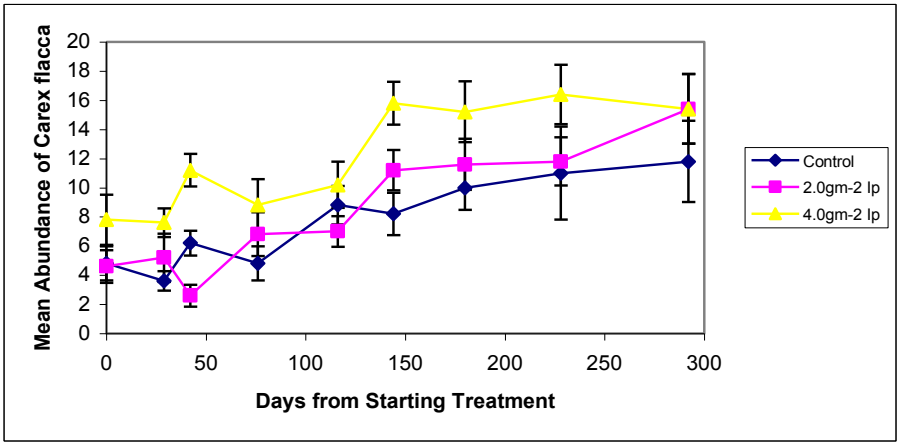


Figure 5.14 - Mean (n = 5 ± 1 S.E) abundance of *Carex flacca* with different doses of Iprodione over a period of 290 days

Examination of the response of individual forb species to the application of Iprodione at the dose rates of 2.0gm^{-2} and 4.0gm^{-2} showed most to respond in a negative manner the exceptions being *Cirsium* species and *Asperula cynanchica* which showed no change. However the low relative abundance (point count) of individual forb species, and large variations in presence between trays, resulted in no individual forb species showing significant difference in means between treatments when tested with using a one-way

ANOVA. *Hieracium pilosella* was typical and the variation of *H. pilosella* with time and treatment is shown in Figure 5.15 to illustrate the point.

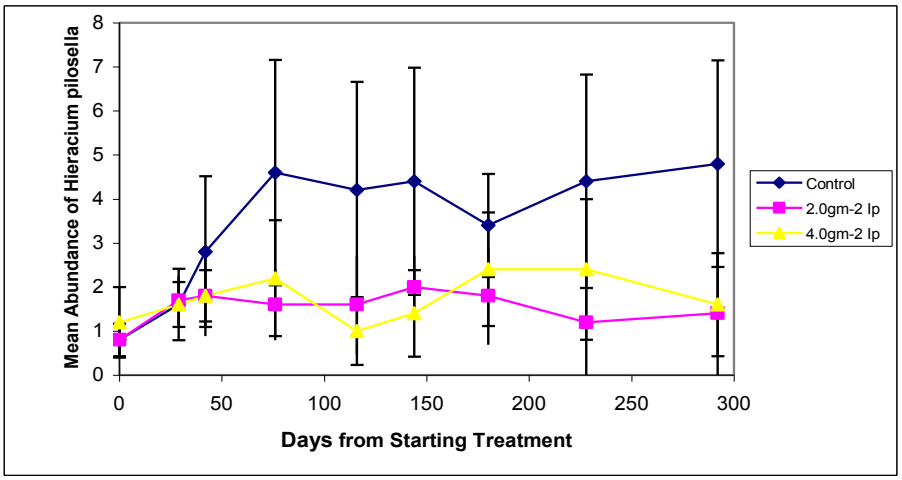


Figure 5.15 - Mean (n = 5 ± 1 S.E) abundance of *Hieracium pilosella* with different doses of Iprodione over a period of 290 days

Combining the changes occurring in the means of forb species as a group gives a stronger result than when forb species are considered individually. Figure 5.16 shows the changes occurring in total forb presence in trays treated as controls or with 2.0gm^{-2} or 4.0gm^{-2} Iprodione.

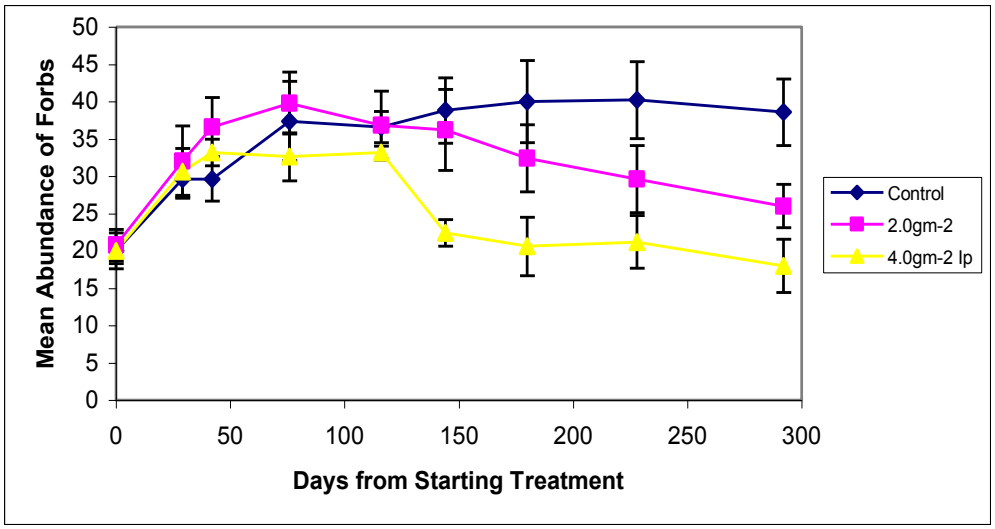


Figure 5.16 - Mean (n = 5 ± 1 S.E) abundance of forb species with different doses of Iprodione over a period of 290 days

In figure 5.16 it can be observed that the mean number of forb species in trays treated with 2.0gm^{-2} and 4.0gm^{-2} Iprodione start to decline relative to that in the control trays after 140

days and this trend continues up to 290 days from the start of treatment. Testing the means of the treatments using a one-way ANOVA shows that, compared to the means in control trays the means in the trays treated with 2.0gm^{-2} Iprodione showed a significant difference at 290 days ($p < 0.05$). The differences between the means in the control trays and those in the trays treated with 4.0gm^{-2} Iprodione were greater than those for the 2.0gm^{-2} treatment. There were significant difference starting at 140 days and continuing through to 290 days ($p < 0.01$).

In Figure 5.17 the results are presented for total grass abundance (point counts) and it can be seen that the trend is opposite to that observed for total forb abundance.

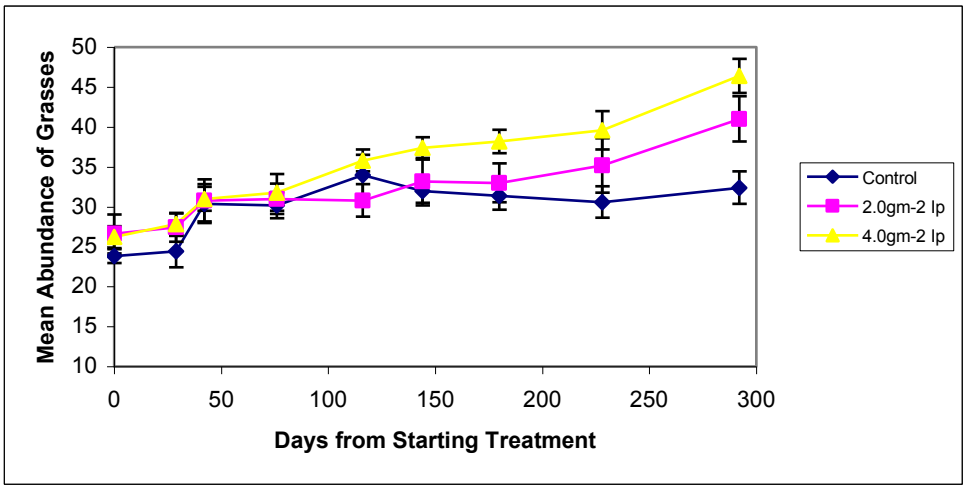


Figure 5.17 - Mean (n = 5 ± 1 S.E) abundance of grass species with different doses of Iprodione over a period of 290 days

It can be observed in Figure 5.17 that the means for the trays treated with fungicide start to separate from those of the controls at about 180 days. Testing for significant difference using a one-way ANOVA shows that for the trays treated with 2.0gm^{-2} Iprodione there is a significant difference in means from the controls at 290 days ($p < 0.05$). In the trays treated with 4.0gm^{-2} Iprodione significant differences from the controls were found earlier at 180 and 230 days ($p < 0.05$) and at 290 days ($p < 0.01$). The results for total abundance grass species are different to that of the 2007 trial (Figure 5.6) where in fungicide treatments up 2.0gm^{-2} showed a decline in grass species abundance. The 2008 trial shows two grass species *B. erectus* and to a lesser extent *F. ovina* increasing in abundance when high dose of Iprodione are applied and a third grass *B. pinnatum* declining in abundance. The

difference between the 2007 and 2008 results might reflect differences in starting abundance of these species.

Figure 5.18 shows the changes in the number of different species (forbs and grasses) in control trays and those treated with fungicide.

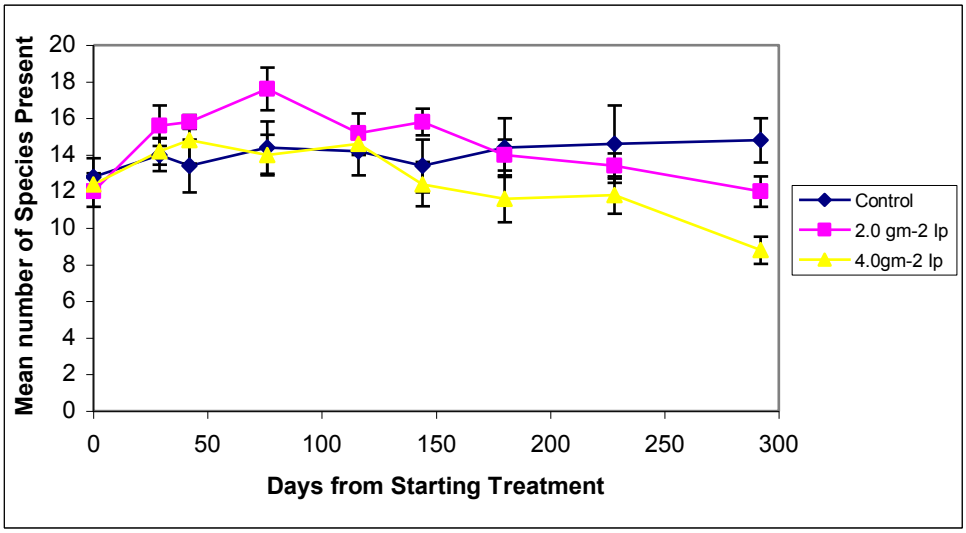


Figure 5.18 - Mean (n = 5 ± 1 S.E) number of species with different doses of Iprodione over a period of 290 days

The results from the 2008 trial are similar to those in 2007 trial (Figure 5.7) with the means for the 2.0gm^{-2} treatment separating from those of the controls at 290 days as do the means for the 4.0gm^{-2} treatment. Testing for significant change in means from fungicide treated trays compared to control s using a one-way ANOVA showed the only significant change to occur in the 4.0gm^{-2} Iprodione treatment at 290 days ($p < 0.01$).

5.4.3.4 Biomass

The weight of dry material collected in the 2008 trial is shown in Table 5.12. The total dry weight of biomass collected in the 2008 trial (290g) was 56% higher than that in the 2007 trial (186g) (Table 5.8). The time in the season producing the highest biomass was also different, that is July which produced the lowest biomass in the 2007 trial (Table 5.8). The increase in biomass in the 2008 trial is consistent with the positive increase in grass abundance in trays treated with Iprodione (Figure 5.17). The most probable explanation of

the increased biomass in the 2008 trial was the need for increased watering during a hot summer.

Table 5.12 - Summary of dry mass of material collected from turf in Trial 3 (2008)

Tray	Treatment	13/05/08 WT (g)	14/07/08 WT (g)	3/09/08 WT (g)	Total WT (g)	Mean WT (g)	± 1 S.E
2A	Control	3.66	5.31	2.57	11.54	16.1	1.67
7A	Control	4.74	10.64	5.39	20.77		
9A	Control	4.37	6.84	2.34	13.55		
12A	Control	6.16	4.83	5.00	15.99		
15A	Control	5.83	6.67	6.14	18.64		
3A	2.0g Ip	9.28	10.38	5.49	25.5	21.26	1.49
5A	2.0g Ip	5.05	7.82	4.35	17.22		
8A	2.0g Ip	6.45	9.98	5.01	21.44		
10A	2.0g Ip	5.63	9.25	4.05	18.93		
14A	2.0g Ip	6.57	10.3	6.55	23.42		
1A	4.0g Ip	5.36	8.12	6.21	19.69	20.65	0.44
4A	4.0g Ip	6.85	8.94	4.92	20.71		
11A	4.0g Ip	7.5	5.38	7.15	20.03		
13A	4.0g Ip	6.44	8.96	6.82	22.2		
16A	4.0g Ip	6.48	7.3	6.81	20.59		
	Total	**90.37**	**120.72**	**78.8**	**289.89**		

Statistical testing of the 2008 data using a one-way ANOVA gave significant difference between the controls and the 2.0gm^{-2} Iprodione treatment ($p < 00.5$).

5.4.3.5 Examination of roots for the presence of AMF

The roots of selected forbs, grasses and sedges, those present with sufficient abundance to allow samples to be taken from several plants, were microscopically examined using the methods described in section 5.3.7. The results are summarised in Table 5.13.

Table 5.13 - Percentage length of roots infected with AMF and percentage length containing vesicles

I – the percentage length of root infected with Hyphae/Arbuscules.

V – the percentage length of root containing vesicles. * - Centaurium erythraea plants 6 – 8 weeks old.

	Control	Control	2.0gm^{-2} Iprodione		4.0gm^{-2} Iprodione	
Species	**I**	**V**	**I**	**V**	**I**	**V**
Hieracium pilosella	81.5	28	57	19	44	22
Succisa pratensis	73	20	49	12	41	14
Leucanthemum vulgare	49.5	12	45	14	30.5	6
Filipendula vulgaris	16	7	11	8	6	5
*Centaurium erythraea**	18	0	Insufficient Material		11	0
Brachypodium pinnatum	48	14	27	5	25	4
Bromus erectus	11	3.5	8	4.5	10.5	5
Carex flacca	1	0.2	3	0.8	1	0

Examination of the data for the controls in Table 5.13 shows that the species can be divided into three groups: those high levels of root infection (>40%), *H. pilosella*, *Succisa pratensis*, *Leucanthemum vulgare* and *B. pinnatum*; those with low levels of infection, *F. vulgaris* and *B. erectus;* and those with very low levels of infection, *C. flacca*. The level of root infection in the forb *Centaurium erythraea* was relatively low but these were only juvenile plants. It can also be seen in Table 5.13 that the species with the highest levels of root infection also appear to have highest levels of vesicles present. This is confirmed by plotting percentage of root infection against percentage length of root containing vesicles (Figure 5.19). It can be seen in Figure 5.19 that there was a strong relationship between the percentage length of roots containing vesicles and total percentage of root infected (R-Sq > 80%). About one third of the root where infection was detected also contained vesicles.

The application of fungicide at the rate of 2.0gm^{-2} and 4.0gm^{-2} had the effect of reducing the percentage levels of root infection (Table 5.13). This was far more apparent in the species with high initial levels of root infection and less or not apparent in species with low initial levels of infection. This is illustrated in Table 5.14 where the percentage levels of AMF infection in plant species receiving the two fungicide treatments are compared with

the values measured in the roots of plants from the control trays (where the infection levels in roots from plants in control trays is set at 100%).

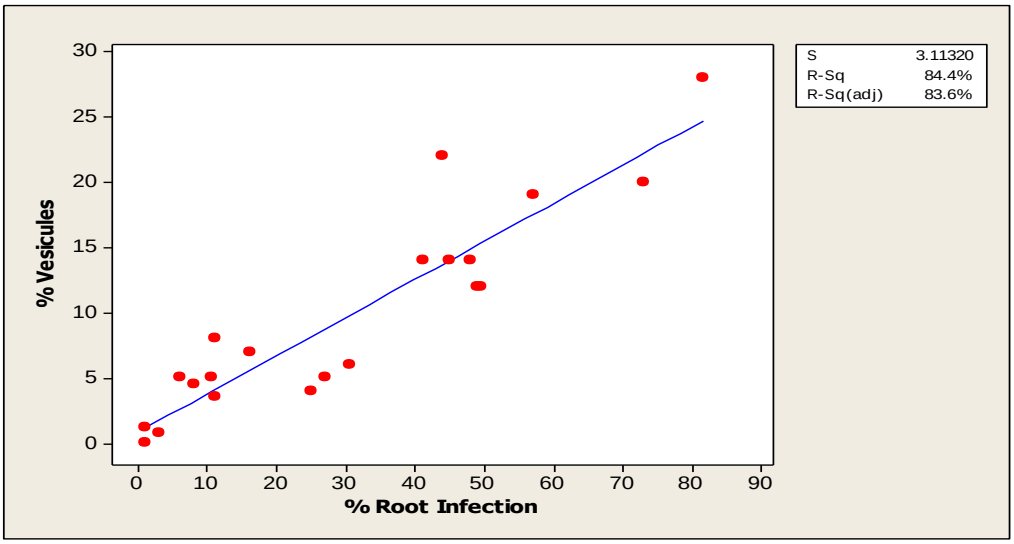


Figure 5.19 - Percentage root length containing vesicles Vs percentage root infection. (% Vesicles = 0.879 + .2923 x percentage length of root infection)

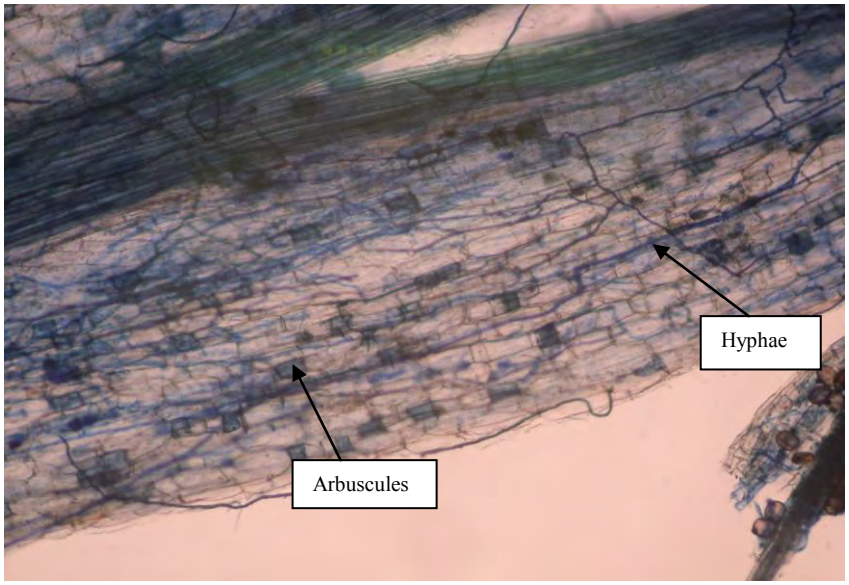
Table 5.14 - Percentage length of root infection in roots from the two treatments compared to the untreated controls

I – the percentage length of root infected with Hyphae/Arbuscules (i.e. 100% for controls)

* - *Centaurium erythraea plants 6 – 8 weeks old.*

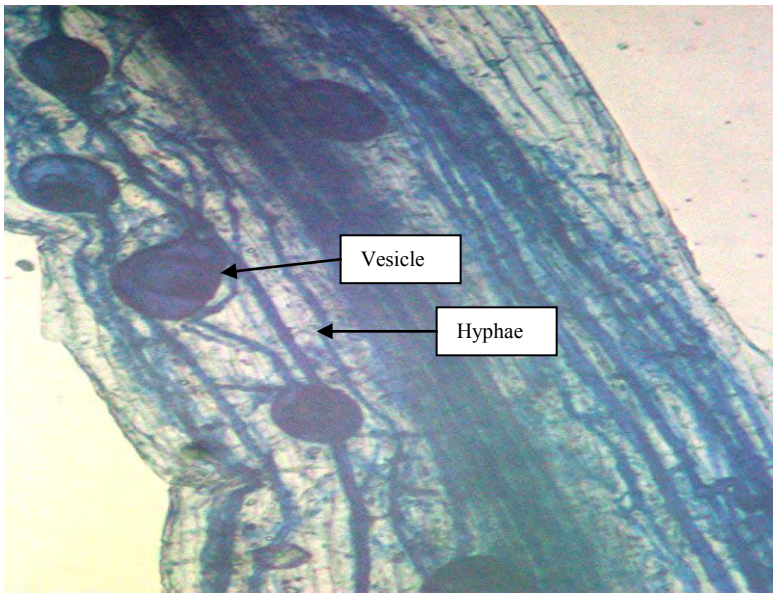
	Control	**2.0gm^{-2} Iprodione**	**4.0gm^{-2} Iprodione**
Species	**I**	**I**	**I**
Hieracium pilosella	100%	70%	54%
Succisa pratensis	100%	67%	56%
Leucanthemum vulgare	100%	91%	61%
Filipendula vulgaris	100%	69%	38%
*Centaurium erythraea**	100%		61%
Brachypodium pinnatum	100%	71%	66%
Bromus erectus	100%	73%	95%
Carex flacca	100%	300%	80%

In Table 5.14 it can be seen that in general the treatment with 4.0gm^{-2} Iprodione reduced the level of root infection to a greater extent than the 2.0gm^{-2} treatments. There was also a reduction in the presence of vesicles consistent with the reduction in the levels of root infection (Table 5.12).



x100

Figure 5.20 - Root of *H. pilosella*. Cleared and stained with ink and vinegar



X 100

Figure 5.21 - Root of *B. pinnatum*. Cleared and stained with ink/vinegar

In Figures 5.20 and 5.21 examples of root infection with AMF in a forb, *H. pilosella* and the grass *B. pinnatum* are shown.

5.5 Discussion

5.5.1 Introduction

An important choice at the start of the turf trials was which fungicide to use. The fungicide Benomyl has been used in field trials in the United States, United Kingdom and Australia (Hartnett and Wilson, 1999; Newsham *et al*. 1995; O' Connor *et al*. 2001) and Iprodione used in the United Kingdom (Gange *et al*. 1993; Ayres *et al*. 2006). Two factors in choosing Iprodione were that its use was allowed in the United Kingdom and it was commercially available. A third factor was that an evaluation of potential adverse phytotoxic effects of Iprodione had been previously carried out (Gange *et al*. 1992). This had established that with exception of one plant species seed germination was not affected by the application of Iprodione. Gange *et al*. (1992) concluded that differences found between controls and treated plots in an earlier trial (Gange *et al*. 1990) were the result of reduced AMF presence and not phytotoxic effects.

The laboratory turf trials, which aim to bridge the gap between research in artificial microcosms and field trials on natural plant communities, were implemented on the premise that it would be feasible to grow the turf for a minimum of one growing season under laboratory conditions. In this respect the trials have been successful, in the 2007 trial the turf was maintained in good condition for about 10 months and in the 2008 trial for about 1 year. The turf trials were probably closer to field trials than those in artificial microcosm as the turf collected should have contained most of the soil biota. In research performed in artificial microcosms the technique adopted was to add identified AMF species to sterilised soil and observe differences in plant characteristics (Grime *et al*. 1987; van der Heijden 2004; van der Heijden *et al.* 1998a).

In field trials the method used has been to reduce the activity of naturally occurring AMF species by the application of fungicide (Gange *et al*. 1993; Hartnett and Wilson, 1999; Daleo *et al*. 2008; Newsham *et al*. 1995; O' Connor *et al*. 2002; Karanika *et al*. 2008), the method adopted in these turf trials.

The relatively small dimensions of individual trays caused edge effects with more rapid drying of the soil and fewer nearest neighbours in turf at the boundaries of the tray.

Moreover the watering regime for the trays differed from that found in the field, with more intense watering required in the trays with more random patterns of rainfall and water retention occurring under field conditions. In the laboratory loss of water from the turf was through evaporation, while in the field both evaporation and drainage through the chalk substrate can occur. The trays in the laboratory were behind south facing windows and were likely to have experienced higher daytime temperatures and warmer nights than in the field. These conditions have the potential to produce a longer growing season than in the field.

5.5.2 Recording of forbs present in trays

The 25 random point surveys of the trays at regular intervals was the primary technique for collecting data on changes in species presence and abundance that could be tested statistically. However recording the presence, approximate size and distribution of forb species present at the start and conclusion of the trial supplemented with regular photographing of the trays has produced some important supplementary data.

Species rich chalk grassland is characterised as fine grained (Mitchley, 1990) i.e. there are many forbs of different species of small stature growing in close proximity. The observation that in control trays and those treated with $0.5gm^{-2}$ and $1.0gm^{-2}$ Iprodione the forbs present start small in January, increase in size during the growing season but remaining approximately uniform in their dimensions conforms to the expected pattern. The observation in the 2007 and particularly in the 2008 trial that in trays treated with $2.0gm^{-2}$ and $4.0gm^{-2}$ Iprodione, the forbs were small of stature at the start of the trials but increased in size and decreased in numbers as the trials progressed (see 5.4.3) does not conform to the expected growth pattern of chalk grassland, that is size inequality was developing. One possible interpretation of these observations is that in chalk grasslands AMF promote size equality in the forbs present by increasing the competitive ability of some mycorrhizal species.

There appears to be little or no information on the role of AMF in promoting or inhibiting plant size inequality in natural communities with many species present, but there is reported research for single species populations (Shumway and Kiode, 1995; Ayres *et al*. 2006; Koide and Dickie 2002). Shumway and Kiode (1995) report on a trial in which

Abutilon theophrasti was grown from seed at different densities with AMF present and absent. In both the presence and absence of AMF plant populations underwent self thinning until a stable density was reached, but overall there was no evidence that size inequality in populations where AMF was present differed from those where AMF was absent. In a review of research into the effects AMF on plant populations Koide and Dickie (2002) identify increased vigour in the offspring of plants infected with AMF (Heppell *et al*. 1998) as a means through which populations can be affected at least in the short term. Koide and Dickie (2002) found that low density populations tended to benefit more from the presence of AMF, than high density populations and this could lead to stabilisation; however there was no evidence that AMF reduced size inequality. In field and glasshouse trials Ayres *et al*. (2006) studied populations *P. lanceolata* with and without natural mycorrhizal colonisation. One of the findings of Ayres *et al*. (2006) was that competition tended to reduce size inequality although the degree depended on the presence of AMF. A possible explanation put forward by Ayres *et al*. (2006) was that that competition between plants was more symmetric with resources more evenly distributed. Increased symmetry of competition between individual plants will inhibit the growth of one plant compared to its neighbours thus reducing size inequality (Ayres *et al*. 2006). Thus the observations in the current turf trials appear broadly consistent with those of Ayres *et al.* (2006) on *P. lanceolata* populations but differ from those Shumway and Kiode (1995) and Koide and Dickie (2002).

5.5.3 The 25 random point surveys

5.5.3.1 Forb species as a group

In all three of the trials, the pilot study in 2006 and the two main studies in 2007 and 2008 (Figures 5.3, 5.5 and 5.16) forbs as a group showed a decrease in abundance, but only when Iprodione was applied at the higher dose rates. At the lower dose rates of $0.5gm^{-2}$ and $1.0gm^{-2}$ there was no significant change in forb abundance. At a dose rate of $2.0gm^{-2}$ in the 2007 trial (Figure 5.5) there was a reduction in forb abundance but it was not significant, whereas in the 2008 trial (Figure 5.16) there was a significant difference ($p < 0.05$) in forb abundance. At the $4.0gm^{-2}$ dose rate in the 2008 trial (Figure 5.16) there was a highly significant ($p < 0.01$) reduction in forb abundance compared to the controls after 140 days of treatment through to completion of the trial at 290 days. These higher dose rates of

Iprodione were also found to be necessary to produce change in community structure in the field trials (see Chapter 6). That higher fungicide dose rates were necessary to produce changes in the structure of chalk grassland communities is consistent with other studies. In a study of the influence of AMF on diversity in tallgrass prairie Smith *et al*. (1999) applied the fungicide Benomyl at a rate of 2.5gm^{-2} every 2 weeks during the growing season. In a study on the effects of AMF populations of *P. lanceolata,* Ayres *et al*. (2006) applied the fungicide Iprodione at the rate of 2gm^{-2} every 2 weeks between March and August and in a study of mountain grassland in Greece, Karanika *et al*. (2008) applied the fungicide Benomyl at a dose rate of 1.25 gm^{-2} every 14 days between April and July.

5.5.3.2 Individual forb species

The result of low species abundance and variations in populations between trays was that significant difference in individual forb abundance could not be demonstrated even at the higher fungicide dose rates. However in both the 2007 and 2008 trials there was a trend for most individual species to decrease in abundance in line with increased fungicide dose. In his paper discussing mechanisms promoting floristic diversity in calcareous grassland Grime (1990) identifies strong mycorrhizal dependence in *H. pilosella*, *C. erythraea*, *L. hispidus* and *Sanguisorba minor* as a factor which increases abundance in these species. In both the 2007 and 2008 turf trials all four of these species showed a decrease in relative abundance in trays where higher doses of fungicide were applied. Also in microcosm trials van der Heijden *et al*. (1998b) found the forbs *H. pilosella, S. minor, Lotus corniculatus* and *Trifolium pratense* produced higher biomass in the presence of AMF, a finding consistent with the results from the turf trials.

5.5.3.3 Grasses and sedges as a group

In the 2006 pilot trial there was a moderate increase in the abundance of grasses and sedges (Figure 5.2). In the 2007 trial (Figure 5.6) there was a significant decrease (p <0.01) in the abundance of grasses and sedges from all three fungicide treatments after 230 days but the difference was only significant for the 1.0gm^{-2} treatment after 300 days (p < 0.05). Examination of figure 5.6 shows that the significant differences between the fungicide treatments and the control were due to an increase in abundance in the control trays, while the abundance in the fungicide trays was essentially constant between 190 and 300 days. In the 2008 trial the difference between the abundance of grasses and sedges in the controls

and the fungicide treated trays was clearly apparent (Figure 5.17). For both the 2.0gm^{-2} and 4.0gm^{-2} treatments there were significant increases in the abundance of grasses and sedges after 290 days from the start of the treatment ($p < 0.05$ and $p < 0.01$). In the trays treated with 4.0gm^{-2} Iprodione a significant difference from the controls occurred 190 days from starting treatment ($p < 0.05$).

Although the data on the effect of the application of fungicide on grasses and sedges was not as unambiguous as that for forbs, the overall interpretation is that for higher dose rates of fungicide there was an increase in the abundance of grasses and sedges. In grassland habitats grasses tend to be the dominant species. Whether these grasses decline with the application of fungicide will depend upon their mycorrhizal dependence. In prairie grassland three of the four dominant grasses are strongly mycorrhizal and the application of fungicide results in a lowering in abundance of grasses and an increase in the abundance of forbs (Hartnett and Wilson, 1999). In chalk grassland, of the dominant grasses only *B. pinnatum* appears to be strongly mycorrhizal (van der Heijden, 2002). Thus the overall effect of applying fungicide at a dose rate 2.0gm^{-2} and above appears to be an increase in the abundance of grasses and sedges.

5.5.3.4 Individual grass and sedge species

The four most abundant grass and sedge species at the research site from which the turf was collected were *B. pinnatum, B. erectus, F. ovina* and *C. flacca.* These four species had differing responses to the application of fungicide at the higher dose rates of 2.0gm^{-2} and 4.0gm^{-2} which can be seen from the results of the 2008 trial. *B. pinnatum* (Figure 5.11) showed significant decreases in relative abundance at both the 2.0gm^{-2} and 4.0gm^{-2} fungicide dose levels ($p < 0.05$ and $p < 0.01$). For *B. erectus* the effect of fungicide application was the opposite to that observed for *B. pinnatum* with significant increases ($p < 0.05$ and $p < 0.01$) in abundance (figure 5.12). *F. ovina* (Figure 5.13) exhibited a small increase in abundance when treated with fungicide at the higher dose rates and *C. flacca* was unaffected by the application of fungicide (Figure 5.14).

The responses of these four species were consistent with the results from microcosm research on calcareous grassland species (van der Heijden *et al*. 1998a; van der Heijden *et al*. 1998b; van der Heijden 2004). In van der Heijden *et al*. (1998b)) the biomass of *B.*

pinnatum produced was shown to be significantly greater with AMF present compared to when it was absent and the weight of biomass produced was dependent on the AMF species present. *B. erectus* produced similar amounts of biomass both in the presence and absence of AMF (van der Heijden *et al*. 1998b) suggesting that *B. erectus* is non mycorrhizal although its seedlings respond to AMF (van der Heijden, 2004). Thus the increased abundance of *B. erectus* observed in the 2008 turf trial appears to result from a reduction in the competitive ability of more mycorrhizal dependent species present in the community. It appears that the main competitor of *B. erectus* in this chalk grassland community was probably *B. pinnatum.* Microcosm research in which *F. ovina* was present has been conducted both by van der Heijden *et al*. (1998a) and van der Heijden *et al.* (1998b) and Grime *et al*. (1987). In all three studies the presence of AMF was found to be mildly antagonistic to *F. ovina* and in an assessment of the mycorrhizal dependence forbs and grasses and sedges van der Heijden (2002) places *F. ovina* in the negative category with only *C. flacca* receiving a more negative score. Van der Heijden *et al*. (1998b) found *C. flacca* to be non mycorrhizal and not to have a symbiotic relationship with AMF a finding which appears to be consistent with the results from the current laboratory trials.

5.5.3.5 Number of species present in trays

The data for the 2007 and 2008 trials (Figures 5.7 and 5.18) show a reduction in the number of forb species present in the trays at the end of the trials (~ 300 days) in trays treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide ($p < 0.05$ and $p < 0.01$). However detailed examination of the species absent from individual trays showed that different species were absent in different trays. It appears that species with small populations were being eliminated from individual trays but no species were being eliminated from all trays.

5.5.3.6 Biomass

The biomass that was collected from trimming the trays during the 2007 and 2008 trial showed variation in both the total weight of material collected and the time in the season at which maximum biomass was produced. The only variable identified as different in the 2007 and 2008 trials was additional watering in 2008. This demonstrates how comparative small changes in environmental conditions can result in changes in community properties.

Statistical testing of the 2008 data using a one-way ANOVA gave significant difference between the controls and the 2.0gm^{-2} Iprodione treatment ($p < 0.05$). However this result needs to be treated with extreme caution due to the small sample sizes of only 5 trays for each treatment. Thus there are not strong grounds to suggest a departure from the general observation that in natural communities, the presence or absence of AMF does not affect overall productivity (O' Connor *et al*. 2001; Hartnett and Wilson, 1999; van der Heijden *et al*. 2006).

5.5.3.7 Examination of roots for the presence of AMF

Examination of the level of root infection in five forb species, two grasses and sedge shows a wide variation in the percentage root length infected with AMF (Table 5.13). The highest percentage infection (81%) was observed in *H. pilosella* and the lowest in *C. flacca* (1%) which is in strong agreement with the classification of mycorrhizal dependence by van der Heijden (2002) where *H. pilosella* was highly dependent and *C. flacca* the least of the species considered. The five forbs and the grass *B. pinnatum* had percentage levels of AMF infection of between 16% and 81% in roots from control trays, which was reduced on the application of fungicide at the dose rates of 2.0gm^{-2} and 4.0gm^{-2}. In Table 5.14 it can be seen that there was a graduated reduction in the percentage levels of root infection, with dose rates of 2.0gm^{-2} reducing root infection by about 30% compared to the control and the 4.0gm^{-2} dose rate by about 40%. Thus even at these high dose rates of Iprodione there was no indication that AMF infection in the roots of these species was close to being eliminated. The reduction of AMF in these species is broadly in line with the reduction of relative abundance observed in trays treated with 2.0gm^{-2} and 4.0gm^{-2} fungicides. The levels of root infection in *B. erectus* (11%) and *C. flacca* (1%) showed little change when treated with fungicide (Table 5.13). This is consistent with an increase in abundance of *B. erectus* and no change in abundance in C. *flacca*. In a microcosm experiment van der Heijden *et al.* (1998a) found a strong positive correlation between the amount of AMF root colonisation in *H. pilosella* and its biomass whereas for *B. erectus* there was no correlation.

The relationship between percentage length infected with AMF and percentage length of root containing vesicles (Figure 5.19) has several important implications. Firstly the percentage length of AMF infected root in which vesicles were present (approximately

30%) appears to be constant across the species examined. This result differs from the findings of van der Heijden *et al*. (1998a) studying *H. pilosella, B. erectus* and *F. ovina* in microcosms where the presence of vesicles varied significantly among different AMF species irrespective of plant species. In the microcosm experiments of van der Heijden *et al*. (1998a) there were only four AMF species present, whereas the turf in the current turf trial would be expected to have many AMF species present (Fitter, 2005; Dumbrell *et al*. 2011). The symbiotic relationship between plant and AMF may not be symmetric (Bever 2002; Bever *et al*. 2002) and optimising the production of vesicles will be of greater benefit to the AMF species. A possible explanation of the uniformity of vesicles presence across plant species is that in natural communities with high species richness many AMF species will also be present. In these conditions AMF species may colonise those plant species which can provide them with optimum benefit, that is a form of biotic niche differentiation by the AMF species (Fitter, 2005) was occurring. A second observation that although treating the plant community with 2.0gm^{-2} and 4.0gm^{-2} Iprodione reduced the length of root infected with AMF, the relationship between percentage root infection and the production of vesicles was not affected.

5.6 Conclusions

The overall aim of the laboratory turf trials was to examine the role of AMF in structuring chalk grassland communities. The first objective in support of the aim was to grow chalk grassland turf in the laboratory for a minimum of one growing season and this objective was achieved in the 2007 and 2008 turf trials. The second objective was to weaken AMF/ plant symbiosis by the regular application of the fungicide Iprodione and observe and measure changes to species presence and abundance. This objective was achieved where the treatment of the plant community with Iprodione at the rates 2.0gm^{-2} and 4.0gm^{-2} did weaken the symbiosis and significant changes in species abundance were measured. The third objective was to measure the presence of AMF in plant roots. This was achieved and showed that treating the plant with Iprodione at a dose rate of 2.0gm^{-2} and 4.0gm^{-2} reduced the percentage length of roots infected with AMF.

The major findings were:

1. That AMF may be important in maintaining size equality in the species rich, close packed structure of chalk grassland communities. Observations of increased plant size inequality in trays sprayed with 2.0gm^{-2} and 4.0gm^{-2} tend support this hypothesis but further and more detailed work is necessary for validation.

2. Measuring changes in species presence and abundance using 25 random point surveys has shown that weakening AMF/plant symbiosis by the application of Iprodione at a dose rate of 2.0gm^{-2} and 4.0gm^{-2} does change chalk grassland community structure. The abundance of forb species as a group was significantly reduced and the abundance of grass species as a group increased. Significant reductions in the abundance of the grass *B. pinnatum* were found accompanied by significant increases in the grass *B. erectus*.

3. Using the technique developed by Vierheilig *et al*. (1998) the roots of selected forbs and grasses were successfully stained to reveal the presence of AMF. Microscopic examination of the roots of the plant species from control trays showed levels of AMF infection consistent with their expected degree of mycorrhizal dependence. The application of Iprodione at dose rates of 2.0gm^{-2} and 4.0gm^{-2} lowered the levels of AMF infection in the roots of some plants but not in others. There was generally a good correspondence between changes in root infection and changes in species abundance. A relationship present across species was found between the percentage lengths of root infected with AMF and the presence of vesicles.

The laboratory turf trials were one component in the study of the role of AMF in structuring chalk grassland communities. The second component was the examination of the role of AMF in structuring chalk grassland in field trials. The results from the field trials are reported in Chapter 6.

Chapter 6 - Influence of AMF on Chalk Grassland Community Structure: Field Trials

6.1 Introduction

The structure of chalk grassland communities growing on the South Downs has been analysed and discussed in Chapter 4. The communities have been shown not to be random collections of species but to conform to repeatable and predictable patterns of association. Chalk grassland communities consist of a matrix of frequently occurring (core) species into which less common species fit. These patterns of association including nestedness and a frequency/abundance relationship are present at a fine scale. AMF/plant symbiosis was identified as a mechanism that could influence the structure of chalk grassland communities at a fine scale.

To investigate the role of AMF/plant symbiosis a series of laboratory turf trials (Chapter 5) were performed. In these trials turf taken from the field site was treated with the fungicide Iprodione and changes to community structure were observed and measured. The main findings were that when fungicide was applied at the higher dose rates of 2.0gm^{-2} and 4.0gm^{-2}, forbs as a group decreased significantly and grasses increased in abundance. Individual forb species decreased in abundance, although not significantly. The grass *Brachypodium pinnatum* decreased significantly in abundance and the grass *Bromus erectus* increased significantly.

In this chapter the results from field trials performed between May 2006 and October 2009 are reported. The field trials evaluate the influence of AMF/plant symbiosis on community structure in semi-natural chalk grassland. These field trials are important for several reasons. The laboratory turf trials provided insight into the role of AMF/plant symbiosis in shaping community structure in semi-artificial systems, but field trials allow an assessment to be made under natural conditions. Few field scale trials have been conducted in natural systems and there have been several calls for the number of this type of study to be increased (Vierheilig *et al*. 1998: Read 2002; Klironomos and Rillig 2008). A search of existing literature has revealed no trials involving the application of fungicide having been carried out on semi-natural calcareous plant communities including chalk grassland The only other semi-natural grassland communities studied with respect to the role of AMF/plant symbiosis on community structure were Prairie grassland in the USA (Hartnett

and Wilson, 1999) a mesotrophic hay meadow in the Czech Republic (Smilauer and Smilauerova, 2000) and acidic montane grassland in Greece (Karanika *et al.* 2008a).

6.2 The Research Site

The field trials were undertaken in the Castle Hill, National Nature Reserve complex on the South Downs. Two research sites were established on the south facing Newmarket Hill site (see Chapter 3 and Figure 3.3). The two sites chosen were at locations where preliminary surveys showed evidence of high species diversity and regular grazing. Site 1 was situated towards the bottom of the south facing slope (TQE 36936 N06859) and Site 2 was situated in a more elevated position half way up the south facing slope (TQE 36861 N 06865).

Figure 6.1 - Site 1 being surveyed in May 2008

There were minor site differences in the vegetation structure and height, with *B. pinnatum* the most abundant species and both were broadly representative of NVC type CG4a *Brachypodium pinnatum – Avenula pratensis - Thymus praecox* sub-community. Each site was enclosed with posts and wire (Figure 6.1) to prevent grazing by livestock, but grazing by rabbits was occurring. The size of the enclosures was approximately 10m x 5m.

6.3 The Aim of the Field Trials

The aim of the field trials was to examine the role of AMF in structuring semi-natural chalk grassland communities.

The methods and approach adopted aimed to;

1. Weaken AMF/plant symbiosis with graduated increases in the dose applied of the fungicide Iprodione over a four year period.

2. Measure species presence and abundance (mean percentage cover) at the start (May) and end (October) of each growing season.

3. Examine the roots of selected species from treated and untreated quadrats at the end of the trial and assess the differences in the percentage length of roots infected with AMF.

6.4 Methods and Materials

6.4.1 Establishing the quadrats and randomising the treatments

The field trial consisted of 32 one metre square quadrats at each of the two sites approximately 100m apart. Each quadrat was separated from the neighbouring quadrat by a minimum distance of 25cm to prevent cross contamination of treatments.

Site 1

17 **4.0gm^{-2}**	**18** **2.0gm^{-2}**	**19**	**20** **4.0gm^{-2}**	**1** **0.5gm^{-2}**	**2** **1.0gm^{-2}**	**3** **Control**	**4** **0.5gm^{-2}**
21 **2.0gm^{-2}**	**22**	**23** **4.0gm^{-2}**	**24** **2.0gm^{-2}**	**5** **1.0gm^{-2}**	**6** **Control**	**7** **0.5gm^{-2}**	**8** **1.0gm^{-2}**
25	**26** **4.0gm^{-2}**	**27** **2.0gm^{-2}**	**28** **4.0gm^{-2}**	**9** **Control**	**10** **0.5gm^{-2}**	**11** **1.0gm^{-2}**	**12** **Control**
29 **4.0gm^{-2}**	**30** **2.0gm^{-2}**	**31**	**32** **2.0gm^{-2}**	**13** **0.5gm^{-2}**	**14** **1.0gm^{-2}**	**15** **Control**	**16** **0.5gm^{-2}**

Site 2

17 **2.0gm^{-2}**	**18** **4.0gm^{-2}**	**19**	**20** **2.0gm^{-2}**	**1** **1.0gm^{-2}**	**2** **Control**	**3** **0.5gm^{-2}**	**4** **1.0gm^{-2}**
21 **4.0gm^{-2}**	**22**	**23** **2.0gm^{-2}**	**24** **4.0gm^{-2}**	**5** **Control**	**6** **0.5gm^{-2}**	**7** **1.0gm^{-2}**	**8** **Control**
25	**26** **2.0gm^{-2}**	**27** **4.0gm^{-2}**	**28** **2.0gm^{-2}**	**9** **0.5gm^{-2}**	**10** **1.0gm^{-2}**	**11** **Control**	**12** **0.5gm^{-2}**
29 **2.0gm^{-2}**	**30** **4.0gm^{-2}**	**31**	**32** **4.0gm^{-2}**	**13** **1.0gm^{-2}**	**14** **Control**	**15** **0.5gm^{-2}**	**16** **1.0gm^{-2}**

Figure 6.2 - Quadrat numbering plan and treatment rates with the fungicide Iprodione (gm^{-2}) for Site 1 and Site 2

Four rates of fungicide; 0.5gm^{-2}, 1.0gm^{-2}, 2.0gm^{-2} and 4.0gm^{-2}, were applied. In the first phase quadrats were treated with fungicide Iprodione at a dose rate of 0.5gm^{-2} and 1.0gm^{-2} over a two year period In the second phase new quadrats were treated with either 2.0gm^{-2} or 4.0gm^{-2}. again over two years The decision to move to higher dose rates of fungicide was based on the observations of minimal change in community structure at low dose rates and reports in the literature (Ayres *et al*. 2006) of higher dose rates of Iprodione being used

in field trials. At each site the quadrats for the controls and each of the four treatments were dispersed horizontally and vertical (Figure 6.2)

Table 6.1 - Dates at which fungicide was applied to quadrats and the prevailing weather conditions

Date	Weather Conditions	Dose Applied.
12/05/06	Hot and sunny	$0.5gm^{-2}$ and $1.0gm^{-2}$
30/06/06	Hot and sunny	$0.5gm^{-2}$ and $1.0gm^{-2}$
01/09/06	Dry, cool and windy	$0.5gm^{-2}$ and $1.0gm^{-2}$
23/10/06	Cloudy, rain ~ 45minutes after spraying	$0.5gm^{-2}$ and $1.0gm^{-2}$
08/03/07	Dry and sunny	$0.5gm^{-2}$ and $1.0gm^{-2}$
04/04/07	Dry, sunny and cool	$0.5gm^{-2}$ and $1.0gm^{-2}$
13/06/07	Dry, sunny and warm.	$0.5gm^{-2}$ and $1.0gm^{-2}$
06/8/07	Dry, sunny with high cloud.	$0.5gm^{-2}$ and $1.0gm^{-2}$
03/09/07	Dry, sunny with some cloud.	$0.5gm^{-2}$ and $1.0gm^{-2}$
05/10/07	Dry, sunny with cool wind.	$0.5gm^{-2}$ and $1.0gm^{-2}$
09/05/08	Dry and sunny	$2.0gm^{-2}$ and $4.0gm^{-2}$
13/06/08	Dry, overcast and cool	$2.0gm^{-2}$ and $4.0gm^{-2}$
08/07/08	Mainly dry. Two light showers	$2.0gm^{-2}$ and $4.0gm^{-2}$
08/08/08	Dry and cool	$2.0gm^{-2}$ and $4.0gm^{-2}$
26/09/08	Dry and sunny	$2.0gm^{-2}$ and $4.0gm^{-2}$
17/10/08	Dry and sunny	$2.0gm^{-2}$ and $4.0gm^{-2}$
09/03/09	Dry and sunny. Cold wind	$2.0gm^{-2}$ and $4.0gm^{-2}$
02/04/09	Dry and Sunny.	$2.0gm^{-2}$ and $4.0gm^{-2}$
24/04/09	Dry sunny and warm wind	$2.0gm^{-2}$ and $4.0gm^{-2}$
29/05/09	Dry sunny and warm wind	$2.0gm^{-2}$ and $4.0gm^{-2}$
09/07/09	Dry and overcast.	$2.0gm^{-2}$ and $4.0gm^{-2}$
06/08/09	Hot and humid.	$2.0gm^{-2}$ and $4.0gm^{-2}$
03/09/09	Cool and blustery.	$2.0gm^{-2}$ and $4.0gm^{-2}$

The relevant quadrats were sprayed with fungicide at approximately monthly intervals from March/May until September/October. Spraying was carried out with a 4.5l

„Hozelock“ (™) sprayer. The fungicide for each quadrat was dissolved in 1.5l of de-ionised water as per manufacture“s recommendation (Bayer Environmental Science, 2009). The control quadrats were sprayed with 1.5l of de-ionised water. Details of spraying dates and weather conditions are given in Table 6.1.

6.4.2 Surveying dates

The quadrats were surveyed in the first two weeks of May and the first two weeks of October over the four years 2006 – 2009. The control plots were surveyed over the four years of the trial. The quadrats treated with $0.5gm^{-2}$ and $1.0gm^{-2}$ fungicide were surveyed in 2006 and 2007 and in May 2008. The quadrats treated with $2.0gm^{-2}$ and $4.0gm^{-2}$ fungicide were surveyed in 2008 and 2009.

6.4.3 Surveying methods

Prior to visiting the sites to perform the survey individual marked sheets were produced for each quadrat to be surveyed. This standardised sheet (Appendix 1) listed the species found at Newmarket Hill in a comprehensive survey carried out in 1991(Steven and Muggeridge 1992). In addition, space was provided for other species found to be recorded. Columns were provided so that both presence and estimates of percentage abundance could be recorded.

Individual quadrats were surveyed by a team of two with expert knowledge of chalk grassland species identification. The method used was to identify all species present, and then to estimate by eye the percentage cover of individual species of grasses, sedges, forbs, lower species, litter and bare earth within each quadrat. Where the species were judged to be present with an abundance of 1-3%, an estimate of the number of individual plants present was also recorded within the categories few, several and many. For species with an estimated cover of 4% or greater only percentage cover was recorded. When all species within a quadrat had been assigned a percentage cover (abundance) value these were totalled with a pre-determined tolerance of 95- 105% set. Outside of this range individual species percentage cover was re-assessed.

6.4.4 Microscopic examination of roots for the presence of AMF

In October 2009, after the end of the experimental application of fungicide and monitoring, plants were removed from the field sites for examination. Plants were taken from quadrats designated as control, and those treated with either 2.0gm^{-2} or 4.0gm^{-2} fungicide. The species chosen for examination had an abundance level that allowed a minimum of five plants to be collected for the three treatments. The species chosen for examination were the grasses *B. pinnatum*, *B. erectus* and *Festuca ovina*, the sedge *Carex flacca* and the forbs *Hieracium pilosella*, *Succisa pratensis*, *Leontodon hispidus* and *Plantago media*. Collected plants were placed in labelled plastic bags, returned to the laboratory and kept refrigerated until the roots could be examined. This took place within a two week period.

The method used to clear, stain and microscopically examine the roots was that used in the laboratory turf trials and fully described in section 5.3.7. In comparison to the roots collected from the laboratory turf trials, roots from plants collected from the field were more fibrous and more difficult to stain. This observation is consistent with the findings of Gange *et al*. (1999) that data collected on AMF following staining can be very variable particularly for specimens collected from the field.

6.5 Analysis of Survey Data

After each of the May and October surveys the data for individual quadrats were collated to produce mean values of species presence and abundance for each of the treatments. At Site 1 there were five quadrats designated as controls, six treated with 0.5gm^{-2} fungicide, five with 1.0gm^{-2} fungicide, six with 2.0gm^{-2} fungicide and six with 4.0gm^{-2} fungicide (Figure 6.2). At Site 2 the allocation of quadrats was five controls, five to the 0.5gm^{-2} treatment and six to each of the 1.0gm^{-2}, 2.0gm^{-2} and 4.0gm^{-2} treatments (Figure 6.2).

Collectively there were ten control quadrats, eleven 0.5gm^{-2} quadrats, eleven 1.0gm^{-2} quadrats, twelve 2.0gm^{-2} quadrats and twelve 4.0gm^{-2} quadrats. In comparing species abundance (percentage cover) for the five treatments over time the data from Sites 1 and 2 were combined to provide a more robust mean.

6.6 Statistical Analysis

The survey data collected over the four year period of the trial were species presence and percentage cover within individual quadrats. These data were analysed using four different techniques to describe the effects of applying fungicide on community structure.

Each of the fungicide treatments was applied for two growing seasons. The $0.5gm^{-2}$ and $1.0gm^{-2}$ fungicide treatments started in May 2006 and finished in October 2007. The $2.0gm^{-2}$ and $4.0gm^{-2}$ fungicide treatments started in May 2008 and finished in October 2009. The mean percentage cover for the $0.5gm^{-2}$ and $1.0gm^{-2}$ fungicide treatments were compared to that of the controls in October 2007 using a one-way ANOVA and Tukey"s test for significance. Similarly mean percentage cover for the $2.0gm^{-2}$ and $4.0\ gm^{-2}$ treatments were compared to that for the controls in October 2009. Due to the potential cumulative effects of time and treatment, the data from controls and fungicide treated quadrats were also tested for significant differences using a General Linear Modelling (GLM) approach (Field, 2009).

The $2.0gm^{-2}$ and $4.0gm^{-2}$ treatments were identified as producing significant differences in species mean cover after two seasons of treatment. In the second analysis mean percentage cover of selected species treated with $2.0gm^{-2}$ and $4.0gm^{-2}$ fungicide was traced from the start of the trial in May 2008 through to the finish in October 2009. The data is presented graphically and the means from the fungicide treated quadrats compared to those from the controls using a one-way ANOVA and Tukey"s test for significance.

An assessment of the rank abundance of species was undertaken on the data collected from the quadrats treated with $2.0gm^{-2}$ and $4.0gm^{-2}$ fungicide. Data from control quadrats was also included to ensure that changes in rank order observed in the fungicide treated quadrats were the result of the weakening of AMF/ plant symbiosis. The structure of chalk grassland communities is determined by the relationship between the dominant and subordinate species present in the community. In this study performed using „Minitab" changes in rank order of dominant and subordinate species were traced from the start of the trial in May 2008, prior to the application of fungicide, through to the conclusion of the trial in October 2009.

Finally, an assessment of the quadrat data for the 35 most abundant species in the community was undertaken using Principal Component Analysis (PCA). PCA provided a multivariate method by which the species data could be examined with respect to the treatment that plots had received during the study. PCA is an ordination method that can be used to construct a series of artificial axes (typically three) which can be used to explain the maximum amount of variance with the fewest number of principal components (Waite, 2000).

PCA, facilitated assessment of the similarity or variation between quadrat data, for the 35 most abundant species. A PCA ordination score plot showed the relationship between treated plots on the basis of species abundance (Minitab v.15).

6.7 Results

The results presented here are divided into two sections. The first section considers the structure of the chalk grassland communities present within Sites 1 and 2 without the application of fungicide. This will be achieved by studying the data from the control quadrats over the four year period of the trial. Differences in species abundance in May and October are highlighted. Using the data from the control quadrats as a base line, changes to community structure resulting from the application of fungicide are then assessed. The second section will present the findings from the examination of the roots of plants collected from Sites 1 and 2 in October 2009. The data show changes to the percentage length of roots infected with AMF when the plants were treated with fungicide.

6.7.1 Results from field surveys

6.7.1.1 Determination of community structure in untreated control quadrats

Determination of the presence and abundance (mean percentage cover) of species present in untreated quadrats over the period of the trial showed that there were differences in species presence and mean percentage cover in the May and October surveys. There was also variation in presence and mean percentage cover between years. The importance of comparing changes resulting from the application of fungicide, with control data from the same point in the treatment cycle must be emphasised.

Table 6.2 - The 35 most abundant species placed into the frequency categories core, intermediate and scarce

(G) – denotes grass. *(S)* – denotes sedge *(F)* – denotes forb

Core	Intermediate	Scarce
Briza media (G)	***Asperula cynanchica (F)***	***Orchid spp (F)***
Bromus erectus (G)	***Brachypodium pinnatum (G***	***Polygala calcarea (F)***
Carex caryophyllea (S)	***Centaurium erythraea (F)***	
Carex flacca (S)	***Euphrasia officinalis agg (F)***	
Centaurea nigra (F)	***Filipendula vulgaris (F)***	
Cirsium acaule (F)	***Hippocrepis comosa (F)***	
Festuca ovina (G)	***Leucanthemum vulgare (F)***	
Galium mollugo (F)	***Phyteuma orbiculare (F)***	
Galium verum (F)	***Rhinanthus minor (F)***	
Hieracium pilosella (F)	***Succisa pratensis (F)***	
Leontodon hispidus (F)	***Viola riviniana (F)***	
Linum catharticum (F)		
Lotus corniculatus (F)		
Pimpinella saxifraga (F)		
Plantago lanceolata (F)		
Plantago media (F)		
Prunella vulgaris (F)		
Ranunculus bulbosus (F)		
Sanguisorba minor (F)		
Thymus praecox (F)		
Trifolium pratense (F)		
Viola hirta (F)		

Examination of the survey data collected over the four year period of the trial showed that a small number of species were present in all quadrats. These were the grasses *B. pinnatum*, *B. erectus* and *F. ovina* and the forb *L. hispidus*. Many other species were present in greater than 50% of quadrats, but often at low levels of mean percentage cover. It is probable that species with a high mean percentage cover will have a greater influence on community structure than those with low levels of cover. Thus the emphasis will be on analysing changes in species with higher levels of mean percentage cover. A total of 35 species (Table 6.2) were recorded with a mean percentage cover of greater than 0.1% over the course of the trial and it is the effect of fungicide treatment on these species that is considered in detail. Species present at mean percentage cover of less than 0.1% are listed in Table 6.3.

It can be seen in Table 6.2 that the 35 most abundant species at Sites 1 and 2 were a mixture of grasses, sedges and forbs at ratios approximate to those found in chalk grassland throughout Sussex. There were twice as many core species (22) as intermediate species (11) and only 2 scarce species. The ratio of core to intermediate to scarce species was might be expected at this scale, i.e. between the quadrat and site scales (see Chapter 4). Therefore the plant communities present at Sites 1 and 2 were typical of *Brachypodium pinnatum* dominated chalk grassland.

Table 6.3 - Alphabetical list of species with mean percentage cover of less than 0.1%

(*G*) – denotes grass *(S)* – denotes sedge (*F*) – denotes forb.

Achillea millifolium (F)	***Cirsium vulgare (F)***	***Origanum vulgare (F)***
Agrimonia eupatoria (F)	***Clinopodium vulgare***	***Picris hiericoides (F)***
Agrostis capillaris (G)	***Dactylis glomerata (G)***	***Plantago major (F)***
Anthylis vulneraria (F)	***Daucus carota (F)***	***Polygala vulgaris (F)***
Avenula pratensis (G)	***Danthonia decumbens (G)***	***Primula veris (F)***
Avenula pubescens (G)	***Galeopsis tetrahit (F)***	***Ranunculus acris (F)***
Bellis perennis (F)	***Gentianella amarella (F)***	***Rosa canina (F)***
Blackstonia perfoliata (F)	***Gentianella anglica (F)***	***Scabiosa columbaria (F)***
Carex panicea (S)	***Hypericum perforatum(F)***	***Senecio jacobaea (F)***
Carlina vulgaris (F)	***Knautia arvensis (F)***	***Sonchus oleraceous (F)***
Cerastium fontanum (F)	***Koeleria macrantha (G)***	***Stachys officinalis (F)***
Cirsium arvense (F)	***Linaria vulgaris (F)***	***Taraxicum officinalis (F)***
Cirsium dissectum (F)	***Medicago lupulina (F)***	***Trifolium repens (F)***
Cirsium eriophorum (F)	***Ononis repens (F)***	

6.7.1.2 Characteristics of control quadrats

Two important characteristics of chalk grassland communities are the presence and abundance of particular species. In the previous section species presence was detailed and here abundance, in the form of mean percentage cover, is also considered.

Table 6.4 - The mean percentage cover and rank order of species recorded in control quadrats in May surveys 2006 - 2009

May	May Mean	SE. Mean	St Dev	Rank
Brachypodium pinnatum	**18.44**	2.46	5.5	**1**
Bromus erectus	**15.4**	1.37	3.06	**2**
Festuca ovina	**12.6**	0.75	1.68	**3**
Cirsium acaule	**4.34**	0.52	1.67	**4**
Leontodon hispidus	**4.16**	0.75	1.68	**5**
Briza media	**3.6**	0.78	1.74	**7**
Carex flacca	**3.0**	0.65	1.45	**6**
Carex caryophyllea	**2.52**	0.58	1.31	**8**
Sanguisorba minor	**2.5**	0.24	0.53	**9**
Hieracium pilosella	**2.34**	0.25	0.56	**10**
Filipendula vulgaris	**2.02**	0.17	0.39	**11**
Succisa pratensis	**1.90**	0.39	0.88	**12**
Hippocrepis comosa	**1.88**	0.25	0.55	**13**
Plantago media	**1.84**	0.17	0.38	**14**
Lotus corniculatus	**1.74**	0.36	0.81	**15**
Centaurea nigra	**1.62**	0.3	0.67	**16**
Plantago lanceolata	**1.3**	0.7	0.16	**17**
Polygala calcarea	**1.22**	0.2	0.45	**18**
Asperula cynanchica	**1.02**	0.04	0.84	**19**
Phyteuma orbiculare	**0.92**	0.37	0.83	**20**
Thymus praecox	**0.9**	0.05	0.12	**21**
Ranunculus bulbosus	**0.82**	0.12	0.26	**22**
Leucanthemum vulgare	**0.68**	0.12	0.24	**23**
Rhinanthus minor	**0.66**	0.13	0.3	**24**
Viola hirta	**0.6**	0.16	0.35	**25**
Linum catharticum	**0.54**	0.16	0.36	**26**
Prunella vulgaris	**0.54**	0.16	0.37	**26**
Viola riviniana	**0.54**	0.07	0.17	**26**
Trifolium pratense	**0.46**	0.15	0.33	**29**
Pimpinella saxifraga	**0.44**	0.09	0.19	**30**
Centaurium erythraea	**0.36**	0.14	0.31	**31**
Orchid spp	**0.32**	0.1	0.23	**32**
Galium verum	**0.24**	0.13	0.29	**33**
Galium mollugo	**0.24**	0.04	0.09	**33**
Euphrasia officinalis agg	**Not recorded in May**			**35**

Mean percentage cover in control quadrats from the May (Table 6.4) and October (Table 6.5) surveys is presented for the period 2006 -2009.

Table 6.5 - The mean percentage cover and rank order of species recorded in control quadrats in October surveys over a four year period

October	October Mean	SE. Mean	St Dev	Rank
Brachypodium pinnatum	**23.55**	3.42	6.84	**1**
Bromus erectus	**16.17**	1.23	2.46	**2**
Festuca ovina	**10**	0.9	1.8	**3**
Leontodon hispidus	**5.68**	0.66	1.31	**4**
Carex flacca	**4.2**	0.25	0.5	**5**
Briza media	**3.98**	1.21	2.42	**6**
Sanguisorba minor	**3.3**	0.11	0.22	**7**
Cirsium acuale	**2.8**	0.15	0.29	**8**
Lotus corniculatus	**2.65**	0.33	0.66	**9**
Succisa pratensis	**2.48**	0.39	0.79	**10**
Hippocrepis comosa	**1.95**	0.43	0.86	**11**
Hieracium pilosella	**1.63**	0.39	0.78	**12**
Filipendula vulgaris	**1.4**	0.15	0.29	**13**
Plantago media	**1.33**	0.12	0.24	**14**
Plantago lanceolata	**1.1**	0.15	0.08	**16**
Asperula cynanchica	**1**	0.04	0.08	**17**
Thymus praecox	**0.95**	0.06	0.13	**17**
Phyteuma orbiculare	**0.9**	0.17	0.33	**18**
Trifolium pratense	**0.9**	0.06	0.12	**18**
Euphrasia officinalis agg	**0.9**	0.39	0.79	**18**
Polygala calcarea	**0.9**	0.04	0.08	**18**
Centaurea nigra	**0.85**	0.1	0.21	**22**
Ranunculus bulbosus	**0.8**	0	0	**23**
Viola riviniana	**0.8**	0.25	0.5	**23**
Pimpinella saxifraga	**0.63**	0.11	0.22	**25**
Leucanthemum vulgare	**0.6**	0.17	0.34	**26**
Linum catharticum	**0.6**	0.16	0.32	**26**
Viola hirta	**0.55**	0.21	0.42	**30**
Prunella vulgaris	**0.6**	0.25	0.5	**26**
Centaurium erythraea	**0.58**	0.21	0.43	**29**
Carex caryophyllea	**0.53**	0.53	1.05	**31**
Galium mollugo	**0.5**	0.08	0.16	**32**
Galium verum	**0.4**	0.04	0.08	**33**
Rhinanthus minor	**0.13**	0.06	0.13	**34**
Orchid spp	**0.1**	0.06	0.12	**35**

Examination of the mean percentage cover and rank order data showed that in both May (Table 6.4) and October (Table 6.5) the community was dominated by the three grasses, *B. pinnatum*, *B. erectus* and *F. ovina*. *B. pinnatum* had the highest mean percentage cover,

between 18% and 23%, *B. erectus* had a cover of 15% to 16 % and *F. ovina* a cover of 10% to 12% i.e. only small seasonal differences. *Briza media* and *C. flacca* were also present, although only at 3% to 4%. The sedge *Carex caryophyllea* was also present in May (at 2.5%) and in October (at 0.5%)

The most abundant forb present was *L. hispidus* which was strongly represented in both the May (Figure 6.4) and October (Figure 6.5) surveys with mean percentage cover values of 4% to 6%. *L. hispidus* was present in all quadrats surveyed. The dwarf thistle *Cirsium acaule* was also strongly represented with a mean cover of about 4% in May and 3% in October and was found in 95% of quadrats. There was a small cohort of forbs whose mean cover in both the May and October survey periods was in the range 2% to 3%. This included *Sanguisorba minor*, *Hieracium pilosella*, *Succisa pratensis* and *Lotus corniculatus*. These forbs were present in 80% - 85% of quadrats surveyed. The mean percentage cover data for May (Table 6.4) clearly shows that only eleven species had a cover of greater than 2% and this was reduced to ten in the October (Table 6.5) survey period.

A group of seven forbs were found to have a mean percentage cover of between 1% and 2% in both May (Figure 6.4) and October (Figure 6.5). These were *Filipendula vulgaris*, *Hippocrepis comosa*, *Plantago media*, *Plantago lanceolata*, *Centaurea nigra*, *Polygala calcarea* and *Asperula cynanchica*. These forbs were generally present in greater than 80% of quadrats, the exception being *A. cynanchica* which was present in about 95% of quadrats.

Nine forbs species were found to have abundance values of between 0.5% and 1% cover, i.e. *Phyteuma orbiculare, Thymus praecox, Ranunculus bulbosus, Leucanthemum vulgare, Rhinanthus minor, Viola hirta, Linum catharticum, Prunella vulgaris and Viola riviniana*. These forbs were present in some 50% of quadrats surveyed. The following forbs were shown to have abundance values of less than 0.5% cover including *Trifolium pratense, Pimpinella saxifrage, Centaurium erythraea, Orchid species, Galium verum, Galium mollugo and Euphrasia officinalis* agg. Moreover these forbs were only present in less than 50% of quadrats surveyed. E. officinalis agg was recorded as present in October but not in May. The majority of forbs showed only small changes in percentage cover between

May and October. Thus although mean percentage cover showed some variation between May and October the same 34 species were present in May and October.

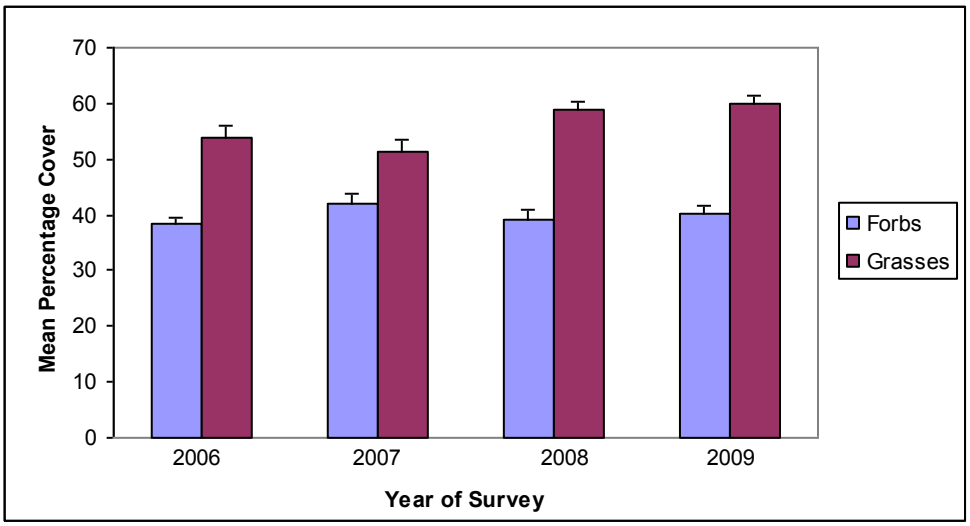


Figure 6.3 - Percentage cover of forbs and grasses/sedges in May surveys 2006 to 2009 of control quadrats (n = 10 ± S.E)

In Figures 6.3 and 6.4 the variation in total percentage cover for grasses/sedges over the four year period of the trial is presented from the data collected in the May and October surveys. For both May and October the data showed that there were fluctuations between the extent of the grass/sedge cover and the forb cover. In May (Figure 6.3), the percentage cover for grasses/sedges ranged from a maximum of 59.9% to a minimum of 51.3 % with a mean value of 56.0 % ± 2.05% (S.E). The percentage cover for forbs in May varied from a maximum of 42.1% to a minimum of 38.3% with a mean value of 39.9 ± 1.04% (S.E).

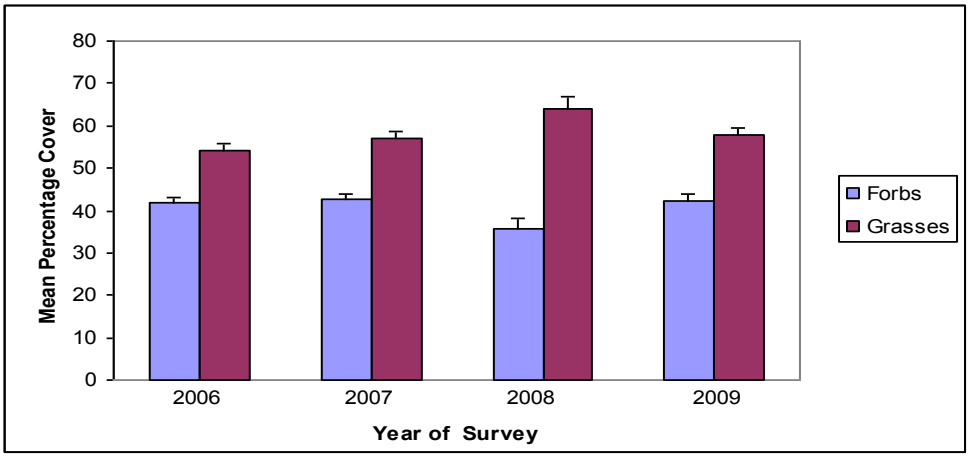


Figure 6.4 - Percentage cover of forbs and grasses/sedges forbs in October surveys 2006 to 2009 of control quadrats (n =10 ± S.E)

The percentage cover in October was slightly higher for both grasses with sedges and forbs, the probable reason being less bare earth present. The percentage grass/sedge cover for October showed a maximum of 64.2% and a minimum of 54.0 %, the mean was 58.3 ± 1.68% (S.E). The maximum cover for forbs was a maximum of 42.2 % and a minimum of 35.5 %, with a mean of 40.3% ± 1.65% (S.E) (Figure 6.4). Between May 2007 and May 2008 testing using a one-way ANOVA gave a significant increase (p = 0.009) in the percentage cover of grasses and sedges of approximately 10%, corresponding to a large increase in the presence of *B. pinnatum*.

6.7.1.3 The effect of fungicide on community structure

In the previous section the structure of the chalk grassland communities at Sites 1 and 2 were characterised using the results of surveys undertaken for control quadrats during the four field seasons of the trial. The surveys identified that there were thirty five species present with a mean percentage cover of greater than 0.1% (Tables 6.2, 6.4 and 6.5). The species with the highest mean abundance was *B. pinnatum*, with a mean cover of 18% in May and 24% in October. It was also shown (Figures 6.3 and 6.4) that the ratio of grasses/sedges to forbs in the community varied between seasons and from year to year. Thus it was imperative that survey data collected from quadrats treated with fungicide be compared with control data from the same survey.

The approach taken was to look at changes in the mean percentage cover of individual species over the course of the trial and particularly at the conclusion of the trial following two seasons of fungicide treatment. The species were then considered within a community context by looking how the application of fungicide changes species rank order in the community and finally in a PCA analysis where the inter-relationship of the 35 most abundant species is considered.

Comparison of mean percentage cover in fungicide treated quadrats with that from control quadrats using a one-way ANOVA showed no significant differences to have occurred during the treatments involving the application of fungicide at the rates of 0.5gm^{-2} and 1.0gm$^{-2.}$ However for the 2.0gm^{-2} and 4.0gm^{-2} treatments 11 species were identified where

significant changes had occurred either during or at the conclusion of the trial. Details of these species are given in the following section. The data for *B. erectus* is also shown.

6.7.1.4 Effect of fungicide on individual species of grasses and sedges

Brachypodium pinnatum

Figure 6.5 shows the mean percentage cover for *B. pinnatum* recorded in control quadrats and those sprayed with 2.0gm^{-2} and 4.0gm^{-2} over a two year period. At the start of the trial there was no significant difference between control quadrats and those designated for treatment. In October 2008 a one-way ANOVA showed a significant difference between the control and 2.0gm^{-2} treatment ($p < 0.05$). In May 2009 there was not a significant difference between control and treated quadrats. In October 2009 mean percentage cover in control quadrats was 27% and that for treated quadrats at less than 20%. In October 2009 using a Tukey test there were significant differences between the control and treated quadrats with a one-way ANOVA giving $p < 0.01$ for the 2.0gm^{-2} treatment and $p < 0.05$ for the 4.0gm^{-2} treatment.

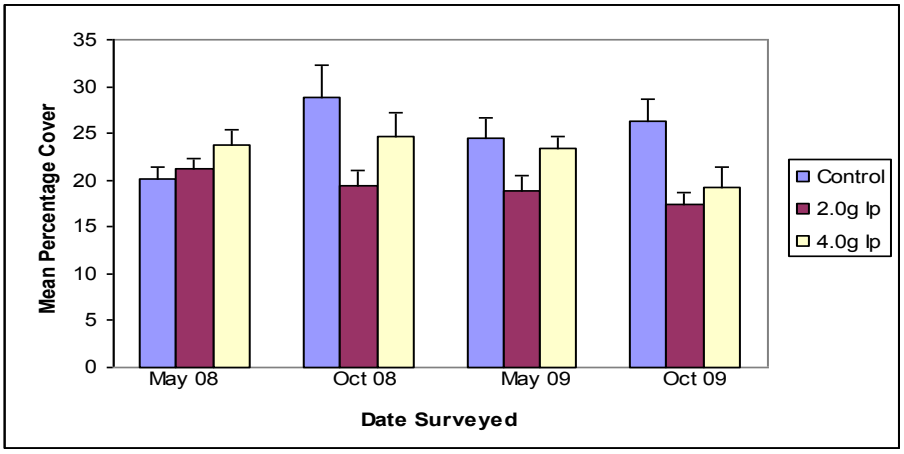


Figure 6.5 - Mean percentage cover (n = 10/12 ± S.E) of *Brachypodium pinnatum* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

Bromus erectus

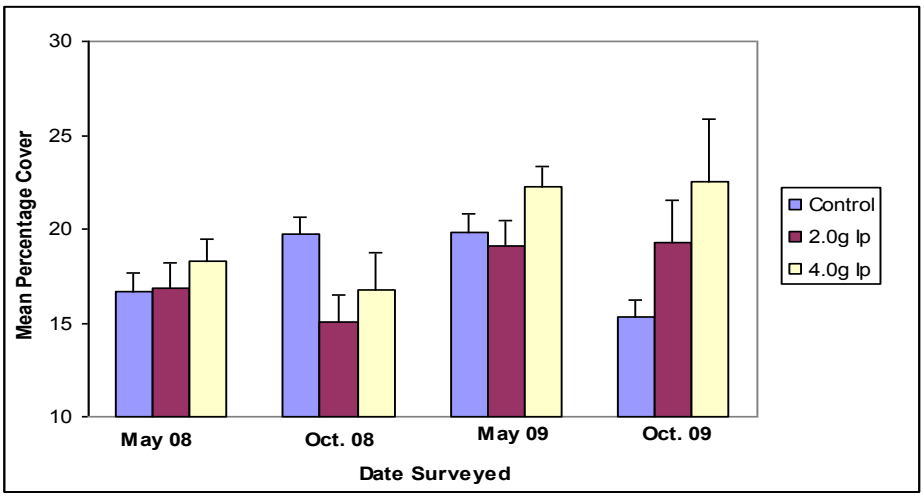


Figure 6.6 - Mean percentage cover (n = 10/12 ± S.E) of *Bromus erectus* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment.

In Figure 6.6 it can be seen that for *B. erectus* at the start of the trial before treatment the mean values for the controls and two fungicide treatments were closely grouped. In October 2008 there was still no significant different between treatments with the mean for the controls higher than the treated quadrats. In May 2009 the mean percentage cover in the quadrats treated with 4.0gm^{-2} fungicide was higher than in the controls and this trend was continued in October 2009 (Figure 6.6). In October 2009 the mean percentage cover in the quadrats treated with 4.0gm^{-2} fungicide was 22.5% compared to 15.3% in the control quadrats. However these differences between the mean percentage cover for controls and quadrats treated with 4.0gm^{-2} were not statistically significant.

Festuca ovina

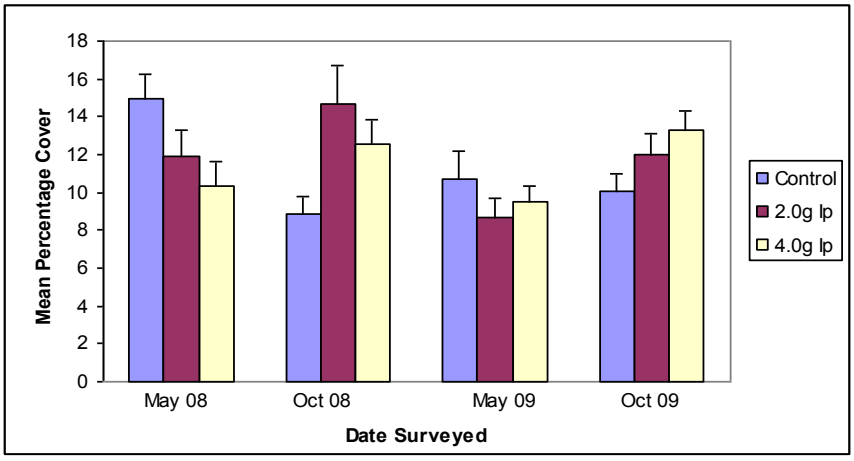


Figure 6.7 - Mean percentage cover (n = 10/12 ± S.E) of *Festuca ovina* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

The data for *F. ovina* (Figure 6.7) shows a strong seasonal effect or interaction. In quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} Iprodione there was an increase in mean percentage cover compared to that in the control quadrats in both of the October surveys. In May 2009 the trend was reversed with mean percentage cover higher in control quadrats. In the October 2008 survey for the 2.0gm^{-2} treatment there was a significant increase compared to that in the control quadrats ($p < 0.05$) and in the October 2009 survey the 4.0gm^{-2} treatment showed a significant difference from the control ($p < 0.05$).

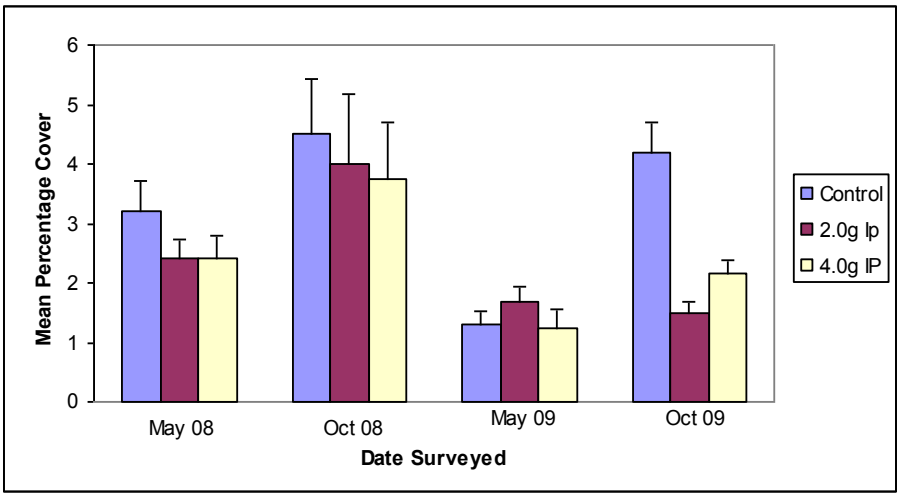


Figure 6.8 - Mean percentage cover (n = 10/12 ± S.E) of *Carex flacca* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

Figure 6.8 shows the levels of *C. flacca* present were low at between 1.5 and 4%. Prior to treatment mean percentage cover in control and treated quadrats was similar and not significantly different. The percentage cover in October was higher than in May. Only in October 2009 was there a significant difference between the quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide and the controls ($p < 0.001$). In October 2009 percentage cover in the control quadrats was similar to that in October 2008, but cover in the treated quadrats remained low. This result was unexpected as levels of AMF infection in the roots of *C. flacca* were found to be low in both the laboratory and field trials.

6.7.1.5 Effect of fungicide on individual species of forbs

It is worth noting that the level of percentage cover in the forb species was considerably lower than that of the grasses with some forbs having very low levels of cover.

Leontodon hispidus

Prior to starting the treatments in May 2008 there was no significant difference in mean percentage cover between the control and treated quadrats (Figure 6.9).

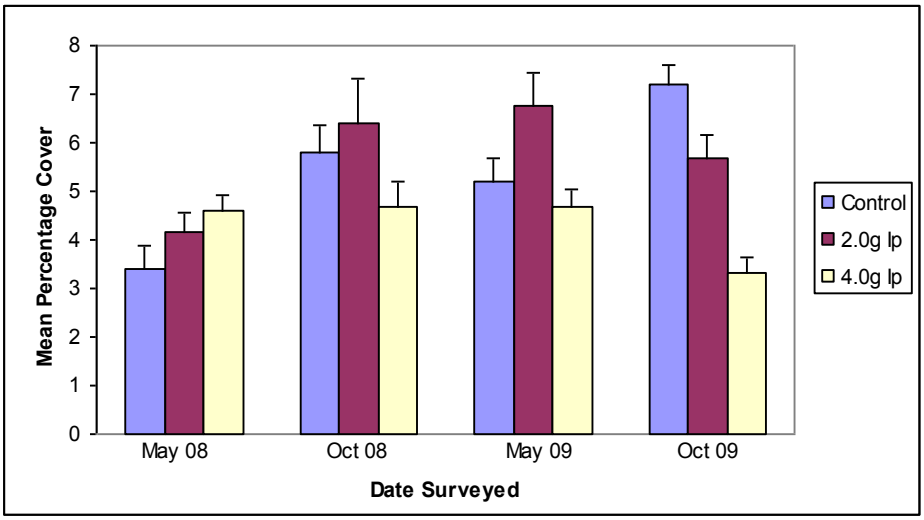


Figure 6.9 - Mean percentage cover (n = 10/12 ± S.E) of *Leontodon hispidus* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

In May 2009 the quadrats treated with fungicide at the rate of 2.0gm^{-2} fungicide had higher levels of mean percentage cover than the controls , but the difference was not significant (Figure 6.9). The survey in October 2009 showed the mean percentage cover in control quadrats had increased and that in the treated quadrats it had decreased. Statistical analysis of the abundance values for October 2009 showed a significant difference between the 2.0gm^{-2} and 4.0gm^{-2} treatments and the controls ($p < 0.05$ and $p < 0.001$ respectively).

Hippocrepis comosa

The response of *H. comosa* to the two fungicide treatments was similar to that of *L. hispidus* but with smaller reductions in mean percentage cover (Figure 6.10). In quadrats treated with 4.0gm^{-2} fungicide there was a significant difference in mean percentage cover from the controls in May 2009 and October 20009 ($p = 0.05$ and $p < 0.05$ respectively) (Figure 6.10). There was no significant difference between quadrats treated with 2.0gm^{-2} fungicide and the controls.

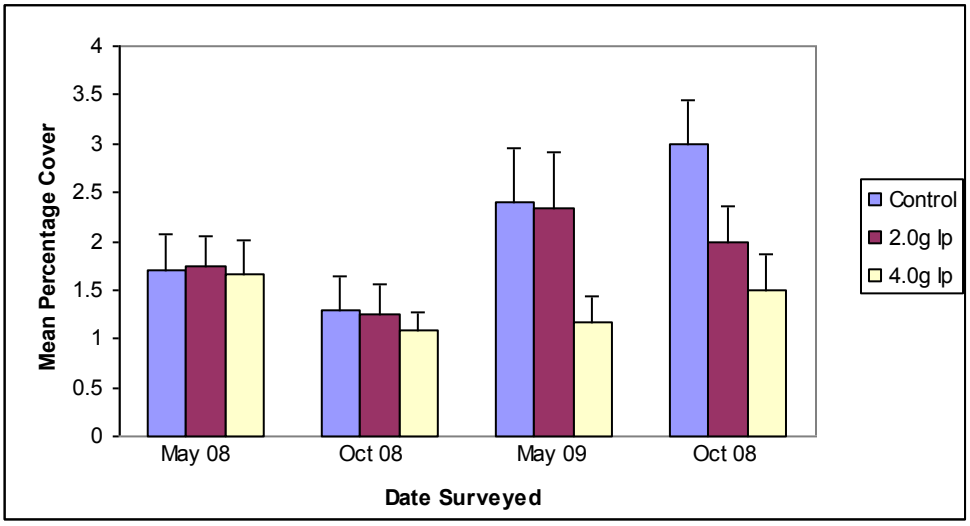


Figure 6.10 - Mean percentage cover (n = 10/12 ± S.E) of *Hippocrepis comosa* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

Hieracium pilosella

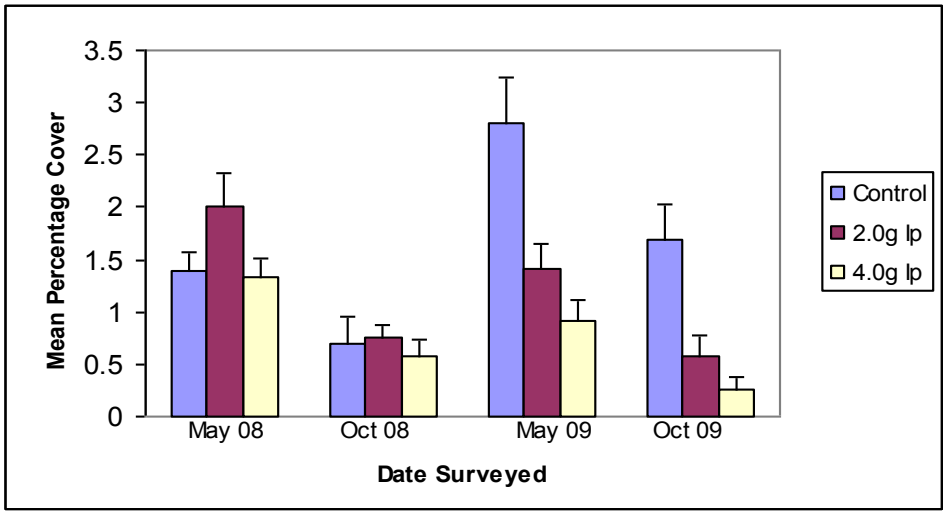


Figure 6.11 - Mean percentage cover (n = 10/12 ± S.E) of *Hieracium pilosella* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

For *H. pilosella* changes in mean percentage cover following fungicide treatment were similar to those for *L. hispidus*, except reduction of *H. pilosella* occurred earlier (that is May 2009) (Figure 6.11). Such was the response of *H. pilosella* to fungicide treatment that statistically significant differences were observed from the controls for both the $2.0gm^{-2}$ and $4.0gm^{-2}$ treatments in May 2009 ($p < 0.001$) and October 2009 ($p < 0.001$). It is worth noting at this point that in both the laboratory and field trials the roots of *H. pilosella* were found to contain high levels of AMF infection.

Centaurea nigra

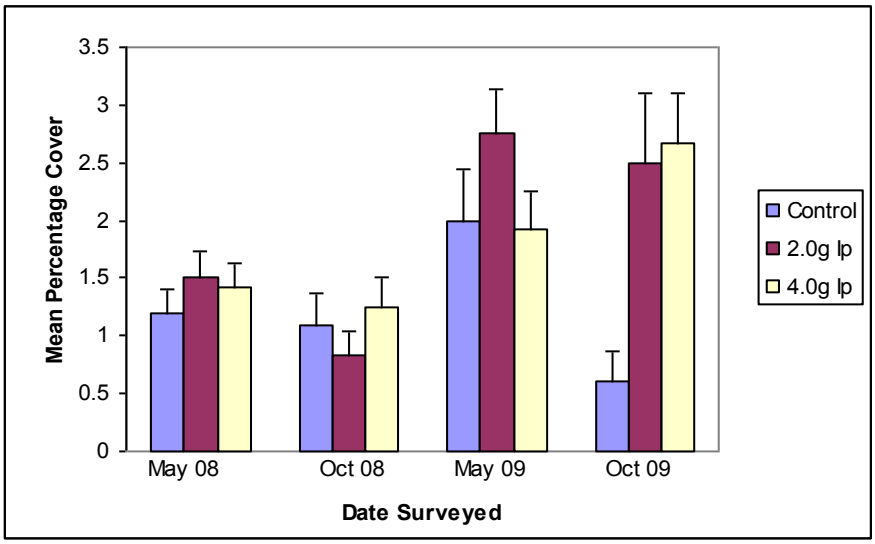


Figure 6.12 - Mean percentage cover (n = 10/12 ± S.E) of *Centaurea nigra* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

C. nigra showed a positive response to spraying with fungicide with increased mean percentage cover compared to the controls (Figure 6.12). The trend was evident in May 2009 and was more pronounced in the October 2009 survey period. In October 2009 analysis confirms a statistically significant difference in mean percentage cover between quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide and that of the controls ($p < 0.05$ and $p < 0.01$ respectively).

Plantago lanceolata

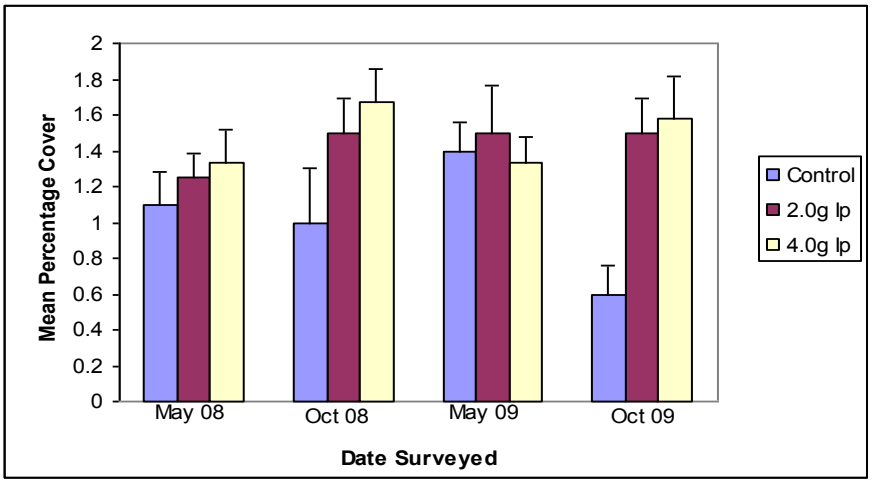


Figure 6.13 - Mean percentage cover (n = 10/12 ± S.E) of *Plantago lanceolata* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

P. lanceolata showed a response (Figure 6.13) similar to that of *C. nigra* (Figure 6.12). In October 2008 there was a positive but not significant response to the application of fungicide. In October 2009 again there was a positive and statistically significant increase in mean percentage cover of quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide compared to the controls ($p < 0.01$) (Figure 6.13).

Lotus corniculatus

L. corniculatus appeared to respond to the application of fungicide, but only at the 2.0gm^{-2} treatment level in October 2009 was the difference in mean percentage cover significantly different to that from the control quadrats ($p < 0.01$) (Figure 6.14).

Trifolium pratense

T. pratense had a very low abundance at less than 1% and was the only species where fungicide treatment resulted in species elimination from the community (Figure 6.15). Over the course of the trial the abundanceof *T. pratense* in the control and 2.0gm^{-2} treatment remained at a similar ratio to that at the start (Figure 6.15). The effect of fungicide treatment at 4.0gm^{-2} was to reduce the percentage cover of *T. pratense* to 0% in October 2008 and again in October 2009. There was a statistically significant difference between control quadrats and those treated with 4.0gm^{-2} fungicide in May 2009 ($p < 0.05$) and October 2009 ($p < 0.01$).

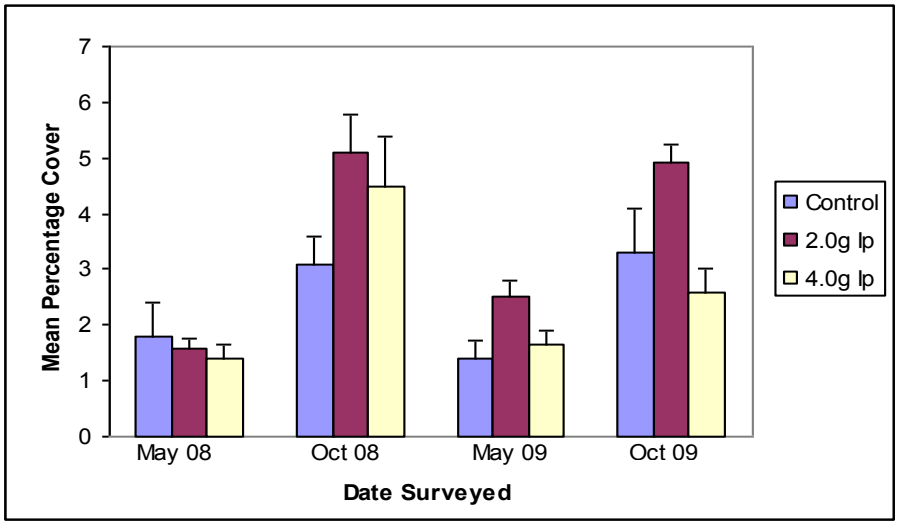


Figure 6.14 - Mean percentage cover (n = 10/12 ± S.E) of *Lotus corniculatus* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment.

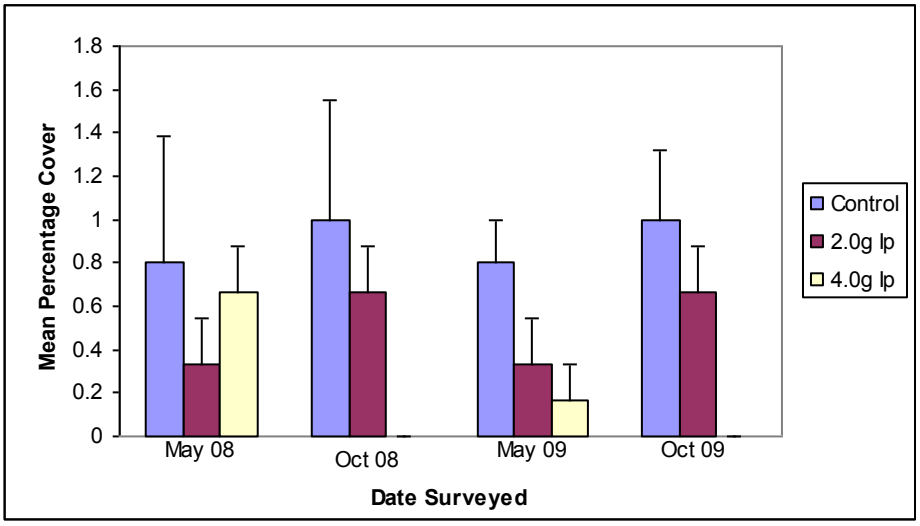


Figure 6.15 - Mean percentage cover (n = 10/12 ± S.E) of *Trifolium pratense* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

Viola riviniana

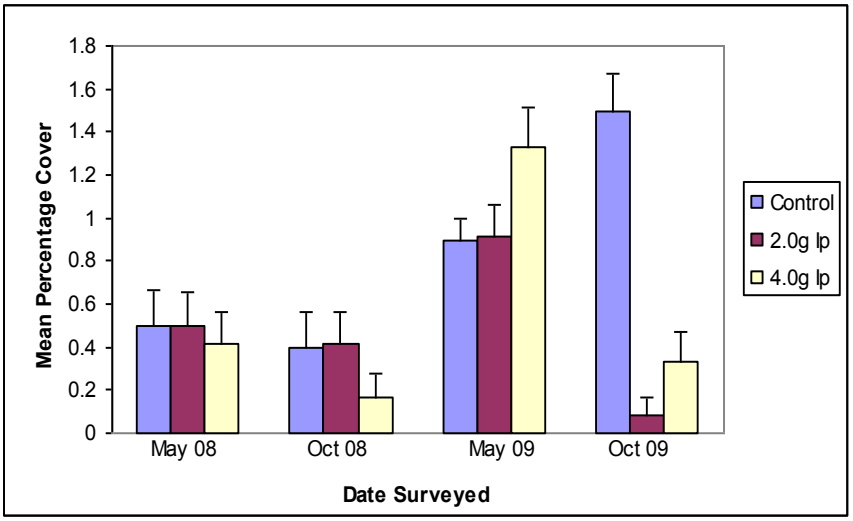


Figure 6.16 - Mean percentage cover (n = 10/12 ± S.E) of *Viola riviniana* with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

V. riviniana was present at a very low level of abundance. The only statistically significant difference in mean percentage cover occurred in October 2009 when abundance in the control quadrats increased and that in the treated quadrats decreased (Figure 6.16). In October 2009 the mean percentage cover in the quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} were significantly lower than in the control quadrats ($p < 0.001$).

Viola hirta

V. hirta was present at very low levels of abundance. The only significant difference in abundance occurred in October 2009 when quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide had significantly higher levels of mean percentage cover compared to the controls ($p < 0.001$) (Figure 6.17).

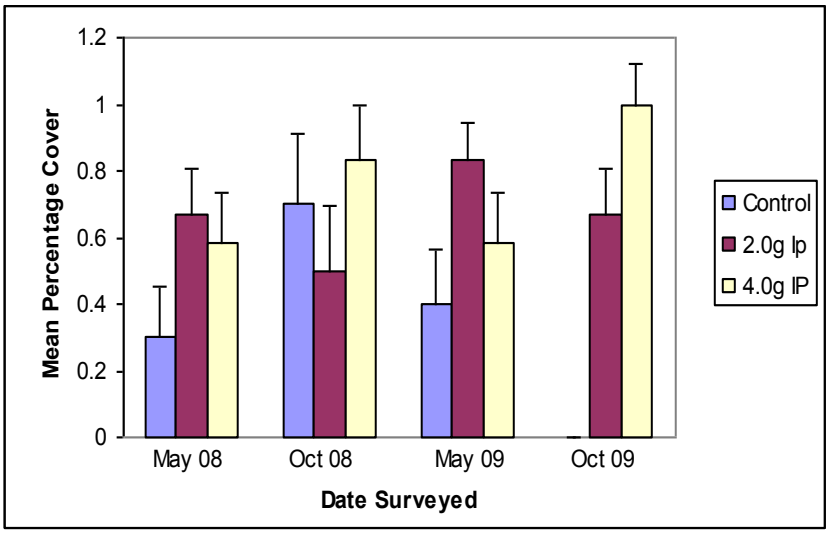


Figure 6.17 - Mean percentage cover (n = 10/12 ± S.E) of Viola hirta with different doses of Iprodione over two growing seasons. Note May 08 values are pre-treatment

6.7.1.6 Effect of fungicide on the species at conclusion of the trial

Examination of changes in mean percentage cover for individual species has shown no significant changes in quadrats treated with 0.5gm^{-2} and 1.0gm^{-2} fungicide. Significant changes were found for some species treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide. The most significant changes were found in the October survey after two seasons of fungicide treatment. The mean percentage covers recorded at the conclusion of the trials for the four treatments are now considered in detail. Mean percentage cover for the species with greater than 0.1% cover (Tables 6.4 and 6.5) were calculated, compared to that in the control quadrats and tested for significant difference using a one-was ANOVA and GLM (repeat measurements). The results are summarised in Table 6.6 and 6.7.

In Table 6.6 the species and treatments which showed significant differences for fungicide treated quadrats when tested with a one-way ANOVA are compared to the significance values obtained using GLM. The calculated values for significance were similar for the two methods such that no difference in the levels of significance was found. Table 6.7

shows the response of the 35 most abundant species to the application of fungicide, i.e. whether they had reacted negatively or positively to two years of fungicide treatment, with levels of significance shown where appropriate.

Table 6.6 - Comparison of p values calculated using GLM (repeat measurements)

Species	Date	GLM Significant (*p* values)		One-way ANOVA Significant (*p* values)	
		Treatment			
		$2gm^{-2}$ Ip	$4gm^{-2}$ Ip	$2gm^{-2}$ Ip	$4gm^{-2}$ Ip
B. pinnatum	Oct. 08	0.014		0.016	
	May 09	0.03		0.047	
	Oct 09	0.004	0.018	< 0.01	< 0.05
B. erectus	Oct 09		0.060		0.074
F. ovina	Oct 08	0.014		0.025	
	Oct 09		0.034		0.023
C. flacca	Oct 09	<0.001	<0.001	< 0.001	< 0.001
L. hispidus	Oct 09	0.024	<0.001	< 0.05	< 0.001
H. comosa	May 09		0.088		0.05
	Oct 09		0.012		< 0.05
H. pilosella	May 09	0.002	<0.001	< 0.001	< 0.001
	Oct 09	0.001	<0.001	< 0.001	< 0.001
C. nigra	Oct 09	0.009	0.005	< 0.05	< 0.01
P. lanceolata	Oct 08		0.048		0.065
	Oct 09	0.004	0.002	< 0. 01	< 0.01
L. corniculatus	May 09	0.011		0.022	
	Oct 09	0.036		0.056	
V. hirta	Oct 09	<0.001	<0.001	< 0.001	< 0.001
V. riviniana	Oct 09	<0.001	<0.001	< 0.001	< 0.001
T. pratense	Oct 08		0.002		0.007
	May 09	0.018	0.002	0.029	< 0.05
	Oct 09		<0.001		< 0.001

In Table 6.7 species, where treatment with fungicide resulted in less than a 25% change in cover from that in the controls, are identified with the letter O, where there was an increase of greater than 25% the letter P is shown and greater than a 25% decrease the letter N is used. Where a significant change has occurred the level of significance is shown.

Table 6.7 - A comparison of mean (n = 12) percentage cover in fungicide treated and control (n = 10) quadrats of the chalk grassland community after two years of treatment

KEY. (G) - Grass (S) – Sedge (F) – Forb. **0** – less than 25% difference (from control).

P – greater than 25% increase (from control). **N** – greater than 25% decrease (from control).

▪ Insufficient data. * Significant at $p < 0.05$, ** Significant at $p < 0.01$, *** Significant at $p < 0.001$ (using one-way ANOVA and GLM)

Species	0.5gm^{-2} Iprodione	1.0gm^{-2} Iprodione	2.0gm^{-2} Iprodione	4.0gm^{-2} Iprodione
Asperula cynanchica(F)	0	0	0	0
Brachypodium pinnatum(G)	0	0	**N** **	**N** *
Briza media (G)	0	0	0	0
Bromus erectus (G)	0	0	**P**	**P**
Carex caryophyllea (S)	0	0	0	0
Carex flacca (S)	0	0	**N****	**N****
Centaurea nigra (F)	0	**P**	**P***	**P****
Centaurium erythraea (F)	0	0	**N**	**N***
Cirsium acaule (F)	0	**P**	0	**N**
Euphrasia officinalis agg (F)	0	0	**P**	**N**
Festuca ovina (G)	0	0	0	**P***
Filipendula vulgaris (F)	0	0	0	0
Galium mollugo (F)	0	0	**P**	**N**
Galium verum (F)	0	0	**P**	**N**
Hieracium pilosella (F)	0	0	**N****	**N****
Hippocrepis comosa(F)	0	0	**N**	**N** *
Leontodon hispidus (F)	0	**N**	**N***	**N****
Leucanthemum vulgare (F)	0	0	0	0
Linum catharticum (F)	0	**N**	0	**N**
Lotus corniculatus (F)	0	0	**P****	0+
Orchid spp (F)	▪	▪	▪	▪
Phyteuma orbiculare (F)	0	0	0	0
Pimpinella saxifrage (F)	**N**	0	**P**	0
Plantago lanceolata (F)	0	0	**P****	**P****
Plantago media (F)	0	0	0	**P**
Polygala calcarea (F)	**P**	**P**	**N**	**N**
Prunella vulgaris (F)	0	0	0	**P**
Ranunculus bulbosus (F)	0	0	**N**	**N**
Rhinanthus minor (F)	0	**N**	0	**P**
Sanguisorba minor (F)	0	0	**P**	**P**
Succisa pratensis (F)	**P**	**P**	0	**N**
Thymus praecox (F)	**P**	0	0	**N***
Trifolium pratense (F)	**N**	0	**N**	**N***
Viola hirta (F)	P	P	**P*****	**P*****
Viola riviniana (F)	**P**	0	**N*****	**N*****
Bromus erectus + Festuca ovina	0	0	**P****	**P*****
Sedges	0	0	**N*****	**N*****
Grasses + Sedges (Total)	0	0	0	0
Forbs (Total)	0	0	0	0

Table 6.7 shows that that the application of fungicide, at the rate of 0.5gm^{-2} and 1.0gm^{-2} had little effect on the percentage cover of all species and there were no significant changes. However examination of the data in the columns representing the application of fungicide at the 2.0gm^{-2} and 4.0gm^{-2} levels shows that respectively 9 and 13 species experienced a significant positive or negative change when compared to the control quadrats (Table 6.7). In the 2.0gm^{-2} treatment group, 5 species (*B. pinnatum, C.flacca*, *H. pilosella*, *L. hispidus* and *V. riviniana*) showed a significant decrease in mean percentage cover and four species (*C. nigra, L. corniculatus*, *P. lanceolata* and *V. hirta*) showed a significant increase in percentage cover.

In the 4.0gm^{-2} group more species were shown to be affected by the application of fungicide, with 9 species showing a significant decrease in mean percentage cover compared to the controls. These included *B. pinnatum, C. flacca*, *C. erythraea*, *H. pilosella*, *H. comosa*, *L. hispidus, T. praecox, T. pratense* and *V. riviniana*. At the 4.0gm^{-2} treatment level (Table 6.7), four species (*F .ovina C. nigra, P. lanceolata* and *V. hirta*) showed a significant positive increase in mean percentage cover when compared to control quadrats. Five species showed a consistent significant decrease in mean percentage cover at both the 2.0gm^{-2} and 4.0gm^{-2} treatment levels, i.e. *B. pinnatum, C. flacca*, *H. pilosella*, *L. hispidus* and *V. riviniana*. A further three species showed significant positive increase at both treatment levels, i.e. *C. nigra, P. lanceolata* and *V. hirta.*

There was a high level of inter-quadrat variability in the cover of *B. erectus* which showed a positive but not significant response to the application of fungicide. However if mean percentage cover of grasses *B. erectus* and *F. ovina* is combined there was a significant difference (Table 6.7), with $p < 0.01$ for the 2.0gm^{-2} treatment and $p < 0.001$ for the 4.0gm^{-2} treatment. Some species showed less than a 25% change in mean cover for all treatments. These were *B. media C. caryophyllea, F. vulgaris, L. vulgare* and *P. orbiculare*.

When considered collectively the results in Table 6.7 showed that when treating chalk grassland with 0.5gm^{-2} and 1.0gm^{-2} Iprodione there was no discernible effects on species mean percentage cover. Treatment with 2.0gm^{-2} Iprodione did produce significant changes in mean percentage cover when compared with the controls and a further five species were affected at the higher dose rate of 4.0gm^{-2}. The results showed more negative, than

positive, effects associated with the application of the fungicide Iprodione. The balance however, between the percentage cover provided by grasses/sedges and forbs does not appear to have been significantly affected by the application of the higher dose rates of fungicide (see bottom of Table 6.7).

6.7.2 Effect of fungicide on species rank order

The rank order of abundance in which species are present in a community are an important characteristic of community structure (Collins *et al*. 2008). This section will focus on the effects associated with the application of fungicide at the rates of 2.0gm^{-2} and 4.0gm^{-2} on species rank order and compare these with rank order in control quadrats.

Rank order of species based on their mean percentage cover is presented based on the field surveys carried out in May 2008, October 2008, May 2009 and October 2009 (Figures 6.18 – 6.21). The approach taken will be to describe the changes in rank order of ten species that showed a negative or positive response to the application of fungicide over the entire survey period (May 2008 to October 2009). The rank order plots present the species in the order of their abundance.

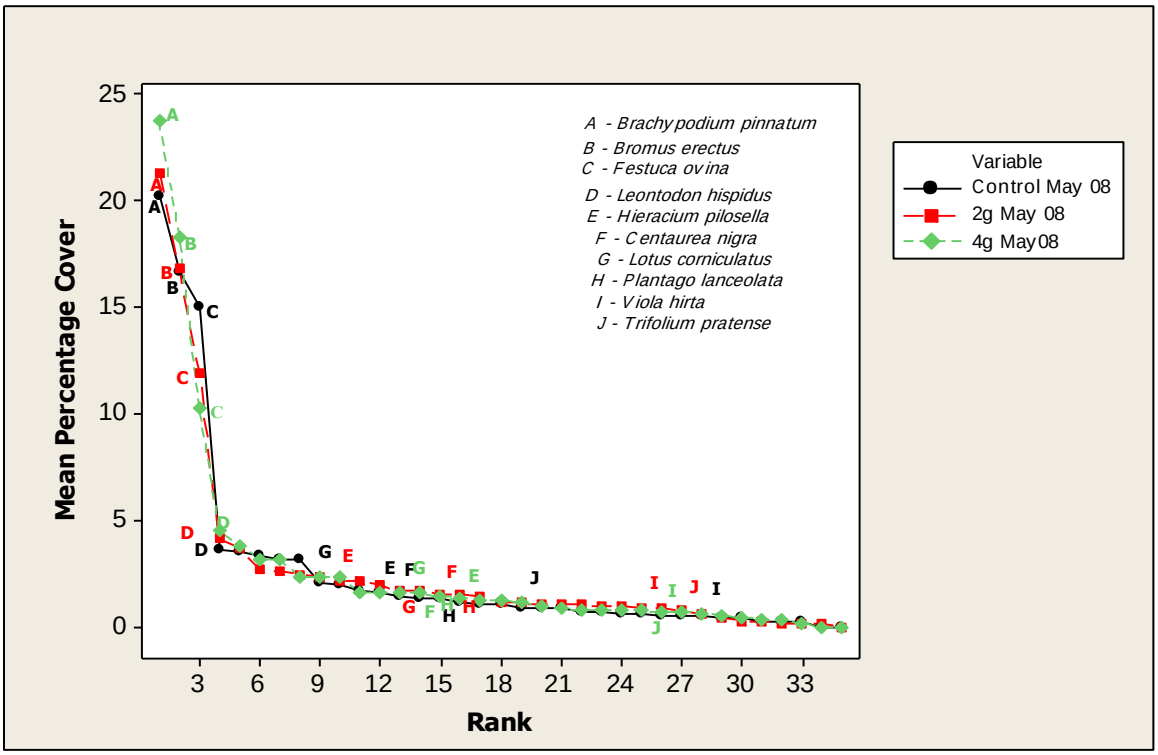


Figure 6.18 - Rank order of species based on mean percentage cover in May 2008 (Pre-treatment)

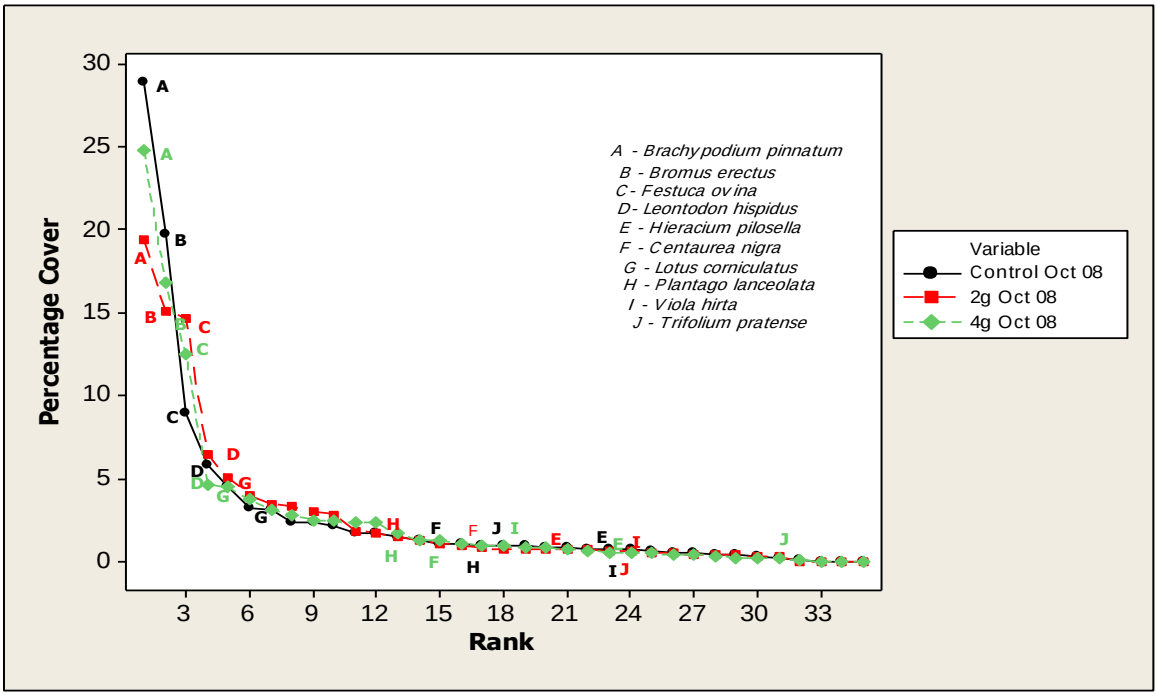


Figure 6.19 -Rank order of species based on mean percentage cover in October 2008

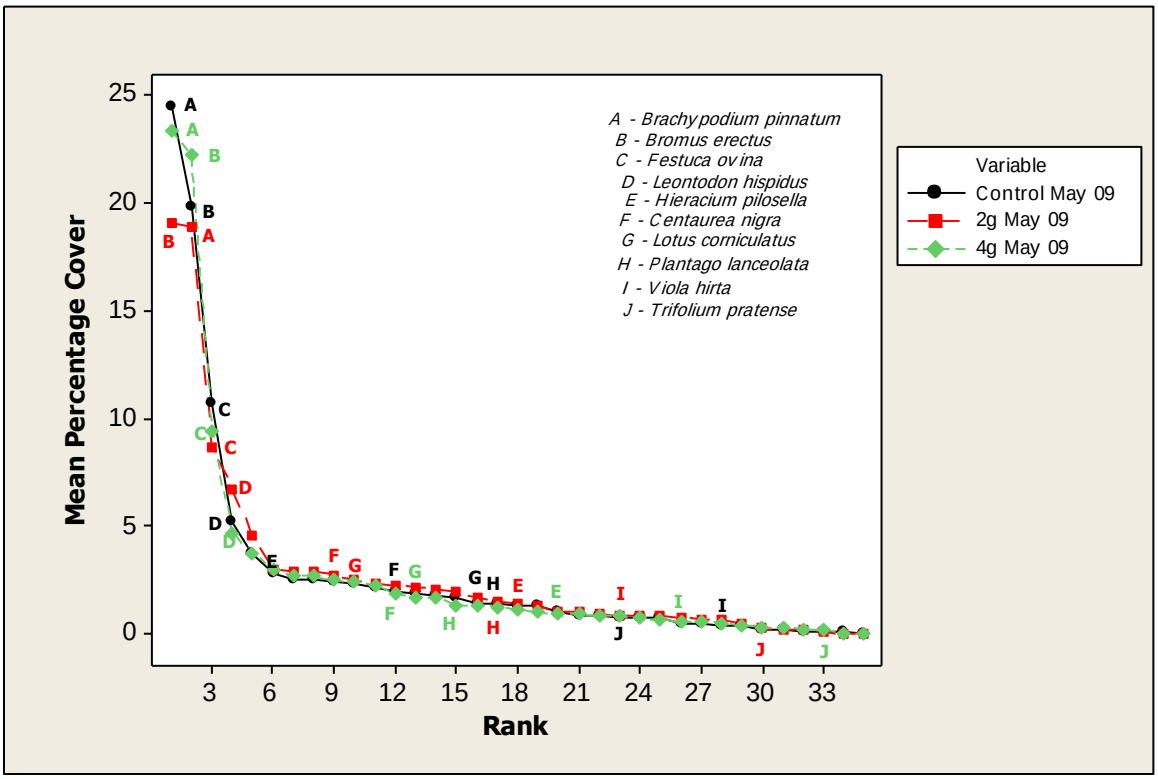


Figure 6.20 - Rank order of species based on mean percentage cover in May 2009

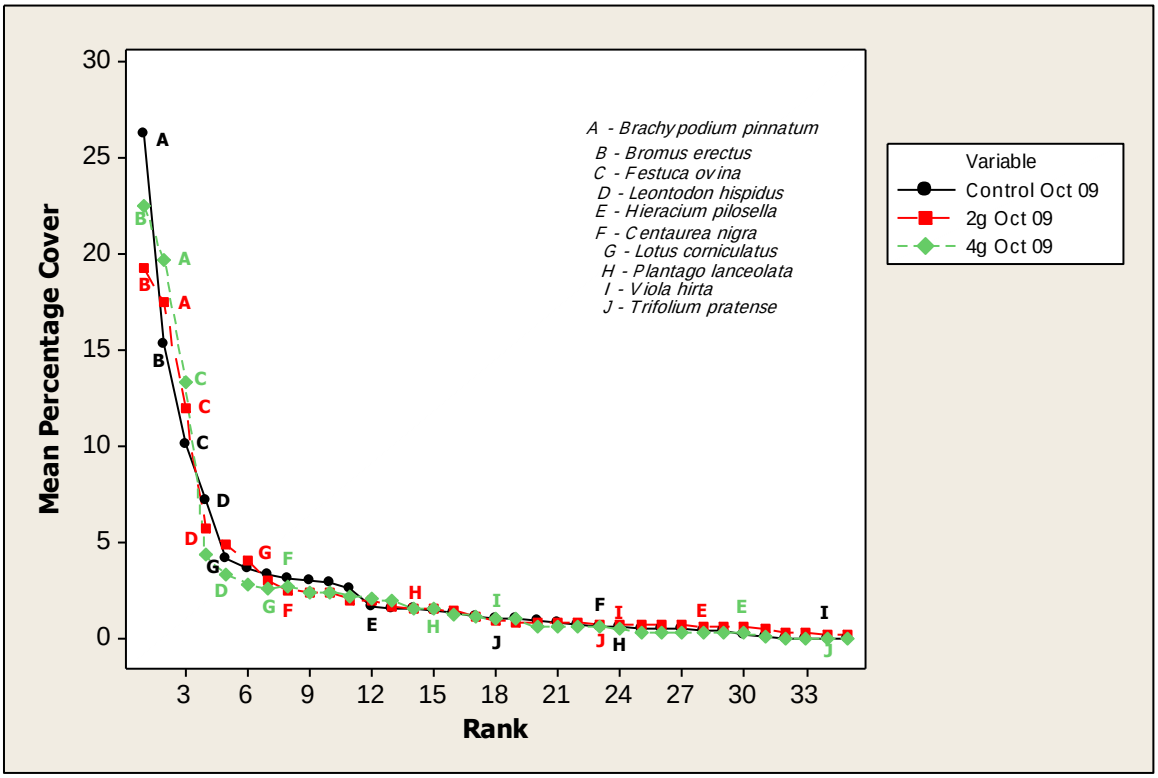


Figure 6.21 - Rank order of species based on mean percentage cover in October 2009

6.7.2.1 The rank order of grasses

The grasses *B. pinnatum*, *B. erectus* and *F. ovina* were ranked 1, 2 and 3 in control and quadrats designated to fungicide treatments at the start of the trial. The response of these dominant grasses to fungicide application will be important in determining the role of AMF/plant symbiosis on chalk grassland community structure.

Brachypodium pinnatum

Examination of the rank order data for the May 2008 survey pre-treatment (Figure 6.18) shows *B. pinnatum* (A) to be ranked 1 in control and quadrats to be treated with fungicide. In October 2008 (Figure 6.19) after one season of fungicide treatment *B. pinnatum* was still ranked 1 for control and fungicide treated quadrats. .

The start of a change in rank order was apparent in the May 2009 survey (Figure 6.20). In the control quadrats mean percentage cover values for the first ranked species *B. pinnatum* and the second ranked *B. erectus* were well separated. However for the quadrats treated with fungicide *B. erectus* had increased in abundance particularly in the quadrats treated

with 4.0gm^{-2} fungicide. In the quadrats treated with 2.0gm^{-2} fungicide *B. erectus* had replaced *B. pinnatum* as the first ranked species.

By the survey of October 2009 (Figure 6.21) the transition of *B. erectus* replacing *B. pinnatum* as the first ranked species in fungicide treated quadrats was complete. In the control quadrats *B. pinnatum* remained ranked 1 with 12% greater mean cover than *B. erectus.*

Bromus erectus

In the previous section describing the changes to *B. pinnatum* it was apparent that the responses of *B. pinnatum* and *B. erectus* to the application of fungicide were linked. As *B. pinnatum* appears to become less competitive in fungicide treated quadrats *B. erectus* (B) becomes more competitive (Figures 6.19, 6.20 and 6.21). At the start of the trial (Figure 6.19) *B. erectus* is clearly the second ranked species in control and quadrats to be treated with fungicide. In quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide there was a decline in the abundance of *B. pinnatum* and an increase in the abundance of *B. erectus*. The process of *B. erectus* displacing *B. pinnatum* as the 1st ranked species, in fungicide treated quadrats, was gradual requiring two seasons of treatment (Figure 6.21) to be complete.

Festuca ovina

F. ovina (C) was the 3rd ranked species in this chalk grassland community. The indications are that *F. ovina* benefits from a reduction in AMF activity but not to the same extent as *B. erectus*. Examination of Figures 6.18 – 6.21 shows that throughout the trial *F. ovina* remained the 3rd ranked species in both the control and fungicide treated quadrats. The interesting characteristic of *F. ovina* was the cyclical response it exhibited to the application of fungicide. In May 2008 pre-treatment (Figure 6.18) the mean percentage cover in control quadrats was higher that those to be treated with fungicide. By October 2008 (Figure 6.19) the quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide had a higher percentage cover than in the controls. However in the May 2009 (Figure 6.20) survey this trend had been reversed with mean percentage cover again higher in the control quadrats. In the October 2009 survey (Figure 6.21), mean percentage cover of *F. ovina was* higher in fungicide treated quadrats when compared to that in the controls. Examination of Figures

6.18 - 6.21 suggests that *F ovina* gained an advantage in fungicide treated quadrats while spraying was taking place, but that much of this advantage was lost in the autumn/winter period while spraying was suspended.

6.7.2.2 The rank order of forbs

Leontodon hispidus

Based on mean percentage cover *L. hispidus* (D) was the major forb species in the community with a rank of 4th. At the start of the trial in May 2008 pre-treatment mean percentage cover in quadrats designated to controls or fungicide treatments were very similar at about 4%. After one season of treatment with fungicide the October 2008 (Figure 6.19) mean percentage cover in controls and fungicide treated quadrats remained similar.

The data for May 2009 (Figure 6.20) showed little change from that of October 2008 (Figure 6.19). In October 2009 (Figure 6.21) after two season of treatment the largest changes in abundance and rank order occurred. The mean percentage cover of *L. hispidus* in control quadrats had risen to 7%, slightly higher than in quadrats treated with 2.0gm^{-2} fungicide. The largest change (Figure 6.21) was observed in quadrats treated with 4.0gm^{-2} fungicide where mean percentage cover had been reduced to 3% and rank order from 4th to 5th.

Hieracium pilosella

In May 2008 *H. pilosella* (E) was ranked 13th in control quadrats, and 17th and 11th in quadrats to be treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide (Figure 6.19). In October 2008 (Figure 6.19) *H. pilosella* had declined in cover and rank in control and treated quadrats.

A major change was observed in May 2009 (Figure 6.20) where *H. pilosella* was ranked 6th and quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide 18th and 20th. In the October 2009 survey (Figure 6.21) the rank for the control was to 12th, and that for the 2.0gm^{-2} and 4.0gm^{-2} 28th and 30th. Thus after two seasons of treatment with fungicide there was a clear separation in the rank order of *H. pilosella* in control and fungicide treated quadrats.

Centaurea nigra

C. nigra was observed to be a mid-ranked species that showed a increase in mean percentage cover following the application of fungicide treatments. In May 2008 the three treatments held similar ranks 14th, 15th and 16th (Figure 6.18). There was little change in rank order in the October 2008 survey period (Figure 6.19). By the May 2009 period there was a small change in order (figure 6.20). The largest change was observed in the October 2009 survey period (Figure 6.21). In October 2009 *C. nigra* in control quadrats was ranked 23rd, in the same sample period the rankings of quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} were both ranked 8th.

Lotus corniculatus

In May 2008 the rankings were relatively similar with *L. corniculatus* ranked 10th in controls, and the quadrats treated wit fungicide 14th and 15th (Figure 6.18). In October 2008 the rank for the controls moved to 7th and the quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} showed a dramatic upward shift in rank to 5th, that is an apparent strong positive effect from the application of fungicide (Figure 6.19).

In May 2009 all three treatments had lower ranks than in October 2008, but quadrats treated with fungicide were higher ranked than the controls (Figure 6.20). In October 2009 a similar result was observed to that seen in October 2008. A large increase in the abundance of *L. corniculatus* was evident, but the control quadrats had a higher rank (5th) than those of the fungicide treated quadrats (7th) (Figure 6.21). These results suggested that over two growing season *L. corniculatus* either responds to the application of fungicide in a complex or the variations in mean percentage cover were due to natural fluctuations in the abundance of *L. corniculatus*.

Plantago lanceolata

In May 2008 prior to treatment, mean percentage cover values in the control, 2.0gm^{-2} and 4.0gm^{-2} treated quadrats yield ranks for *P. lanceolata* of 16th, 16th and 17th (Figure 6.18). In October 2008 the rank for the control quadrats was 17th, but the ranks for the two fungicide treatments had increased to 13th (Figure 6.19).

In May 2009 *P. lanceolata's* rank returned to those of May 2008, with the controls ranked 17th and the 2.0gm^{-2} and 4.0gm^{-2} fungicide treated quadrats ranked 15th and 17th respectively (Figure 6.20). In October 2009 the largest change in the response of *P. lanceolata* was observed (Figure 6.21). The rank for the control quadrats decreased to 24th, while that for the quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} treatments had increased marginally to 14th and 15th. This appears to suggest that with the application of fungicide *P. lanceolata* maintained a rank around 15th. The control quadrants, however, showed a decline in ranked abundance.

Viola hirta

V. hirta was part of a group of forbs that were often found in quadrats, but with low mean percentage cover (e.g. less than 1%) (Table 6.4). At the start of the trial in May 2008 the mean percentage cover for the control and two treatments were similar, i.e. 29th, 26th and 27th (Figure 6.18). In October 2008 (Figure 6.19) and May 2009 (Figure 6.20) there was little change in the order or position of the rankings. In October 2009 there was a large change in the ranking of the controls and the 4.0gm^{-2} treatment (6.21). In the control quadrats *V. hirta* had dropped in rank from 28th to 34th and the 4.0gm-^{2} treatment had increased in rank from 26th to 18th.

Trifolium pratense

As with *V. hirta* the mean percentage cover and populations of *T. pratense* were very low, in the study reported here. At the start of the trial, pre-treatment, in May 2008, the rank of the control quadrats was higher than those to be treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide (that was 20th, 28th and 26th) (Figure 6.18). In October 2008 the rank of *T. pratense* in control quadrats and 2.0gm^{-2} treatments had increased slightly (18th and 24th respectively). The rank, however, for the quadrats treated with 4.0gm^{-2} fungicide declined to rank 31st (Figure 6.21).

In May 2009 the rank of *T. pratense* dropped in all three treatments to below that of the 2008 values (23rd, 30th and 33rd respectively) (Figure 6.20). In October 2009 the rank for

the controls and the 2.0gm^{-2} treatment had returned to 18th and 25th respectively. While the 4.0gm^{-2} treatment showed a further decline in rank abundance to 34th (Figure 6.21).

6.7.3 Principal component analysis of community structure

Abundance data (mean percentage cover) for the 35 most abundant species in the community (Table 6.2) was investigated using Principal Component Analysis (PCA). The ordination plot describes the species data effectively. Species abundance data were used to evaluate the changes in plots following application of fungicide at the survey points of May and October over a two year period.

The points representing treatments were separated clearly along the first axis, which described 27.9% (eigenvalue 9.7493) of the variation in the data (Figure 6.22). The first axis was shown to be significantly correlated with time ($r = 0.969$; $p < 0.001$), and clearly illustrated a seasonal response, present in the abundance of species in the chalk grassland community. For example species such as *C. caryophyllea*, *L. vulgare*, *C. erythraea, R. bulbosus* and *R. minor* had maximum abundance in the spring (May surveys) (Figure 6.23). In contrast *C. flacca*, *P. saxifraga*, *S. minor*, *E. officinalis*, *L. corniculatus*, *G. verum* and *A. cynanchica* had maximum abundance in the autumn (October surveys) (Figure 6.23).

The points were also separated well along the second axis, which described a further 19.9% (eigenvalue 6.9811) of the variation in the data (Figure 6.22). The second axis was shown to be significantly positively correlated with fungicide treatment scores ($r = 0.598$; $p = 0.04$). The ordination plot clearly shows a fungicide treatment gradient, with more heavily treated plots shown to have higher PC scores on the second axis. When these values were considered with the loading plot (Figure 6.23) it highlighted species which responded either positively or negatively to treatment with the fungicide Iprodione. For example, *H. comosa, V .riviniana and T .praecox* were shown to have a very low axis 2 score, and were also shown to respond negatively to fungicide application. Other species that reacted negatively to the application of fungicide were *B. pinnatum*, *L. hispidus* and *S. pratensis,* which also tended to have increased cover in October (Figure 6.23). *H. pilosella* and *P. calcarea* also had a negative response to fungicide but had maximum abundance in May (Figure 6.23). Of the species identified by PCA as being negatively affected by fungicide treatment *T. praecox* and *P. calcarea* are of particular note as one-way ANOVA

and GLM analysis (Table 6.7) had shown a negative but not significant response to fungicide application.

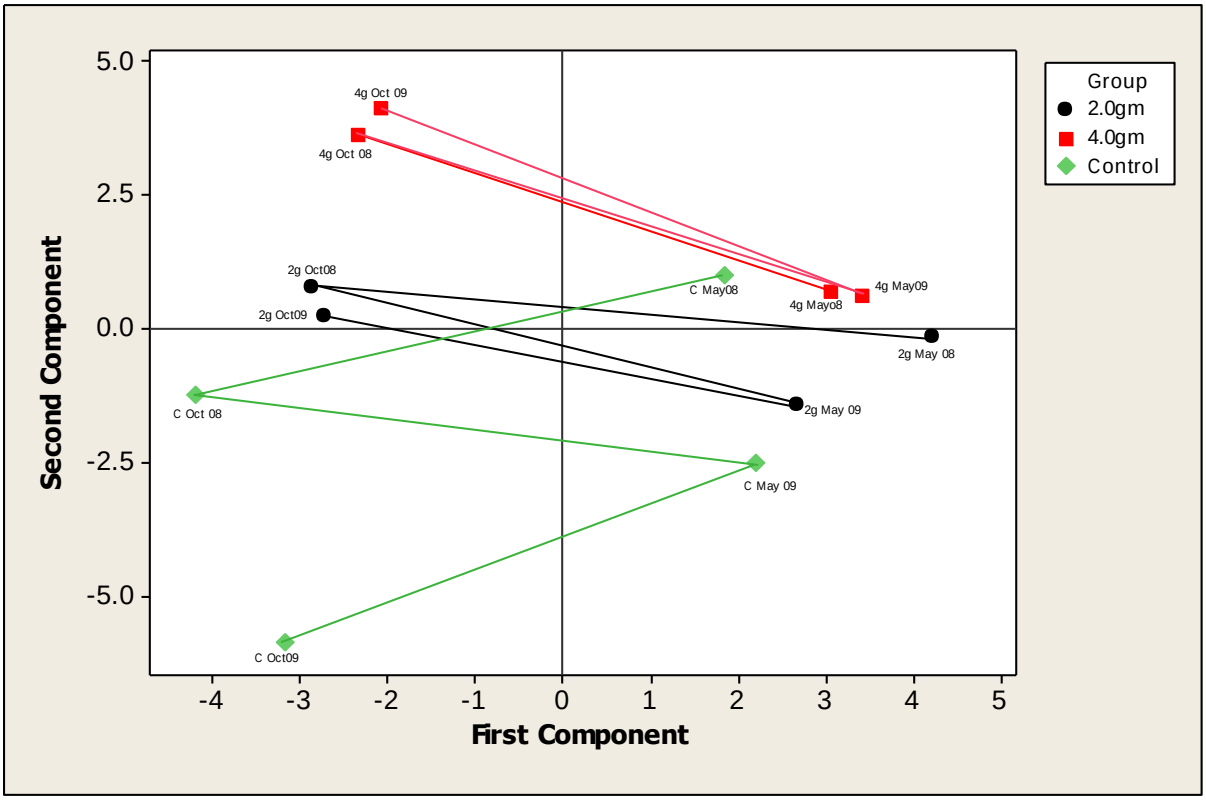


Figure 6.22 - Score plot for the 35 most abundant species for the period May 2008 to October 2009

The score plot (Figure 6.22) and the loading plot (Figure 6.23) also shows that there were strong positive responses to the application of fungicide. *P. lanceolata*, *V. hirta* and *P. vulgaris* are identified as having a strong positive response to the application of fungicide, whereas in the one-way ANOVA analysis *P. vulgaris* was found to have a positive but not significant response to fungicide application (Table 6.7). The dominant grass *B. erectus* gave a positive response with maximum abundance in May and *F. ovina* a positive response with maximum abundance in October. The forb *C. nigra* showed a significant increase in abundance following the application of fungicide when one-way ANOVA analysis was performed. The loading plot (Figure 6.23) suggests a slightly weaker response with the tendency for maximum abundance to occur in May..

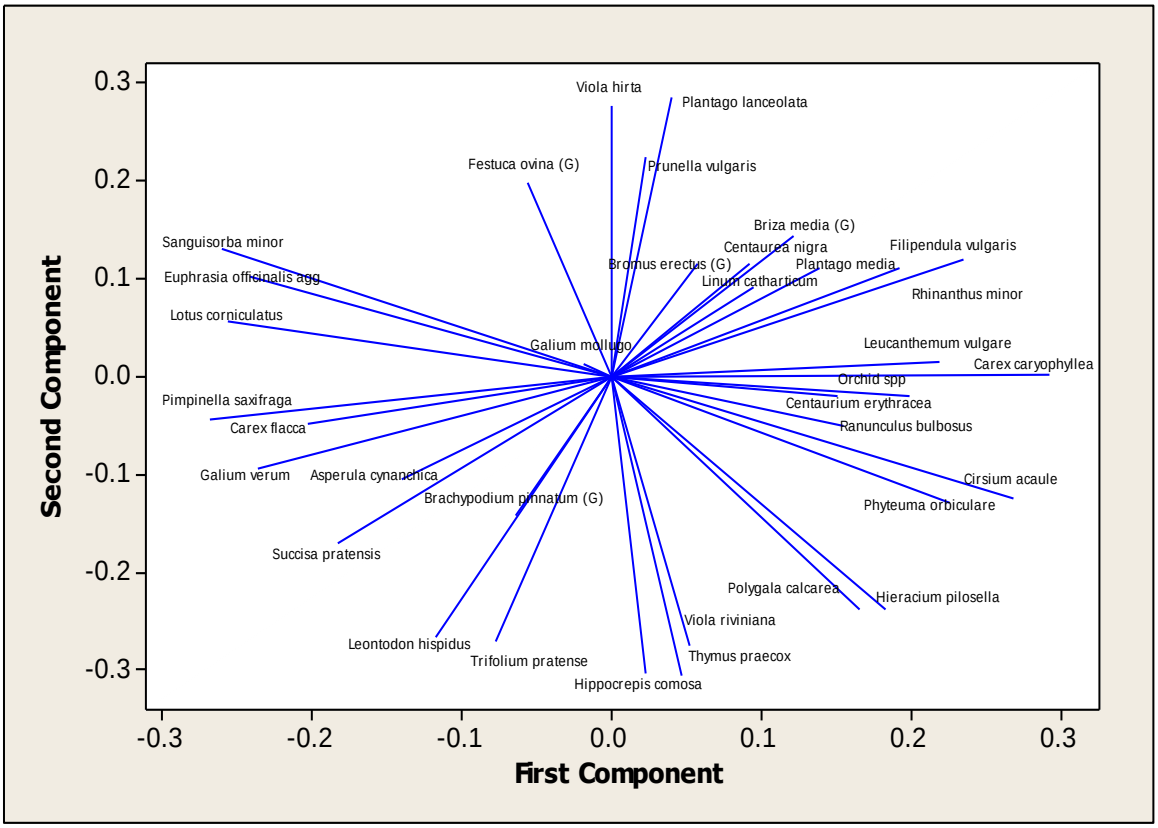


Figure 6.23 - Loading plot for the 35 most abundant species for the period May 2008 to October 2009

Finally, the analysis of change of species abundance with time, and the analysis of changes in rank order over the two years of the trial, have both suggested that for some species there was recovery from the effects of spraying in the period October to May when fungicide was not being applied. Examination of the score plot in Figure 6.22 tends to support these observations, with the May values for the three treatments clustering towards the middle of the second component axis while the October scores have greater spread.

6.7.4 Microscopic examination of the selected species for the presence of AMF

The roots of selected forbs, grasses and sedges were examined using the methods outlined in section 6.4.4, with a more detailed account of the methodology given in the previous chapter in section 5.4.6. The results are summarised in Table 6.8

Table 6.8 - The percentage length of root infected with AMF and percentage length of root containing vesicles.

I – the percentage length of root infected with hyphae/arbuscules.

V – the percentage length of root containing vesicles.

	Control	Control	2.0gm^{-2} Iprodione		4.0gm^{-2} Iprodione	
Species	**I**	**V**	**I**	**V**	**I**	**V**
Hieracium pilosella	58.2	5	49.2	11.5	42.7	14.8
Succisa pratensis	47.7	6.7	47.1	4.5	38	4.9
Leontodon hispidus	31.6	2.35	26.6	6.9	22.2	5.8
Plantago media	24.9	1.67	29.2	6.3	25.5	5.8
Festuca ovina	34.3	2.1	42.8	5.0	40.7	2.8
Brachypodium pinnatum	18.9	0.95	13.7	0.43	11.9	0.43
Bromus erectus	7.7	0.36	11.9	0.45	12.2	1.25
Carex flacca	2.9	0.0	2.9	0.0	2.1	0.0

Examination of Table 6.8 shows that in four of the species examined (*H. pilosella, S. pratensis, L. hispidus* and *B. pinnatum*) the effect of the application of fungicide was to reduce the levels of root infection when compared to root infection in the controls. It is also apparent that the levels of infection in roots of these species treated with fungicide at the rate of 4.0gm^{-2} was lower than those treated with 2.0gm^{-2} fungicide.

Three of the species (*P. media, F. ovina* and *B. erectus)* showed higher values of root infection where they had been treated with fungicide, although the increases were all less than 10% (Table 6.8).

The final species, (*C. flacca*) had very low levels of root infection in both controls and plants treated with fungicide, although the level of root infection was marginally lower in the roots from plants treated with 4.0gm^{-2} fungicide (Table 6.8).

The examination of roots from the laboratory turf trials (Chapter 5) showed a strong relationship (Figure 5.17) between the total lengths of root infected with AMF and the length of root in which vesicles were observed.

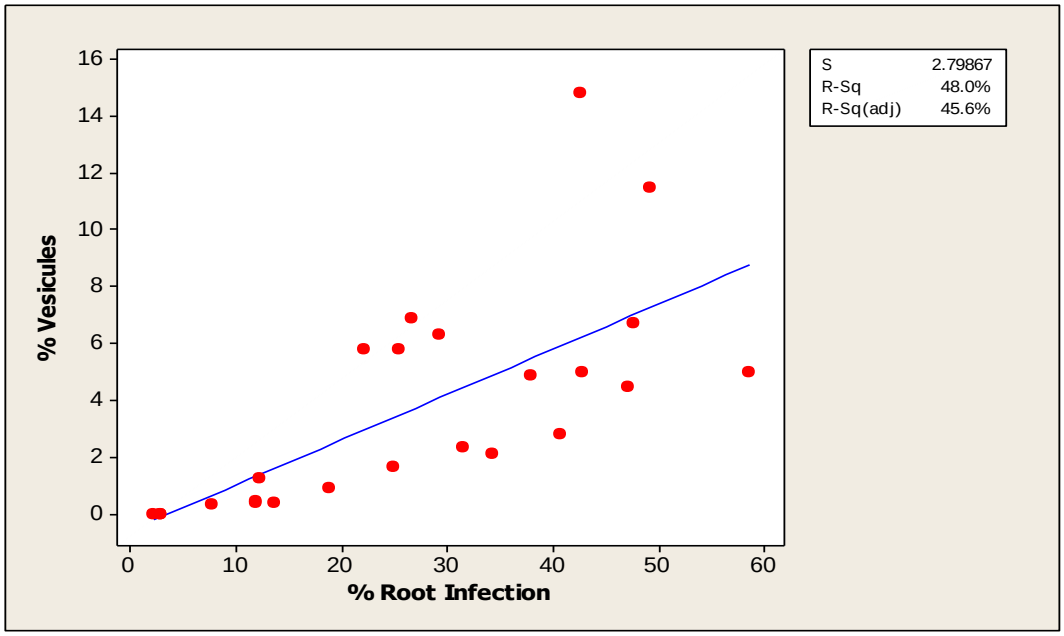


Figure 6.24 - Plot of percentage root length containing vesicles Vs percentage root infection. (Percentage length of root containing vesicles = - 0.520 + 0.159 x percentage length of root infection)

Plotting percentage length of root infected with AMF against length containing vesicles produced a linear relationship (Figure 6.24). Regression analysis provided an R-Sq value of 45.6% ($p < 0.001$). This coefficient of determination value (R-Sq) was about half that observed in the roots of plants from the laboratory turf trials (laboratory trials yielded an R-Sq value of *circa* 80%). Examination of Figure 6.24, and the gradient, shows about 16% of root length infected with AMF contained vesicles compared to about 29% in roots from the laboratory turf trials (Figure 5.19).

Referring back to Chapter 5, examination of the percentage length of root infected with AMF in plants from the laboratory turf trials (Table 5.13) showed them to be generally higher than in the roots of plants from the field trials (Table 6.8). In the laboratory turf and field trials five of the species from which roots were taken, (*H. pilosella, S. pratensis, B. pinnatum, B. erectus* and *C. flacca*) were common to both trials. Calculation of the mean percentage root infection for these five species, taken from control quadrats, gives values of 42.9% for the laboratory turf trials and 27.1% for the field trials.

Table 6.8 gives the percentage length of root infected with AMF in each of the species for the controls and plants treated with $2.0gm^{-2}$ or $4.0gm^{-2}$ fungicide. However it can also be instructive to see the effect of the fungicide treatments on the level of root infection

compared to that observed in the controls. This is shown in Table 6.9 were the level of infection in the control is 100% and the values for the fungicide treated plants is the percentage root infection compared to that in the control.

Table 6.9 - Percentage root infection in plants treated with fungicide compared to that in the controls (100%).

I– the percentage length of root infected with Hyphae/ Arbuscules.

	Control	2.0gm^{-2} Iprodione	4.0gm^{-2} Iprodione
Species	I	I	I
Hieracium pilosella	100%	84%	73%
Succisa pratensis	100%	99%	80%
Leontodon hispidus	100%	84%	70%
Plantago media	100%	117%	101%
Festuca ovina	100%	125%	119%
Brachypodium pinnatum	100%	72%	63%
Bromus erectus	100%	154%	158%
Carex flacca	100%	100%	72%

Reference to Tables 5.13 and 5.14 in Chapter 5 and Tables 6.8 and 6.9 in this chapter shows that, although levels of root infection in plants from the laboratory trials were higher compared to the field trials, the effect of fungicide produced a greater percentage reduction in infection in roots from the laboratory trials.

6.8 Discussion

The aim of the field trials was to examine the role of AMF in structuring semi-natural chalk grassland communities. This was to be achieved by weakening AMF/plant symbiosis by the application of graduated doses of the fungicide Iprodione and analysing changes to species presence and abundance.

This trial was one of a small number of trials (Gange *et al*. 1993; Newsham *et al*. 1995; Hartnett and Wilson 1999: Smilauer and Smilauerova 2000; O' Connor *et al*. 2002; Karanika *et al*. 2008a;) that have used fungicides to weaken AMF/plant symbiosis and study the role of AMF in structuring plant communities in the field. The trial reported here appears to be the first on semi-natural chalk grassland. Previous trials on grassland communities have been on Prairie grassland (Hartnett and Wilson, 1999), on a mesotrophic

hay meadow in the Czech Republic (Smilauer and Smilauerova, 2000), and on acidic montane grassland in Greece (Karanika *et al*. 2008a).

The trial on chalk grassland was conducted between 2006 and 2009 and used four fungicide dose levels. Low levels of fungicide application did not produce significant changes in community structure. However, higher dose rates of 2.0gm^{-2} and 4.0gm^{-2} fungicide did produce significant changes in community structure. Examination of selected grasses, sedges and forbs has shown a graduated effect of fungicide application on the levels of AMF present in the roots.

6.8.1 Species presence and abundance in control quadrats

The surveys, conducted over four years, allowed a summary of species presence within the community to be compiled. In Table 6.2 the 35 species that were always or nearly always present over the four year period are listed. These species were found to be present in control quadrats with a mean percentage cover of greater than 0.1%. Consideration of the 35 species listed in Table 6.2, within the broader context of chalk grassland composition, demonstrates that they were a mixture of grasses, sedges and forbs representative of the frequency classes, core, intermediate and scarce (see Chapter 4). Thus it can be argued that these 35 species were representative of species typically found at chalk grassland sites. A further 42 species were recorded as being occasionally present, with a percentage cover of less than 0.1%. These „scarce or rare" species are unlikely to be important within the community functional processes (Phoenix *et al*. 2008), but increase species richness and conservation value (Willems, 2007).

All 35 of the most abundant species (Table 6.2) contribute to the structure and functioning of the chalk grassland community studied in this trial. However it is probable that the most abundant species will have a greater effect on community structure and functional processes (Phoenix *et al*. 2008) including those involving AMF/plant symbiosis. Usually in grassland communities one or more grass or sedge will be the species with the highest abundance. In the chalk grassland communities being studied in this trial the three most abundant species were *B. pinnatum*, *B. erectus* and *F. ovina.* These three grass species have high above ground biomass and an extensive underground system of fine roots. Wahl and Ryser (2000) quote the mean diameter of *B. pinnatum* roots as 0.21mm^2, *B. erectus* as

0.27 mm^2 and *F. ovina* as 0.17mm^2. In this study of the role of AMF/plant symbiosis on the structure of chalk grassland communities the mycorrhizal characteristics of these three dominant grasses are important. The only forb with a mean percentage cover of greater than 4% was *L. hispidus*. The next three most abundant species were the grass *B. media* and the sedges *C. flacca* and *C. caryophyllea*. Thus six of the seven most abundant species were grasses or sedges making up ~ 50% to 60% of the mean percentage cover (Figures 6.3 and 6.4).

While the most abundant species were with one exception grasses and sedges the remainder of species with cover of greater than 0.1% were all forbs (Table 6.2). However only 10 of these (*S. minor, C. acaule, L. corniculatus, S. pratensis, H. comosa, H. pilosella, P. lanceolata, P. media, F. vulgaris and A. cynanchica*) had mean percentage covers of greater than 1% throughout the growing season. While the roots of the forbs selected for examination of AMF took different forms, in general the root systems of the forbs were both thicker and shorter than those of the grass species. Therefore both individually and collectively the number of AMF connections between the roots of forbs was probably lower than that between grasses and sedges, but are still likely to be important in determining community structure at a fine scale.

Examination of the mean percentage covers of the 35 most abundant species in May (Table 6.2) and October (Table 6.3) show most species to exhibit seasonal variation dependent on their phenological cycle. Thus when assessing the effects of fungicide application on community structure, it is important to compare values of mean percentage cover with those of the controls at the same point in the temporal cycle. The survey data also showed that year to year variation was even greater than seasonal variation. This is illustrated in Figures 6.3 and 6.4, which plots the relative percentage cover in May and October over the four years of the trial. While the mean cover for grasses and sedges is similar for May and October 56.2% ± 2.05% and 58.3% ± 1.68% the difference between the maximum cover observed (64.2%) and the minimum (51.3%) was relatively large. When the abundance of grasses/sedges is high the corresponding cover of forbs is reduced. Thus there were natural variations in community structure over the period of the trials. This effect serves to illustrate that the structure of plant communities is not static but exist in a form of dynamic equilibrium (van der Maarel and Sykes, 1993).

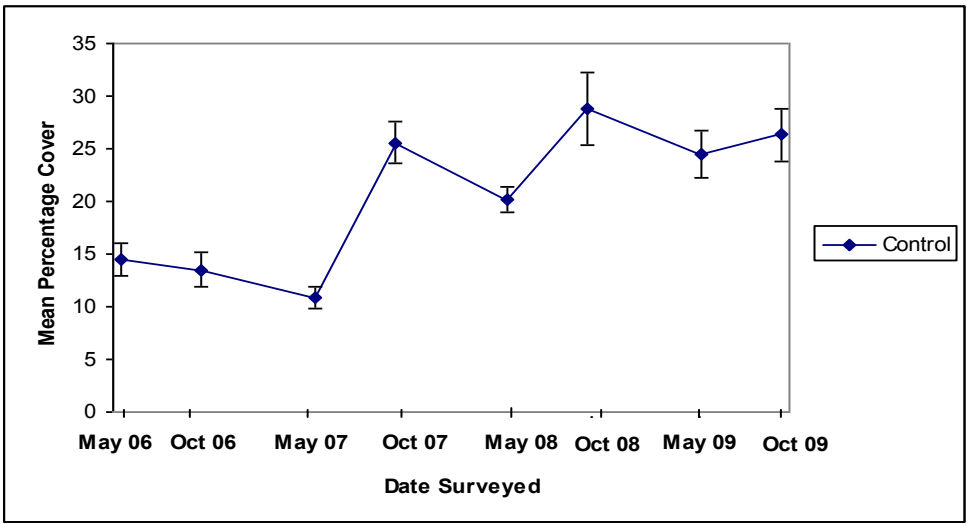


Figure 6.25 - Mean percentage cover (n =10 ± S.E) of *Brachypodium pinnatum* between May 2006 and October 2009

Examination of the survey data for grasses and sedges in the control quadrats showed the grass *B. pinnatum* to be the largest contributor to seasonal and year on year fluctuations. Figure 6.25 shows the variation in mean percentage cover of *B. pinnatum* over the four year period of the trial. It can be seen from Figure 6.25 that between May 2007 and October 2007 there was a significant increase ($p < 0.001$) in the mean percentage cover of *B. pinnatum* and that the increased level of cover was maintained over the following two years.

Several factors appear to be important in determining the presence and abundance of *B. pinnatum*. These are site aspect, rainfall and soil nitrogen content with all three variables closely linked. Studies of calcareous grassland in the United Kingdom (Grime and Curtis, 1976; Buckland *et al*. 1997; Bennie *et al*. 2006) have found that drought prone south facing slopes discourage the growth of coarser grasses including *B. pinnatum* . In northern Hungary in a study of xeric grassland Endresz *et al*. (2005) found that *B. pinnatum* occupied microhabitats exposed to full sun on north and east facing slopes but avoided adjacent grassland on south and west facing slopes. Endresz *et al*. (2005) conclude that it was the dry conditions that discouraged *B. pinnatum* on south and west facing slopes. Endresz *et al*. (2005) also observed that autumn rain triggered growth of *B. pinnatum* on a south-west slope but not on a north-east one. Rainfall and retained water in the soil appear to be important for the presence and growth of *B. pinnatum*. The significant increase observed in the abundance of *B. pinnatum* at Sites 1 and 2 between May and October 2007

(Figure 6.25) corresponded to a doubling of normal rainfall in this period (See Chapter 3). Additional rainfall also has the potential to increase the deposition of nitrogen pollution from the atmosphere.

Field studies have found that increased nitrogen in the soil promotes the growth of *B. pinnatum* (Bobbink, 1991; Willems *et al.* 1993; Ryser *et al*. 1997; Denyer *et al*. 2007) but a microcosm experiment conducted by Wilson *et al*. (1995) found that high nitrogen levels did not enhance its growth. Hurst and John (1999) found high nitrogen levels under the roots of *B. pinnatum* which is consistent with localised high levels of nitrate measured at Sites 1 and 2 (Owen, 2008). Research by van der Heijden *et al*. (2008) has found that AMF have the ability to mitigate the effects of nitrogen enrichment in dune grassland.

In this study the most abundant species were *B. pinnatum* and *B. erectus*, but this has occurred comparatively recently (Table 6.10)

Table 6.10 – A comparison of the rank order of species abundance from surveys carried out in 1982 by Grubb (1986b), in 1991 Steven and Muggeridge (1992) and 2009 (this study) at Newmarket Hill

Abundance/Rank	1982	October 1991	October 2009
1	*Sanguisorba minor*	*Festuca ovina /rubra*	*Brachypodium pinnatum*
2	*Festuca ovina /rubra*	*Brachypodium pinnatum*	*Bromus erectus*
3	*Brachypodium pinnatum*	*Sanguisorba minor*	*Festuca ovina*
4	*Carex flacca*	*Leontodon hispidus*	*Leontodon hispidus*
5	*Bromus erectus*	*Cirsium acaule*	*Carex flacca*
6	*Leontodon hispidus*	*Filipendula vulgaris*	*Briza media*
7	*Cirsium acaule*	*Bromus erectus*	*Sanguisorba minor*
8	*Briza media*	*Lotus corniculatus*	*Cirsium acaule*
9	*Thymus praecox*	*Briza media*	*Lotus corniculatus*
10	*Hippocrepis comosa*	*Carex flacca*	*Succisa pratensis*

It can be seen (Table 6.10) that in 1982 the forb *S. minor* was the most abundant species followed by the grasses *F. ovina /rubra.* In 1992 *F. ovina /rubra* was the 1st ranked species and *S. minor* was ranked 3rd. In 2009 *B. pinnatum* was the most abundant species followed by *B. erectus* with *S. minor* declining to 7th. Thus between 1981 and 2009 both *B. pinnatum* and *B. erectus* have increased in rank and abundance with *F.ovina* displaced to 3rd. Although the rank order has change the same five grasses and sedges were present in 1982 and 2009. Three of the forbs (*S. minor*, *L. hispidus* and *C. acaule*) were present in

both 1982 and 2009. The forbs *T. praecox* and *H. comosa* present in 1982 were replaced by *L. corniculatus* and *S. pratensis* in 2009. However there has been a strong constancy of species over nearly a thirty year period. In general there has been an increase in the abundance of grasses and sedges and a decline in the abundance of forbs. As already discussed, increases in nitrate levels from the atmosphere may be partially responsible as may be a relaxation in the grazing regime (Emery, 2009). Also the surveys in 1986, 1991 and 2009 were carried out by different survey teams.

While the plants in the community have their characteristics of presence and diversity the same will be true for the AMF species present. A number of studies have considered the diversity and characteristics of AMF species. A study by Johnson *et al*. (2005) found that AMF diversity was related to the composition and age of the plant species present. Oehl et al. (2010) found that soil type and land use intensity affected AMF diversity. Regardless of the type of soil present, greater AMF diversity was found in grassland when compared to arable soils (Oehl *et al*. 2010). However in a microcosm experiment using soil taken from the top 2cm of a calcareous grassland site Johnson *et al*. (2003) found greater AMF diversity in bare soil compared to that in a 12 plant species community. An interesting observation in the context of the conditions pertaining to Newmarket Hill was made by Eriksson (2001). In a study of semi-natural grassland in Sweden Eriksson (2001) found that the highest levels of AMF colonisation occurred at sites with a long history of management, such as the chalk grassland at Newmarket Hill. Furthermore the higher levels of AMF colonisation was correlated with increasing plant species diversity at the $1m^2$ scale (Eriksson 2001).

According to Gibson and Brown (1991) the age of chalk grassland can be estimated from the species present. *B. media*, *C. flacca*, *Carlina vulgaris*, *C. erythraea*, *C. acaule*, *H. pilosella*, *Linum catharticum, L. corniculatus*, *Pimpinella saxifrage*, *S. minor*, *T. praecox* and *V. hirta* are often found in chalk grassland in excess of 100 years since formation and *A. cynanchica*, *B. erectus* and *G. verum* are normally only found in chalk grassland that have been in existence for longer than 100 years. In a more recent study of dry calcareous grassland in Germany, Karlik and Poschlod (2009) investigated the species composition of ancient (continuous pasture since 1830) and more recent grassland. Strong indicators of ancient grassland were the species *C. caryophyllea*, *C. flacca*, *C. vulgaris*, *C. acaule*, *H. comosa* and *Scabiosa columbria*. All of these species identified by Gibson and Brown

(1991) and Karlik and Poschlod (2009) as indicators of ancient grassland were present at Newmarket Hill. This suggests that the chalk grassland communities at Site 1 and Site 2 have been pasture for a period of at least 100 years and probably much longer. Based on the findings of Oehl *et al*. (2010) and Eriksson (2001) AMF diversity at Sites 1 and 2 should be high and a strong relationship exist between the plants present and AMF, resulting in increased levels of root colonisation.

6.8.2 The effect of fungicide on community structure

6.8.2.1 Changes during the trial

Here changes in mean percentage cover from the start to the finish of the trial are addressed. Only species that showed significant change following the application of fungicide at the higher dose rates are considered.

Grasses and Sedges

The changes in mean percentage cover with time for *B. pinnatum, B. erectus, F. ovina* and *C. flacca* are shown in Figures 6.5, 6.6, 6.7 and 6.8. The strong mycorrhizal dependence of *B. pinnatum* in this field trial was consistent with the results of experiments performed by Grime *et al*. (1987); van der Heijden *et al*. (1998a); van der Heijden (2004) in microcosms and also with the results from laboratory trials (Chapter 5) in this study. In the microcosm experiments *B. pinnatum*, grown with other calcareous species, was shown to produce greater biomass in the presence of AMF compared to conditions in which AMF was absent.

The results (Figures 6.6 and 6.7) showing *B. erectus* and *F. ovina* to benefit from the reduction of AMF are consistent with the findings of Grime *et al*. (1987); van der Heijden *et al.* (1998a); van der Heijden (2004) in microcosm research and with the results from the laboratory trials in this study. Thus the results of mycorrhizal research carried out on *B. pinnatum*, *B. erectus* and *F. ovina* carried out in artificial microcosms are shown to translate to field conditions involving more complex communities.

The results for *C. flacca* (Figure 6.8) in October 2008 and May 2009 are consistent with those with microcosm research (van der Heijden *et al*. 1998b) in which *C. flacca* was shown to be non mycorrhizal. However in October 2009 in this study there was a large increase in mean percentage cover in control quadrats but not those treated with fungicide. One possible explanation is that *C. flacca* is more competitive with *B. erectus* and *F. ovina* in strongly mycorrhizal conditions.

Forbs

Eleven forb species showed significant changes in mean percentage cover, of which seven had a negative and four a positive response (Table 6.10). Three of the species *L. hispidus, H. pilosella* and *H. comosa* showed declines in mean percentage cover in May 2008 one year after starting treatment (Figures 6.9, 6.10 and 6.11). Both *L. hispidus* and *H. pilosella* have been shown to be strongly mycorrhizal in microcosm experiments (Grime *et al*. 1987; Grime 1990; van der Heijden *et al*. 1998b). In this study *T. pratense* was negatively affected by the application of fungicide at the $4.0gm^{-2}$ dose level as early as May 2008 (Figure 6.15). The strong reaction of *T. pratense* to the application of fungicide is consistent with the findings of van der Heijden *et al*. (1998b), who found almost no growth in *T. pratense* in the absence of AMF.

C. nigra was the forb that showed the greatest positive response to the application of fungicide (Figure 6.12). This result would not have been expected on the basis of microcosm experiments conducted by Grime *et al*. (1987) which found *C. nigra* to be strongly mycorrhizal. Both *P. lanceolata* and *V. hirta* responded positive to the application of fungicide (Figures 6.13 and 6.17). Research by Grime *et al*. (1987) suggests that *P. lanceolata* benefits from the presence of AMF, while that of Ayres et al. (2006) suggests different behaviour under varying environmental conditions, while a third study by Newsham *et al.* (1995) reported *P. lanceolata* to benefit from the reduction of AMF.

For most of the forbs, significant changes in mean percentage cover did not occur until the second year of treatment. This is similar to that for other field trials where the effects of fungicide application took several season to become significant (Hartnett and Wilson 1999; Karanika *et al*. 2008a). The response of individual species to fungicide treatment

was in general consistent with the results from microcosms (Grime *et al*. 1987; van der Heijden *et al*. 1998a; van der Heijden *et al*. 1998b). However the observation in this study of plants categorised as mycorrhizal responding positively to the application of fungicide appears to differ from that found in microcosm research. However in field trials other researchers have also reported „mycorrhizal species" responding positively to the application of fungicide (Newsham *et al*. 1995; Smilauer and Smilauerova 2000; Karanika *et al*. 2008a). Why this positive response is observed in the field and not in artificial communities grown in microcosms is unclear. However one fundamental difference is that the AMF used in microcosm trials are few in number and are common generalist species (Grime *et al*. 1987; van der Heijden *et al*. 1998a; van der Heijden *et al*. 1998b). In field trials there is a high diversity of AMF species. Fitter (2005) and Johnson *et al*. (2005) found that AMF diversity was related to the composition and age of the plant species present. Oehl *et al*. (2010) reports that the number and colonising characteristic of AMF was found to be high in grassland under long term management. In a study of a woodland community the effect of fungicide on AMF present in the roots of the forb *Ajuga reptans* was to decrease the presence of specialist AMF and promote the growth of generalist AMF (Helgason *et al*. 2007). Thus greater AMF diversity and a broader range of AMF functionality, and different response to the application of fungicide, may contribute to the different behaviour observed in field trials when compared to microcosm research.

6.8.2.2 Changes after two seasons

At the lower dose rates of $0.5gm^{-2}$ and $1.0gm^{-2}$ two seasons of treatment were completed in October 2007 and for the higher dose rates of $2.0gm^{-2}$ and $4.0gm^{-2}$ in October 2009. Survey data for the 35 most abundant species (Table 6.2) was used to compare fungicide treated quadrats with those of the controls. From this analysis Table 6.7 was constructed. Only dose rates $2.0gm^{-2}$ and $4.0gm^{-2}$ resulted in significant changes to mean percentage cover. Therefore this discussion will focus on the analysis from survey data collected for these higher dose rates.

Examination of Table 6.7 shows that for the $2.0gm^{-2}$ and 4.0 gm^{-2} treatments there was a full range of responses, from very significant negative, to no response, to very significant positive ($p < 0.001$). Species that showed significant negative or positive response to the application of fungicide are summarised and highlighted in Table 6.11.

Table 6.11 shows that 14 of the 35 most abundant species had significant responses to the application of fungicide. Ten were core species and four intermediate species. This equates to about 50% of the core species in the community and 36% of the intermediate species. Intermediate species as a group have lower frequency of occurrence and abundance in chalk grassland as a whole (see Chapter 4) and could be considered subordinates (although *B. pinnatum* was the dominant species in this study).

Inspection of Table 6.11 shows that all four intermediate species gave a negative response to the application of fungicide that is they benefit from symbiosis with AMF. Of the 10 core species 5 gave a negative and 5 a positive response to the application of fungicide. These data suggests that intermediate (subordinate) species may be more AMF dependent than core species.

Table 6.11 - Species that have shown the greatest response to the application of fungicide at the rate of 2.0gm^{-2} and 4.0gm^{-2}

O – less than 25% difference (from control). **P** – greater than 25% increase (from control).

N – greater than 25% decrease (from control). * Significant at ***p*** = < 0.05,

** Significant at ***p*** = < 0.01, *** Significant at ***p*** = < 0.001 **(C)** – Core species **(I)** – Intermediate species.

Species	2.0gm^{-2}Treatment	4.0gm^{-2} treatment
***Brachypodium pinnatum*(I)**	**N****	**N***
***Festuca ovina* (C)**	**P**	**P***
***Carex flacca* (C)**	**N*****	**N*****
***Centaurea nigra* (C)**	**P***	**P*****
***Centaurium erythraea* (I)**	**N**	**N***
***Hieracium pilosella* (C)**	**N*****	**N*****
***Hippocrepis comosa* (I)**	**N**	**N***
***Leontodon hispidus* (C)**	**N***	**N*****
***Lotus corniculatus* (C)**	**P****	**0**
Plantago lanceolata (C)	**P****	**P****
***Thymus praecox* (C)**	**0**	**N***
***Trifolium pratense* (C)**	**N**	**N***
***Viola hirta* (C)**	**P*****	**P*****
***Viola riviniana* (I)**	**N*****	**N*****

The effect of fungicide application was generally greater at the higher dose level of 4.0gm^{-2} than at 2.0gm^{-2} consistent with a greater level of AMF reduction with the higher dose. There were almost twice as many significant negative responses than positive responses,

but 20 of the 35 most abundant species showed either no change or small positive or negative changes to mean percentage cover in response to fungicide treatment.

The observations of some plant species increasing in cover with the application of fungicide suggest competitive release from more mycorrhizal species (Hartnett and Wilson, 2002). An alternative explanation is that some AMF benefit from the application of fungicide. In a study of AMF in a natural woodland community Helgason *et al*. (2007) found that the application of the fungicide Benomyl reduced the presence of specialist AMF in the roots of the forb *Ajuga reptans* and increased the presence of generalist AMF. An interpretation of this result could be that generalist AMF provide less P than specialist AMF, but this could not validated from this particular study (Helgason *et al*. 2007).

The observation of negative, neutral and positive responses of chalk grassland species to the application of fungicide is consistent with the finding from field trials on other grassland habitats. In Prairie grassland Hartnett and Wilson (1999) found that the application of Benomyl reduced the abundance of the dominant strongly mycorrhizal grasses *Andropodon gerardii*, *Sorghastrum nutans* and *Andropogon scoparius*, while a dominant weakly mycorrhizal grass was unaffected. The main beneficiaries of fungicide application were the subordinate weakly or non mycorrhizal grasses and forbs (Hartnett and Wilson 1999). The subordinates appear to gain in abundance from competitive release from the dominant mycorrhizal grasses (Hartnett and Wilson 2002). In a trial on traditional Hay meadows in the Czech Republic (Smilauer and Smilauerova 2000) the general effect of the application of Benomyl was to reduce the abundance of forb species the exception was the forb *Achillea millifolium* which increased in abundance. Karanika *et al*. (2008a) conducted a field trial in acidic grassland growing above 1000m in northern Greece. There were two dominant species; the grass *Agrostis capillaris* and an annual forb *Galium lucidum*, both of which were judged to be weakly mycorrhizal. The rest of the community consisting of forbs and legumes was considered to be mycorrhizal. The effect of applying the fungicide Benomyl was much greater after two years of application. As expected the reduction of AMF increased the abundance of the two dominant species and reduced the abundance of the forbs and legumes; some being eliminated from the community. The unexpected observation was that a perennial legume, *Doricnium herbaceum,* increased significantly in abundance despite having high levels of AMF colonisation in untreated plants (Karanika *et al*. 2008a).

6.8.3 Changes in species rank order after the application of fungicide

Examining the changes in species rank order over a period of time provides an alternative way to assess the effective of fungicide treatment on community structure. As stated by Collins *et al*. (2008) rank abundance statistics provides a graphical framework for quantifying community dynamics and may provide insights hidden by univariate analysis. While the earlier analysis considered the absolute changes in mean percentage cover for species, the study of rank abundance (Figures 6.18 - 6.21) looks at the changes to the competitive hierarchy of species resulting from the application of fungicide.

6.8.3.1 Changes in rank order of grass species

The largest changes in community structure resulting from the application of fungicide were in the rank order of *B. pinnatum* and *B. erectus*. *B. pinnatum* started out in May 2008 as the 1st rank species in both the control quadrats and those to be treated with fungicide. *B. erectus* was ranked 2nd in all quadrats. By October 2009 *B. pinnatum* remained as 1st ranked in control quadrats, but in quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} *B. erectus* was now the 1st ranked species. This change in rank resulting from the application of fungicide represents a large change in community structure. Examination of the rank order of the ten most abundant species (Table 6.10) from data collected in 1982 (Grubb 1986a; Mitchley and Grubb 1986), and in 1991 (Steven and Muggeridge 1992) and from the current study, that is over an approximately 30 year period, has shown *B. pinnatum* to be more highly ranked than *B. erectus*. That the application of fungicide over two growing seasons leads to a reversal of a long term and stable rank order in *B. pinnatum* and *B. erectus*, demonstrates the strength of the role of AMF in determining the structure of this community.

F. ovina did not change in rank order with the application of fungicide but there was clear evidence that it benefited from the application of fungicide over the summer, but that the benefit was lost over the winter when spraying was not conducted. Evidence of *F. ovina* not benefiting from an association with AMF has been provided by Grime et al. (1987) in microcosm experiments and in field trials (Newsham *et al*. 1995). As recently as 1991 *F. ovina* was the highest ranked grass species at Newmarket Hill (Table 6.10). A relaxation in the grazing regime (Emery, 2009) may have been a factor in the decline of *F. ovina* and

increased abundance of *B. pinnatum*. At Newmarket hill, high AMF diversity associated with mature communities (Johnson *et al*. 2005) and AMF effectiveness associated with long term management of grassland (Oehl *et al*. 2010) may also favour *B. pinnatum* in competition with *F. ovina.*

The magnitude of the change in rank order between *B. pinnatum* and *B. erectus* following the application of fungicide can be gauged by the change to the NVC classification (Rodwell, 1990) of the communities being studied at Newmarket Hill At the start of the trial in 2006 the surveys of Sites 1 and 2 showed *B. pinnatum* to be dominant with the high levels of strong calcicoles present, suggesting the sub community CG4a (*Brachypodium pinnatum – Avenula pratensis – Thymus praecox*) as the most consistent. The effect of applying fungicide over a two year period has been to modify the community in fungicide treated quadrats from a NVC classification of CG4a (*Brachypodium pinnatum* dominated) to CG3b (*Bromus erectus* dominated) while the community in control quadrats was unchanged.

6.8.3.2 Changes in rank order of forb species

Examination of the rank order data in Figures 6.18 – 6.21 shows that, with the possible exception of *L. hispidus*, the forbs were subordinate to the grass species in the community.

A number of microcosm studies of the mycorrhizal dependence of species found in calcareous grassland have been carried out (Grime *et al*. 1987; van der Heijden *et al*. 1998a). Using biomass data generated by these studies, van der Heijden (2002) was able to construct a table of mycorrhizal dependence for twenty five calcareous grassland species. In Table 6.12 the species in van der Heijden (2002) which were also present at Sites 1 and 2 of the present study are reproduced. These data can be used to assess changes observed in the rank order of the forbs in this study. van der Heijden (2002) suggests that *T. pratense* was the most mycorrhizal dependent species of those assessed and the changes in rank of *T. pratense* in the current study was consistent with this assessment. *T. pratense* had low levels of cover in the control quadrats with ranks in the low twenties, but the effect of fungicide at the higher dose rate was to reduce the rank to 34th with *T. pratense* eliminated from the community.

Table 6.12 – The mycorrhizal dependence of calcareous plant species grown in artificial microcosms. (The ranks are from van der Heijden, 2002)

Notes. (P) – denotes a positive mycorrhizal dependence. (N) – denotes a negative mycorrhizal dependence. Species at the top of the table have the highest mycorrhizal dependence.

Rank	Species.
1	***Trifolium pratense* (P)**
2	***Centaurium erythraea* (P)**
3	***Sanguisorba minor* (P)**
5	***Lotus corniculatus* (P)**
6	***Hieracium pilosella* (P)**
7	***Centaurea nigra* (P)**
8	***Plantago lanceolata* (P)**
10	***Prunella vulgaris* (P)**
11	***Brachypodium pinnatum*(P)**
12	***Galium verum* (P)**
13	***Leontodon hispidus* (P)**
15	***Briza media* (P)**
21	***Bromus erectus* (N)**
24	***Festuca ovina* (N)**
25	***Carex flacca* (N)**

H. pilosella was ranked the 6th most mycorrhizal species by van der Heijden (2002) and the changes in rank following the application of fungicide reflect this assessment. In October 2009 *H. pilosella* was ranked 12th in control quadrats and 28th and 30th in quadrats treated with 2.0gm^{-2} and 4.0gm^{-2} fungicide. Treatment of *L. hispidus* with fungicide, (which was ranked 13th by van der Heijden (2002)) did not produce a change of rank in the 2.0gm^{-2} treatment and the 4.0gm^{-2} treatment lowered rank by only one place.

Three of the forbs (*L. corniculatus*, *C. nigra* and *P. lanceolata*) showed increases in rank in treated quadrats when compared to the rank of the controls. Based upon the assessment of mycorrhizal dependence *L. corniculatus* (5th), *C. nigra* (7th) and *P. lanceolata* (8th) (van der Heijden 2002) these species might have been expected to decrease in rank following the application of fungicide, but showed an increase. However as discussed earlier these results are consistent with observations in other field trials (Newsham *et al*. 1995; Smilauer and Smilauerova 2000; Karanika *et al*. 2008a).

6.8.4 Community Variation (PCA)

There were strong seasonal changes in the community with some species strongly represented in May and others in October. This finding highlights the need to conduct surveys in the spring and in the autumn as was emphasised by Hartnett and Wilson (1999) when studying Prairie grassland. The graduated effect of fungicide dose on levels of mean percentage cover was apparent in the $4.0gm^{-2}$ treatment, which showed a stronger response than the $2.0gm^{-2}$ treatment, with both treatments producing a stronger response than in the control quadrats (Figure 6.22). The effect of fungicide application was stronger in October after 6 months of spraying with some evidence of recovery during the winter months. In addition to the species identified as having a significant negative or positive response to the application of fungicide (Table 6.11), two forbs with low percentage cover were also identified. These were *P. vulgaris* which responded positively and *P. calcarea* which responded negatively to the application of fungicide. The PCA analysis also revealed a strong positive response to the fungicide treatments in *B. erectus* and *F. ovina* separately, rather than when combined (see Table 6.7).

6.8.5 Microscopic examination of the presence of AMF

The roots of a total of eight species, four forbs and three grasses and a sedge, were examined for the presence of AMF (Table 6.8). The percentage root infections in the forb species were generally higher than in the grasses and sedge (Table 6.8). Three of the forbs *H. pilosella*, *S. pratensis* and *L. hispidus* and the grass *B. pinnatum* showed a graduated reduction in the presence of AMF in their roots when treated with $2.0gm^{-2}$ and $4.0gm^{-2}$ fungicide. These four species had been identified by van der Heijden (2002) as being strongly mycorrhizal. The forb *P. media*, the grasses *B. erectus* and *F. ovina,* and the sedge *C. flacca* did not show a reduction in the presence of AMF in their roots when treated with fungicide. van der Heijden (2002) has classified the two grasses and the sedge as weakly mycorrhizal. The reduction of AMF in the roots of strongly mycorrhizal plant species when treated with fungicide has been observed in other field trials. In Prairie grassland (Hartnett *et al*. 1993; Hartnett and Wilson 1999) the application of fungicide reduced the level of AMF in mycorrhizal grasses by 75%. Fungicide also reduced the presence of AMF in mycorrhizal species in other field trials, for example in a lichen rich community (Newsham *et al*. 1995) in a semi-arid herb land (O' Connor *et al*. 2002) and in

montane grassland (Karanika *et al*. 2008b). It was also noted in these field trials that other species, some of which were considered mycorrhizal, did not show a significant reduction in AMF presence when treated with fungicide, e.g. *P. lanceolata* (Newsham *et al*. 1995).

It is also possible to compare the levels of root infection in individual species with those from other trials. In Table 6.13 the percentage level of AMF root infection found in untreated *F. ovina* in six studies including this one is shown.

It can be seen in Table 6.13 that the results from the current study were in good agreement with three of the other trials, while two of the trials showed very high levels of root infection. This suggests that the level of root infection is influenced by the conditions in which plants are grown. Other measurements of root infection include 4% in *C. flacca* (Johnson *et al.* 2003) compared to 3% in this study. Van der Heijden *et al*. 1998a measured 25% root infection in *B. erectus* and 80% in *H. pilosella,* which compares with o 8% and 58% for these species in this study. The results suggest that levels of infection are generally lower in field conditions compared to microcosms.

Table 6.13 - Percentage AMF infection in roots of untreated *Festuca ovina.*

Study (Type)	***Festuca ovina*** **(Percentage root infection)**
This study (Field trial)	34%
(Newsham *et al*. 1995) (Field trial)	15%
(Karanika *et al*. 2008b) (Microcosm – field soil)	30% ± 10%
(van der Heijden *et al.* 1998a) (Pot Trial)	25%
(Grime *et al*. 1987) (Microcosm)	89%
(Johnson *et al*. 2003) (Microcosm – monoculture)	70%

In this study the level of AMF infection was generally higher in forb species compared with grasses and sedges. This observation is consistent with a study by Karanika *et al*. (2008b) of a trial of montane grassland species grown in natural soil. Karanika *et al*. (2008b) found that the levels of AMF infection were lowest in grass species, higher in forb species and highest in legumes including *L .corniculatus* and *T. pratense*. Karanika *et al*. (2008b) ascribe the low levels of AMF infection to finer root architecture and lower requirement for the nutrient P in grass species. Lower levels of AMF in grass species

appear to be a general observation, having also been reported in Prairie grassland species (Hartnett and Wilson, 1999). Lower levels of AMF in grass species were also found in a field study by Wearn and Gange (2007). Wearn and Gange (2007) measured the percentage AMF infection in three grasses, *Anthoxanthum oderatum*, *Holcus lanatus* and *Agrostis tenuis*. In samples taken in October the values for rabbit grazed plots were in the range 17 to 22%, this is similar to the value for *B. pinnatum* (19%) measured this study

The supply of P to plant species by AMF through a network of external hyphae is the major symbiotic process considered to be important when P availability in the soil is low (Koide, 1991). More recent studies have considered the P requirements of individual species and how P levels in the soil affect levels of AMF colonisation. In a study of montane grassland in Greece where the levels of soil P are low Karanika *et al*. (2008b) found that adding P to the soil increased AMF root colonisation in the high P demanding forb *Galium lucidum* but reduced colonisation in the forb *P. lanceolata* and the grass *Agrostis capillaries*. A meta-analysis incorporating a range of studies of field trials by Treseder (2004) found that nitrogen fertilisation reduced AMF infection levels by 15% and phosphorous fertilisation by 32%. In a study of community structure in Prairie grassland Collins and Foster (2009) found that where P levels in the soil were low then AMF influenced plant community structure, but at high P levels the influence of AMF on community structure was low.

The levels of AMF colonisation in the roots of individual species should be related to the supply of nutrients and the effect this has on the plant. In a paper by Gange and Ayres (1999) a model was proposed that predicted a general curvilinear relation ship between the level of AMF colonisation and the benefit received by the plant. Beyond the optimum level of colonisation benefit to the plant would decrease. The level of optimum root colonisation would be different for individual plants and vary with the conditions under which the symbiosis was occurring. In a field trial in which the forb *P. lanceolata* was being studied maximum benefit occurred at about 30% root colonisation (Ayres *et al*. 2006).

In the current study four of the species, *H. pilosella*, *S. pratensis* and *L. hispidus* and *B. pinnatum,* showed levels of AMF root colonisation which was substantially reduced by the application of fungicide (Table 6.8). Examination of Figure 6.23 suggests that this could be related to plant benefit because mean percentage cover of all four species responded

negatively to the application of fungicide. While it is not possible to determine if the plants in the control quadrats were receiving maximum benefit from AMF colonisation, the benefit must have been sufficiently high for the application of fungicide to produce a substantial loss of benefit. In microcosm studies the levels of AMF root colonisation and biomass correlated for *H. pilosella* but not for *B. erectus* and *F. ovina* (van der Heijden et al. 1998a).

Four species generally with lower levels of root infection, *P. media*, *F. ovina*, *B. erectus* and *C. flacca,* did not show a reduction in the AMF root levels, but in most cases a small increase. Inspection of Figure 6.23 shows three of the species had an increased mean percentage cover following the application of fungicide and the fourth, *C. flacca,* showed only a small negative decrease. This suggests that these plant species in control quadrats were not receiving significant nutrient benefit from the AMF present in their roots. It also suggests that the AMF species present were not reduced by Iprodione at the dose rates administered. It is possible that the AMF in these species benefited from competitive release from AMF species more sensitive to fungicide, as suggested by Helgason et al. (2007) in a study of a natural woodland community. The increase in cover probable results from increased nutrient availability arising from loss of AMF nutrient benefit in the more mycorrhizal dependent species (Hartnett and Wilson, 2002).

The relationship between length of root colonised and length of root in which vesicles were present may reflect the mutual benefit between individual plant species and the AMF species colonising them. The results from the current study showed that 16% of the root length infected with AMF contained vesicles. This agrees quite well with the study of Wearn and Gange (2007) on the grasses *Anthoxanthum oderatum*, *Holcus lanatus* and *Agrostis tenuis* where, depending on the treatment samples collected in October, gave percentage values for the presence of vesicles in AMF infected roots in the range 10% to 30%. The relationship between vesicles and length of root colonised suggest that AMF receive more benefit from the plant, the greater the length of root colonised. If the relationship is mutually beneficial this implies that the plant receives greater benefit from increased AMF colonisation, but as pointed out by Johnson *et al*. (1997) there is likely to be a continuum of relationships ranging from beneficial to antagonistic.

6.9 Conclusions

A field trial was conducted on semi-natural chalk grassland where the community being studied had the grass *B. pinnatum* as the most abundant species. The closest NVC match to the community tested was the sub-community CG4a (*Brachypodium pinnatum – Avenula pratensis – Thymus praecox*). The presence of indicator species at the research site suggests that this community has developed for a period in excess of 100 years. This, and the high plant species richness, may also suggest that a high diversity of AMF species was also present with the potential for a wide range of symbiotic relationships to develop. Thus the results of this trial should be applicable to other chalk grassland communities of this type.

The trial conducted over a period of four years examined the effect of applying the fungicide Iprodione at four dose rates. Appling fungicide at the dose rate of $0.5gm^{-2}$ and $1.0gm^{-2}$ produced no significant change to species presence or abundance in the community. At the higher dose rates of $2.0gm^{-2}$ and $4.0gm^{-2}$ applied over a two year period there were changes in the abundance of some plant species. However even at the higher fungicide dose rate of $4.0gm^{-2}$, only about a 30% reduction in AMF root colonisation was observed.

Of the 35 most abundant species present in the community, 15 showed significant changes in mean percentage cover following two years treatment at the higher dose rates.
Of the species affected by the application of fungicide the ratio of those negatively to positively affected was 2:1. The species that showed a strong negative response were those that previous microcosm research had identified as strongly mycorrhizal.

There was good consistency between the results of the field trials and that for individual species in earlier microcosm research. The main change in abundance in the field trials was that the strongly mycorrhizal grass *B. pinnatum* was replaced as the first ranked species by the weakly mycorrhizal grass *B. erectus*. In effect the result of the application of fungicide at the higher dose rates was to change the NVC classification of the community in fungicide treated quadrats from CG4a (*Brachypodium pinnatum* dominated) to CG3b (*Bromus erectus* dominated).

While there was good agreement between this field trial study and previous research in artificial microcosms, the field trials revealed an additional characteristic. The application of fungicide to a species rich community such as chalk grassland resulted in an increase in abundance of some species where a decrease would have been expected. These are species which microcosm research had categorised as strongly mycorrhizal. The probable mechanism is competitive release from species that are even more mycorrhizal.

The study shows that the community structure of chalk grassland can be altered through the application of fungicide causing the reduction of AMF activity in some species. The changes in species abundance have been achieved by relatively small reductions in the levels of AMF in species reacting negatively to the application of fungicide. This suggests that longer periods of fungicide application or higher dose rates would produce even greater changes to community structure. The aim of the field trial was to examine the role of AMF in structuring semi-natural chalk grassland communities. This has been completed and has shown that AMF/plant symbiosis has a substantial role in structuring chalk grassland communities.

Chapter 7 - Discussion

7.1 Introduction

In this chapter the broader implications of the findings from the analysis Chapters 4, 5 and 6 are considered. Initially the chalk grassland of the South Downs is considered at the landscape scale in conjunction with the composition of the regional species pool that generates the different chalk grassland communities. The concept of core species and their importance in defining community structure is discussed. The identification of nestedness in chalk grassland is considered in terms of how it relates to species entering and leaving the community. A comparison between nestedness in chalk grassland and in other nested communities is made.

The main aim of this research project was to investigate the role of AMF in structuring chalk grassland communities. This was achieved using two approaches: a laboratory trial using natural chalk grassland turf, and field trials. The rationale for the two approaches is discussed. The results are compared for commonality and differences. Where differences are found, possible reasons are advanced.

In the following two sections the results from the laboratory and field trials are firstly compared to those from previous research on calcareous plant species using artificial microcosms and secondly with results from other field trials on natural or semi-natural communities.

The final section interprets the findings of this study within the context of current ideas on the mechanisms operating in the complex conditions of multiple interactions between AMF species and plant species.

7.2 The Landscape, Species Pool, Core Species and Nestedness

Individual landscape types, for example deciduous woodland, salt marsh and upland moors, have their own visual characteristics. This allows them to be recognised without detailed analysis of the structural composition of the vegetation present. This is also true of chalk grassland found on the slopes of the South Downs. However detailed analysis of

plant survey data, collected in 1991 (Steven, 1992; Steven and Muggeridge, 1992) for chalk grassland in Sussex, shows repeating patterns of species presence and abundance.

Details of the analysis of survey data collected by Steven (1992) and Steven and Muggeridge (1992) are described in Chapter 4. The analysis has shown that in 1991 the regional species pool consisted of 226 different plant species present in chalk grassland in Sussex. Of these 68% were forbs, 13% grasses and sedges, 17% mosses and lichens and 2% shrubs. Many of the 226 species were present infrequently in chalk grassland communities with 94 species present at ≤ 5% of sites and a further 44 between 6 and 20% of sites. These species, while contributing to the character of chalk grassland and important in the context of conservation, will not have a major role in structuring chalk grassland communities. This role falls to the 33 core species (in this study defined as being present at > 70% of sites) of which 10 were grasses and the remainder forbs. Not only are these the most frequently occurring species in the community, the relationship between frequency and abundance (Figure 4.12), suggests that on average these core species will have higher small (quadrat) scale levels of abundance.

Analysis of the Steven (1992) and Steven and Muggeridge (1992) data for fragmented chalk grassland sites in Sussex, shows that plant species tend to be present, join or are lost from the communities in an ordered manner, that is it is more probable that a scarce or rare species with a low level of abundance is absent than a species with high abundance. This is illustrated by the strong associations found between adjacent frequency classes, that is between core and intermediate and between intermediate and scarce/rare. The consequence of these inter-class relationships is that chalk grassland community, which does not have a high complement of core species, will have few intermediate species. In the absence of a substantial number of intermediate species, scarce and rare species are likely to be absent. This relationship between frequency classes is present at both the site and quadrat scale. This relationship between frequency classes and the calculation of a strongly ordered „cool temperature", (see Chapter 4) for the chalk grassland sub-group community strong calcicoles, demonstrates that the plant species are nested.

The presence of nestedness in chalk grassland species on the South Downs is consistent with the observation of nestedness in other fragmented grassland landscapes, for example improved and unimproved meadows (Myklestad and Sætersdal, 2004), Alvar grasslands

(Partel *et al*. 2001), sedge meadows (Mathews, 2004) and in Californian grasslands (Elmendorf and Harrison, 2009). The form of the nestedness found in chalk grassland was imperfect, consistent with the theoretical predictions of Fischer and Lindenmayer (2002 and 2005). For perfect nestedness to occur species would be present in the order of their overall frequency of occurrence with no missing species. For the chalk grassland studied some core species were absent both at individual sites and in quadrats. Imperfect nestedness was most apparent at the quadrat scale. Here the number of missing species, that is gaps in the rank order, increases when moving from core to intermediate to scarce to rare. However the analysis has shown the ratios of core species to intermediate species and intermediate to scarce/rare species to be approximately constant, indicating that there are repeating patterns in the formation of chalk grassland communities.

This observed phenomenon of nestedness in chalk grassland probably results from underlying processes and mechanisms. At the larger scale, site area and site heterogeneity have been shown to be important (Honnay *et al*. 1999; Hylander *et al*. 2005), affecting species population sizes and extinction processes. Environmental, topographic and edaphic factors may also influence the presence or absence of a species. At a local scale, particularly the quadrat level, biotic processes become increasingly relevant. A number of studies have shown that frequency of grazing including abandonment (Enyedi *et al*. 2008; Denyer *et al*. 2009; Hannus and von Numers, 2010) and grazing intensity (Bacon, 1990; Pykälä, 2004; Sankaran and McNauhton, 2005) are important in maintaining species richness, by preventing extinction of species of low stature being out-competed in grassland communities. A biotic process that could contribute towards nestedness is AMF/plant symbiosis (Verdu and Valiente-Banuet, 2008). With their potential to mediate inter (van der Heijden *et al*. 1998a) and intra (Ayres *et al*. 2006) specific competition and aid seedling establishment (van der Heijden, 2004; van der Heijden and Horton, 2009), the presence of diverse (possibly nested) AMF species (Johnson *et al*. 2005; Oehl *et al*. 2010) could play an important role in the process of species leaving and entering the community.

7.3 Rationale for the Trials and Comparison of Results of Laboratory and Field Trials

7.3.1 Rationale for trials

The major aim of this research project was to demonstrate that AMF/plant symbiosis plays a substantial role in structuring semi-natural chalk grassland communities. Previous experimentation with microcosms (Grime *et al*. 1987; van der Heijden *et al*. 1998a); van der Heijden 2004) has shown that some plant species frequently found in chalk grassland, range in their mycorrhizal dependence from strongly beneficial to antagonistic. Thus trials testing mycorrhizal dependence of these and other chalk grassland plants under laboratory and field conditions were likely to show differences in mycorrhizal behaviour and associations. In earlier field trials on other plant communities it was demonstrated that the action of the fungicides Iprodione (Gange *et al*. 1992) and Benomyl (Smith *et al*. 2000) was essentially to lower mycorrhizal activity and that the impact on other soil organisms was low. In a review of the role of mycorrhizas in plant community dynamics, Hartnett and Wilson (2002) stated that, the suppression of AMF with fungicide was the best approach currently available to assess the role of AMF in natural communities. In the present study the approach was to weaken the AMF/ plant symbiosis by the application of the fungicide Iprodione. There is precedence for this approach which has been used to study plant communities in early succession (Gange *et al*. 1993) in which Iprodione was used, and in the study of Prairie grassland (Hartnett and Wilson, 1999) in which Benomyl was used. Since then Benomyl has been used in field trials testing the role of AMF in other grassland communities (Smilauer and Smilauerova, 2000; Karanika *et al*. 2008a).

The current research study was in two parts; the laboratory turf trials described in Chapter 5 and the field trials described in Chapter 6.

7.3.2 Comparison of laboratory and field trials

In the laboratory trials the chalk grassland turf was grown in trays for one season compared with two seasons of treatment in the field trials. In the laboratory trials the starting condition of the turf was broadly similar to that in the field trials, i.e. the plant, AMF and microbial species present in the turf were representative of those present in the field. However the environmental conditions were different in the two trials. In the laboratory trials temperatures experienced by the turf were those of spring and summer without night time lows. In high summer the turf in the laboratory was probably warmer than in the field with no chalk substrate to conduct heat away. An advantage of the laboratory trials was that the trays could be surveyed in great detail on a regular basis. Small changes in the

presence and abundance of species could be monitored and the growth of individual plants within trays observed.

The strength of the field trials was that they were conducted under natural environmental conditions. The effect of two seasons of treatment was measured and the combined turf area over the two sites was approximately 25 times greater for each treatment than that of the laboratory trials. Although not necessarily a disadvantage, abundance (percentage cover) was estimated in the field trial, and therefore lacked the precision of the laboratory trials.

Although the results from the laboratory and field trials differed in detail, there were many areas of consistency. In both trials, treatment with fungicide at the $0.5gm^{-2}$ and $1.0gm^{-2}$ levels had no significant effect on species presence and abundance. At the higher dose rates of $2.0gm^{-2}$ and $4.0gm^{-2}$ there were significant changes in community structure in both trials. The requirement to apply high doses of fungicide to significantly impair AMF/ plant symbiosis has been found by other researchers using both Iprodione (Ayres *et al*. 2006) and Benomyl (Hartnett and Wilson, 1999; Karanika *et al*. 2008a). In the current research, even at the highest dose rate, the reduction in symbiosis, as indicated by percentage reduction (~50 % compared to the control) in root infection was modest. There were no indications that the AMF species present were close to being eliminated, even at the higher dose rates of fungicide.

A notable difference between the two trials was the timescales at which changes to community structure occurred. In the laboratory trials significant changes to presence and abundance were measurable in less than 6 months, whereas in the field trials changes generally took two seasons to become significant. Longer times for the treatments to take effect in the field are consistent with the observations of Hartnett and Wilson (1999) when studying Prairie grassland, where it took 5 years for changes to develop fully.

Not only did changes in species presence and abundance occur more quickly in the laboratory trials and changes tended to be more significant. This may suggest that in the laboratory trials the species present were under greater environmental stress. It is also possible that under field conditions natural rainfall and drainage through the substrate may have weakened the effect of the fungicide. Thus in the laboratory trials both species

richness and abundance of forbs was significantly reduced at the conclusion of the trials at the higher dose rates. In the field trials, while the abundance of individual forb species changed, the overall percentage cover of forbs remained essentially constant. There was no firm evidence of species loss from the community in the field trials with the possible exceptions of *Centaurium erythraea* and *Trifolium pratense*. The observed differences between the two types of trial are consistent with the observations from other mycorrhizal research, for example Ayres *et al.* (2006) that small changes in environmental conditions can result in different plant behaviour. In a study of a grass, a sedge and four forbs from a semi natural grassland in North Carolina USA, Pringle and Bever (2011) examined the growth of the species in the laboratory and in the field. The species *Allium vineale, Anthoxanthum odoratum, Cerastium glomeratum, Plantago lanceolata, Rumex acetosella* and *Veronica arvensis* were grown in the presence of different AMF species. The growth of species in the laboratory was correlated with that in the field, with a stronger association for the more mycorrhizal responsive species.

In the study reported here, there was strong agreement from both the laboratory and field trials, concerning the behaviour of the dominant grasses *Brachypodium pinnatum*, *Bromus erectus* and *Festuca ovina.* It was found that treating the chalk grassland community with high levels of fungicide decreased the abundance of the mycorrhizal dependent grass *B. pinnatum* and increased the abundance of weakly/non mycorrhizal dependent grass *B. erectus* and to a lesser extent the non/antagonistic mycorrhizal grass *F. ovina*. The characteristics of these three grasses are important in chalk grassland community assemblage, forming the matrix into which the other species, predominantly forbs, fit (Grubb, 1986 ; Zobel *et al*. 2010).

7.4 Comparison of Current Research Results with those from Microcosm Studies

In general there is good agreement with the results of earlier mycorrhizal research conducted on calcareous plant species grown in artificial microcosms (Grime *et al*. 1987; van der Heijden *et al*. 1998a; van der Heijden, 2004; van der Heijden *et al*. 2003). In previous research, *B. pinnatum*, *Centaurium erythraea, Hieracium pilosella, Leontodon hispidus, Sanguisorba minor* and *T. pratense* were found to be strongly mycorrhizal and the results of the current study were consistent with these findings. However the forbs

Centaurea nigra, Plantago lanceolata and *Prunella vulgaris* were shown to be mycorrhizal in microcosms, but increased in abundance when treated with Iprodione in the current field trial. This may be due to changes in competitive behaviour with other plant species when AMF levels are reduced by spraying (Hartnett and Wilson 2002). In another trial *P. lanceolata* has shown ambiguous behaviour, with AMF being both beneficial and antagonistic under varying environmental conditions (Ayres *et al*. 2006). In a microcosm experiment *P. lanceolata* was grown as a monoculture and in competition with a legume and a grass and the response of *P. lanceolata* to AMF presence was low in both (Scheublin *et al*. 2007) suggesting that in this trial AMF did not affect the competitive ability of *P. lanceolata*

In microcosms, *B. erectus* and *F. ovina* were shown not to benefit from association with AMF and Grime *et al*. (1987) suggests that AMF may be antagonistic to *F. ovina*. In a microcosm experiment by Scheublin *et al*. (2007) *F. ovina* grown as a monoculture benefited from the presence of AMF, but in competition with *Lotus corniculatus* the biomass of *F. ovina* was significantly reduced in the presence of AMF. Both the laboratory and field trials in this research have confirmed that a reduction in AMF activity has a beneficial effect on the growth response of *B. erectus* and *F. ovina*. *Carex flacca* was shown to be non-mycorrhizal in microcosm research (van der Heijden *et al*. 1998b) and levels of root infection from the laboratory and field trials support this. However in the field trials the abundance of *C. flacca* reduced significantly after the application of fungicide. This may be an indirect effect resulting from a lowering of AMF presence in the community (Hartnett and Wilson, 2002). This may have lead to changes in the competitive relationships of *C. flacca* with other species.

7.5 Comparison of Current Results with Those from Other Field Trials

Comparing the results of the current field trials, with those of other field trials (Gange *et al*. 1993; Hartnett and Wilson, 1999; O' Connor *et al*. 2001; Newsham et al. 1995; Smilauer and Smilauerova, 2000; Karanika *et al*. 2008a) it is evident that there some common factors. In the current trials and other field trials, the application of either Iprodione or Benomyl, usually at relatively high concentrations, has produced changes in plant community structure. In some cases the patterns of change emerge after several years of treatment (Hartnett and Wilson, 1999; Karanika *et al*. 2008a). In mature plant communities

in late succession the effect of reducing AMF presence was to reduce the fitness of one (or more) strongly mycorrhizal dominant specie and promote the growth of other dominants or subordinate species, as in this research and that of Hartnett and Wilson (1999). The ability of AMF to affect the competitive ability of dominant species shows its potential importance in determining the abundance of matrix species in a community in addition to the interstial subordinate species.

7.5.1 Comparison with individual trials

In a trial performed by Gange *et al*. (1993) on a plant community in very early succession, the results showed that AMF were a factor in the survival of individual plant species. The role of AMF in seedling survival was shown to be important in establishing and maintaining the community and allowing subordinate species to prosper. In the absence of AMF species, perennial forbs in particular, were lost from the community. While AMF have been shown to be important in this early successional community, the short timescale of community regeneration, that is completely from seed, probably precludes the development of complex relationships between AMF and plant species including positive and negative feedback likely to be found in mature communities such as chalk grassland (Bever, 2002; Bever *et al*. 2002; Reynolds *et al*. 2003; Zhang *et al*. 2010; Bever *et al*. 2010). Mature communities in late succession such as the chalk grassland in this study are also likely to have high AMF diversity (Johnson *et al*. 2005; Oehl *et al.* 2010). The mycorrhizal immaturity of early succession communities compared to more mature communities may make them more vulnerable to environmental stress. In this context it is worth noting that the results from the field trials carried out by Gange *et al*. (1993) correspond more closely with the laboratory turf trials than the field trials in the current research. This is based on the contention that plants in the laboratory trials were more stressed than those in the field trials.

In field trials on a lichen/moss community in Suffolk, U.K, treatment with the fungicide Benomyl over a three year period allowed the moss *Ceratodon pupureus* to become dominant, eliminating the lichen *Cladonia rangiformi* from the community (Newsham *et al*. 1995). Of greater relevance to the current research, an increase in abundance of the grass *F. ovina* and forb *P. lanceolata* was also observed, as was found in the current field

trials. Thus there appears to be accumulating evidence that *F. ovina* and *P. lanceolata* may benefit from the reduction in AMF in field conditions (Newsham *et al*. 1995).

Between 1991 and 1995 a field trial exploring the role of mycorrhizal fungi in shaping the community structure in Prairie grassland in Kansas USA was carried out (Hartnett and Wilson, 1999). The effective dosage of fungicide used on the Prairie grassland, Benomyl at 1.25g m^{-2}, applied at 2 week intervals throughout the growing season, was similar to the 2.0gm^{-2} and 4.0gm^{-2} of Iprodione applied monthly in this chalk grassland trial. The initial community structure for the Prairie trial differed from that of the chalk grassland community in that three out of the four dominant C4 warm season grasses (*Andropogon gerardii, Sorghastrum nutans* and *Andropogon scorparius*) were strongly mycorrhizal. These Prairie grasses showed a 25% reduction in cover after two season of treatment with Benomyl, a similar value to that for *B. pinnatum* in chalk grassland. One Prairie dominant grass, *Panicum virgatum* was unaffected by the application of fungicide. In the Prairie grassland trial subordinate weakly mycorrhizal grasses and forbs benefited from the reduction in fitness of the three dominant strongly mycorrhizal grasses. In this chalk grassland the weakly mycorrhizal co-dominant grasses *B. erectus* and *F. ovina* were the main beneficiaries in the reduction of fitness of *B. pinnatum*. Although the changes observed in the two types of grassland differ in detail, there is a clear pattern of response in both. During the Prairie field trials the percentage root infection in the dominant grasses was measured. After the first season of treatment the levels of root infection fell from a level of 15 - 20% in grasses from control plots to 2 - 5% in fungicide treated plots. These low levels of root infection both in the controls and treated roots are similar to those measured in grasses and sedges in this study. Thus the levels of infection in these graminoids from different geographical regions were similar and much lower than those generally observed in forbs.

A field trial was conducted between 1994 and 1998 on a hay meadow in the Czech Republic (Smilauer and Smilauerova, 2000). The meadow was of low nutrient status, cut once in June and had been under the same management for more than 120 years. The fungicide Benomyl was applied every five weeks at a rate of 4.5 gm^{-2} in 1994/5 and 8gm^{-2} in 1996/7/8. The application of fungicide had little effect on the biomass of grasses and sedges, but reduced the biomass of forbs including *P. lanceolata* by 25%. The main change was in the biomass of the forb *Achillea millefolium*, which increased by 75%. There are

some similarities between the study of Smilauer and Smilauerova (2000) and the current study, but because Smilauer and Smilauerova (2000) treated the grasses and sedges as a group and identified only three forbs individually only limited comparisons can be made. The most relevant observation was the increased biomass of *Achillea millefolium*, which reflects observations in the current study where the forb *Centaurea nigra* increased in abundance in quadrats sprayed with fungicide. Smilauer and Smilauerova (2000) suggest elimination of root pathogens by the fungicide as an explanation, but competitive release as suggested by Hartnett and Wilson (2002) would appear to be equally valid.

A trial in southern Australia on a semi-arid herbland using Benomyl as the fungicide resulted in an approximate 75% reduction in the dominant mycorrhizal herb *Medicago minima* and significant increases in the biomass of the low/non mycorrhizal herbs *Salvia verbenaca* and *Carrichtera annua* (O' Connor *et al*. 2001). There appears to be a parallel in the responses of these semi-arid herbs to the application of fungicide and those of the chalk grassland species. The mycorrhizal grass *B. pinnatum* was reduced in abundance by the application of fungicide and the weakly mycorrhizal *B. erectus* and *F. ovina* increased in abundance. This suggests that the mechanics of competition may be similar in the two communities. Glasshouse trials performed in conjunction with the field trials had shown that in addition to the dominant specie *Medicago minima,* two subordinate species *(Vittadinia gracilis* and *Velleia arguta*) were also strongly responsive to the presence of AMF (O' Connor *et al*. 2001). However in the field trials the biomass of *V. gracilis* and *V. arguta* was not reduced by the application of fungicide. O' Connor *et al*. (2001) argue that for *V. gracilis* and *V. arguta* the decrease in benefit from the reduction in the presence of AMF is offset by the decline in competitiveness of the dominant *M. minima*. They suggest that the AMF response of a plant grown in isolation is not a good predictor of its response in the competitive environment of a plant community. In the current field trials *C. nigra* showed similar trends, increasing in abundance with the application of fungicide, despite results from microcosms showing it to be strongly mycorrhizal (Grime *et al*. 1987). In a trial on semi-arid herblands (O' Connor *et al*. 2001), the application of fungicide reduced the percentage root infection in the herbs to about 70% of that in the controls, which is a similar reduction to that in the current laboratory and field trials.

A field trial has been conducted on Montane grassland in Greece (Karanika *et al*. 2008a). The mycorrhizal characteristics of this grassland are different to that in Prairie grassland

(Hartnett and Wilson, 1999) in that the dominant grass (*Agrostis capillaris*) and forb (*Galium lucidum*) are non mycorrhizal and the subordinate forbs in the community are mycorrhizal. In this study of chalk grassland one dominant grass and the forbs are mycorrhizal. The trial in Greece was conducted over two growing seasons (Karanika *et al*. 2008a). The grassland was growing in condition of low P and other nutrient levels in the soil. Mycorrhizal activity was reduced by the application of Benomyl at the rate of $1.25gm^{-2}$ at 2 week intervals throughout the growing season (April to July). The main changes to community structure were observed after the second season of fungicide treatment. As expected *A. capillaris* and *G. lucidum* were not affected by the application of fungicide. Subordinate forbs and legumes were reduced in abundance, some being eliminated from the community. However some species benefited from the reduction of AMF activity. These were sedges as a group and the perennial legume *Doricnium herbaceum*. The increase in biomass of *D. herbaceum* occurred despite studies of individual species from this grassland showing legumes to have the highest levels of AMF colonisation (Karanika *et al*. 2008b). The positive response of *D. herbaceum* to the application of fungicide is consistent with observations on other mycorrhizal species in the competitive environment of the community and again suggests competitive release (Hartnett and Wilson, 2002).

Comparison of the current research on chalk grassland with trials from other diverse plant communities has shown a number of areas of commonality. All of the trials have shown changes in community structure following the application of fungicide. It has also been generally demonstrated that repeated application of fungicide reduces the level of AMF in plant roots and results in greater changes to community structure. In a number of studies there have been unexpected increases in the abundance of individual species following the application of fungicide. It can be concluded that in untreated plant communities AMF/plant symbiosis is one of the important factors that structure plant communities.

7.6 Community Dynamics and Mechanisms

The evidence from the current study and other experiments and field trials has shown AMF to have an important role in structuring chalk grassland communities. Within a recent review on the importance mycorrhizal networks for facilitation in natural ecosystems, van der Heijden and Horton (2009) describe the role of AMF in seedling establishment adjacent to larger plants. Their findings were that 48% of plants benefited from the

presence of AMF, 25% showed negative effects and 27% no effects (van der Heijden and Horton, 2009). In the current field trials on chalk grassland the results for the quadrats sprayed with 4.0gm^{-2} showed that 50% of species reacted negatively to spraying (i.e. benefited from AMF), 26.5% reacted positively (i.e. did not benefit from AMF) and 23.5% were unaffected (see Table 6.5) which were similar to those of van der Heijden and Horton (2009). In the current field trials it is not possible to determine contributions of cover from seedling establishment and cover derived from changes in mature or clonal plants. However a possible explanation for the strong agreement of the two sets of results is that plants that are strongly mycorrhizal as seedlings retain this characteristic into maturity. Both sets of data suggest that in complex plant communities species with a range of AMF dependence will be present. It is possible that this diversity in AMF/plant relationships is important in maintaining long term stability in community structure, in the case of chalk grassland for periods in excess of 100 years (Gibson and Brown, 1991; Karlik and Poschlod, 2009).

Many of the trials carried out in microcosms involved three way testing of species, usually of strong, intermediate and low mycorrhizal dependence. A common feature, for example in a trial involving *Festuca ovina*, *Lotus corniculatus* and *Plantago lanceolata* (Scheublin *et al*. 2007), was that the mycorrhizal dependence of species grown separately, that is their fundamental AMF/plant niche, was different to the realised mycorrhizal niche when the plant was grown in competition. Thus within a community containing many species, individual plants of the same species may have different realised mycorrhizal niches depending on who their immediate neighbours and competitors are and which species of AMF have colonised their roots. There is evidence that plant neighbours are able to affect the species and behaviour of the AMF present (Hausmann and Hawkes, 2009). While the concept of carbon transfer between plants remains controversial there is recent evidence of the transfer of phosphate between plants via hyphae. Wilson *et al*. (2006) report that in a trial involving interactions between tallgrass Prairie species there was greater flow of the phosphorus isotope ^{32}P from a facultative mycotrophic forb (*Artemisia ludoviciana*) to a an obligate mycotrophic grass (*Sorgastrum nutans*) via hyphae than occurred in the reverse direction. This transfer of a nutrient from one species to another via a hyphal network could be a mechanism that contributes to the survival of mycorrhizal subordinates, mainly forbs, in chalk grassland communities.

The role of soil organisms, including AMF, in promoting positive and negative feedback processes is now considered very important in structuring plant communities (Bever *et al*. 2010). While positive feedback from soil organisms tends to increase the abundance of already dominant species, negative feedback restricts dominants and increases benefit to subordinates, increasing diversity (Bever *et al*. 2010). In the current study on chalk grassland, the three dominant species in order of their abundance were *B. pinnatum*, *B. erectus* and *F. ovina*. Of these three species, both the laboratory experiments and field trials have shown *B. pinnatum* to be strongly mycorrhizal in comparison to the other two. On this basis it can be argued that *B. pinnatum* will be the species that has the greatest „mycorrhizal" effect on the structure of this community. *B. pinnatum* with highest abundance and a fine root system will contribute many hyphae to the overall AMF hyphal network. Thus it can be postulated that *B. pinnatum* will be highly connected to the roots of the subordinate species within the community. However Bever *et al*. (2010) suggest that transfer of material between plants via a hyphal network will be of secondary importance compared to positive and negative feedback. There is also a high probability that for individual forbs of a particular species, that tend to be distributed throughout the community rather than closely bunched, one of their nearest neighbours will be *B. pinnatum*. Thus individual forbs may be in competition with *B. pinnatum*, particularly for light, while at the same time being in a mycorrhizal symbiotic relationship where *B. pinnatum* influences the AMF species present and the colonising characteristics of the forb (Hausmann and Hawkes, 2009). It therefore appears possible that *B. pinnatum* could have a dual role within the community. Ungrazed or uncut it will overtop the community and reduce forb survival, but held in check by grazing, which may also modify its relationship with AMF and competitive ability (Pietikainen and Kytoviita, 2007), *B. pinnatum* may also have a role in the survival of some subordinate forb species contributing to diversity.

Research on other species rich communities suggests that in chalk grassland there should be many species of AMF present (Fitter, 2005; Johnson *et al*. 2005). A model where all species of AMF were interacting with all of the plant species invokes a system of immense complexity and potential for changeable competitive relationships, whereas chalk grassland can remain stable for long periods of time (Karlik and Poschlod, 2009) and nestedness suggests a hierarchy of competitive relationships. A more realistic scenario may be for smaller units of two to four plants to interact, including *B. pinnatum* where some benefit from the presence of AMF and others do not. Indeed, observations in this study

(e.g. section 5.5.3.1) show that forb species in trays tended to be present in clusters of 4 to 7 plants. The interaction in and between these sub-units may provide the dynamics for the contribution that AMF make to community structure. Spraying in the field trials reduced individual species abundance in proportion to their pre-treatment abundance, i.e. species starting with low abundance were not eliminated from the community. This suggests that the community reacts as a whole and not necessarily as individual species.

A feature of chalk grassland is its species richness at a fine scale with forbs in particular remaining small in stature. It seems probable that interactive AMF/plant symbiosis within interconnected hyphae networks play a role in maintaining size equality between plants and this could be evidence of negative feedback (Bever *et al*. 2010). If some plants are able to out grow and out compete their neighbours, their neighbour‟s survival will be threatened. Although difficult to determine if this was happening in the field trials, in the laboratory trials at the higher fungicide dose rates some plants were growing large and others disappearing from the trays.

The main aim of this research project was to investigate the role of AMF/ plant symbiosis in structuring chalk grassland communities. This research project has demonstrated that AMF/plant symbiosis has a very significant role in determining the structure of chalk grassland communities and allowed insights into the underlying dynamics and mechanism involved.

Chapter 8 - Conclusions

8.1 Conclusions

The principle aim of this PhD thesis was to evaluate the role AMF play in structuring chalk grassland communities. This was achieved through meeting the following objectives.

1. The structure of the chalk grassland communities was determined by the analysis of detailed survey data. In particular the relationship between frequency classes, nestedness and frequency/abundance relationships were considered to describe the structure of chalk grassland communities.

2. The role of AMF in structuring these chalk grassland communities was assessed using both laboratory based and field trials. In these trials the symbiotic relationship between AMF and chalk grassland plants was weakened by the application of the fungicide Iprodione. Detailed survey and analysis of species presence and abundance showed that AMF/ plant symbiosis has a significant role in determining the structure of chalk grassland communities.

The analysis reported in Chapter 4 showed that chalk grassland on the South Downs in Sussex had a local species pool of 226 species, of which 68% were forbs, 11% grasses or sedges and 17% mosses or lichens. Thirty two species were identified as belonging to the functional group strong calcicoles. Comparison of the 1991 survey data of (Steven and Muggeridge, 1992; Steven, 1992) with survey data collected in 1926 by (Tansley and Adamson, 1926) showed that the balance between forbs, grasses and sedges, and mosses and lichens was largely unchanged, although both *Brachypodium pinnatum* and *Bromus erectus* had increased in abundance.

Examination of the relationship between the frequency classes core, intermediate and scarce/rare showed that the total species present at a site and in a quadrat was related to the number of core species present. This relationship was particularly significant at the small (quadrat) scale. It can be noted from the literature (e.g. Hanski, 1982; Collins and Glenn, 1997)) that emphasis is put on the relationship between core and satellite species. The results of this study suggest that the „intermediate" group of species are equally important,

particularly when examining the role of underlying factors such as AMF on structuring plant communities.

Using the functional group strong calcicoles as representative of the local species pool it was shown that in common with other fragmented grassland communities, chalk grassland species were nested at both the site and quadrat scales. Thus colonisation and extinction were occurring at the quadrat scale, where competition and interaction between individual plants from different species was occurring as well as at the site scale. A strong frequency/abundance relationship was found where the most frequently observed species (core) also had the highest abundance.

The current research has shown chalk grassland communities not to be random collections of plant speciesthat is with core species having the highest probability of being present and scarce/rare the lowest. These patterns of species presence suggest that community structure is determined by underlying mechanisms and rules. That chalk grassland communities are structured at the small scale, where plant interactions take place, strengthens the validity of research into the role of AMF/plant symbiosis in structuring chalk grassland communities.

In laboratory turf trials (Chapter 5) the initial objective was to keep the turf alive and growing for a minimum of one season. This was achieved in 2007 and 2008. Prior to starting the trial it was noted that forb species tended to grow in small mixed groups of 4 to 7 plants. At the start of the trial it was unclear how high the dose rate of Iprodione would need to be to substantially weaken AMF/plant symbiosis. From changes in species abundance and levels of AMF infection in plant roots, a dose rate of $2.0gm^{-2}$ - $4.0gm^{-2}$ Iprodione per month was found to be effective in significantly weakening AMF/plant symbiosis and changing community structure.

The two significant changes in species abundance followed the application of fungicide at $2.0gm^{-2}$ and $4.0gm^{-2}$ were that *B. pinnatum* reduced in abundance and *B. erectus* increased in abundance. Examination of the roots of forbs, grasses and sedges showed the levels of root infection consistent with their predicted levels of mycorrhizal dependence (van der Heijden, 2002). The application of Iprodione at the dose rate of $2.0gm^{-2}$ and $4.0gm^{-2}$ per month reduced the levels of root infection in „mycorrhizal" species but not in „non-mycorrhizal" species.

Field trials (Chapter 6) were conducted on a chalk grassland community where *B. pinnatum* was the most abundant species and the closest match to the National Vegetation Classification was *Brachypodium pinnatum* sub-community CG4a (*Avenula pratensis* - *Thymus praecox*). The chalk grassland studied conformed to the specification for ancient chalk grassland (Gibson and Brown, 1991) which indicated that it had been developing for a period in excess of 100 years.

B. erectus replaced *B. pinnatum* as the most abundant species following the application of $2.0gm^{-2}$ and $4.0gm^{-2}$ Iprodione at monthly intervals over two growing seasons. Of the 35 most abundant species approximately 50% reacted negatively to the application of fungicide (i.e. benefit from an association with AMF). About 25% reacted positively to the application of fungicide and a further 25% were unaffected. In total nine species showed a significant negative and four a significant positive response to the application of fungicide. Some of the species that gave a positive response to the application of fungicide had previously been found to benefit from the presence of AMF when grown alone or in microcosms. The unexpected positive response of some species to the application of fungicide has been observed in other grassland communities (Smilauer and Smilauerova 2000; Karanika *et al*. 2008). These positive reactions are probably the result of competitive release (Hartnett and Wilson, 2002), although reduced levels of root pathogens has also been suggested (Smilauer and Smilauerova, 2000). The levels of root infection in the forbs and grasses examined were consistent with changes in abundance of these species as highlighted by multivariate analysis (Chapter 6).

Overall this study of the of semi-natural grassland communities on the South Downs has shown them to be structured in an ordered manner. The presence and abundance of individual species has been shown to conform to a pattern where the presence of core species is more probable than that of scarce or rare. This suggest that underlying abiotic (for example pH and rainfall) and biotic factors (for example grazing and AMF) have an important role in determining community structure. Laboratory and field trials where semi-natural chalk grassland communities were treated with fungicide have shown that by weakening AMF/plant symbiosis community structure (species presence and abundance) can be significantly changed. In general the changes observed for individual species were consistent with the findings of microcosm research. An additional finding from the field

trials was that within the complex interactions of a species rich community, some mycorrhizal plants can benefit from a reduction in AMF activity. Thus this research has demonstrated that AMF has a significant role in structuring semi-natural chalk grassland communities.

8.2 Limitations

The research was performed on one type of chalk grassland community: *B. pinnatum* sub-community CG4a (*Avenula pratensis - Thymus praecox*). Other chalk grassland communities have *B. erectus* or *F .ovina* as the most abundant species. From the research presented in this thesis it has been shown that both *B. erectus* and *F. ovina* are more weakly mycorrhizal dependent than *B. pinnatum*. Treating *B. erectus* and *F. ovina* dominated communities with fungicide would therefore be expected to produce different changes in community structure. An understanding of the role of AMF in structuring a range of communities would give a more sophisticated appreciation of how AMF structure semi-natural chalk grassland communities.

The field trial ran for only two years at the higher fungicide dose rates of $2.0gm^{-2}$ and $4.0gm^{-2}$. Conducting the trial over a longer period, or at a higher dose rate, might have led to the elimination of some species from the community or other species to show a significant positive response. It might also have been possible to collect evidence of plant size inequality developing, the prevention of which may be an important role of AMF in structuring chalk grassland communities. Thus a longer trial period would not change the conclusions of this research, but might give a more detailed understanding of the role of AMF in of respect of individual species.

Statistical analysis of the data collected in the laboratory and field trials has shown AMF to have a significant role in structuring chalk grassland communities. However it is acknowledged that more sophisticated statistical analysis of this data may reveal additional inter plant relationships.

8.3 Recommendations

The analysis described in Chapter 4 was based on surveys of 136 sites in Sussex carried out in 1991. It is now 20 years since the last comprehensive survey and the results from Newmarket Hill suggest that changes in rank abundance have occurred. Re-surveying all 136 sites would be both expensive and require many hours of surveying. A more feasible plan might be to identify a smaller number of sites (that is about 20) representative of the original 136, to be re-surveyed. More up to date information on the current structure of chalk grassland communities would be a valuable input into conservation and restoration strategies. The creation of the new South Downs National Park could provide the impetus for this to be done.

The triangular relationship between *B. pinnatum*, *B. erectus* and *F. ovina* is clearly important in the structuring of chalk grassland. While this could be studied by further field trials a laboratory based experiment would be more feasible. Using soil from the field containing a full compliment of AMF, *B. pinnatum*, *B. erectus* and *F. ovina* could be grown in different combinations. The fungicide Iprodione would be applied to weaken AMF/plant symbiosis and changes in abundance measured.

Recently reported research suggests that plant neighbours are important in determining which AMF species are present and their colonising characteristics ((Hausmann and Hawkes, 2009). Results from the laboratory trials in this study showed that prior to treatment plants of different species tended to cluster in groups of 4 – 7. Thus field work at a number of chalk grassland sites using small scale (for example 10cm x 10cm) quadrats and recording neighbouring species has the potential to reveal community structure at the inter-plant scale.

Large areas of chalk grassland have been lost to agriculture and development over the last sixty years and conservation and restoration of semi-natural chalk grassland is important in arresting and reversing this trend. The analysis in Chapter 4 showed that core species must be present in the community for less frequent species to be incorporated. Chalk grassland is very difficult to re-create. A small scale field trial in which core species are grown from seed in the presence of chalk grassland AMF, and allowed to become established, with intermediate and scarce species introduced sequentially is a prospective strategy that could be used in restoration.

Chapter 9 - Bibliography

ADASTRA, (2010) An annual review of wildlife recordings in Sussex. The Sussex Biodiversity Centre.

Allison, V. J. (2002) Nutrients, arbuscular mycorrhizas and competition interact to influence seed production and germination success in *Achillea millefolium*. *Functional Ecology,* **16**, 742-749.

Atmar, W. and Patterson, B.D. (1993) The Measure of Order and Disorder in the Distribution of Species in Fragmented Habitats. *Oecologia*, **96**, 373-382.

Atmar, W and Patterson, B.D. (1995) The nested temperature calculator: a visual basic programme, including 294 presence-absence matrices. AICS Research, INC., University Park, N.M and The Field Museum, Chicago, IL. Report

Auge, R.M., Sylvia, D.M., Park, S., Buttery, B.R., Saxton, A.M., Moore, .L. and Cho, K.H. (2004) Partitioning mycorrhizal influence on water relations of *Phaseolus vulgaris* into soil and water components. *Canadian Journal of Botany-Revue*, **82**, 503-514.

Ayres, R.L., Gange, A.C., and Aplin, D. M. (2006) Interactions between arbuscular mycorrhizal fungi and interspecific competition affect size and size inequality of *Plantago lanceolata* L. *Journal of Ecology,* **94**, 285-294.

Bacon, T. C. (1990) The Use of Livestock in Calcareous Grassland Management. *Calcareous Grassland - Ecology and Management.* (ed. by S. H. Hillier, W. D. E. Walton, and D. A. Wells), pp. 121-127. Bluntisham Books., Huntingdon.

Barrett, G., Campbell, C.D., Fitter, A.H. and Hodge, A. (2011) The arbuscular mycorrhizal fungus *Glomus hoi* can capture and transfer nitrogen from organic patches to its associated host plant at low temperature. *Applied Soil Ecology*, **48**, 102-105.

Bennie, A., Hill, M.O., Baxter, R., and Huntley, B. (2006) Influence of slope and aspect on long-term vegetation change in British chalk grassland. *Journal of Ecology,* **94**, 355-368.

Bever, J.D. (2002) Negative feedback within a mutualism: host specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings Royal Society,* **269**, 2595-2601.

Bever, J.D., Pringle, A., and Schulz, P.A. (2002) Dynamics within the Plant - Arbuscular Mycorrhizal Fungal Mutualism: Testing the Nature of Community Feedback. *Mycorrhizal Ecology* pp. 267-290. Springer.

Bever, J.D., Westover, K.M., and Antonovics, J. (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology,* **85**, 561-573.

Bever, J.D., Dickie, I.A., Facelli, E., Facelli, J.M., Klironomos, J.N., Moora, M., Rillig, M.C., Stock, W.D., Tibbett, M. and Zobel, M. (2010) Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology and Evolution*, **25**, 468-478.

Biodiversity Action Group (2000) Habitat Action Plan for Sussex -Chalk Grassland.

Bobbink, R. (1991) Effect of Nutrient Enrichment in Dutch Chalk Grassland. *Journal of Applied Ecology.* **28**, 28-41.

Bonis, A., Grubb, P.J. and Coomes, D.A. (1997) Requirements of gap-demanding species in chalk grassland: reduction of root competition versus nutrient enrichment by animals. *Journal of Ecology*, **85**, 625-633.

Bossuyt, B., De Fre, B. and Hoffman,M. (2005) Abundance and flowering success patterns in short-term grazing grasland:early evidence of facillitation. *Journal of Ecology*, **93**, 1104-1114.

Brundett, M.C, Piche, Y. and Peterson,, R.L. (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Canadian Journal of Botany*, **62**, 2128-2134.

Bruun, H.H. and Moen, J. (2003) Nested communities of alpine plants on isolated mountains: relative importance of colonisation and extinction. *Journal of Biogeography*, **30**, 297-303.

Brys, R., Jacquemyn, H., Endels, P., De Blust, G., and Hermy, M. (2004) The effects of grassland management on plant performance and demography in the perennial herb *Primula veris. Journal of Applied Ecology,* **41**, 1080-1091.

Bucking, H. and Shachar-Hill, Y. (2005) Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. *New Phytologist,* **165**, 899-912.

Buckland, S.M., Grime, J.P., Hodgson, J.G., and Thompson, K. (1997) A comparison of plant responses to the extreme drought of 1995 in northern England. *Journal of Ecology,* **85,** 875-882.

Burnside, N.G. (2000) Ecological Management of the South Downs: Application of GIS and Landscape Ecology. PhD Thesis, University of Brighton..

Burnside, N.G., Smith, R.F., and Waite, S. (2003) Recent historical land use change on the South Downs, United Kingdom. *Environmental Conservation,* **30**, 52-60.

Burnside, N.G., Smith, R.F., and Waite, S. (2002) Habitat suitability modelling for calcareous grassland restoration on the South Downs, United Kingdom. *Journal of Environmental Management.,* **65**, 209-221.

Burnside, N.G. (2005) GIS representation of NVC communities at Newmarket Hill.

Burnside, N.G. (2008) Slope and Aspect of Newmarket Hill, Castle Hill SSSI.

Chen, X., Tang, J., Zhi, G., and Hu, S. (2005) Arbuscular mycorrhizal colonisation and phosphorus acquisition of plants: Effects of coexisting species. *Applied Soil Ecology,* **28**, 259-269.

Chytry, M., Tichy, M. and Rolecek, J. (2003) Local and regional patterns of species richness in central European vegetation types along a pH/calcium gradient. *Folia Geobotanica*, **38**, 429-442.

Chytry, M., Danihelka, K., Ermakov, N., Hajek, M., Hajkova, P., Koci, M., Kubesova, S., Lusyk, P., Otypkova, Z., Popov, D., Rolecek, M., Reznickova, M., Smarda, P. and Valachovic, M. (2007) Plant species richness in continental southern Siberia: effects of pH and climate in the context of species pool hypothesis. *Global Ecology and Biogeography*, **16**, 668-678.

Clements, F. E. (1916) *Plant Succession. An analysis of the Development of Vegetation.* Washington.

Collins, S.L. and Glenn, S.M. (1990) A Hierarchical Analysis of Species Abundance Pattern In Grassland Vegetations. *The American Naturalist.*, **135**, 633-648.

Collins, S.L. and Glenn, S.M. (1997) Effect of organismal and distance scaling on analysis of species distribution and abundance. *Ecological Applications.*, **7**, 543-551.

Collins, S.L., Suding, K.N., Cleland, E.E., Batty, M., Pennings, S.C., Gross, K.L., Grace, J.B., Gough, L., Fargione, J.E. and Clarrk, C.M. (2008) Rank clocks and community dynamics. *Ecology*, **89**, 3534-3541.

Collins, C.D. and Foster, B.L. (2009) Community-level consequences of mycorrhizae depend on phosphorous availability. *Ecology*, **90**, 2567-2576.

Crawley, M.J. (1997a) The Structure of Plant Communities. *Plant Ecology.* (ed. by M.J. Crawley), pp. 475-531. Blackwell Science.

Crawley, M. J. (1997b) Plant - herbivore Dynamics. *Plant Ecology* (ed. by M. J. Crawley), pp. 401-474.

Dahl, E. and Hadac, E. (1941) Strandgesellschaften der Insel Ostøy im Oslofjord. *Nyt. Mag. Naturv,* **82**, 251.

Daleo, P., Alberti, J., Canepuccia, A., Escapa, M., Fanjul, E., Silliman, B.R., Bertness, M.D. and Iribarne, O. (2008) Mycorrhizal fungi determine salt-marsh plant zonation depending on nutrient supply. *Journal of Ecology*, **96**, 431-437.

Denyer, J. L. (2005) Interaction between Rabbits, Plants and Soil and their Consequences for Chalk Grassland and Chalk Heath Vegetation. University of Sussex, Brighton.

Denyer, J.L., Hartley, S.E. and John, E.A. (2007) Small mammalian herbivore determines vegetation response to patchy nutrient inputs. *Oikos*, **116**, 1186-1192.

Denyer, J.L., Hartley, S.E. and John, E.A. (2009) Both bottom-up and top-down processes contribute to plant diversity maintenance in an edaphically heterogeneous ecosystem. *Journal of Ecology*, **98**, 498-508.

Department of Environment, Food and Rural Affairs. The Natural Choice: securing the value of nature. (2011). White Paper. ISBN 9780101808224. The Stationary Office Limited.

Dumbrell, A.J., Ashton, P.D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A.H. and Helgason, T. (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist*, **190**, 794-804.

Ellenberg, H., Weber, H. E., Dull, R., Wirth, V., Werner, W., and Pauliben, D. (1991) Ziegerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica,* **18**, 1-248.

Elmendorf, S.C. and Harrison, S.P. (2009) Temporal variability and nestedness in California grassland species composition. *Ecology*, **90**, 1492-1497.

Emery, M. Grazing at Newmarket Hill. 2009. Personal Communication

Enyedi, Z.M., Ruprecht, E. and Deak, M. (2008) Long-term effects of the abandonment of grazing on steppe-like grassland. *Applied Vegetation Science*, **11**, 55-62.

Eriksson, A. (2001) Arbuscular mycorrhiza in relationship to management history, soil nutrients and plant species diversity. *Plant Ecology*, **155**, 129-137.

Ewald, J. (2003) The calcareous riddle: Why are there so many calciphilous species in central European flora. *Folia Geobotanica,* **38**, 357-366.

Field, A. (2009) Repeat-measures designs (GLM 4). *Discovering Statistics Using SPSS.* (Third Edition) pp. 457 - 505 Sage Publications.

Fischer, M. and Stocklin, J. (1997) Local Extinctions of Plants in Remnants of Extensively Used Calcareous Grasslands 1950-1985. *Conservation Biology*, **11**, 727-737.

Fischer, J. and Lindenmayer, D.B. (2002) Treating the nested temperature as a 'black box' can lead to false conclusions. *Oikos*, **99**, 193-199.

Fischer, J. and Lindenmayer, D.B. (2005) Perfectly nested or significantly nested - an important difference for conservation management. *Oikos*, **109**, 485-494.

Fitter, A. (1997) Nutrient Acquisition. *Plant Ecology* (ed. by M. J. Crawley), pp. 51-72.

Fitter, A.H. (2005) Darkness Visible: reflections on underground ecology. *Ecology,* **93**, 231-243.

Fitter, A.H., Graves, J.D., Watkins, N.K., Robinson, D., and Scrimgeour, S. (1998) Carbon transfer between plants and its control in networks of arbuscular mycorrhizal fungi. *Functional Ecology,* **12**, 406-412.

Feddermann, N., Finlay, R., Boller, T. and Elfstrand, M. (2010) Functional diversity in arbuscular mycorrhizal fungi - the role of gene expression, phosphorous nutrition and symbiotic efficiency. *Fungal Ecology*, **3**, 1-8.

Fleisman, E., Betrus, C.J., Blair, R.B., MacNally, R. and Murphy, D.D. (2002) Nestedness analysis and conservation planning: the importance of place, environment, and life history across taxonomic groups. *Ocecologia*, **133**, 78-89.

Gange, A.C., Brown, V. K., and Farmer, L. M. (1990) A test of mycorrhizal benefit in an early successional plat community. *New Phytologist,* **115**, 85-91.

Gange, A.C., Brown, V.K. and Farmer, L.M. (1992) Effects of pesticides on the germination of weed seeds: implications for manipulative experiments. *Journal of Applied Ecology.*, **29**, 303-310.

Gange, A.C., Brown, V.K., and Sinclair, G.S. (1993) Vesicular-arbuscular mycorrhizal fungi - A determinator of plant community structure in early succession. *Functional Ecology,* **7**, 616-622.

Gange, A.C. and Ayres, R.L. (1999) On the relationship between arbuscular mycorrhizal colonisation and plant 'benefit'. *Oikos*, **87**, 615-621.

Gange, A.C., Bower, E., Stagg, P.G., Aplin, D.M., Gilliam, A.E. and Bracken, M. (1999) A comparison of visualisation techniques for recording arbuscular mycorrhizal colonisation. *New Phytologist*, **142**, 123-132.

Gange, A.C., Brown, V.K., and Aplin, D.M. (2005) Ecological Specificity of Arbuscular Mycorrhizal: Evidence from Foliar and Seed-Feeding Insects. *Ecology,* **86**, 603-611.

Gange, A.C. and Smith, A.K. (2005) Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects. *Ecological Entomology*, **30**, 600-606.

Gaston, K.J. (1996) The multiple forms of interspecific abundance- distribution relationship. *Oikos*, **76**, 211-220.

Gaston, K.J., Blackburn, T.M. and Lawton, J.H. (1997) Interspecific abundance - range size relationships: an appraisal of Mechanisms. *Journal of Animal Ecology*, **66**, 579-601.

Gaston, K.J., Blackburn, T.M., Greenwood, J.J.D., Gregory, R.D., Quinn, R.M. and Lawton, J.H. (2000) Abundance--occupancy relationships. *Journal of Applied Ecology*, **37**, 39-59.

Gay, P.E., Grubb, P.J., and Hudson, H. J. (1982) Seasonal Changes in the Concentration of Nitrogen, Phosphorous and Potassium, and in the Density of Mycorrhiza, in Biennial and Matrix-Forming Perennial Species of Closed Turf. *Journal of Ecology,* **70**, 571-593.

Gibson, C.W.D., Watt, T.A. and Brown, V.K. (1987) The Use of Sheep Grazing to Recreate Species-rich Grassland from Abandoned Arable Land. *Biological Conservation*, **42**, 165-183.

Gibson, C.W.D. and Brown, V.K. (1991) The Nature and Rate of Development of Calcareous Grassland in Southern Britain. *Biological Conservation*, **58**, 297-316.

Gibson, D.J., Ely, J.S. and Collins, S.L. (1999) The core-satellite species hypothesis provides a theoretical basis for Grime's classification of dominant, subordinate and transient species. *Journal of Ecology*, **87**, 1064-1067.

Gleason, H.A. (1926) The individualistic concept of the .plant association. *Bulletin of the Torrey Botanical Club.,* **53**, 1-20.

Green, B.H. (1990) Agricultural intensification and the loss of habitat, species and amenity in British grasslands. *Grass and Forage Science,* **45**, 365-372.

Grime, J. P. and Curtis, A.V. (1976) The Interaction of Drought and Mineral Nutrient Stress in Calcareous Grassland. *Journal of Ecology,* **64**, 975-988.

Grime, J. P., Mackay, M. L., Hillier, S. H., and Read, D. J (1987) Letters to Nature. Floristic diversity in model system using experimental microcosms. *Nature,* **338**, 420-422.

Grime, J. P. (1990) Mechanisms promoting floristic diversity in calcareous grasslands. *Calcareous Grassland - Ecology and Management.* (ed. by S. H. Hillier, W. D. E. Walton, and D. A. Wells), pp. 51-56. Bluntisham Books., Huntingdon.

Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, **86**, 902-910.

Grubb, P.J. (1977) Maintenance of Species-Richness in Plant Communities - Importance of Regeneration Niche,. Biological Reviews of the Cambridge Philosophical Society **52** 107-145.

Grubb, P. J. (1986) Problems Posed by Sparse and Patchily Distributed Species in Species Rich Plant Communities. *Community Ecology* (ed. by J. Diamond and P. J. Case), pp. 207-226. Harper and Row, New York.

Haines-Young, R., Watkins, C., Wale, C., and Murdock, A. (2006) Modelling natural capital: The case of landscape restoration on the South Downs, England. *Landscape and Urban Planning,* **75**, 244-264.

Hajek, M., Hajkova, P., Apostolova, I., Horsak, M., Plasek, V., Shaw, B. and Lazarova, M. (2009) Disjunct Occurrences of Plant Species in Refugial Mires of Bulgaria. *Folia Geobotanica*, **44**, 365-386.

Hanley, M.E., Fenner, M. and Edwards, P.J. (1995) An experimental field study of the effects of mollusc grazing on seedling recruitment and survival in grassland. *Journal of Ecology*, **83**, 621-627.

Hanski, I. (1982) Dynamics of regional distribution: the core and satellite species hypothesis. *Oikos*, **38**, 210-221.

Hansson, L. (1998) Nestedness as a conservation tool: plants and birds of oak-hazel woodland in Sweden. *Ecology Letters*, **1**, 142-145.

Hartnett, D.C., Hetrick, B. A.D., Wilson, G.W.T., and Gibson, D.J. (1993) Mycorrhizal influence on intra- and interspecific neighbour interactions amoung co-occurring prairie grasses. *Journal of Ecology,* **81**, 787-795.

Hartnett, D.C. and Wilson, G.W.T. (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology,* **80**, 1187-1196.

Hartnett, D.C. and Wilson, G.W.T. (2002) The role of mycorrhizas in plant community structures and dynamics: lessons from grasslands. *Plant and Soil*, **244**, 319-331.

Hannus, J.J. and von Numers, M. (2010) Temporal changes in the island flora at different scales in the archipelago of S W Finland. *Applied Vegetation Science*, **13**, 531-545.

Hausmann, N.T. and Hawkes, C.V. (2009) Plant neighbourhood control of arbuscular mycorrhizal community composition. *New Phytologist*, **83**, 1188-1200.

Helgason, T., Merryweather, J.W., Young, J.P.W. and Fitter, A.H. (2007) Specifity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *Journal of Ecology*, **95**, 623-630.

Helgason, T. and Fitter, A.H. (2009) Natural selection and evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). *Journal of Experimental Botany*, **60**, 2465-2480.

Heppell, K.B., Shumway, D.L. and Koide, R.T. (1998) The effect of mycorrhizal infection of *Abutilon theophrasti* on competativeness of offspring. *Functional Ecology*, **12**, 171-175.

Hillier, S.J. (1990) Gaps, Seed Banks, and Plant Species Diversity in Calcareous Grassland. *Calcareous Grassland - Ecology and Management.* (eds S. H. Hillier, W. D. E. Walton and D. A. Wells), pp. 57-66. Bluntisham Books, Huntingdon.

Hessle, A., Wissman, J., Bertilsson, J. and Burstedt, E. (2008) The effect of breed of cattle and season on diet selection and defoliation of competitive plant species in semi-natural grasslands. *Grass and Forage Science*, **63**, 86-93.

Heubes, J., Retzer, V. and Schmidtlein, S.B.C. (2011) Historical Land Use Explains Current Distribution of Calcareous Grassland Species. *Folia Geobotanica*, **46**, 1-16.

Hodgson, J.G., Grime, J.P., Wilson, P.J., Thompson, K., and Band, S.R. (2005) The Impacts of agricultural change (1963-2003) on the grassland flora of Central England: processes and prospects. *Basic and Applied Ecology,* **6**, 107-118.

Hokkanen, P.J., Kouki, J. and Komonen, A. (2009) Nestedness, SLOSS and conservation networks of boreal herb-rich forests. *Applied Vegetation Science*, **12**, 295-303.

Holm, R.A., (2006). Photograph of chalk grassland on the South Downs.

Holt, A.R., Gaston, K.J. and He, F. (2002) Occupancy-abundance relationships and spatial distribution: A review. *Basic and Applied Ecology*, **3**, 1-13.

Holt, A.R., Gaston, K.J. and He, F. (2002) Occupancy-abundance relationships and spatial distribution: A review. *Basic and Applied Ecology*, **3**, 1-13.

Honnay, O., Hermy, M. and Coppin, P. (1999) Nested plant communities in deciduous forest fragments: species relaxation or nested habitats? *Oikos*, **84**, 119-129.

Hope-Simpson, J.F. (1940) Studies of the Vegetation of the English Chalk:Late Stages in Succession Leading to Chalk Grassland. *Journal of Ecology*, **28**, 386-400.

Hurst, A.H. and John, E. (1999) The biotic and abiotic changes associated with *Brachypodium pinnatum* dominance in chalk grassland in south-east England. *Biological Conservation,* **88**, 75-84.

Hutchings, M.J. (1987) The Population Biology of the Early Spider Orchid, *Ophrys sphegodes* Mill.I. A Demographic Study from 1975 to 1984. *Journal of Ecology*, **75**, 711-727.

Hutchings, M.J. and Booth, K.D. (1996a) Studies of the feasibility of re-creating chalk grassland vegetation on ex-arable land. II. Germination and survivorship of seedlings under different management regimes. *Journal of Applied Ecology.,* **33**, 1182-1190.

Hutchings, M. J. and Booth, K. D. (1996b) Studies on the feasibility of re-creating chalk grassland vegetation on ex-arable land.
1. The potential roles of seed bank and seed rain. *Journal of Applied Ecology.,* **33**, 1171-1181.

Hutchingson, G. E. (1957) Concluding Remarks. Cold Spring Harbour Symposium on Quantitative Biology.

Hylander, K., Nilsson, B., Bengt, G.J. and Tove, G. (2005) Differences in habitat quality explain nestedness in land snail meta-community. *Oikos*, **108**, 351-361.

Invam (2008). International Culture Collection of Arbuscular Mycorrhizal Fungi.

Jacquemyn, H., Brys, R., and Hermy, M. (2003) Short-term effects of different management regimes on the response of calcareous grassland vegetation to increased nitrogen. *Biological Conservation,* **111**, 137-147.

Jakobsen, I. (1999) Transpoprt of Phosphorous and Carbon in Arbuscular Mycorrhizas. *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. 2nd Edition.* (ed. by A. Varma and R.T. Kiode), pp. 305-332. Springer-Verlag, Berlin Heidleberg.

Johnson, N.C., Graham, J.H. and Smith, F.A. (1997) Functioning of mycorrhizal associations along the mutual - parasitism continuum. *New Phytologist*, **135**, 575-585.

Johnson, D., Vandenkoornhuyse, P.J., Leake, J.R., Gilbert, L., Booth, R.E., Grime, J.P., Young, J.P.W. and Read, D.J. (2003) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist*, **161**, 503-515.

Johnson, D., Ijdo, M., Genney, D.R., Anderson, I.C. and Alexander, I.J. (2005) How do plants regulate function, community structure, and diversity of mycorrhizal fungi? *Journal of Experimental Botany*, **56**, 1751-1760.

Joint Nature Conservation Committee. UK Biodiversity Group Tranche 2 Action Plans - Volume II : Terrestrial and freshwater habitats. 1998 Report

Karandashov, V., Nagy, R., Wegmuller, S., Amrhein, N., and Bucher, M. (2004) Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis. *Plant Biology,* **101**, 6285-6290.

Karanika, E.D., Mamolos, A.P., Alifragris, D.A., Kalburtji, K.L. & Veresoglou, D.S. (2008a) Arbuscular mycorrhizas contribute to nutrition, productivity, structure and diversity of plant communities in mountainous herbaceous grassland of northern Greece. *Plant Ecology*, **199**, 225-234.

Karanika, E.D., Voulgari, O.K., Mamolos, A.P., Demetrios, A.A. & Demetrios, D.S. (2008b) Arbuscular mycorrhizal fungi in northern Greece and influence of soil resources on their colonisation. *Pedobiologia*, **51**, 409-418.

Karlik,P. and Poschlod, P. (2009) History or abiotic filter: which is more important in determining species composition in calcareous grassland? *Preslia*, **81**, 321-340.

Keymer, R.J. and Leach, S.J. (1990) Calcareous grassland - a limited resource in Britain, Nature Conservancy Council. In. *Calcareous Grassland - Ecology and Management* (ed. by S. H. Hillier, W. D. E. Walton, and D. A. Wells), pp. 11-17. Bluntisham Books, Huntingdon.

Klironomos, J.N., McCune, J., and Neville, J. (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters,* **3**, 137-141.

Klironomos, J.N and Rillig, M.C. Incorporating complexity in the study of plant-microbial interactions. Plant-Microbial Interactions 2008. 2008. 2-7-0008. Conference Proceeding

Klironomos, J.N., Zobel, M., Tibbett, M., Stock, W.D., Rillig, M.C., Parrent, J.L., Moora, M., Koch, A.M., Facelli, J.M., Facelli, E., Dickie, I.A. and Bever, J.D. (2011). Forces that structure plant communities: quantifying the importance of the mycorrhizal symbiosis. *New Phytologist*, **189**, 366-370.

Koch, A.M., Kuhn, G., Fumagalli, L., Goudet, J., and Sanders, I.R. (2004) High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *PNSA,* **101**, 2369-2374.

Koide, R.T. (1991) Tansley Review No. 29. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist,* **117**, 365-386.

Kiode, R.T. and Kabir, Z. (2000) Extraradical hyphae of mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist*, **148**, 511-517.

Koide, R.T. and Dickie, I.A. (2002) Effect of mycorrhizal fungi on plant populations. *Plant and Soil,* **244**, 307-317.

Koske, R.E. and Gemma, J.N. (1989) A modified procedure for staining roots to detect V A mycorrhizas. *Mycol.Res.*, **92**, 486-489.

Leps, J. (2005) Diversity and ecosystem function. *Vegetation Ecology* (ed. by E. van de Maarel), pp. 199-237.

Lewis, J.D. and Koide, R.T. (1990) Phosphorous supply, mycorrhizal infection and plant offsping vigour. *Functional Ecology,* **4**, 695-702.

Liu, A., Hamel, C., Hamilton , R.I., Ma, B.L. and Smith, D.L. (2000) Acquisition of Cu, Zn, Mn, and Fe by mycorrhizal maise (Zea mays L) grown in soil at different P and micronutrient levels. *Mycorrhiza.*, **9**, 331-336.

Mamolos, A.P., Veresoglou, D.S. and Barbayiannis, N. (1995) Plant species abundance and tissue concentrations of limiting nutrients in low nutrient grasslands: a test of competition theory. *Journal of Ecology*, **83**, 485-495.

Marulanda, A., Azcon, R. and Ruiz-Lozano, M. (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. *Physiologia-Plantarium*, **119**, 526-533.

Mathews, J.W. (2004) Effect of site and species characteristics on nested patterns of species composition i sedge meadows. *Plant Ecology*, **174**, 271-278.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, D.L. and Swan, J.A. (1990) A New method which gives an objective measure of colonisation of roots by vesicular - arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495-501.

McLaren, J.R. and Turkington, R. (2011) Biomass compensation and plant responses to 7 years of plant functional group removal. *Journal of Vegetation Science*, **22**, 503-515.

Meeching(2008) Annual Rainfall at Newhaven East Sussex.www.meeching.com/wx/rain.Accessed 10/12/10

Merryweather, J. and Fitter, A. (1995a) Arbuscular mycorrhiza and phosphorous as controlling factors in the life history of *Hyacinthoides non-scipta* Chouard ex Rothm. *New Phytologist,* **129**, 629-636.

Merryweather, J. and Fitter, A. (1995b) Phosphorous and carbon budgets: mycorrhizal contribution in *Hyacinthoides non-scripta* (L.) Chouard ex Rothm, under natural conditions. *New Phytologist,* **129**, 619-627.

Met Office (2008) Details of Sunshine on South Coast of UK.. www.metoffice.gov.uk/climate/uk/location/england.Accessed 10/01/08

Mitchley, J. and Grubb, P.J. (1986) Control of relative abundance of perennials in chalk in southern England. 1. Constance in rank order and results of pot and field experiments on the role of interference. *Journal of Ecology,* **74**, 1139-1166.

Mitchley, J. (1988a) Control of Relative Abundance of Perennials in Chalk Grassland in Southern England. 2. Vertical Canopy Structure. *Journal of Ecology,* **76**, 341-350.

Mitchley, J. (1988b) Control of Relative Abundance of Perennials in Chalk Grassland in Southern England. 3. Shoot Phenology. *Journal of Ecology,* **76**, 607-616.

Mitchley, J. (1990) Control of Relative Abundance of Perennial Dicotyledons in Chalk Grassland. *Calcareous Grassland - Ecology and Management* (eds S. H. Hillier, W. D. E. Walton and D. A. Wells), pp. 67-73. Bluntisham Books, Huntingdon.

Morris, M.G., Thomas, J.A., Ward, L. K., Snazell, R.G., Pywell, R.F., Stevenson, M. J., and Optam, P. (1997) LANDECONET. Department of Landscape Ecology. Institute of Terrestrial Ecology.

Morris, M.G. (1990) The Effects of Management on the Invertebrate Community of Calcareous Grassland. *Calcareous Grassland - Ecology and Management.* (eds S. H. Hillier, W. D. E. Walton and D. A. Wells), pp. 128-133. Bluntisham Books, Huntingdon.

Murakami, M. and Hirao, T. (2010) Nestedness of insect assemblages on small Bahamian islands: importance of spatial processes. *Insect Conservation and Diversity*, **3**, 229-235.

Myklestad, A. and Sætersdal, M. (2003) The importance of traditional meadow management techniques for conservation of vascular plant species richness in Norway. *Biological Conservation*, **118**, 133-139.

Myklestad, A. and Sætersdal, M. (2004) The importance of traditional meadow management techniques for conservation of vascular plant species richness in Norway. *Biological Conservation*, **118**, 133-139.

Natural England (2008) Castle Hill SSSI. www.jncc.gov.uk/ProtectedSites. Accessed 10/01/08.

Newsham, K.K., Watkinson, A.R., West, H.M., and Fitter, A.H. (1995) Symbiotic fungi determine plant community structure: changes in lichen rich community induced by fungicide application. *Functional Ecology,* **9**, 442-447.

O' Connor, P.J., Smith, S.E. and Smith, F.A. (2001) Arbuscular mycorrhizas influence plant diversity and community structure in semiarid herbland. *New Phytologist*, **154**, 209-218.

Olff, H. and Bakker, J.P. (1998) Do intrinsically dominant and subordinate species exist? A test statistic for field data. *Applied Vegetation Science*, **1**, 15-20.

Owen, I.J. (2008) Analysis of soil samples taken from Newmarket Hill East Sussex. The Macaulay (Soil) Institute., Cragiebuckler

Partel, M. and Zobel, M. (1999) Small-scale plant species richness in calcareous grasslands determined by the species pool, community age and shoot density. *Ecography*, **22**, 153-159.

Pärtel, M., Moora, M. and Zobel, M. (2001) Variation in species richness within and between calcareous (alvar) grassland stands: the role of core and satellite species. *Plant Ecology*, **157**, 203-211.

Pärtel, M., Helm, A., Ingerpruu, N., Reier, U., and Tuvi, E.L. (2004) Conservation of .Northern European plant diversity: the correspondence with soil pH. *Biological Conservation,* **120**, 525-531.

Patterson, B.D. and Atmar, W. (1986) Nested subsets and structure of insular mamalian faunas and archipelagos. *Biological Journal of the Linnean Society*, **28**, 65-82.

Patterson, B.D. and Atmar, W. Analyzing species composition in fragments. Rhieinwald, G. Isolated vertebrate Communities in the Tropics. Proceedings of 4th International Symposium. pp. 10-24. 2000. Bonn. Conference Proceeding

Perring, F. (1958a) A Theoretical Approach to the Study of Chalk Grassland. *Journal of Ecology,* 665-679.

Perring, F. (1958b) Topographical Gradients of Chalk Grassland. *Journal of Ecology,* 447-481.

Perring, F. (1959) Climate Gradients of Chalk Grassland. *Journal of Ecology,* 415-442.

Phillips, J.M. and Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasitic and vesicular-mycorrhizal fungi for rapid assessment of infection. *Trans.Br.Mycol.Soc.*, **55**, 158-161.

Pietikainen, A. and Kytoviita, M.M. (2007) Defoliation changes mycorrhizal benefit and competitive interactions between seedlings and adult plants. *Journal of Ecology,* **95**, 639-647.

Poschlod, P. and WallisDeViries, M.F. (2002) The historical and socioeconomic perspective of calcareous grassland - lessons from the distant and recent past. *Biological Conservation,* **104**, 361-376.

Poschlod, P., Bakker, J.P., and Kahmen, S. (2005) Changing land use and its impact on biodiversity. *Basic and Applied Ecology,* **6**, 93-98.

Pringle, A. and Bever, J.D. (2011) Analogous effects of arbuscular mycorrhizal fungi in the laboratory and a North Carolina field. *New Phytologist*, **180**, 162-175.

Pykälä, J. (2004) Cattle grazing increases plant species richness of most species trait groups in mesic semi-natural grasslands. *Plant Ecology,* **175**, 217-226.

Pykälä, J., Luoto, M., Heikkinen, R.K., and Kontula, T. (2005) Plant species richness and persistence of rare plants in abandoned semi-natural grassland in northern Europe. *Basic and Applied Ecology,* **6**, 25-33.

Raunikiaer, C. (1934) *The Life Forms of Plants and plant statistical Geography.* Clarendon Press, Oxford.

Read, D.J. (1999) Mycorrhiza - The State of the Art. *Mycorrhiza: Structure, Function, Molecular Biology and Biotecnology. 2nd Ed.* (ed. by A. Varma and B. Hock), pp. 3-34. Springer-Verlag, Berlin Heidelberg.

Read, D.J. (2002) Towards Ecological Relevance - Progress and Pitfalls in the Path Towards an Understanding of Mycorrhizal Functions in Nature. *Mycorrhizal Ecology.* (ed. by M.G.A.van der Heijden and I.R.Sanders), pp. 3-24. Springer.

Reitalu, T., Sykes, M.T., Johansson, L.J., Lonn, M., Hall, K., Vandewalle, M. and Prentice, H.C. (2009) Small-scale plant species richness and evenness in semi-natural grasslands respond differently to habitat fragmentation. *Biological Conservation*, **142**, 899-908.

Reynolds, H.L., Packer, A., Bever, J.D. and Clay, K. (2003) Grassroots Ecology: Plant-Microbial-Soil Interactions as Drivers of Plant Community Structure and Dynamics. *Ecology*, **84**, 2281-2291.

Rillig, M.C. and Mummey, D.L. (2006) Tansley Review. Mycorrhizas and soil structure. *New Phytologist*, **171**, 41-53.

Robinson, D. and Fitter, A. (1999) The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. *Journal of Experimental Botany,* **50**, 9-13.

Rodwell, J. (1990) Types of Calcareous Grassland. *Calcareous Grassland - Ecology and Management.* (eds S. H. Hillier, W. D. E. Walton & D. A. Wells), pp. 29-34. Bluntisham Books, Huntingdon.

Rodwell, J. (1991) *British Plant Communities. Vol 1.* Cambridge University Press., Cambridge.

Ryser, P., Verduyn, B. and Lambers, H. (1997) Phosphorous allocation and utilization in three grass species with contrasting response to N and P supply. *New Phytologist*, **137**, 293-302.

Sankaran, M. and McNaughton, S.J. (2005) Terrestrial plant - herbivore interactions: integrating across multiple determinants and trophic levels. *Vegetation Ecology* (ed. by E. van de Maarel), pp. 265-285.

Scheublin, T.R., van Logtestijn, R.S.P., and van der Heijden, M. G. A. (2007) Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species. *Journal of Ecology,* **95**, 631-638.

Shumway, D.L. and Kiode, R.T. (1995) Size and reproductive in equality in mycorrhizal and non-mycorrhizal populations of *Abutilon theophrasti. Journal of Ecology*, **83**, 613-620.

Sikes, B.A., Cottenie, K. and Klironomos, J.N. (2009) Plant and fungal identities determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology*, **97**, 1274-1280.

Silvertown, J. and Wilson, J.B. (1994) Community structure in a Desert Perennial Community. *Ecology*, **75**, 409-417.

Smilauer, P. and Smilauerova, M. (2000) Effect of AM Symbiosis Exclusion on Grassland Community Composition. *Folia Geobotanica*, **35**, 13-25

Smith, R.S. and Rushton, S.P. (1994) The effect of grazing management on the vegetation of mesotrophic (meadow) grassland in Northern England. *Journal of Applied Ecology,* **34**, 13-24.

Smith, S.E. and Read, D.J. (1997a) Growth and carbon economy of VA mycorrhizal plants. *Mycorrhizal Symbiosis* pp. 105-125. Academic Press.

Smith, S.E. and Read, D.J. (1997b) Introduction. *Mycorrhizal Symbiosis* Academic Press.

Smith, S.E. and Read, D.J. (1997c) The symbionts forming VA mycorrhizas. *Mycorrhizal Symbiosis* pp. 11-32. Academic Press.

Smith, M.D., Hartnett, D.C. and Wilson, G.W.T. (1999) Interacting influence of mycorrhizal symbiosis and competition on plant diversity in tallgrass prairie. *Oecologia*, **121**, 574-582.

Smith, M.D., Hartnett, D.C. and Rice, C.W. (2000) Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. *Soil Biology and Biochemistry*, **32**, 935-946.

Steven, G.A (1992) Botanical Survey of Unimproved Grassland on the South Downs in West Sussex. English Nature (South-East England) Report.

Steven, G. and Muggeridge, N (1992) Botanical Survey of Unimproved Grassland on the South Downs in East Sussex. English Nature (South-East England) Report.

Stiles, A. and Scheiner, S.M. (2008) Nestedness of remnant Sonoran Desert plant communities in metropolitan Phoenix. Arizona. *Ecology*, **98**, 2473-2481.

Streitwolf-Engel, R., Boller, T., Weimken, A., and Sanders, I.R. (1997) Clonal growth traits of two *Prunella* species are determined by co-occurring arbuscular mycorrhizal fungi from a calcareous grassland. *Journal of Ecology,* **85**, 181-191.

Subramanian, K.S. and Charest, C. (1999) Acquisition of N by external hyphae of an arbuscular mycorrhizal fungi and its impact on phytosociological responses in maize under drought -stress and well watered conditions. *Mycorrhiza.,* **9**, 69-75.

Sun, Y., Li, X.L. and Feng, G. (2008) Effect of arbuscular mycorrhizal colonisation on ecological functional traits of ephemerals in the Gurbantonggut deserts. *Symbiosis*, **46**, 121-127.

Sussex Downs Conservation Board (1996) *Management Strategy for the Sussex Downs Area of Outstanding Natural Beauty.* Sussex Downs Conservation Board and the Countryside Commission..

Sussex Wildlife Trust. (1995) Chalk Grassland and Chalk Heath. *Vision for Wildlife in Sussex.* (ed. by Sussex Wildlife Trust.), Gemini Press..

Tansley, A.G. and Adamson, R.S. (1925) Studies of the vegetation of the English chalk.III The chalk grassland of the Hampshire-Sussex border. *Journal of Ecology*, **13**, 177.

Tansley, A.G. and Adamson, R.S. (1926) Studies of Vegetation of the English Chalk. 1v. A Preliminary Survey of Chalk Grasslands of the South Downs. *Journal of Ecology*, **x1v**, 1-31.

Thomas, J.A. (1990) The Conservation of Adonis Blue and Lulworth Skipper Butterflies - Two Sides of the Same Coin. *Calcareous Grassland - Ecology and Management.* (ed. by S. J. Hillier, W. D. E. Walton, and D. A. Wells), pp. 112-117. Bluntisham Books., Huntingdon.

Tilman, D. (1997) Mechanisms of Plant Competition. *Plant Ecology (Second Edition)* (ed. by M. J. Crawley), pp. 239-261. Blackwell Science.

Tofts, R. and Silvertown, J. (2000) A phylogenic approach to community assembly from a local species pool. *Proceedings Royal Society*, **267**, 363-369.

van Andel, J. (2005) Species interactions structuring plant communities. *Vegetation Ecology.* (ed. by E. van de Maarel), pp. 238-264.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., and Sanders, I.R. (1998) Mycorrhizal fungal diversity determines plant biodiversity ecosystem variability and productivity. *Nature,* **396**, 69-72.

van der Heijden, M.G.A., Boller, T., Wiemken, A. and Sanders, I.R. (1998) Different arbuscular mycorrhizal species are potential determinants of plant community structure. *Ecology*, **79**, 2082-2091.

van der Heijden, M.G.A. (2002) Arbuscular Mycorrhizal Fungi as a Determinant of Plant Diversity: In Search of Underlying Mechanisms and General Principles. *Mycorrhizal Ecology* (ed. by M. G. A. van der Heijden and I. R. Sanders), pp. 243-261. Springer.

van der Heijden, M.G.A., Wiemken, A. and Sanders, I.R. (2003) Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. *New Phytologist*, **157**, 569-578.

van der Heijden, M. G. A. (2004) Arbuscular mycorrhizal fungi as support systems for seeding establishment in grassland. *Ecology Letters,* **7**, 293-303.

van der Heijden, M.G.A., Verkade, S. and de Bruin, S.J. (2008) Mycorrhizal fungi reduce the negative effects of nitrogen enrichment on plant community structure in dune grassland. *Global Change Biology.*, **14**, 2626-2635.

van der Maarel, E. (2005) Vegetation Ecology - an overview. *Vegetation Ecology* (ed. by van de Maarel E), pp. 1-51. Blackwell Publishing.

van der Maarel, E. and Sykes, M.T. (1993) Small-scale species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *Journal of Vegetation Science,* **4**, 179-188.

Verdu, M and Valiente-Banuet, A. (2008) The Nested Assembly of Plant Facilitation Networks Prevents Species Extinctions. *American Naturalist*, **172**, 551-760.

Vierheilig, H., Coughlan, A.P., Wyss, U., and Piche, Y. (1998) Ink and Vinegar, a Simple Staining Technique for Arbuscular Mycorrhizal Fungi. *Applied and Environmental Microbiology.,* **December**, 5004-5007.

Wahl, S. and Ryser, P. (2000) Root tissue structure is linked to ecological strategies of grass. *New Phytologist*, **148**, 459-471. Pearson Education Limited.

Waite, S. (2000) *Statistical Ecology in Practice (A Guide to Analysing Environmental and Ecological Field Data)*

Ward, L.K. (1990) Management of Grassland - Scrub Mosaics. *Calcareous Grassland - Ecology and Management.* (eds S. J. Hillier, W. D. E. Walton and D. A. Wells), pp. 134-139. Bluntisham Books, Huntingdon.

Wearn, J.A. and Gange, A.C. (2007) Above-ground herbivory causes rapid and sustained changes in mycorrhizal colonization of grasses. *Oecologia*, **153**, 959-971.

West, H.M. (1996) Influence of arbuscular mycorrhizal infection on competition between *Holcus lanatus* and *Dactylis glomerata*. *Journal of Ecology,* **84**, 429-438.

Westhoff, V. and van de Maarel, E. (1978) The Braun-Blanket Approach. *Classification of Plant Communities* (ed. by R. H. Whittiker), pp. 287-297. Junk, The Hague.

Willems, J.H. (1990) Calcareous Grassland in Continental Europe. *Calcareous Grassland - Ecology and Management.* (ed. by S. H. Hillier, W. D. E. Walton, and D. A. Wells), pp. 3-10. Blutisham Books, Huntingdon.

Willems, J.H., Peet, R.K. and Bik, L. (1993) Changes in chalk grassland structure and species richness resulting from selective nutrient additions. *Journal of Vegetation Science*, **4**, 203-212.

Willems, J.H. (2002) Approaches and Results in Restoration of Dutch Calcareous Grasslands During the last 30 years. *Restoration Ecology*, **9,** 147-154.

Wohlgemuth, T. and Gigon, A. (2003) Calcicole plant diversity in Switzerland may reflect a variety of habitat templates. *Folia Geobotanica*, **38**, 443-452.

Wolfe, B.E., Husband, B.C., and Klironomos, N. (2005) Effect of a belowground mutualism on an aboveground mutualism. *Ecology Letters,* **8**, 218-223.

Wilson, G.W.T., Hartnett, D.C. and Rice, C.W. (2006) Mycorrhizal-mediated phosphorus transfer between tall grass prairie plants *Sorghastrum nutans* and *Artemisia ludoviciania*. *Functional Ecology*, **20**, 427-435.

Woodcock, B.A., Pywell, R.F., Roy, D.B., Rose, R.J., and Bell, D. (2005) Grazing management of calcareous grasslands and its implications for the conservation of beetle communities. *Biological Conservation,* **125**, 193-202.

Wright, D.H. and Reeves, J.H. (1992) On the meaning and measurement of nestedness of species assemblages. *Oecologia*, **92**, 416-428.

Zabinski, C.A., Quinn, l., and Callaway, R.M. (2002) Phosphorus uptake, not carbon transfer, explains arbuscular mycorrhizal enhancement of *Centaurea maculosa* in the presence of native grassland species'. *Functional Ecology,* **16**, 758-765.

Zhang, Q., Yang, R., Tang, J., Yang, H., Hu, S. and Chen, X. (2010) Positive Feedback between Mycorrhizal Fungi and Plant Influences Plant Invasion Success and Resistance to Inaction. *PloS One*, **5**, 1-10.

Zohlen, A. and Tyler, G. (2000) Immobilisation of tissue iron on calcareous soil: differences between calcicole and calcifuge plants. *Oikos*, **89**, 95-106.

Zobel, M., van der Maarel, E., and Dupre, C. (1998) Species pool: the concept, its determination and significance for community restoration. *Applied Vegetation Science,* **1**, 55-66.

Zobel, M., Moora, M.and Herben, T. (2010) Clonal mobility and its implications for spatio-temporal patterns of plant communities: what we need to know next? *Oikos*, **119**, 802-806.

Appendices

Appendix 1 - Recording sheet for field surveys

Survey Data		Newmarket Hill	Date		
Site No.	Quadrat No.				
Species	Presence	Abundance (%)	Species	Presence	Abundance (%
Achillea millefolium			Hypericum perforatum		
Agrostis stolonifera			Hypnum cupressiforme		
Anthyllis vulneraria			Koeleria macrantha		
Asperula cynanchica			Leontodon hispidus		
Avenula pratensis			Leontodon taraxacoides		
Avenula pubescens			Leucanthemum vulgare		
Bellis perennis			Linum catharticum		
Blackstonia perfoliata			Lotus corniculatus		
Brachypodium pinnatum			Medicargo lupulina		
Briza media			Neckara crispa		
Bromus erectus			Orchis mascula		
Bryum capillare			Phyteuma orbiculare		
Calliergon cuspidatum			Picris hieracoides		
Campanuala rotundifolia			Pimpinella saxifraga		
Carex caryophyllea			Plagiomnium undulatum		
Carex flacca			Plantago lanceolata		
Carlina vulgaris			Plantago media		
Centaurea nigra			Polygala calcarea		
Centaurium erythraea			Polygala vulgaris		
Centaurium pulchellum			Primula veris		
Cerastium fontanum			Prunella vulgaris		
Cirsium acaule			Pseudoscleropodium puru		
Cladonia rangiformis			Ranunculus acris		
Clinopodium vulgare			Ranunculus bulbosus		
Crataegus monogyna			Rhianthus minor		
Ctenidium molluscum			Sanguisorba minor		
Dactylis glomerata			Scabiosa columbria		
Euphrasia officinalis agg.			Senecio integrifolius		
Festuca ovina			Senecio jacobaea		
Filipendula vulgaris			Stachys officinallis		
Fissidens sp			Succisa pratensis		
Galium mollugo			Thymus praecox arcticus		
Galium verum			Trifolium pratense		
Gentianella amarelle			Viola hirta		
Gymnadenia conopsea			Viola reichenbachiana		
Hieracium pilosella gr			Viola riviniana		
Hippocrepis comosa			Weissia sp		
Homalothecium lutescens					