Epidemiology and chemical control of Fusarium ear and seedling blight of wheat

Thesis

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EPIDEMIOLOGY AND CHEMICAL CONTROL OF FUSARIUM EAR AND SEEDLING BLIGHT OF WHEAT.

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Abstract

The aim of the study was to determine a link between the applications of fungicides, ear blight (caused by *Microdochium nivale* and *Fusarium culmorum*), grain quality and the subsequent emergence of infected seed. In order to determine this a series of field and glasshouse trials were carried out. In field situations the pathogen causing ear blight symptoms is often unknown. Azoxystrobin is reported to be less effective against *F. culmorum* (Dardis & Walsh, 2000) and the results from this study agree with this work. The trials concluded that for chemical control in the field a mixture of the fungicides azoxystrobin and metconazole provided the most significant reduction of ear blight severity and grain infection when compared with the control treatments.

The emergence trials concluded that the use of a seed treatment (fludioxonil) can significantly improve the emergence of an infected crop. It is known that *M. nivale* may be present in a seed crop with the absence of visible symptoms on the seed (Hare *et al*, 1999). For plots drilled with out a seed treatment, the ones that had received an ear spray of azoxystrobin alone or a mixture of azoxystrobin and metconazole showed a higher percentage emergence regardless of the pathogen. The emergence trials proved the infection of seed can be significantly reduced by the use of ear sprays. If the seed is to be saved on the farm, the use of a seed treatment will improve the emergence of the new crop.

Trials on the effect of increasing inoculum load on the symptoms of ear blight and the infection of grain. It was found that increasing the inoculum load for *F. culmorum* either alone or in a mixture of pathogens gave a reduction in yield and thousand-grain weight but this was not so with *M. nivale* this agrees with Hare *et al*,(1999) who

studies the relationship between wheat seed weight infected with *F. culmorum or M. nivale.* When grain form these trial was drilled in the emergence trials it was shown that where the seed contained *F. culmorum* a significant improvement in emergence was seen when a seed treatment of fludioxonil was used.

Glasshouse studies were conducted on point inoculation of ears with pathogens and found that there is a relationship between grain infection and seed weight for *F. culmorum* but not for *M. nivale.* Thus, grain weight can not be used on farm as a measure of infection when assessing seed health as *M. nivale* can be symptomless.

List of Publications

Conference presentations:

Winson, S.J., Hare, M.C. & Jenkinson, P. 2000. The relationship between point of inoculation of wheat ears and the infection of grain by *Fusarium culmorum* and *Microdochium nivale*. In: *Proceedings of 6th European Fusarium Seminar*, Berlin, Germany, September 11-16 2000. pp 70-71.

Winson, S.J., Hare, M.C. & Jenkinson, P. 2000. The interaction between ear sprays and seed treatment for the control of Fusarium seedling blight in wheat. In: Symposium Proceedings No 76: Seed treatment, challenges & opportunities, The Belfry, Warwickshire, UK. 26-27th February 2001. pp 251-256.

Winson, S.J., Hare, M.C. & Jenkinson, P. 2003. Effect of inoculum load on the development of *Fusarium* ear blight in wheat. In: Proceedings of the 8th International Congress on Plant Pathology, Christchurch, New Zealand, 2-7th February 2003. pp 110.

Winson, S.J., Hare, M.C. & Jenkinson, P. 2003. Interaction between point inoculation of wheat ears, fungicide sprays and grain infection by *Fusarium culmorum* and *Microdochium nivale*. In: Proceedings of the 8th International Congress on Plant Pathology, Christchurch, New Zealand, 2-7th February 2003. pp 64.

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Chapter 1

Introduction and Literature Review.

Introduction.

Fusarium species have been isolated and identified from all over the world. One of the first written descriptions of ear rot of maize caused by *Fusarium moniliforme* was described from native Aztec descriptions in the sixteenth century by a Franciscan friar in Mexico (Booth, 1984). *Fusarium* spp. can cause disease in most genera of plants; important diseases include vascular wilt of tomatoes caused by *Fusarium oxysporum f.* sp. *lycopersici:* vascular wilts of corms, bulbs and tubers of beans, peanuts and soybean caused by *Fusarium solani* (Agrios, 1988).

Fusarium species cause disease in all species of cereals (wheat, barley, rye, oats, triticale) and grasses, grown in the UK. The earliest description of *Fusarium* disease in the UK was recorded in "Diseases of Field and Garden Crops" by W. G. Smith, 1884, in which he referred to the disease as *Fusiosporium culmorum.* In 1934 Bennett isolated and identified 14 different species of *Fusarium* from cereals in Britain. The majority of diseases can however be attributed to one of five species, *F. avenaceum, F. culmorum, F. graminearum, F. poae* and *Microdochium nivale* (formally known as *Fusarium nivale*) (Mueller, 1977). This review will be confined to ear blight and seedling blight of small grain cereals caused by these five species.

Causal organisms and geographical distribution.

As stated earlier *Fusarium* ear blight in wheat is caused by five major species, as listed in Table 1.1, although Mesterhazy (1984b) identified a further twelve causal organisms associated with the disease. The disease has been recorded world-wide (Parry *et al.,* 1995). The causes of ear blight are dominated by five species and the international distribution of

these species is dependent on their temperature requirements. For example, *F. graminearum* is generally dominant in regions including USA, Canada, Australia and Central Europe because they have prolonged spells of hot weather and the species has optimal growth at temperatures of 20-25°C (Cook, 1981). In the regions with a cooler maritime climate *F. culmorum* is the predominant species with *F. poae* and *M. nivale* becoming increasingly more important (Polley & Turner, 1991). *Fusarium avenaceum* represents in general a much smaller proportion of the species isolated from the ears but is present over a much wider area.

Table 1.1. Imperfect (Anamorph) and perfect (Teleomorph) states of five common *Fusarium* species causing disease of cereals.

Imperfect (Conidial) State	Perfect (Perithecial) State
Fusarium avenaceum (Corda ex Fr.) Sacc.	*Gibberella avenacea* Cook
Fusarium culmorum (W.G. Smith)	Not known
Fusarium graminearum Schwabe	*Gibberella zeae* (Schw.) Petch
Fusarium poae (Peck) Wollenwebber	Not known
Fusarium nivale (Fr.) Ces. = *Microdochium nivale* Samuals and Hallet	*Calonectria nivalis* Schaffnit = *Monographella nivalis* (Schaffit) E. Müller

In the USA during 1994 McMullen and Nelson (1995) surveyed 161 fields of wheat in North Dakota. They observed that 60% of fields surveyed in the North East district of the state were affected by scab and of these up to 76% of ears in some fields were affected.

Wong *et al.* (1992) surveyed fields in Manitoba between 1989 and 1991; of the 436 fields observed 70% of plants exhibited symptoms. The severity of disease (measured in percentage kernels infected) varied significantly between years.

Daamen *et al.* (1991) conducted a national survey of the Netherlands from 1974 to 1986 and found an average of 51% of fields were infected with Fusarium ear blight with an average of 1.2% glumes infected.

Turner *et al.* (1999) showed in a survey of 256 UK wheat crops that more than 60% of the crops were infected with ear blight with an average of 12% ears infected in each field. In 1998 Jennings *et al.*(2000) took grain samples from 53 severely infected wheat fields (6 in Wales and 47 in England). They found the main ear blight pathogen was *M. nivale*, present in 96% of samples. Other ear blight pathogens were found as follows; *F. avenaceum* 43%, *F. graminearum 40%, F. poae 34% and F. culmorum 21%.*

According to Bottalico and Logrieco (2001), the predominant species for causing ear blight in Europe are *F. culmorum, F. graminearum* and *F. avenaceum.* However, work by others including; Adler *et al.* (1990) in Austria, Obst *et al.* (2000) in Germany and Pasquini *et al.* (2001) in Italy have all stated that the frequency of the species may vary with the geographical location of the country.

Moore (1948) reported in surveys that *F. graminearum* was the primary causal agent of ear blight in the UK in wheat in 1946 and oats in Wales in 1948. Contradictory to this were the findings of McKay (1957) in his report of a disease survey of oats and wheat in Ireland (1930-54). He found that the most common species causing seedling blight, foot rot, brown foot rot and ear blight were *F. culmorum* and *M. nivale*. He also suggested that *F. avenaceum* may be involved in certain cases, but unfortunately no data were presented to show the percentage of ears infected by the individual species. Polley and Turner (1991) found wheat grain infected with 3.4% *M. nivale*, 1.0% *F. avenaceum* and 5.34% *F.culmorum*. A repeat of the survey in the following year produced similar results. In Scotland 2.5 % of grain was infected with *F. poae*, 4.0% with *M. nivale*, 1.0% *F. avenaceum* and less than 1.0% with *F. culmorum*.

In 1966, Hewett examined wheat seed infection of variety trials from the 1965 harvest. Seed infection by *M. nivale* was found to be high, a mean of 20% compared to 4 and 7% in 1963 and 1964 respectively. Infections of *F. culmorum* and *F. avenaceum* were also high with a mean of 39% (9 and 10% in 1963 and 1964). Particularly high incidences were found to be present in plots where lodging had occurred. Similar material harvested in 1969, 1970 and 1973 showed mean seed infections of 1-2%. Hewett pointed out that very few samples of seed intended for sowing contained appreciable levels of *M. nivale*, whereas *Stagonospora nodorum* infection was much more common.

Symptoms of *Fusarium.*

Fusarium culmorum can either be seed-borne or survive in the soil as chlamydospores (long lived spores), on organic matter and crop debris. The fungus can cause the death of seedlings either pre- or post-emergence. Brown stem lesions may be present on plants that

survive. They are usually not serious but occasionally may cause a stem rot severe enough to cause a white head; such stems are usually rotten at one of the stem internodes, *Fusarium culmorum* does not usually cause a leaf disease, but will cause *Fusarium* ear blight (FEB). The first visible sign of FEB are pale brown water soaked lesions on the glume, which spreads to the site of attachment of the glume to the rachis and possibly beyond until the whole ear is infected. Affected spikelets or florets assume a bleached appearance, which is very noticeable, when the ear is green (Figure 1.1). Infection of the rachis leads to blighting of the whole ear above the infection site. Atanasoff (1920) thought this to be due to restricted transport of water and nutrients to the ear but this has not been confirmed experimentally.

In the advanced stages of infection the ear bears the pink cottony mycelial growth of the fungus. If weather conditions are conducive, the mycelial growth will cover the whole ear. Grain sites infected during flowering will have no grain formed in infected spikelets. Later attacks will cause grain shrivelling, it can also attack the grain directly. The infected grains are shrunken and misshapen. Abramson *et al.* (1987), described severe ear blight symptoms caused by *F. graminearum* or *F. culmorum* as being the cause of the development of 'tombstone' kernels in the grain, small shrivelled grains white to pale pink in colour (Figure 1.2). Seed infected with *M. nivale* may appear small and shrivelled but will not have the colour change. Seed-borne infections can be partially controlled by seed treatments, enough to prevent severe seedling losses.

Fusarium avenaceum causes diseases very similar to those described for *F. culmorum* and the disease cycle is the same except *F. avenaceum* does not form chlamydospores in the soil and is generally regarded as less virulent than *F. culmorum* (Jenkins *et al.*, 1988).

Figure 1.1 Symptoms of *Fusarium* ear blight on winter wheat

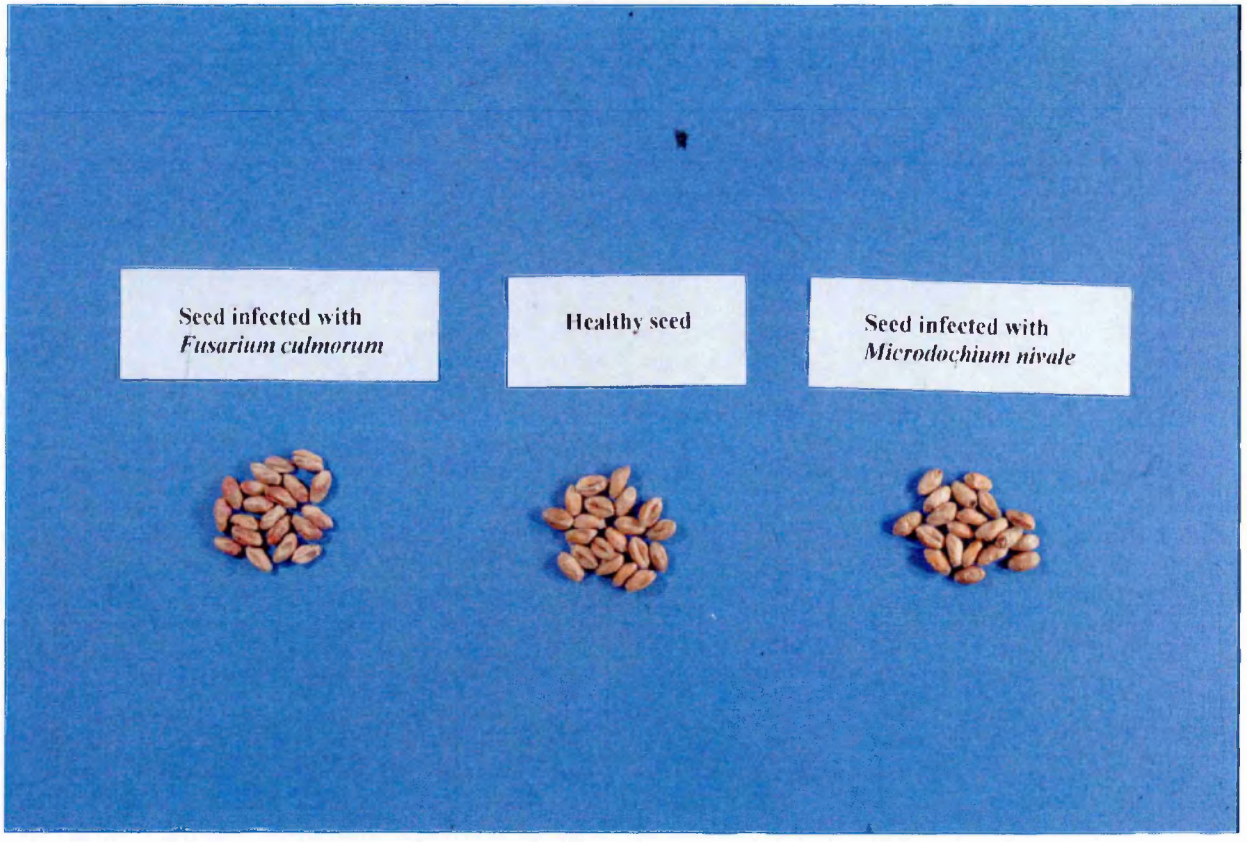

Figure 1.2. Visible symptoms of Fusarium infected wheat grains. Healthy grain (centre), grain infected with *F. culmorum* (left) showing pink colouring and shrivelled grains and *M. nivale* showing slight shrivelling (right).

Fusarium graminearum also causes diseases but in the ear blight phase, superficial fungal growth is more evident and purple-black perithecia (*Gibberella zeae*) are embedded in it. Affected grains may be shrunken and coloured red in appearance. The fungus can form chlamydospores but these as yet have not been proved to be long lived.

Microdochium nivale favours much cooler temperatures than the three previous pathogens. Unlike these it survives on crop debris and organic matter rather than forming chlamydospores. In Scotland, amongst other countries, it causes a disease called snow mould on cereals when the crop is lying under a covering of snow, this causes leaf disease and may kill the crowns of plants. *Microdochium nivale* causes discolouration of the lower nodes and can be the cause of foot rot (Jenkins *et al.,* 1988). It can cause FEB like the other *Fusarium* species, but the symptoms are less severe. *Microdochium nivale* is seed-borne and is the most common cause of pre-and post- emergence seedling death in the UK. Work by Snyder and Nash (1968) stated that seedlings may become infected as a result of seed being sown into infected soil or later in the growing season airborne conidia or ascospores may infect the ears (Francis and Burgess, 1977). The resultant infected grain then completes the disease cycle by causing seedling blight (Cook, 1980).

Seedling Blight.

Fusarium avenaceum, *F. culmorum*, *F. graminearum* and *M. nivale* can all cause pre- and post-emergence damage to cereal seedlings. The visible symptoms can range from death soon after germination to superficial stem lesions on the emerged plants, all symptoms are characteristic to the four pathogens and the cause can only be identified by isolating and identifying the pathogen in the laboratory (Jenkins *et al.,* 1988). In 1928 Bennett, from fieldwork, regarded *F. culmorum* and *F. avenaceum* as predominant pathogens with *F.*

culmorum causing in the most severe cases, death of the plant. Colhoun and Park (1964) worked on plants in pots under glasshouse conditions and found that *F. culmorum* caused the pre-emergence death of the coleoptile, often just before emergence. Pre-emergence and post-emergence damage was found to be most severe in dry soils and increased with increased temperature (in the range of 8 – 23°C). All species of *Fusarium* can cause brown lesions on stems without killing the plants, but in favourable conditions both *F. graminearum* and *F. culmorum* can cause complete seedling death within 14 days of sowing.

Greaney and Machacek (1942) studied the prevalence of seed-borne fungi on cereals in certain seed inspection districts of Canada. In the years 1939 and 1940 they tested 2,200 and 1,556 samples respectively of wheat, barley and rye and tested them for seed-borne diseases. Many species of bacteria and fungi were isolated, *Alternaria* was by far the most commonly isolated fungus but species of *Helminthosporium* and *Fusarium* were the most important disease-producing organisms. They also found that seed treatment with an organic mercury dust had no appreciable effect on the germination of healthy (uninjured seed virtually free of pathogenic fungi) seed of wheat, oats and barley. In both years the treatment of healthy wheat seed only produced a small improvement in germination (in 1939, 95.1 to 95.9% and 1940 from 95.1 to 95.5%). However, the germination of infected seed was improved by over 5% in both years by the use of a seed treatment (New Improved Ceresan and Talc), in 1939 – 90.5 to 95.2% and 1940 88.0 to 96.0%. They concluded that treating the seed of wheat, barley and oats infected with species of *Helminthosporium* and *Fusarium* with mercurial compound dust improved seed germination and gave almost complete control of seedling blight, seedling foot rot, leaf spot and leaf blotch.

Interactions between environmental factors have been shown to be an important factor in seedling diseases. Colhoun *et al.* (1968) found the most influential factors to be soil moisture, temperature and the level of seed-borne inoculum. Work on wheat showed little pre or post-emergence death or stem lesion development at any temperature or soil moisture until the spore load reached high (10^5 spores/ 25g seed) levels. High temperatures (above 23°C) favoured disease, but in dry soils a high spore load compensated for lower temperatures (e.g. 18°C) to give severe disease. In cold wet soils there was little or no damage even at high seed spore loads. In wet soils, high temperature combined with high spore loads did result in increases in stem lesions.

Microdochium nivale causes seedling diseases similar to those described above but with several important differences. Sowing seed infected by *M. nivale* can result in up to 80% death of seedlings soon after germination. Later symptoms include browning of the coleoptile, which may be deformed, and often the first leaf takes on a shredded appearance or may be seen lying on the ground. In some cases, lens shaped pale brown or auburn lesions can be seen on the first and second leaves (Millar and Colhoun, 1969a).

The disease favours dry soils and lower temperatures, for example in dryer soils there was more disease at 6.1°C than at other temperatures up to 16.4°C. At all soil temperatures the amount and severity of disease was reduced as the soil moisture increased. There was more disease at higher seed spore loads but a very low spore load gave considerable disease when the soil was dry. Millar and Colhoun (1969b) also noted that disease frequency increased with sowing depth. This work confirmed earlier findings of Noble and Montgomery (1956) who found that deep sowing especially in heavy soils resulted in severely damaged coleoptiles and greater pre-emergence death of oat seedlings.

Foot Rot.

Atanasoff (1920) described foot rot as being apparent on stem-bases of cereals as a browning and rotting of roots, crown and scale leaves. Parry *et al.* (1994) state that of the stems of plants grown, the roots and stem close to the ground may become infected around anthesis causing rotting of the tissue and a pink or yellow/ brown discolouration may occur. This could cause an interference with water and nutrient movement within the plant resulting in the possible occurrence of whiteheads.

Cereal foot rots can often occur as a complex of several diseases but there seems to be little published work on the interaction between the diseases. Millar and Colhoun (1969a) state that the soil micoflora can inhibit *M nivale* by preventing infection developing from inoculated seed. Work by Bateman (1979) on relationships between *M. nivale* and other micro-organisms on wheat and barley; found that different isolates of *Alternaria* spp. and *Epicoccum* spp. affected the pathogen differently. The *Alternaria* spp. were found to be the most antagonistic to *M. nivale*. This work confirmed earlier findings of Ponchet (1966) that saprophytic micro-organisms affect the development of *M. nivale* during seed germination and early seedling growth and during seed colonisation. Parry *et al.* (1995) reported that in field plots of winter wheat inoculated with *F. culmorum* and *Pseudocercosporella herpotrichoides* (cause of eyespot) singly and in combination, the proportion of plants infected with *F. culmorum* increased from 60% in plots inoculated with *F. culmorum* alone to 90% in plots inoculated with both fungi. This may mean that *F. culmorum* colonisation was enhanced by the presence of the eyespot fungus. Plants affected by both pathogens had the most severe symptoms and so would be the most likely to lodge. Work by Bateman (1993) on the development of disease symptoms and fungal pathogens agree with this. However, this is in contrast to work in Germany by Fehrmann

and Duben (1980), which indicated that as the eyespot index decreased there was a slight increase in the *Fusarium* diseases.

There has been work done to suggest that some *Fusarium* species may grow or move systemically within the plant. Jordan and Fielding (1988) inoculated spring wheat below ground level with *F. avenaceum, F. culmorum, F. graminearum* and *M. nivale* singly and in combinations. Subsequently *F. culmorum* was isolated from all internodes and some ears, and from this they concluded that the fungus had progressed internally.

Contrary to this Bennett in 1928 studied the height to which *F. avenaceum* and / or *F. culmorum* had progressed up the stems of plants grown in pots or in the field. From these plants the pathogen was isolated 44 times from the internode immediately above ground level, and 15 times from the second internode, but none from above this level.

Snijders (1990) studied the systemic growth of *F. culmorum* in winter wheat precluding conditions that allowed infection by water splash. *Fusarium culmorum* was isolated from stem tissue of both wounded and soil inoculated plants. Snijders concluded that foot-rot can therefore lead to infection of higher stem internodes under conditions not suitable for *Fusarium* dispersal. However, no evidence was found for systemic fungal growth leading to infected heads.

The work of Clement and Parry (1988) agrees with that of Snijders, who studied the fungal colonisation of wheat by *F. culmorum, F. graminearum* and *M. nivale*. Wheat plants were grown in inoculated compost to evaluate the possibility that systemic growth may transfer infection from the stem-base to the ear. Each fungus was recovered from stem tissues

above soil level in some, apparently symptomless plants. Symptoms of *Fusarium* foot rot were seen in an increased proportion of plants during grain-fill and desiccation. *Fusarium culmorum* was the most frequently isolated pathogen and was also found highest from the soil level. It was found that only 3% of plants were colonised above the second node and none of the fungal species were recovered from the fifth node or the ear. They concluded that the colonisation and systemic growth from *Fusarium* inoculated compost is unlikely to contribute to the development of ear blight symptoms.

More recently, work by Bateman (2002) states that in the absence of artificial inoculum of the ears *F. culmorum* caused ear blight only when weather conditions (a warm dry period in early summer) allowed brown foot rot to develop. This only happened where surface inoculum was present on infected plant material. He found that infected seed caused no disease and concluded that conditions favouring foot rot development and subsequently, moist conditions during anthesis must be present for ear blight to develop.

Ear Blight.

All five of the species of *Fusarium* covered in this review cause disease on the ears of wheat. Infection may occur at any time from ear emergence to maturity but ears are most susceptible to infection and most damage is caused during anthesis (flowering period). The *Fusarium* pathogen first infects the extruded anthers because substances such as choline and betaine present in the anthers stimulate fungal growth (Strange and Smith, 1978). Several factors influence infection, including extrusion or retention of anthers, contact of inoculum with host anthers or floral tissue other than anthers, stage of plant maturity and environmental conditions, chiefly humidity.

The earliest visible signs of ear blight infection in wheat are small water-soaked lesions on the outer glumes. Infection may remain on the glumes but under favourable conditions the disease will spread and affect the floret or more usually the whole spikelet (Figure 1.1). The infected tissue becomes bleached in appearance and is easily picked out in contrast with healthy green spikelets. The glumes of an infected spikelet may become bound together with the growth of the fungus within the spikelet and the base of the spikelet may become covered with a pink mass of sporulating fungus. Under moist conditions the whole spikelet can be covered in mycelial growth. The disease can spread to adjacent glumes but the most severe effect is when the pathogen grows into the rachis and cuts off supplies of nutrients and water to the parts above which in turn also become bleached. Affected ears take on the appearance of premature ripeness but may become darker in colour due to the growth of saprophytic moulds on them (McKay, 1957).

The effect of *Fusarium* ear diseases on the grain depends on the timing of infection. As stated previously, infection at early anthesis can cause bleached spikelets, which do not contain any grain, or grain that is shrivelled and less viable. Infection later in the flowering period may show no visible symptoms but infection of the grain may still happen.

In 1942 Dickson described grains infected with *F. graminearum* as shrivelled with a scabby appearance. Tufty mycelial growths appear white, pink or brown coloured depending on the time of infection and the weather conditions. Work by Colhoun (1972) on *F. graminearum* states that although the grain may be shrivelled in appearance the fungus rarely, if ever, penetrates far enough into the seed to reach the embryo. The fungal hyphae can be found on the outer and inner seed coat. Bateman (1983) isolated *M. nivale* from the seed coat, endosperm and embryo but infections were found to be concentrated in

the space beneath the epidermis.

Fusarium Disease cycle and epidemiology.

The disease cycles of the *Fusarium* diseases of cereals (seedling blight, foot rot and ear blight) are important in the understanding of the disease progression and therefore control. A generalised disease-cycle of the *Fusarium* species found on cereal crops is illustrated in Figure 1.3.

Central to the disease cycle is the initial source of inoculum which is common in the soil. Nelson *et al.* (1983) stated that it was rare to find a necrotic root of a plant in most agricultural soils that is not colonised by at least one *Fusarium* species. Soil-borne inoculum survives either as saprophytic mycelium or as thick-walled resting spores (chlamydospores) depending on the *Fusarium* species (Parry *et al.,* 1994). Sowing cereals into *Fusarium* infested soil may result in the infection of plants and the development of both seedling blight and foot rot. Zinkernagel *et al.* (1997) found that later in the growing season the pathogen in the form of conidia may infect the plant by rain splash dispersal step-wise, leaf by leaf as is known to be the case with *Septoria tritici* and *Stagonospora nodorum.* Air-borne ascospores, where produced, are an additional source of inoculum for ear infection. *Fusarium*-infected grain resulting from the development of FEB can, if used for seed, provide an important source of inoculum for the development of seedling blight and completion of the disease cycle (Parry *et al.,* 1995). Duthie and Hall (1987) concluded

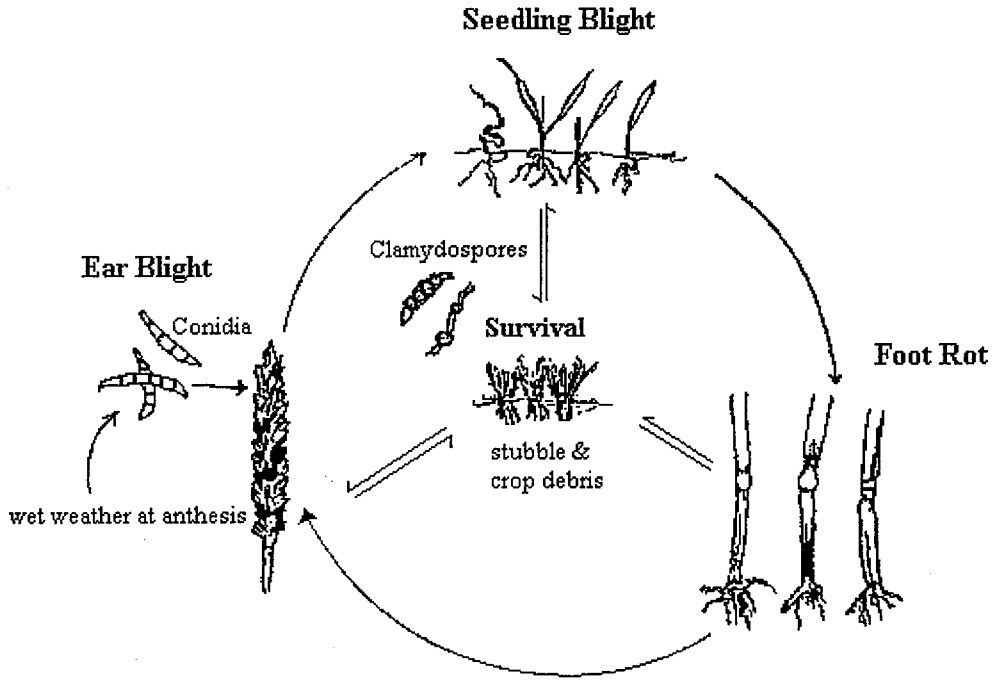

Figure 1.3. Generalised disease cycle of *Fusarium* on small grain cereals (Parry *et al.*, 1995).

that infection of the stem-bases of winter wheat by *F. graminearum* was directly related to the incidence of seed infection at sowing. They stated that the efficiency of transmission of the pathogen from seed to stem ranged from 55 - 94% over four sampling dates in two field trials. They found that all transmission of the disease happened in the autumn and that germination of seed and stand density decreased as the incidence of infected seed increased.

Significance of *Fusarium* ear blight.

Ear blight is of economic importance since it can significantly reduce the yield of cereals, it can also affect grain and seed quality.

Effect on yield.

The effect of *Fusarium* on grain yield has been studied using the three components that contribute to the yield of cereals: the number of ears per unit area, the number of grains per ear and the individual grain weight (thousand-grain weight).

There is much literature, from all over the world regarding the effect of FEB on yield. Much of the work is based on estimated yield losses in the field as a result of severe epidemics. Parry *et al.* (1995) cite work by Dickson and Mains (1929), who recorded crop losses of 8×10^6 bushels of wheat due to a severe epidemic of FEB in the USA in 1919. In India, Chaudhary *et al.* (1990) reported losses due to *F. avenaceum* of between 15 and 29% as a result of natural field infection. However, no mention of how crop losses were calculated is made in the report.

Hewett (1966) conducted tests on variety trials for the National Institute of Agricultural Biology (NIAB) and found that levels of *F. culmorum* and *F. avenaceum* were erratic but there was clearly an association between grain infection levels and plot conditions particularly with lodging.

More precise data have been obtained using trials where plants have been artificially inoculated in the field. Bennett (1933) found that plants inoculated at mid-anthesis with either *F. culmorum* or *F. avenaceum* failed to produce grain. Ears inoculated post-anthesis produced mycelium-covered grain, which bore sporodochia. By inoculating individual ears of wheat in the field to mimic natural infection, a situation was established with centres of disease within the crop. The disease was found to spread in the direction of the prevailing wind, with the number of affected plants and severity of attack falling in proportion to the distance from the centre of infection forming a dispersal gradient. The number of grains per ear was recorded, from the untreated (control) plots (57.6 grains per ear) and this was compared with neighbouring plots infected with *F. culmorum*, (35.6 grains per ear) and *F. avenaceum*, (39.6 grains per ear). The presence of *Fusarium* in the grain reduced the germination capacity of the seed. Healthy samples had a germination capacity of 89% and this was reduced to 35% and 49% for grain infected with *F. culmorum* and *F. avenaceum* respectively. This suggests that the use of contaminated seed can have a severe effect on the establishment of cereal crops. These results must, however, be regarded with caution as only 10 ears per plot were taken as a representative plot sample.

Hani (1981) working in Switzerland inoculated ears of wheat with *F. culmorum* and *M. nivale*, and observed yield reductions of 60 and 15% respectively. Saur (1991), reported

reductions in yield of between 6.4 and 39.2%, when over 500 wheat genotypes were inoculated with *F. culmorum* and *F. graminearum*. On field trials in Manitoba by Wong *et et al.* (1992) ears were inoculated with *F. graminearum* and results showed that when 28% of grains were infected, the grain yield was reduced by 32% and the thousand grain weight by 34%. Although grain weight per ear was reduced by 36% there was no reduction in total grain number.

Inoculated field trials have been used to examine the resistance of cereal cultivars to FEB and also to understand the relationship between disease and yield. Miedaner *et al.*(1993), researching resistance in winter rye breeding lines to FEB, found that yields were reduced between 27.4 and 48.7% when inoculated with *F. culmorum* and 38 - 51.7% for *F. graminearum*. In similar work by Snijders and Perkowski (1990), on winter wheat, found that thousand grain weight reductions of between 2.8 and 22.4% were recorded after inoculation with *F. culmorum* depending on the wheat genotype, which were comparable to the yield reductions found by Saur (1991).

Reduced seed quality.

Commercial seed samples are selected and screened for infection levels in the field. High levels of *M. nivale* were found in winter wheat harvested in 1980 and this was associated with low emergence when untreated seed was sown (Hewett, 1983). It was also pointed out that there is a difficulty in testing naturally-infected seed samples for emergence in the field since both *Fusarium* species and *Stagonospora nodorum* are usually present and can cause similar emergence problems. Colhoun (1970) recorded losses of up to 18 and 20% when plants were grown from seed inoculated with both *M. nivale* and *F. culmorum*.

Losses were only apparent when a sufficient number of plants were killed and the surviving plants did not compensate for these. It was found however, that the surviving plants did tiller more frequently.

Richardson (1974) found a good relationship in field trials with oats, between seed infection with *M. nivale* and seedling emergence, and also yields. The effect on yield was thought to be due to a reduction in panicle numbers resulting from a low seedling population. Colhoun (1970) states that losses of this kind are unlikely to occur often in commercial crops; his experiments showed that when conditions are unfavourable for seedling disease (this included the use of treated seed) little or no loss of yield occurred.

Bechtel *et al.* (1985) conducted tests on grain infected with *F. culmorum* to determine the damage caused by the fungal infection. Results revealed that the fungus is an aggressive invader destroying starch granules, storage proteins and cell walls. The fungus was found to be most prevalent in aleurone and pericarp tissues, but hyphae were found throughout the starchy endosperm. In all but the heavily infected grains, the endosperm was spared infection but even seed with lightly infected endosperm exhibited reduced germination and vigour. Microscopic studies revealed in lightly-infected grains that were germinating with extensive invasion of the scutellum and embryonic axis, indicating renewed fungal growth during imbibition.

Rennie *et al.* (1990) state the incidence of *M. nivale*, the main seed-borne *Fusarium* species on seed harvested in Scotland in 1987 and 1988, was 38 and 35% in seed intended for sowing as certified or farm-saved seed respectively. Treatment with an organomercury seed dressing increased germination from 70 to 91% in 1987 and from 73 to 87% in 1988.

More recently Cockerell and Jacks (2001) analysed winter wheat seed samples submitted to the Official Seed Testing Station for Scotland between 1991 and 2000. They state that the incidence of seed borne *M. nivale* fluctuated from year to year ranging from 3% in 1995 to 42% in 1997, in eight of the ten years sampled the average infection measured was higher than the 5% advisory threshold. They also state that there appeared to be no regional differences in the incidence of *M. nivale* seed infection across Scotland. However, the mean level of infection generally increased with increasing rainfall, but the year 2000 proved to be an exception to this with low infection in a year with high rainfall. They also concluded that there did not appear to be a relationship between mean *M. nivale* levels and mean temperature for the same period each year.

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Reduced grain quality and mycotoxins.

The genus *Fusarium* is one of the most prolific mycotoxin-producing genera (Rotter *et al.*, 1996), producing a large number of compounds including the T2 toxin, zearalenone, deoxynivalenol and moniliformin. These mycotoxins pose a threat to animal and human health where the grain is used for animal feed or human consumption. For example

Fusarium mycotoxins produced by *F. poae* and *F. sporotrichioides* found in over-wintered cereals have been associated with the development of Alimentary Toxic Aleukia (ATA) in humans (Joffe, 1978).

When infected grain is fed to livestock various toxicological and immunotoxic effects may result. Pigs fed with *Fusarium* infected grains have shown reduced food intake (Rotter *et al.*, 1995). Long *et al.* (1982) demonstrated that when young pigs were fed cereals contaminated with *F. culmorum* or *F. graminearum*, the mycotoxin zearalenone resulted in vaginal prolapses and vula vaginitis. Damage to the oesophageal part of the stomach in pigs was found by Trenholm *et al.* (1984). Grain infected by *F. poae*, *F. culmorum* or *F. graminearum*, when fed to poultry resulted in poor feathering and stunted growth (Hoerr *et al.*, 1982).

Work by Berova and Mladenov (1974) in Bulgaria reported that grain protein and gluten, and also baking qualities of flour were reduced when the wheat used was infected by *F. graminearum*. Due to the health risks associated with mycotoxins it is unlikely that *Fusarium* contaminated grain would be deemed suitable for baking under normal circumstances. In the USA and Canada standards are set for the contamination of grain by mycotoxins due to the health risks to humans (van Egmond, 1989). In the European Union standards are being discussed, Pricket (2000) has reported they are likely to be 500 μg DON kg^{-1} for finished products e.g. breakfast cereals, pasta and bread and 750 μg DON kg^{-1} for raw flour and grain.

In the premium grain market where it is used for brewing beer, contamination of wheat and barley with *Fusarium* spp. is also believed to cause problems of 'gushing' in the beer

making process (Narziß *et al.*, 1990). Schwarz *et al.* (1996) micro-malted fifty barley samples displaying a range of 0 - 100% seeds infected with *Fusarium*, and found that those samples which were infected with *Fusarium* tended to 'gush'. It was also found that levels of deoxynivalenol and ergosterol were found to be strongly correlated with the amount of gushing observed.

Control of *Fusarium*.

Management options for controlling ear blight include biological and cultural control, crop rotations, genetic resistance and fungicide applications. Research has however, shown that no one management strategy has been effective in controlling FEB (Parry *et al.*, 1995 and Bai and Shanner, 1996a).

Biological control.

There is some evidence that *Fusarium* species can be controlled by other micro-organisms. For example Tveit and Wood (1955) obtained control of seedlings with some isolates of *Chaetomium cochliodes* and *C. globosum* added to the seed or the soil in which seeds naturally infected with *Fusarium* pathogens, mainly *M. nivale*, were sown. Glasshouse trials gave results that showed control equivalent to the organomercury seed treatment but trials in the field proved less successful. Millar and Colhoun (1969b) found that seed (infected with *M. nivale*) sown into some soils developed little or no disease. They concluded that this was due to the presence in the soil of inhibiting or competitive micro-organisms.

Diamond and Cooke (2003) pre-inoculated wheat ears at anthesis in the glasshouse with

two non-host pathogens and the cell free germination fluids of three FEB pathogens. It resulted in significant reductions in disease development and severity caused by *Fusarium culmorum, F. avenaceum, F. poae* and *M. nivale*. Ears inoculated with *Phoma betae* and challenged with *F. culmorum* showed a 60% reduction in symptoms when compared with the control treatment after 25 days. There was an increase of 140% in the incubation period for the *P. ultimum* pre-inoculated *M. nivale* treatment and a 75% increase in the latent period in the *M. nivale* germination fluid pre-inoculation treatment challenged with conidia of the same species when compared with the control treatments. These results show that control of ear blight with *P. ultimum* and *P. betae* combined with their ability to increase the length of both the incubation and latent periods is possible and requires further investigation.

Johansson *et al.* (2003) studied the suppression of wheat seedling disease caused by *M. nivale* and *F. culmorum* using a bacterial seed treatment in Sweden. During five growing seasons 164 bacterial isolates were tested under field conditions against both pathogens as causes of seedling blight. Results showed the most efficient isolates, three fluorescent *pseudomonas* and a species of *Pantoea*, suppressed disease equal to an application of the fungicide guazatine, with respect to both crop stand and yield. A seed treatment with *Pantoea sp.* (isolate MF 626) increased yield by an average of more than 500 kg ha^{-1} in six field trials.

Cultural control.

There are various methods of cultural control that can be implemented to reduce the severity of FEB. Cultural control techniques such as crop rotation, appropriate use of fertilisers, irrigation and weed control may all contribute to a reduction in the amount of

Fusarium inoculum available for dispersal (Parry *et al.*, 1995).

Crop rotation.

The high incidence of FEB in cereals has received the attention of researchers all over the world for many decades. Koehler *et al.* (1924) noted a high incidence of FEB in plots where continuous wheat had been grown or where a maize-wheat rotation was implemented. During 1919 seven USA states were surveyed and Koehler found the incidence of FEB was highest (over 40%) when wheat was grown after maize. Wheat following wheat gave an incidence of just under 30% and the lowest incidence (below 15%) was when wheat followed a clover and timothy mixture. He concluded that the increased incidence after maize was due to the perithecia on corn stalks of susceptible cultivars and on stubble left by the previous crop. The author suggested that corn stubble should be buried or removed to reduce the incidence of FEB in the following wheat crop and sowing wheat after maize should be avoided. He recommended a rotation of four years for maize in the corn belt of the USA to decrease the risk of an ear blight epidemic.

Teich and Nelson (1984) also found the previous crop to be an important factor in the incidence of FEB in their survey of wheat fields in Canada. Sutton (1982) in his review of the epidemiology of *F. graminearum* identified the principal inoculum reservoir as host debris: stalks and ears of maize and cereal stubble. This agrees with the earlier work by Koehler *et al.* (1924). Teich and Nelson (1984) state that the average incidence of head blight on wheat following maize was 6-7 times greater than that of wheat following soybeans or cereals (wheat, barley and oats).

Land preparation.

Destruction of the debris left by a previous crop is an important way of reducing soil-borne inoculum. Booth and Taylor (1976) conducted two experiments on the infection of wheat plants with *M. nivale* from soil-borne straw debris. In experiment 1 the stubble was removed, ploughed in or left standing. Experiment 2 investigated the infectivity of straw debris after burial in the soil for varying periods of time. Infection of wheat plants from straw debris occurred in both experiments. The ploughing in of the previous crop debris was found to reduce (though not statistically significantly) the mean number of heads with *Fusarium* symptoms per 100,000 plants from 8.5 to 5.0 in Canada during 1983 (Teich and Nelson, 1984). This work agrees with Pereyra (1999) who tested survival and inoculum potential of *F. graminearum* under four different conditions – chisel plough, residues on the soil surface, chisel plough residue buried at 7.5 – 10 cm and 15 – 20 cm and mouldboard residue buried at 15 – 20 cm. It was found that surface residues, which decomposed more slowly, seemed to provide a nutrient source for *F. graminearum* for a longer period of time than buried residues. Wilcoxon *et al.* (1992) as a result of field experiments on winter wheat inoculated with *F. graminearum* warned against the use of minimal tillage as this failed to bury or destroy the maize debris. The best suggested method of destruction was stubble burning. This has not been possible in Europe since the ban on stubble burning was introduced. In a review of FEB, Parry *et al.* (1995) also suggested that direct drilling or minimal cultivation operations are likely to cause higher risks of FEB developing. Booth and Taylor (1976) concluded that *M. nivale* could exist in the soil without crop debris because 47% of seedlings became infected in plots where stubble had been carefully removed by hand. Snyder and Nash (1968) studied the occurrence of *Fusarium* in plots at Rothamsted Experimental Station, UK. It was found that *F. culmorum* existed as chlamydospores in the soil in the absence of crop debris. Crop

debris may act as a source of inoculum for seedling disease but it is not the only source.

Yi (2002) studied the effect of surface crop residues on *F. graminearum*. They studied the effects of residue type (maize or wheat), incorporation depth (5, 10, 15 cm), and soil amendment (calcium ammonium nitrate or nitrolime). It was concluded that burying the residues deeper in the soil can effectively reduce *F. graminearum* populations, but as decomposition is slowed down the pathogen may survive for longer periods in the soil.

Soil Fertility and Nitrogen Inputs.

Teich and Nelson (1984) found that fields under different cultural practices in Canada with the recommended 90 kg ha^{-1} or less of actual nitrogen, showed statistically significantly higher levels of *Fusarium* ear blight than those with extra nitrogen: 8.8 heads per 100,000 plants compared with 4.6 heads.

In fields fertilised with nitrates, head blight incidence was not significantly higher than those fertilised with urea, urea and nitrate and manure. Phosphorus ratings of high or medium-low gave significantly different results in the amount of disease as heads infected per 100,000 ears, high phosphorus 4.4 and 7.9 for medium to low phosphorus application. Actual figures for the amount of phosphorus applied were not presented. The authors concluded that nutrient stress may have increased susceptibility to *Fusarium* infection, or produced confusing symptoms such as chlorophyll loss from glumes.

In contrast to the above, research by Martin *et al.* (1991) investigated the effects of production inputs on the incidence of infection by *Fusarium* species on cereal seed. Nitrogen was applied as ammonium nitrate at various growth stages to wheat, barley and

triticale. It was found by the authors that the high yielding inputs (including nitrogen top dressing and the plant growth regulator ethephon (Cerone 280 g a.i ha $^{-1}$)) favoured yield but also increased *Fusarium* infection of seed. For example, on barley, the standard application of nitrogen and ammonium nitrate and foliar fertiliser with ethepon increased the incidence of *F. avenaceum* from 10.4% for the standard application (70 Kg ha^{-1} N) to 22.2%.

The impact of nitrogen on the development of FEB in wheat and the resulting deoxnivalenol in the kernel was studied by Lemmens *et al.* (2004). They applied five different types of nitrogen at five input rates and observed a significant increase of disease intensity as the nitrogen input was increased, while the type of nitrogen input had little or no effect on ear blight. In a second series of trials, spring wheat varieties were inoculated with *F. culmorum* and *F. graminearum*. It was found that in the higher rates, comparable with contemporary crop production, disease intensity and DON contamination remained at constant levels. They concluded that the adaptation of nitrogen fertilization is of no use in managing ear blight in wheat cultivation.

Weeds.

Teich and Nelson (1984) compared wheat crops in fields that either had or had not received herbicide treatment. Ear blight symptoms were significantly increased in fields where weed density was high, although no data on the numbers of weeds present were given.

Jenkinson and Parry (1994) surveyed weeds from fallow fields in Shropshire, UK. Over 70 isolates of *Fusarium* spp. were found and each tested for pathogenicity to the winter wheat

cultivar Mercia. Several common weeds including *Capsella bursa-pastoris* (Shepherd's purse), *Matricaria spp.* (Mayweed) and *Viola arvensis* (field pansy) were shown to provide an alternative host to five of the species of *Fusarium* isolated; these were also found to be pathogenic to wheat. These results demonstrate that weed species are a source of inoculum for the development of Fusarium disease, although they may only contribute to foot rot as there is not direct evidence for a link between weeds and FEB from this study, and weed control may be useful for the reduction of inoculum. However, Parry *et al.* (1995) states that the role of weeds in the development of FEB remains unclear and the efficacy of weed control in reducing FEB is debatable.

Genetic Resistance.

It was first noted by Arthur (1891) that some early-maturing wheat cultivars tended to be more resistant to FEB than late-maturing ones. Since then there has been a considerable amount of screening work involving thousands of breeding lines to identify sources of FEB resistance in wheat. Couture (1982) determined the incidence of *Fusarium* species in 34 lines of spring cereals (13 varieties of wheat, 13 varieties of oats and 8 varieties of barley). Significant differences in infection were found between cultivars in all three cereals, the most marked in wheat and the least marked in barley. He found a significant negative correlation between plant height and seed infection of the lines involved of the three species. Life-span of the crop was also significantly related to infection of the wheat lines. It was also found that lines of hard wheat were all less infected than lines of soft wheat and malting barley cultivars were less infected than feed barley types. The development of genetic resistance of cereals to FEB has been reviewed extensively by Parry *et al.* (1995) and Snijders (1990 and 1994).

Bai & Shanner (1996b) tested the varietal resistance of nine cultivars of wheat to *F. graminearum*. They inoculated the ears at the central floret and found that the fungus spread to non- inoculated spikelets in less than 20% of the plants in resistant cultivars and the spread was not seen until twelve days after inoculation. All susceptible cultivars showed spread of the pathogen by eight days after inoculation. They concluded that the measurement of spread of visible symptoms within a spike is a stable and a reliable estimate of cultivar resistance. Bai *et al.* (2001) conducted a further experiment to study the resistance of wheat and the accumulation of deoxynivalenol in the grain. Results showed a significant correlation between symptom ratings, seed quality and DON levels in the grain. They concluded that the percentage of scabbed spikelets and grains can generally be used to predict DON levels in harvested grain and in breeding programmes; selection of plants having few scabbed spikelets and grains is most likely to result in low DON levels. The results of Miedaner's (1997) work show the correlations between resistant traits and mycotoxin levels are not consistent and highly dependant on the environment, and that further experiments are required to clarify whether greater resistance will lead to a correlated reduction of the mycotoxin content of grain under natural infection.

Weather.

The influence of climatic factors on *Fusarium* species pathogenic to cereals was reviewed by Doohan *et al.* (2003). They stated that the main species differ in their climatic distribution and in the optimum climatic conditions required for their persistence; and that they rarely exist in isolation, but occur as a complex with each other *Fusarium* and other fungal genera. Climatic conditions will influence the competition between them, and the predominance of different fungi within the complex.

Rossi *et al.* (2002) studied the dynamics of airborne *Fusarium* macroconidia in wheat fields naturally affected by ear blight. They counted the number of spores m^{-3} and related it to the meteorological conditions. It was found that there was an association between rainfall and peaks of the macroconidia sampled. No or very few conidia were sampled before rainfall, but the numbers progressively increased during rainfall; in the presence of high humidity, conidia continued to be sampled at high densities for some hours after rainfall had ceased and they reached their peak under these conditions. The density of the airborne conidia rapidly decreased when the relative humidity dropped.

The effect of temperature on ear blight infection of different cultivars was studied by Brennan *et al.* (2005) they concluded that temperature did have an effect on the pathogen although they only conducted experiments in the glasshouse and at two temperatures (16 and 20°C). *Fusarium culmorum*-inoculated ears showed greater visual disease symptoms at 20°C than at 16°C both overall and at an individual cultivar level while the results for *F. graminearum* were the opposite. Similar amounts of pathogen DNA were found in contaminated ears at both temperatures for *F. culmorum* while for *F. graminearum* inoculated plants higher levels of pathogen DNA were found at 20°C.

Bateman (2001) found seasonal variations in populations of *Fusarium* species in wheat field soils. They found that there was usually a decrease in *F. culmorum* in late autumn or early winter as temperatures decreased and soil moisture increased. Populations then increased in the spring and summer with a rise in temperature. If conditions in each year were unsuitable for the development of foot rot or for rapid increases in populations of *F. culmorum* then the pathogen population was lower the following year.

Chemical control.

Seedling Blight.

Chemical control of seedling blight using seed treatments to sterilise the surface of the seed has been practised for many years. Seed treatments have contained copper carbonate, nickel sulphide, iodine-infusorial earth and organo-mercury compounds, of these Machacek and Greaney (1935) found those containing organo-mercury to be the most effective. Significant increases in the yield of wheat were achieved when treated seed was sown into heavily *F. culmorum* contaminated soil. Hewett (1966) concluded from a series of NIAB variety trials that seed treatment is effective in restoring the seedling emergence of diseased samples but, in view of the general agreement between leaf attack and final seed infection, it seems unlikely that there is much subsequent benefit. Bateman (1976) tested organo-mercury seed treatments in pot experiments and found phenyl mercury acetate (PMA) to give the best control of *M. nivale*. The control was better on wheat than barley and he concluded that this was due to the disease being more accessible to the seed treatment on wheat than barley, due to the barley hulls (chaff adhering to the grain) acting as a barrier. Bateman (1983) studied the response of *M. nivale* in wheat and barley caryopses to organo-mercury treatment in relation to the site of infection. He established that in wheat seed treated with PMA there was considerably less viable infection in the outer epidermis but the fungus could still be isolated from the inner seed coat, the endosperm and the embryo. It was concluded that the partial control by organo-mercury seed treatment against *M. nivale* can be explained by the depth of infection and the inaccessibility of the fungicide to the fungus e.g. in the crease.

According to Colhoun (1972), Millar (1962) found the use of the organo-mercurial seed dressing Agrosan gave a significant reduced incidence of infection with *M. nivale* but,

stated that it can not be expected that the disease can be eliminated from crops. Colhoun also states that Malalasekera and Colhoun (1967) using the organo-mercrial dust Ceresan for control of *F. culmorum* seedling blight obtained similar results.

Richardson (1974) studied the effects of infection and organo-mercury treatment on oat emergence. Seedling disease and eventual yield of oats were also determined. He concluded that seed stocks infected with *M. nivale* responded well to treatment and seed treatment was frequently beneficial with 75% of the stocks giving an economic return on treatment cost even in the absence of significant levels of seed-borne inoculum.

The effects of organo-mercury seed treatment on barley artificially inoculated with *Fusarium* spp. was studied by Clarke (1981). Field plots of spring barley infected with low levels of seed-borne *Fusarium*, untreated or treated with Ceresol (PMA) were sown. Following emergence, pathogenic isolates of *M. nivale* or *F. roseum* were introduced to some plots into the soil in a mulch. The seed treatment had no effect on emergence in 1978 but increased emergence by 23% in 1979. In both years seed treatment markedly raised yield in the absence of inoculum, 1978 by 26% and 1979 by almost 25%. Only in one year out of two were barley yields damaged by inoculation and then only by *F. roseum* and only then, when a seed treatment was used. How the loss of 27% occurred is not clear and it was concluded that it may be due to other yield components (number of fertile tillers or grains per ear) that were not recorded in these experiments. In 1979 seed treatment increased emergence by 23%, but yield by only 12% overall. In 1978 significant yield increases were obtained for all treatments except with *F. roseum* where a loss occurred. Clarke concluded that the findings show that the economic benefit of seed treatment is proven.

Until 1992 the use of organo-mercury seed treatment was common place. However, following the implementation of the EU Commission directive 79/117/EC (Anon, 1979) mercury containing seed treatments have not been registered for use in the UK.

Frohberg (1978) and Wainwright *et al.* (1979) conducted trials using triadimenol (from the demethylation inhibitor (DMI) group of fungicides) as a seed treatment. They concluded that although triadimenol alone gave useful control of seedling death caused by *M. nivale* both pre- and post-emergence death could be significantly reduced by the addition of fuberidazole (from the MBC group).

There is now evidence that *M. nivale* isolates are resistant to the MBC fungicides. Locke *et al.* (1987) obtained 704 isolates from 109 winter wheat crops in England and Wales. Of these isolates 82% were *M. nivale* and 92.1% of these were found to be resistant to benomyl. Scheinpflug and Durben (1988) reported reduced control from Baytan (triademenol and fuberidazole) after twelve years use in areas of Northern Germany. They suggested that in a seed treatment, MBC should not be the only component for the control of *M. nivale*. They suggested that a mixture of ingredients against which there is no cross resistance would be a more successful means of control. In extensive surveys of wheat stem-base isolates of *M. nivale* by Pettitt *et al.* (1993) and Parry *et al.* (1995) the existence of *M. nivale* isolates resistant to MBCs has been confirmed in the UK.

Compounds from other chemical groups have also been shown to have activity against *Fusarium* seedling blight. In 1993 Jones evaluated five seed treatments for the control of *M. nivale* on seed. All five treatments – Ceresol (PMA, ICI), Baytan (triadimenol and fuberidzole, Bayer), Ferrax (fluriafol and ethirimol and thiabendazole, ICI), Cerevax

(carboxin and thiabendazole, ICI) and Panoctine (guazatine, Rhone-Poulenc) gave significant increases in plant populations over the untreated. All treatments reduced the percentage of diseased plants and increased the number of healthy plants. Overall, the most effective treatment was Panoctine followed by Baytan and Cerevax.

Mihuta–Grimm & Foster (1989) evaluated seed treatments for the eradication of *Fusarium* species. Their study included isolating and identifying the species of *Fusarium* from infested fields, then testing various seed treatments for the control of seedling blight. Six seed treatments were compared and all gave a significant reduction in the level of seed-borne *Fusarium* when compared in the laboratory with the untreated control. However, under field conditions stand counts of seedlings were not significantly different from those of the untreated controls, this conflict with other research on seed treatments and may be due to the low numbers of seeds tested. In laboratory tests only 25 grains per treatment and 100 grains per treatment were sown in the field.

Martin *et al.* (1998) studied the effect of different levels of *Fusarium* seed infection and three fungicide seed treatments (triazole plus imidazole, fuberidazole and carboxin plus thiram) on barley establishment and yield. He states that without seed treatments cultivars with an average of 48% seed infection gave between 9% and 23% lower establishment than cultivars with 7% seed infection.

Gaurilcikiene (2000) investigated the effects of fungicide seed treatments on the incidence of seed-borne *F. graminearum* infection and foot rot on the germination of triticale. He concluded that different fungicides gave control in the laboratory (iminoctadine, sisthane and iprodione plus thiram) to the glasshouse (iminoctadine, tebuconazole, prochloraz and

fenbuconazole). In the field he tested seed samples of wheat and triticale with the purpose of determining the role of natural inoculation of *Monographella nivalis* and *Fusarium* spp, in and on the seeds. He stated that infected seeds did not germinate or gave rise to abnormal seedlings or visibly diseased plants.

Ear blight.

Ear blight is caused by a complex of fungi, for example, Mesterhazy (1984), found seventeen species associated with the disease in Hungary between 1970 and 1983. These included *F. graminearum, F. avenaceum, F. poae* and *M. nivale.* This could be one reason why the control of FEB by fungicide sprays has so far been poor and inconsistent. The activity of a fungicide against a sensitive species may be masked by the subsequent infection by a non-sensitive species. Such observations were made by Bateman (1993) on stem-base pathogens isolated from plots of wheat either untreated or treated with carbendazim (0.25 kg a.i. ha^{-1}), prochloraz (0.4 kg a.i. ha^{-1}), or carbendazim plus prochloraz (as Sportak Alpha, 0.15 kg a.i. ha^{-1} + 0.4 kg a.i. ha^{-1}). He found that following prochloraz application, the incidence of *Rhizoctonia cerealis* isolations increased whereas isolations of *Pseudocercosporella herpotrichoides* decreased.

Polley *et al.* (1991) stated there was no substantial evidence that fungicides currently then in use gave effective and consistent control of stem-base diseases caused by *Fusarium* species. The authors tested three fungicides at two concentrations on winter wheat seed taken from the ADAS National winter wheat survey in 1989 and 1990, benomyl 5 and 20 mg a.i. l^{-1}, prochloraz 2 and 0.05 mg a.i. l^{-1}, flusilazole 16 and 1.6 mg a.i. l^{-1} and one concentration of iprodione (5 mg a.i. l^{-1}). They measured the growth rate of *F. culmorum* and *M. nivale* on PDA amended with or without the fungicides. A significant difference

was observed between *F. culmorum* and *M. nivale* in sensitivity to benomyl and iprodione. Isolates of *M. nivale* were significantly less sensitive to benomyl and significantly more sensitive to iprodione than *F. culmorum*. Prochloraz was found to be the most effective fungicide tested against *Fusarium* spp. Flusilazole showed similar levels of activity both being slightly more effective against *F. culmorum* than *M. nivale*. In 1990 a sub-population of *M. nivale* was found to have a marked decrease in sensitivity to benomyl. Of 31 isolates, only 41.9% were found to be completely sensitive to benomyl. Polley *et al.* (1989) concluded that resistance detected in *in vitro* tests is not necessarily related to a loss of performance in the field and therefore isolates showing lower sensitivity in tests carried out in this survey were not classed as resistant as a loss of field performance was not demonstrated. Widespread resistance to MBC (methylbenzimidazole carbamate) fungicides, such as benomyl, by populations of *M. nivale* has been reported by Locke *et al.* (1987) and Pettitt *et al.* (1993). Locke *et al.* suggested that there was little justification for the continuing use of MBC fungicides in wheat, except against FEB, which may be caused by a species other than *M. nivale*.

Jacobsen (1977) demonstrated the effect of four fungicide treatments on *Septoria* leaf and glume blotch and FEB caused by *F. graminearum* on grain yield and thousand grain weight of wheat. Benomyl (0.55 kg a.i. ha^{-1}), mancozeb (1.75 kg a.i. ha^{-1}), mancozeb plus benomyl (1.1 kg a.i. ha^{-1} plus 0.2 kg a.i. ha^{-1}) and benomyl plus carbendazim (0.55 kg a.i. ha^{-1} plus 1.1 kg a.i. ha^{-1}) were applied at growth stage 55 and again 10 days later. Applications of benomyl alone or in combination with carbendazim or mancozeb gave 'cleaner' heads and reduced the incidence of FEB. At harvest fungicide application resulted in a reduction of infected grain and increased thousand grain weights (up 2% for

benomyl alone, 1.2% for mancozeb plus benomyl and 1.7% for mancozeb alone). It is unlikely though that these small increases were statistically different from the control. Ear blight was also reduced by up to 50% compared with the control. This experiment shows good control by fungicides of FEB; however the results were not confirmed by other studies using different locations and with different disease pressures.

Studies by Martin and Johnston (1982) on naturally infected trials in the Atlantic Provinces of Canada, showed that plots which received propiconazole at a rate of 259 g a.i. ha $^{-1}$ applied at growth stages 50 and 75 significantly reduced the incidence of FEB by 41% compared with the untreated control and gave an increase in yield of 34%. The fungicide treatments did not however reduce the concentration of deoxynivalenol (DON) in the harvested grain. It should be remembered that Jacobsen (1977) used applications of propiconazole to control not only FEB but also *Stagonospora nodorum* on the upper leaves, so it is difficult to relate the yield increase directly to FEB control.

Fehrmann and Ahrens (1984) artificially inoculated plants in the field with *F. culmorum* at mid-anthesis. They found that applications of prochloraz (1.2 l ha^{-1}) before or after inoculation resulted in yield losses of 34% and 26% respectively, compared with a yield loss of 38% for the untreated control plots. For plants inoculated with *F. graminearum*, a yield loss of 48% (in control plots) was reduced to 36% after two applications of prochloraz. Unfortunately the authors failed to show if such reductions were statistically significant.

Hutcheon and Jordan (1992) investigated fungicide timing and performance for *Fusarium* control in wheat. Glasshouse-grown plants were inoculated with *M. nivale*, *F. culmorum*, *F. graminearum* or *F. avenaceum* either at the seedling stage or at flowering. Applications of UK264 (tebuconazole 250 l ha^{-1} and triadimenol 125 l ha^{-1}) effectively reduced the percentage of ear tissue infected by the pathogens when applied 2, 6 or 14 days after inoculation at anthesis. In the control plots 62.4% of spikelets were infected which yielded 1.388 g of grain per ear. Plants inoculated at GS 49 and sprayed 3 days later were shown to have ears, which had 33.6% of spikelets infected and yielded 1.543g of grain per ear. Plots inoculated at mid-anthesis and sprayed three days later resulted in ears with 18.4% of spikelets infected and a yield of 1.498 g per ear. The authors suggest that seed treatments can reduce disease at the early stages of plant development, but is unlikely to prevent FEB later due to the pressure from external inoculum. Late season foliar sprays also have the potential to reduce yield losses caused by severe ear blight disease, so a combination of seed treatment and late season sprays were considered by the authors to be the best method of control for FEB. Further work under field conditions would be required to confirm the results of this work.

Field trial work by Jennings *et al.* (2000) using azoxystrobin, tebuconazole, metconazole and carbendazim applied to the ear showed that applications of tebuconazole, metconazole and carbendazim resulted in a significant reduction in the extent of grain colonisation by *Fusarium* species and DON concentration. Conversely, applications of the same fungicides resulted in an increase in the extent of grain colonisation by *M. nivale*. In the first year of the study the application of azoxystrobin caused a reduction in competition between *Fusarium* spp. and *M. nivale* and as a result greater colonisation by *Fusarium* spp. was

observed and DON contamination was increased by 41%. In the second year *M. nivale* was not present on the ears and DON concentration was not increased after an application of azoxystrobin. More recently, Simpson *et al.* (2001) stated that in a series of field trials, both naturally and artificially inoculated, applications of fungicides (tebuconazole and azoxystrobin) gave differential control of the pathogens (*Fusarium* spp. and *M. nivale*). Tebuconazole selectively controlled *F. culmorum* and *F. avenaceum* and reduced levels of DON concentration. Application of azoxystrobin, however, selectively controlled *M. nivale* and allowed greater colonisation of the ear by *Fusarium* spp. This also led to higher levels of DON being detected. They concluded that their results indicated a potential risk of increasing DON contamination of grain following azoxystrobin to control ear blight in susceptible cultivars.

In contrast to the observations of Jennings *et al.* (2000) and Simpson *et al.* (2001), Jones (2000) stated that applications of azoxystrobin during field trials between 1995 and 1997 inoculated with *F. graminearum* caused a decrease in ear blight severity of 12% compared with the unsprayed control plots and a reduction in DON contamination of 12%. Siranidou and Bachenauer (2001) found that applications of azoxystrobin against ear blight caused by *F. culmorum* reduced disease severity significantly; however, the DON concentrations in grain remained unchanged when compared with the untreated. In 2001 Cromey *et al.* demonstrated that azoxystrobin could provide some control of ear blight without increasing the DON content of grain. Naturally-infected plots of winter wheat were sprayed at GS 59 or 65 with azoxystrobin, tebuconazole or carbendazim. Tebuconazole reduced disease severity by 41% whilst azoxystrobin and carbendazim both reduced ear blight by 29% when compared to the unsprayed control treatment. Both tebuconazole and carbendazim

significantly reduced levels of DON in the grain whilst azoxystrobin did not have any effect on the levels of mycotoxins investigated.

The effectiveness of flutriafol + carbendazim, fenpropimorph and diclobutrazol in combination with prochloraz at full and half rate were tested in the field against *F. graminearum* by Korptis *et al.* (2002). They found that full rate applications were effective against the pathogen and green leaf area (GLA) was extended in all cases by the application of the fungicides. Green leaf area was found to be negatively correlated with disease severity and positively correlated with yield. High GLA and an extended green leaf area duration (GLAD) also increased grain weight and yield. Grain was collected from ears at GS 85 and at harvest and drilled to measure emergence. Significant differences were found in emergence from healthy and FEB infected ears, seedlings from infected grains were characterized by reduced coleoptile elongation and seedling death. They concluded that fungicide treatments might reduce inoculum and increase yield but are not sufficient to reduce *Fusarium* contamination in the grain of wheat.

Mesterhazy (2003) stated that attempts to control FEB with fungicides have been highly variable. Cultivar resistance, fungicide efficacy, fungicide coverage, and timing and pathogen aggressiveness cause this. He studied the effect of different fungicides on wheat varieties of different resistances using isolates of *F. culmorum* and *F. graminearum.* It was found that in most cases that the application of a fungicide reduced visual symptoms, yield loss and DON concentration. It was concluded that further research was required to develop more resistant cultivars, better spraying technology and more effective fungicides.

Aims of the project.

- *To investigate the epidemiological link between ear blight and its control with the fungicides azoxystrobin and metconazole, and the incidence and severity of subsequent seedling blight.*

Objectives.

- *To determine the effect of applications of azoxystrobin and metconazole on the development of Fusarium ear blight symptoms, grain infection and yield.*

- *To investigate the effect of different inoculum loads of M. nivale and or F. culmorum on the development of Fusarium ear blight symptoms, grain infection and yield.*

- *To investigate the effect of inoculation with M. nivale and or F. culmorum at a given point on the ear on the development of Fusarium ear blight symptoms, grain infection and yield.*

- *To determine the effect of fludioxonil seed treatment applied to infected grain harvested from ear blight trails on the incidence of seedling blight in the field.*

Chapter 2

General Materials and Methods.

Pathogens.

Isolates of *Fusarium culmorum* and *Microdochium nivale* used in the experimental work undertaken in this project are detailed in Table 2.1. All *Fusarium* and *Microdochium isolates* were obtained from the culture collection at Harper Adams University College, Newport, Shropshire, UK.

Table 2.1. Isolates used in experimental work.

Fusarium & Microdochium	**Isolate**
Fusarium culmorum	*Fu* 42, *Fu* 36, *Fu* 5, *Fu* 302, *Fc* 95
Microdochium nivale var. *majus*	EST 11, MW 3, AV 9, SO54, SO 47
Microdochium nivale var. *nivale*	W 71, *Mn* 1/1, SO 28, SO33, SO 47

Host Cultivar.

Winter wheat (*Triticum aestivum*) cultivars: Cadenza, Equinox and Riband were used in the experimental work.

Culture of Pathogens.

Aseptic techniques.

All glasshouse materials, pathogen growth media, distilled water and other (autoclavable) materials were sterilised by heat treatment at 121°C and 103.4 KPa for 20 minutes in an autoclave. All aseptic operations were performed in a laminar flow cabinet following sterilisation of all surfaces with 90% ethanol.

Spore Production.

The isolates were taken from the CERC pathogen collection where all isolates are maintained as spore suspensions in 10% glycerol at -80°C. All isolates were sub-cultured onto PDA (potato dextrose agar. Merck KgaA. Darmstadt, Germany). Sub-cultures of the isolate were produced by transferring a 5 mm plug of mycelium from the edge of actively growing cultures, using a sterile cork borer, to a Petri dish containing 15 ml of PDA. The plates were sealed with 'Parafilm' (American Can Company, Greenwich, USA). After 14 days in the incubator at 20°C, (+/-2°C), the cultures were moved into a refrigerator at 4°C. After 12 weeks all isolates were sub-cultured onto fresh media to maintain pathogenicity. Subcultures were subjected to specific light regimes for 14 days to induce sporulation. Cultures of *M. nivale* were illuminated by near ultra-violet fluorescent tubes on a 12-hour photoperiod at 15°C (+/- 2°C). Cultures of *F. culmorum* were placed in a dark incubator at 20°C (+/- 2°C).

Preparation of Experimental Spore Suspension.

Macroconidia were dislodged and washed from actively growing cultures using a sterile microbiology loop and 5 ml of sterile distilled water (SDW). The resultant spore suspension was then passed through two layers of sterile muslin to remove hyphal

fragments. The concentration of spores in the suspension was determined using a haemocytometer (Improved Neubauer, Webber Scientific International Limited, Teddington, Middlesex, UK). The suspension was then frozen and stored or adjusted to the required concentration with SDW and applied to wheat plants.

Preparation of Host Plants.

For glasshouse experiments wheat seed (cv. Cadenza) was treated with Beret Gold (fludioxonil at a rate of 4 ml kg^{-1} of seed (Syngenta Crop Protection UK Ltd, Cambridge UK)) and sown into trays of peat based compost (John Innes No.2. Arthur Bower's, Lincoln, UK). Seeds were allowed to germinate at 20°C (+/- 2°C) on a glasshouse bench for 7 – 10 days. They were then transplanted into 15 cm diameter pots at a rate of 5 plants per pot. Plants were grown in the cool (10°C +/- 2°C) bay of the glasshouse under a photoperiod of 12 hours for 4 weeks in order to encourage tillering. Plants were watered daily and, after Zadoks growth stage (GS) 32 (Zadoks *et al.*, 1974) had been passed, they were fed weekly with an application of foliar fertiliser (10% N, 10% P_2O_5, 27% K_2O as Phostrogen (Phostrogen Ltd, Clwyd, UK). After 4 weeks the temperature in the bay was increased to 22°C (+/-3°C) and the photoperiod to 16 hours. When necessary, the plants were sprayed with Fortress (quinoxyfen 500g/l, (Dow Elanco Ltd, Hitchin, UK.)) to eradicate powdery mildew, and Aphox (primicarb 50%w/w, (Zeneca Crop Protection. Fernhurst, UK)) for aphid control. If the glasshouse became infested with aphids Nicotine shreds (Nicotine 40% Shreds, Dow Agrosciences, Hitchin, UK.) were used according to the manufacturers instructions.

Inoculation of Plants.

During glasshouse experiments plants were artificially inoculated at GS 65 mid-anthesis. Plants were inoculated by the application of approximately 2 ml of prepared conidial suspension, at a concentration of 1 $x10^5$ conidia ml^{-1} of water per ear as a fine atomised spray until run-off using a hand held sprayer. Point inoculation of ears both in the field and glasshouse was achieved by placing 25 µl of spore suspension (1 x 10^5 spores ml^{-1}) using a pipette, between the lemma and palea of spikelet ten according to the procedure used by Hilton (1999) (Figure 2.1). Following inoculation, ears were placed in clear plastic bags for 48 hours to increase humidity surrounding the ears and induce optimum conditions for infection.

In the field, spores were applied using a pressurised hand-held knapsack sprayer (Bastion 15, Application Technique Ltd, Herts, UK) at the rate of 1 x 10^5 spores ml^{-1} in 33 ml m^{-2}, see individual Chapter material and methods. Immediately after artificial inoculation, in order to produce conditions to encourage ear blight, all plots were subjected to overhead mist irrigation applied for 1 minute every 20 minutes between the hours of 8.00am and 6.00pm BST. This continued for 21 days following inoculation (Figure 2.2).

Fungicide Application.

In the glasshouse fungicides were applied using a precision pot sprayer situated in the glasshouse at Harper Adams custom built for Harper Adams University College by J. Reader, using Lurmark 110° flat fan nozzles (Lurmark, Cambridge, UK) and applying fungicides at 210 l ha^{-1}. In the field fungicides were applied using a pressurised knapsack

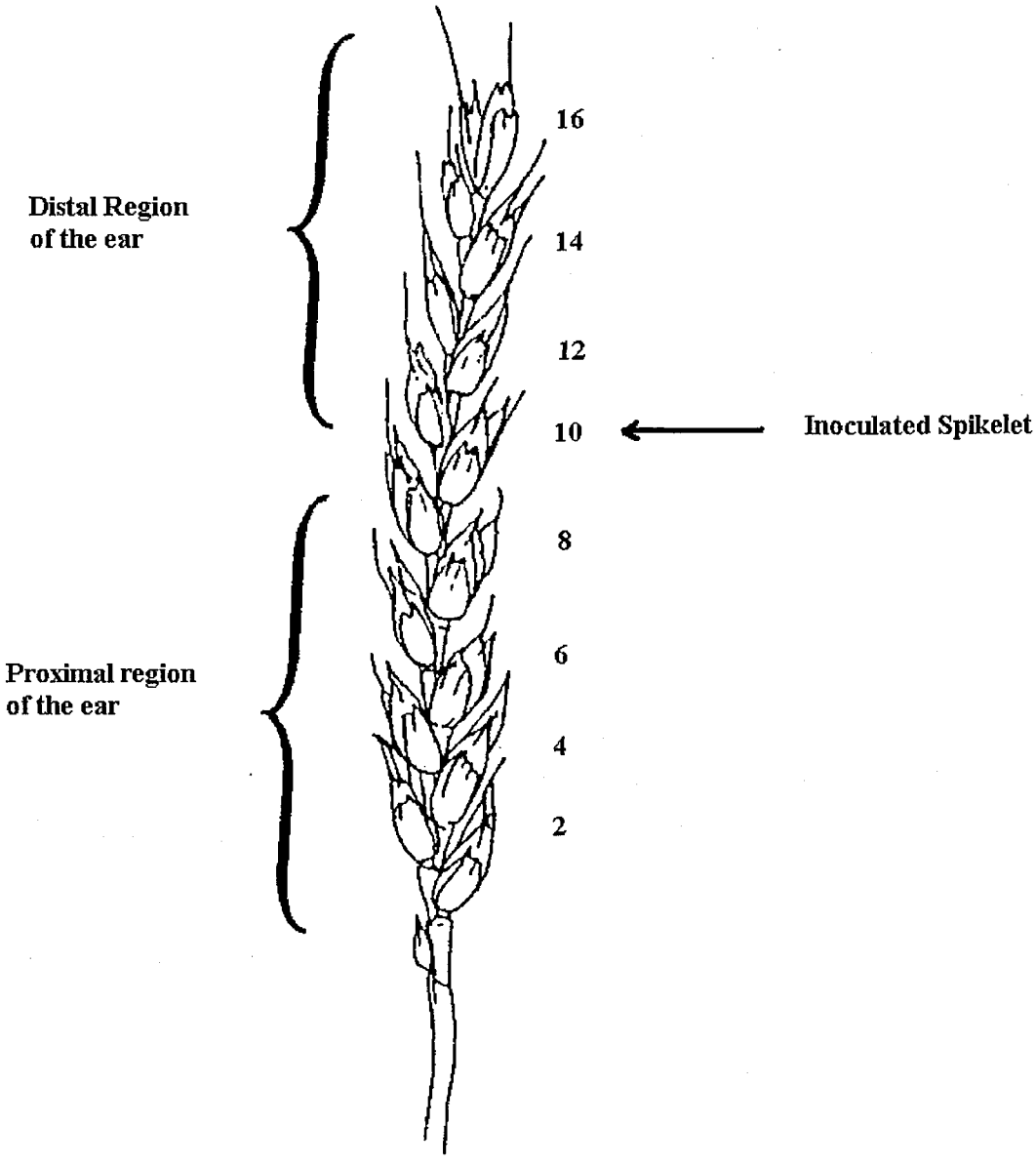


Figure 2.1. Diagrammatic representation of the inoculated spikelet (10^{th} from the base of the ear showing the distal (above the point of inoculation) and proximal (below the point of inoculation) regions of the ear.

sprayer (Safer s.p.a, Italy) with four nozzles (03-F110 flat fan nozzles, Lurmark, Cambridge, UK); applications were made at 3 bar pressure at a flow rate of 2.16 l min^{-1} to apply 200 l ha^{-1}. The fungicides tested during the glasshouse and field ear blight experiments are shown in Table 2.2.

Table 2.2. Fungicides used in glasshouse and field ear blight trials.

Active Ingredient	Product	Company Details
azoxystrobin 250 g l^{-1} SC	Amistar	Syngenta Crop Protection, Cambridge, UK
metconazole 60 g l^{-1} SL	Caramba	BASF. Agricultural Division, Cheadle Hulme, UK
tebuconazole 120 g l^{-1} EC	Folicur	Bayer PLC. Bury St Edmunds, UK

Figure 2.2. Overhead mist irrigation of field plots at Harper Adams University College, Shropshire, following inoculation at GS 65.

Seed Treatment Application.

The seed treatment fludioxonil (Beret Gold, Novartis Crop Protection) was applied at the manufacture's full recommended dose of 2.0 l tonne^{-1}, using a Mini-Rotostat (Marline Ltd, Norfolk, UK) at Harper Adams.

Emergence Assessments.

Emergence was measured daily by counting the number of seedlings between two markers 0.5 m apart, this is done twice in each plot. This figure was then used to calculate the number emerged m^{-2}. Where percentage emergence is used this was calculated on 100% of the drilled seed (drilled at 200 seeds m^{-2}) emerging. Final emergence was defined as complete when no further seedlings had emerged for five consecutive days.

Disease Assessments.

Seedling Disease:

The severity of seedling disease was assessed in three ways. Firstly, the most severe symptom was pre-emergence death of the seedling (non-emergence). The second most severe symptom was post-emergence death of the seedling. The third and least severe symptom of disease was an assessment of necrosis on the emerged seedling.

Ear assessments:

In both field and glasshouse experiments ears were assessed for symptoms of ear blight. In the field, in each plot, 100 ears were visually assessed at random, in the glasshouse all ears were assessed in the trial. The number of bleached spikelets per ear and the total number of spikelets were recorded in order that the percentage of spikelets infected could be

calculated. Thus incidence (mean percentage of infected ears) and severity (mean percentage of infected spikelets) measurements were made for each plot.

In the field, assessments of disease severity were performed at approximately GS 75 and 85 – approximately 21 and 28 days after inoculation respectively. In glasshouse experiments disease assessments were undertaken at GS 75.

In experiment 1, 10 ears were selected at random from each plot and harvested whole for spore washing.

Yield assessments.

When ripe (GS 92) all field plots were harvested using a Seedmaster plot combine (Wintersteiger, Ried in Innkreis, Austria). Total grain yield and percentage grain moisture content were recorded during harvest. A sample of approximately 2 kg was taken from each plot and used to determine thousand-grain weight and specific weight. From each 2 kg sample a sub-sample of approximately 200 g was taken using a ripple grain divider (Novaliter Laboratories, UK). Grain from this sub-sample was used for the grain infection and quality assessments.

Grain infection and quality assessments.

An assessment of the degree of seed infection was performed for each experiment by calculating the percentage of seeds from which isolates of *M. nivale* or *F. culmorum* were recovered. For *M. nivale* MBC-amended PDA plate tests were used. The MBC fungicide was added to the agar to reduce the growth of other fungal pathogens (Pettit *et al.*, 1983). For *F. culmorum* moist blotters were used in accordance with the International rules for

seed testing as laid down by the International Seed Testing Agency (ISTA) (Anon., 1985).

A seed viability test was performed on seed from each field experiment using the tetrazolium viability test in accordance with the International rules for seed testing as laid down by the ISTA (Anon., 1985). One hundred seeds were sampled from each plot sample and tested for viability. Laboratory germination tests were carried out on one hundred randomly-selected seeds from each plot using the moist blotter method as detailed by ISTA (Anon., 1985).

Statistical Analyses.

Where appropriate, ANOVA was carried out on all data using the statistical software package Genstat 5.1 (Lawes Agricultural Trust. IACR. Rothamsted, UK). Where appropriate, percentages were arc sine transformed, and regression analysis was also used where appropriate.

Chapter 3

Investigations into the effect of selected fungicides on the development of *Fusarium* ear blight and the subsequent development of seedling blight caused by *Microdochium nivale* and *Fusarium culmorum.*

Introduction.

There are numerous records that identify FEB as an economically important disease world-wide but despite this research shows conflicting opinions into the effectiveness of fungicidal control. For example, Boyacioglu *et al.* (1992) reported that when field trial plots were inoculated with *F. graminearum* at anthesis the fungicide triadimefon effectively reduced grain infection and DON concentration when applied at three timings: two days pre-inoculation, at the time of inoculation and two days post-inoculation of ears. It reduced grain and DON infection by between 65 – 97% and disease severity by 50 – 62% at the second and third time of application. The application of propiconazole at the time of inoculation and two days post-inoculation of the ears reduced *F. graminearum* grain infection by 39 – 56%. In contrast the fungicide thiabendazole had no effect on grain infection but was most effective at reducing DON concentration (by 83%) when applied two days pre-inoculation. Homdork *et al.* (2000) reported that an application of tebuconazole on ears three days before inoculation with *F. culmorum* reduced disease severity by 92% when compared with the control treatment. When applied five days after inoculation the same fungicide reduced head blight by 57%.

Similarly as previously discussed in Chapter 1, work on the effectiveness of azoxystrobin and tebuconazole against ear blight pathogens shows conflicting results. The observations of Jennings *et al.* (2000), Jones (2000) and Simpson *et al.* (2001) stated that control of *Fusarium* spp. is poor and inconsistent with the risk of increasing DON concentrations in the grain. In contrast to these findings Cromey *et al.* (2001) demonstrated that azoxystrobin could provide some control of ear blight

with out increasing the DON content of grain when naturally infected plots of winter wheat were sprayed at GS 59 or 65 with azoxystrobin, tebuconazole or carbendazim.

In contrast to the above findings work by, amongst others, Martin and Johnston (1982) and Milus and Parsons (1994) shows the control of FEB in the field to be poor and inconsistent.

Infected grain can provide a primary source of inoculum for the subsequent development of *Fusarium* seedling and ear blight (Hewett, 1983). Seedling blight can also be caused by soil-borne inoculum (Bateman, 1977), however, under UK field conditions soil- borne inoculum is thought to be of little importance (Paveley *et al.*, 1996). If severe, *Fusarium* seedling blight can result in low seedling establishment, and yield reductions of up to 40% in the field have been observed in the UK (Noon and Jackson, 1992). Seedling blight is usually controlled by the use of fungicide seed treatments and an advisory threshold for treatment set by the NIAB of 5% infected seeds exists in the UK. In 1993/4 over 90% of wheat seed samples were infected by *M. nivale* and of those more than 90% exceeded the 5% advisory limit (Reeves and Wray, 1994). It is clear that in seed crops the control of FEB and grain infection is of great importance. Humphries *et al.* (1995 and 1997) found that there was a significant correlation between seed-borne *M. nivale*, establishment and grain yield of both winter wheat (1995) and winter barley (1997). In laboratory tests they concluded that the seed-borne *M. nivale* caused reductions in emergence which could in turn reduce grain yield and so indicated the importance of effective control of seed-borne *M. nivale* in winter wheat and barley in Ireland.

Infection of harvested grain by *Fusarium* species is important in terms of seed germination. In 1966, Hewett examined seed infection of variety trials from the 1965 harvest. Seed infection by *M. nivale* was found to be high, a mean of 20% compared to 4 and 7% in 1963 and 1964. Infections of *F. culmorum* and *F. avenaceum* were also high with a mean of 39% (9 and 10% in 1963 and 1964). Particularly high incidences were found to be present in plots where lodging had occurred. Similar material harvested in 1969, 1970 and 1973 showed mean seed infections of 1-2%. Hewett points out that very few samples of seed intended for sowing contained appreciable levels of *M. nivale; Stagonospora nodorum* infection was much more common.

High levels of *M. nivale* were found in winter wheat harvested in 1980 and this was associated with low emergence when untreated seed was sown (Hewett, 1983). It was also pointed out that there was a difficulty in testing naturally infected seed samples for emergence in the field since both *Fusarium* species and *S. nodorum* are usually present and can cause similar emergence problems. Colhoun (1970) recorded losses of up to 18 and 20% when plants were grown from seed inoculated with both *M. nivale* and *F. culmorum.* Losses were only apparent when a sufficient number of plants were killed and the surviving plants did not compensate for these. It was, however, found that the surviving plants did tiller more frequently.

Rennie *et al.* (1990) stated the incidence of *M. nivale,* the main seed-borne *Fusarium* seedling blight pathogen, on seed harvested in Scotland in 1987 and 1988, that was intended for sowing as certified or farm saved seed as 38 and 35% respectively.

Treatment with an organomercury seed dressing increased germination from 70 to 91 % in 1987 and from 73 to 87% in 1988.

To date there has been no published work on the link between controlling ear blight with fungicide applications and the use of this grain as a seed crop with or without a seed dressing, and the incidence of seedling blight symptoms.

This chapter contains the results of two years of field trials; details are given in Table 3.1. The first field-based experiment (experiment 1) was designed and executed in the harvest year of 1999 with the aim of investigating the chemical control of FEB using azoxystrobin (Amistar) and metconazole (Caramba). The trial was conducted on plots of winter wheat (cv. Equinox), which were inoculated with *Fusarium culmorum* and *Microdochium nivale* conidia at anthesis. Seed from this was cleaned and drilled to assess the emergence with or without a seed dressing (experiment 2). Two field trials (experiments 3 & 4) in the harvest year of 2000 were undertaken to determine the chemical control of FEB using azoxystrobin and metconazole. The trials were conducted on plots of winter wheat (cv. Equinox), which were inoculated with *Microdochium nivale* (experiment 3) or *Fusarium culmorum* (experiment 4) conidia at anthesis. Seed from these trials were also cleaned and drilled to assess the emergence with or without a seed dressing (experiments 5 & 6).

Aims.

1. To determine the effect of fungicide applications of azoxystrobin and metconazole alone and in combination on the severity of FEB symptoms, grain quality and yield.
2. To determine the effect of a seed treatment with fludioxonil applied to infected

grain harvested from the ear blight trials on the incidence of seedling blight under field conditions.

Objectives.

1. To evaluate the effect of azoxystrobin and metconazole alone and in combination on the incidence and severity of ear blight symptoms caused by *M. nivale* and *F. culmorum*.
2. To determine the effect of azoxystrobin and metconazole alone and in combination on grain yield.
3. To determine the effect of azoxystrobin and metconazole alone and in combination on the infection of harvested grain and grain quality.
4. To evaluate the effect of a fludioxonil seed treatment when applied to infected grain harvested from the ear blight trials on the incidence of seedling blight under field conditions.

Null hypotheses.

1. The application of azoxystrobin or metconazole alone and in combination in the field has no effect on the incidence and severity of FEB symptoms, grain yield or the infection of harvested grain and grain quality.
2. The application of fludioxonil has no effect on the emergence of seedling blight caused by sowing seed infected with *Fusarium culmorum* and *Microdochium nivale* alone and in combination.

Table 3.1. Overall outline of the experiments detailed in Chapter 3 - effect of fungicide applications at manufacturer's maximum rate on the development of ear blight and emergence experiments carried out under field conditions.

Experiment Number	Hypothesis Tested	Location	Year	Pathogen	Target
1	1	Field	1999	*F.c & M.n*	Ear blight
2	2	Field	1999	*F.c & M.n*	Seedling blight
3	1	Field	2000	*M.n*	Ear blight
4	1	Field	2000	*F.c*	Ear blight
5	2	Field	2000	*M.n*	Seedling blight
6	2	Field	2000	*F.c*	Seedling blight

M.n – M. nivale *F.c – F. culmorum*

Method.

A field trial (experiment 1) was conducted in the harvest year 1999 at Harper Adams University College in plots of winter wheat cv. Equinox. The trial was drilled as a first cereal following potatoes in a sandy loam soil. The trial contained twelve treatments (Table 3.2) that were laid out in a randomised block design with treatments allocated to plots (size 2 x 8m) at random. Five replicates of each treatment were used giving five blocks and 60 plots. An agronomic diary is given in Appendix 1.

After the grain was harvested a bulk sample of seed for each field trial treatment was produced, by taking a 1 kg sample of seed harvested from field plots and combining the samples from individual plots. This seed was used to drill an emergence trial – experiment, experiment 2.

The emergence trial (experiment 2) was made up of the 12 ear spray treatments either with or without the seed treatment of fludioxonil (50 g a.i. t^{-1}) (Beret Gold. Syngenta Crop Protection. Whittlesford, Cambridgeshire. UK), (Table 3.3). Five replicates of each treatment were drilled at a rate of 400 seeds m^{-2}, giving five blocks and 120 plots. Assessments of emergence (as described in detail in Chapter 2) were performed every two days from the first emerged coleoptile until emergence was complete.

Two field trials (experiments 3 & 4) were conducted in the harvest year 2000 at Harper Adams University College in plots of winter wheat cv. Equinox. The plots (10 x 2m) were drilled into a sandy loam following potatoes. Each trial contained four treatments that were laid out in a randomised block design with treatments (Tables 3.4 and 3.5) allocated to plots at random. Six replicates of each treatment were used giving six blocks and 24 plots. An agronomic diary is given in Appendix 2.

In both years the plots in experiments 1, 3 and 4 were inoculated at GS 65 (mid anthesis) with a water-based conidial suspension of either *F. culmorum* only, *M. nivale* only (2000) or a mixture of both pathogens (1999 only). The spore suspension was applied with a hand held plot sprayer, at the rate of 100,000 spores per ml^{-1} in 33 ml of water m^{-2}. The spore suspension contained five isolates of *F. culmorum* in equal proportions. The *M. nivale* only suspension contained five isolates of *M. nivale* var. *majus* and five isolates of *M. nivale* var. *nivale* in equal proportions see Table 2.1 in Chapter 2. The mixture of the two pathogens contained 50% of each of the mixtures above. After inoculation the plots were mist irrigated for 21 days as described in detail in Chapter 2 (Figure 2.2). Fungicide treatments (Table 3.3 for 1999 and Table 3.4 and 3.5 for 2000) were applied at GS59 using a gas pressurised knapsack sprayer

(Safer Spa, Italy) with 4 nozzles (03-F110, Lurmark, Long Stanton, Cambridge, UK) at a volume of 200 l ha^{-1} water.

For each field trial, the incidence (percentage of infected ears) and severity (percentage of infected spikelets per ear) of FEB was calculated at 21 and 28 days after inoculation (approximately GS75 and 85) by randomly selecting and assessing 100 ears per plot. In field trial 1, 10 ears were selected at random from each plot and harvested whole for spore washing.

Table 3.2. Azoxystobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate to winter wheat (cv. Equinox), experiment 1, ear blight field trial 1999, prior to inoculation with *M. nivale*, *F. culmorum* or a mixture of both pathogens.

Treatment No.	Fungicide Treatment – applied at Growth Stage 59 (g a.i ha^{-1})	Inoculum – applied at Growth Stage 65
1	No Fungicide	*F.c & M.n*
2	Az. (250)	*F.c & M.n*
3	Az. (250) & Met. (45)	*F.c & M.n*
4	Met. (90)	*F.c & M.n*
5	No Fungicide	*M.n*
6	Az. (250)	*M.n*
7	Az. (250) & Met. (45)	*M.n*
8	Met. (90)	*M.n*
9	No Fungicide	*F.c*
10	Az. (250)	*F.c*
11	Az. (250) & Met. (45)	*F.c*
12	Met. (90)	*F.c*

Az. – azoxystrobin Met. – metconazole

F.c – Fusarium culmorum *M.n – Microdochium nivale*

Table 3.3. Percentage of seed-borne inoculum (*F. culmorum* and *M. nivale*) following no ear spray, ear sprays of fungicides at manufacturers' full rates or a mixture containing metconazole at half rate and seed treatment of fludioxonil for seedling blight under field conditions 1999. (Experiment 2)

Treatment No.	Seed-borne Inoculum (%)	Seed Treatment
1	*F.c (24.8) & M.n (14.1)*	Untreated
2	*F.c (26.4) & M.n (6.6)*	Untreated
3	*F.c (20.7) & M.n (4.3)*	Untreated
4	*F.c (16.6) & M.n (19.7)*	Untreated
5	*F.c (17.3) & M.n (28.9)*	Untreated
6	*F.c (12.9) & M.n (16.1)*	Untreated
7	*F.c (12.4) & M.n (14.6)*	Untreated
8	*F.c (9.3) & M.n (17.2)*	Untreated
9	*F.c (24.1) & M.n (19.2)*	Untreated
10	*F.c (26.7) & M.n (10.2)*	Untreated
11	*F.c (18.0) & M.n (11.9)*	Untreated
12	*F.c (16.1) & M.n (18.4)*	Untreated
13	*F.c (24.8) & M.n (14.1)*	Fludioxonil
14	*F.c (26.4) & M.n (6.6)*	Fludioxonil
15	*F.c (20.7) & M.n (4.3)*	Fludioxonil
16	*F.c (16.6) & M.n (19.7)*	Fludioxonil
17	*F.c (17.3) & M.n (28.9)*	Fludioxonil
18	*F.c (12.9) & M.n (16.1)*	Fludioxonil
19	*F.c (12.4) & M.n (14.6)*	Fludioxonil
20	*F.c (9.3) & M.n (17.2)*	Fludioxonil
21	*F.c (24.1) & M.n (19.2)*	Fludioxonil
22	*F.c (26.7) & M.n (10.2)*	Fludioxonil
23	*F.c (18.0) & M.n (11.9)*	Fludioxonil
24	*F.c (16.1) & M.n (18.4)*	Fludioxonil

F.c – Fusarium culmorum *M.n – Microdochium nivale*

Table 3.4 Treatment numbers and fungicides applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate for *M. nivale* ear blight trial 2000 (experiment 3).

Treatment	Fungicide Treatment (g a.i ha^{-1})	Inoculum
1	No Fungicide	*M.n*
2	Az. (250)	*M.n*
3	Met. (90)	*M.n*
4	Az. (250) & Met. (45)	*M.n*

Az. – azoxystrobin Met. – metconazole *M.n – M. nivale*

Table 3.5. Treatment numbers and fungicides applied at manufacturer's full rate and in a mixture of azoxystobin at full rate and metconazole half rate rate for *F. culmorum* ear blight trial 2000 (experiment 4).

Treatment	Fungicide Treatment (g a.i ha^{-1})	Inoculum
1	No Fungicide	*F.c*
2	Az. (250)	*F.c*
3	Met. (90)	*F.c*
4	Az. (250) & Met. (45)	*F.c*

Az. – azoxystrobin Met. – metconazole *F.c – F. culmorum*

All trials plots were harvested when ripe (approximately GS 92) and the grain moisture content and the yield was recorded on the combine in the field. A two-

kilogram sample of grain was taken from each plot and the specific weight and thousand grain weight were also determined. Grain contamination was examined by plating out 200 grains from each plot onto PDA as described in detail in Chapter 2. An assessment of seed viability was performed for each trial using the tetrazolium viability test in accordance with international rules on seed testing as laid down by ISTA (Anon, 1985). Laboratory germination tests were carried out on one hundred randomly selected seeds using the moist blotter method as laid down by ISTA (Anon, 1998).

All data was statistically analysed using factorial ANOVA (where appropriate) and the statistical software package Genstat 5.1 (Lawes Agricultural Trust. IACR. Rothamsted, UK). Where the distribution of data was not found to be normal percentages were transformed, and regression analysis was used where appropriate.

Results.

Experiment 1: Effect of azoxystrobin and metconazole on the development of *Fusarium* ear blight caused by *Microdochium nivale* and *Fusarium culmorum* and the subsequent infection of harvested grain.

Incidence of diseased ears.

Diseased ears were seen in all plots at each assessment date and the incidence of individual diseased ears increased from a maximum of 71.3% 21 DAI to 82.3% 28 DAI. Statistical analysis of the data revealed a significant ($P < 0.01$) interaction at 21

and 28 days after inoculation (DAI) between the factors fungicide and pathogen. This interaction can be explained by the fact that significant control from azoxystrobin or metconazole, whether alone or in mixture, was only observed when *M. nivale* was the inoculated pathogen, with azoxystrobin giving numerically better control than metconazole. However, greater numerical control was seen from metconazole when *F. culmorum* was the inoculated pathogen and from the mixture of the two fungicides when both *M. nivale* and *F. culmorum* had been inoculated in mixture. Individual treatment means are presented in Table 3.6.

Spikelet infection per ear.

Infected spikelets were seen in all plots at each assessment. The percentage of infected spikelets per ear increased from a maximum of 9.7%, 21 DAI to a maximum of 20.1%, 28 DAI. Analysis of the data revealed a significant (P = 0.01) interaction between both fungicide and pathogen at 21 DAI and P=0.04 at 28 DAI. At 21 DAI azoxystrobin gave control of *M. nivale* when inoculated on its own but it did not control *F. culmorum* or the mixture of the pathogens. However, metconazole gave significant control of both *F. culmorum* and *M. nivale* when inoculated separately. The mixture of the two fungicides was effective against each pathogen treatment. At 28 DAI the interaction between factors appears to be less clear but is probably due to the difference between the two fungicides against the mixed inoculum; metconazole was significantly better than azoxystrobin. No other significant differences were seen between the fungicide treatments and few fungicide treatments were significantly different from the untreated controls. Individual treatment means for the percentage of infected spikelets per ear are presented in Table 3.6.

Table 3.6. Treatment means for the effect of azoxystobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate maximum rates on incidence and severity (21 and 28 days after inoculation) of FEB, grain yield, thousand grain weight and specific weight for winter wheat field trial (Experiment 1) inoculated with *F. culmorum* and/or *M. nivale*. *Numbers in parentheses are back transformed means.

		Disease assessments				Yield data		
		21 days after inoculation		28 days after inoculation				
Treatments (g a.i ha^{-1})	Inoculum	Incidence of diseased ears %	Arcsine % spikelet infection *	Incidence of diseased ears %	Arcsine % spikelet infection *	Grain yield @ 15% M.C (t ha^{-1})	TGW (g)	Specific weight (kg hl^{-1})
1 No fungicide	*F.c & M.n*	64.3	19.1 (10.8)	71.5	17.5 (9.04)	4.2	47.3	59.6
2 Az (250)	*F.c & M.n*	70.9	19.3 (11.2)	82.3	19.1 (10.8)	4.9	47.1	60.4
3 Az (250) & Met (45)	*F.c & M.n*	57.8	13.2 (4.3)	71.8	18.5 (10.1)	4.4	51.0	62.5
4 Met (90)	*F.c & M.n*	67.8	16.4 (8.0)	68.2	15.7 (7.4)	4.4	50.9	62.9
5 No fungicide	*M.n*	69.8	18.8 (10.4)	72.6	16.9 (8.5)	4.9	53.0	62.8
6 Az (250)	*M.n*	48.5	11.2 (3.8)	67.4	16.3 (8.2)	5.2	53.6	64.6
7 Az (250) & Met (45)	*M.n*	45.6	9.7 (2.9)	68.7	14.8 (6.7)	4.9	54.2	63.9
8 Met (90)	*M.n*	56.8	13.5 (6.0)	66.7	15.2 (7.0)	5.1	53.9	63.8
9 No fungicide	*F.c*	69.4	17.6 (9.3)	77.0	19.4 (11.1)	3.8	49.2	62.2
10 Az (250)	*F.c*	71.3	18.6 (10.2)	78.4	20.1 (11.9)	4.6	49.5	61.3
11 Az (250) & Met (45)	*F.c*	62.7	12.8 (5.3)	71.9	17.1 (8.7)	4.8	52.7	62.6
12 Met (90)	*F.c*	60.0	13.8 (5.8)	71.1	17.5 (9.1)	4.7	52.2	63.0
	P values	0.01	0.01	0.05	0.04	0.01	0.02	0.02
	LSD	11.9	3.2	8.5	2.6	0.9	3.0	1.5
	SED	5.9	1.6	4.2	1.3	0.4	1.5	0.7
	CV	15.1	16.6	9.2	11.8	15.	4.6	1.9

Az – Azoxystrobin Met – Metconazole *F.c – F. culmorum* *M.n – M. nival*

Grain Yield.

The only significant differences (P = 0.05) for fungicide treatments for mean grain yield (3.8 – 5.2t ha^{-1}), were when the inoculated pathogen was *F. culmorum* only. Then significant increases in yield (P = 0.01) were shown when the untreated was compared with azoxystrobin / metconazole mixture and metconazole individually. However, azoxystrobin gave no significant yield increase. There was a significant (P = 0.01) interaction between treatments due to the fact that *F. culmorum* reduced grain yield where as *M. nivale* did not. Treatment means for grain yield are presented in Table 3.6.

Thousand Grain Weight.

Analysis of thousand-grain weight (47.3 – 54.2 g) showed significant (P = 0.02) differences between the factors fungicide and treatment. Treatment means for TGW are presented in Table 3.6.

Specific Weight

Analysis of specific weights (59.5 – 64.6 kg hl^{-1}) revealed a significant (P = 0.02) interaction between the factors fungicide and pathogen. Results show that azoxystrobin gave a significant increase when the inoculated pathogen was *M. nivale*. When the pathogen was a mixture of *M. nivale* and *F. culmorum* significant increases were given by the mixture of azoxystrobin and metconazole. Treatment means for specific weight are presented in Table 3.6.

Seed Infection Data

Seed infection data showed the both *F.culmorum* and *M. nivale* were isolated from all

plots irrespective of inoculum applied indicating the presence of natural inoculum or spread of inoculum between plots. However, where single species were inoculated they were the dominant species on plots not treated with a fungicide.

Analysis of the seed infection data revealed a significant (*F.c* - P =0.04. *M.n* - P = 0.02) interaction between the fungicide and pathogen. For seed infection with *F. culmorum*, ear sprays containing metconazole alone gave significant control in all cases and the azoxystrobin / metconazole mixture gave a significant increase when the pathogen was the mixture of *M. nivale* and *F. culmorum* or *F. culmorum* only. Ear treatment with azoxystrobin alone gave no significant improvement in seed infection over the untreated for any of the inoculated pathogens. For seed infection with *M. nivale* ear treatments with azoxystrobin either alone or in the mixture gave a significant reduction in all cases regardless of the inoculation received. In plots where *M. nivale* was the inoculated pathogen metconazole alone also gave a significant reduction in seed infection with *M. nivale*. Overall the best treatment appeared to be the mixture of the fungicides. Treatment means for seed infection are given in Table 3.7.

Table 3.7. Mean values for seed infection with *F. culmorum* and *M. nivale* after ear sprays with azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate for winter wheat field trial (experiment 1) inoculated with *F. culmorum* and/or *M. nivale*. Numbers in parentheses are back transformed data.

Treatment No.	Treatment (g a.i ha^{-1})	Inoculum	Arc sine % infection	
			F.c	*M.n*
1	No fungicide	*F.c & M.n.*	24.8 (18.0)	14.1 (6.5)
2	Az (250)	*F.c & M.n*	26.4 (20.0)	6.6 (1.8)
3	Az (250) & Met (45)	*F.c & M.n*	20.7 (12.7)	4.3 (0.8)
4	Met (90)	*F.c & M.n*	16.6 (8.3)	19.7 (10.3)
5	No fungicide	*M.n*	17.3 (9.3)	28.9 (23.5)
6	Az (250)	*M.n*	12.9 (5.0)	16.1 (8.3)
7	Az (250) & Met (45)	*M.n*	12.4 (12.8)	14.6 (7.3)
8	Met (90)	*M.n*	9.3 (2.8)	17.2 (9.3)
9	No fungicide	*F.c*	24.1 (17.0)	19.2 (10.0)
10	Az (250)	*F.c*	26.1 (20.5)	10.2 (3.0)
11	Az (250) & Met (45)	*F.c*	18.0 (9.8)	11.9 (3.5)
12	Met (90)	*F.c*	16.1 (8.0)	18.4 (4.0)
		P values	0.04	0.02
		LSD	6.0	5.7
		SED	1.9	2.1
		CV	22.2	26.1

Met	metconazole	*M.n*	*Microdochium nivale*
Az	azoxystrobin	*F.c*	*Fusarium culmorum*

Sooty mould control.

Analysis of the data revealed a significant interaction between fungicide and pathogens on sooty mould (*Cladosporium* and *Alternaria*) incidence on the ears. The best control of sooty mould by azoxystrobin and metconazole, when used alone, was

following inoculation with *M. nivale*. However, when the fungicides were applied in the mixture greatest control was achieved following the mixed inoculation of both pathogens. Treatment means are presented in Table 3.8. All chemical treatments gave control over their respective untreated apart from the mixture of azoxystrobin and metconazole following inoculation with *F. culmorum* on its own.

Table 3.8. Percentage incidence of sooty mould means for winter wheat field trial (experiment 1) inoculated with *F. culmorum* and/or *M. nivale*.

	Unt (%)	**Az.** 250 g a.i ha^{-1} (%)	**Az. & Met.** 250 & 45 g a.i ha^{-1} (%)	**Met.** 90g a.i ha^{-1} (%)
F.c & M.n	59.1	47.5	14.2	48.2
M.n only	46.2	13.2	26.5	13.6
F.c only	73.8	44.4	74.0	45.1
P value 0.04				
LSD 1.5				
SED 0.7				
CV 0.3				

Unt – untreated plots. Az. – azoxystrobin. Met. – metconazole.

Seed Weight.

The mean seed weight (44.9 to 59.8 mg) of individual seeds are presented in Table 3.9, the results were comparable to the TGW measured at harvest (Table 3.6). Factorial analysis of seed weight showed no significant differences ($P < 0.05$) between any treatments or pathogens. Seed weight frequency distributions for experiment 1 are presented in Appendix 3.

Table 3.9. Mean seed weight of 200 seeds per plot taken from the 1999 harvested field trial after ear sprays of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate and inoculation with *M. nivale* and or *F. culmorum* and ear spray with azoxystrobin and or metconazole. (Experiment 1).

Treatment No.	Treatment (g a.i ha^{-1})	Inoculum	Seed Weight (mg)
1	No fungicide	*F.c & M.n.*	44.9
2	Az (250)	*F.c & M.n*	48.8
3	Az (250) & Met (45)	*F.c & M.n*	59.8
4	Met (90)	*F.c & M.n*	53.6
5	No fungicide	*M.n*	56.9
6	Az (250)	*M.n*	50.6
7	Az (250) & Met (45)	*M.n*	55.7
8	Met (90)	*M.n*	57.7
9	No fungicide	*F.c*	48.9
10	Az (250)	*F.c*	52.4
11	Az (250) & Met (45)	*F.c*	56.0
12	Met (90)	*F.c*	56.7
		P value	5.02
		LSD	23.9
		SED	12.2
		CV	22.9

Met	Metconazole	*M.n*	*Microdochium nivale*
Az	Azoxystrobin	*F.c*	*Fusarium culmorum*

Seed viability

Analysis of seed viability data showed a significant ($P < 0.01$) interaction between fungicide and inoculated pathogen. Each fungicide treatment significantly increased seed viability when *M. nivale* was inoculated on its own. However, only the mixture of azoxystrobin + metconazole resulted in a significant increase following treatment with the mixture of the pathogens and no significant increase was observed when

plots were inoculated with *F. culmorum*. In addition, azoxystrobin applied alone and in mixture resulted in a significant reduction in seed viability when *F. culmorum* was the inoculated pathogen. Treatment means for seed viability are given in Table 3.10.

Table 3.10. Pathogen treatment means for seed viability, after ear sprays of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate and inoculation with either *M. nivale* or *F. culmorum* (experiment 1).

	Unt.	**Az.** 250 g a.i ha^{-1}	**Az & Met.** 250 & 45 g a.i ha^{-1}	**Met.** 90 g a.i ha^{-1}
Mix	64.0	68.0	75.0	60.0
M. nivale only	66.0	75.0	84.0	78.0
F. culmorum only	81.0	54.0	60.0	76.0
P value	<0.01			
SEM	0.2			
LSD	4.3			
CV	4.3			

Met - metconazole Az – azoxystrobin Untrt - Untreated

Experiment 2: Effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate applied at GS 59 and fludioxonil seed treatment on the emergence of winter wheat after inoculation of parent crop with *Microdochium nivale* and *Fusarium culmorum* at GS 65.

Seedling emergence

Analysis of the emergence data revealed a significant ($P < 0.01$) interaction between the fungicide applied to the ear, the inoculated pathogen and seed treatment. The emergence data showed that treatment with fludioxonil resulted in a numerical increase in seed emergence for each ear / fungicide / pathogen treatment when compared with that which had no seed treatment applied before drilling. These numerical increases were statistically significant ($P < 0.01$) except where azoxystrobin was used following inoculation with *M. nivale* either alone or in mixture. This suggests that there was no significant statistical evidence for the need to treat seed following the application of azoxystrobin when *M. nivale* was present, whereas in all cases when *F. culmorum* was the inoculated pathogen the seed treatment gave a significant benefit. The mean emergence values are presented in Table 3.11.

The highest emergence (72.8%) was found to be from the seed that was treated with fludioxonil and had the lowest but one combined seed infection (26.5%) with *M. nivale* and *F. culmorum.* The lowest emergence was from seed with no treatment and with the highest seed infection (46.2%) of *M. nivale* and *F. culmorum.* The relationship between seed infection and seedling emergence for experiment 2 is shown in Figure 3.1. Photographs of the emergence trial are shown in Appendix 4.

Table 3.11. Percentage seedling emergence and infection figures for each seed lot in the 1999 emergence trial, after ear sprays or either azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate and inoculation with either *M. nivale* or *F. culmorum.* Experiment 2.

Ear Spray	Pathogen Applied	% Emergence	
		Fludioxonil	Untreated
Untreated	*M.n*	66.0	29.1
Az.	*M.n*	70.2	62.7
Met.	*M.n*	72.8	42.6
Az. & Met.	*M.n*	66.0	62.4
Untreated	*F.c*	60.7	40.2
Az.	*F.c*	60.0	45.3
Met.	*F.c*	70.5	42.6
Az. & Met.	*F.c*	70.8	49.5
Untreated	*F.c & M.n*	63.0	43.2
Az.	*F.c & M.n*	67.1	54.6
Met.	*F.c & M.n*	65.4	38.4
Az. & Met.	*F.c & M.n*	69.0	54.3
	P values	0.015	
	LSD	15.7	
	SED	21.9	
	CV	21.9	

Az. azoxystrobin Met. metconazole

F.c *F. culmorum* *M.n* *M. nivale*

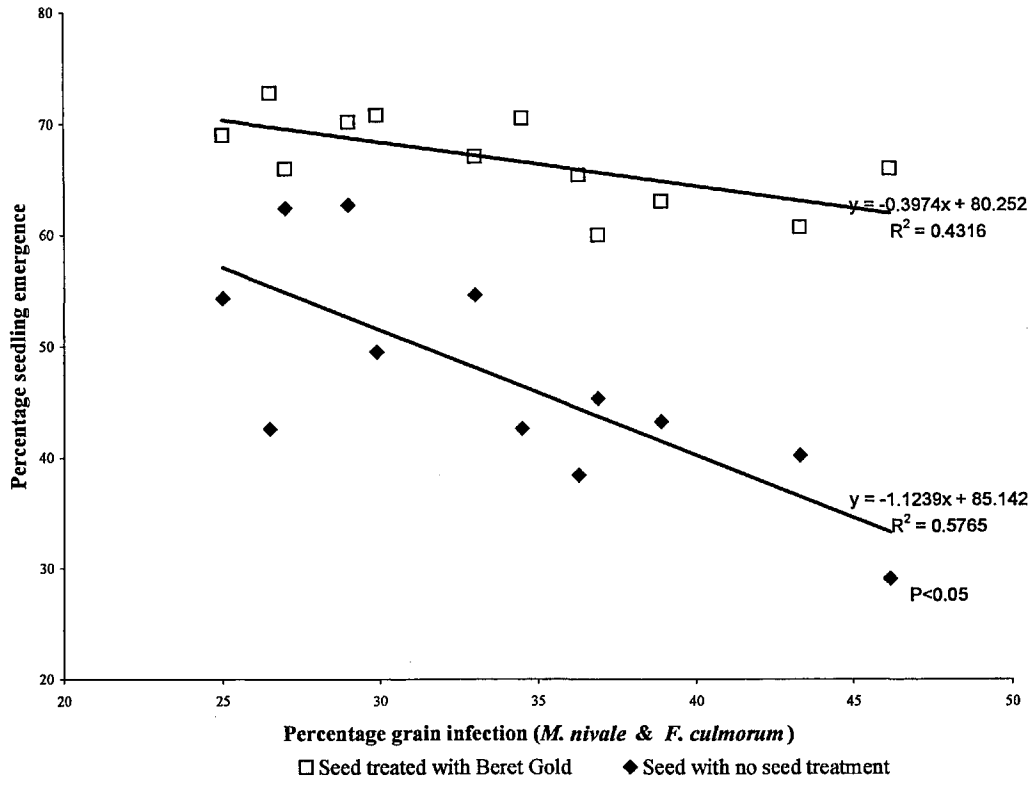


Figure 3.1. Relationship between seed infection and seedling emergence for the 1999 field trial emergence trial, after ear sprays or either azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate and inoculation with *M. nivale* and or *F. culmorum.* (Experiment 2).

Post emergence symptoms of seedling blight were not seen in the field during these experiments.

Experiment 3: The effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate at GS 59 on the development of ear blight caused by *Microdochium nivale* and the subsequent infection of grain.

Incidence of diseased ears.

Diseased ears were seen in all plots at each assessment date, analysis of the data revealed a significant ($P < 0.01$) difference between the fungicides. Treatment means for incidence of diseased ears for both assessment dates are presented in Table 3.12. At 21 DAI a significant ($P = 0.002$) reduction in incidence was seen for the azoxystrobin treatment when compared with the untreated and both the metconazole and the azoxystrobin / metconazole mixture. By 28 DAI only plots treated with azoxystrobin showed a significant reduction in incidence of diseased spikelets when compared with the untreated plots. The azoxystrobin treated plots also had a significantly ($P < 0.05$) lower incidence of diseased ears than those treated with metconazole

Spikelet infection per ear.

Significant ($P < 0.05$) control of spikelet infection over the untreated was shown by all three fungicide treatments at 21 and 28 DAI. There were also significant differences in control between fungicide treatments at 21 DAI. Significant ($P < 0.05$) differences were seen between metconazole and azoxystrobin and the azoxystrobin / metconazole mixture but not between azoxystrobin and the mixture. This trend was repeated at 28 DAI with metconazole and azoxystrobin and the azoxystrobin / metconazole mixture. Treatment means for spikelet infection per ear for both

assessment dates are presented in Table 3.12.

Grain yield.

No statistically significant differences (P = 0.83) between treatments were shown for grain yield (8.4 – 8.7 t ha^{-1}), (Table 3.12).

Thousand grain weight.

Thousand grain weights ranged from 61.5 g – 65.5 g. All fungicides gave an improved TGW but the increases were not significantly (P = 0.81) different, (Table 3.12).

Specific weight.

Specific weight ranged from 60.6 kg hl^{-1} – 63.8 kg hl^{-1}, with all fungicide treatments giving a significant ($P < 0.05$) improvement when compared with the untreated. Significant ($P < 0.05$) differences were also seen between fungicide treatments, azoxystrobin alone and the azoxystrobin / metconazole mixture were significantly better than metconazole only, (Table 3.12).

Seed infection.

Significant differences ($P < 0.05$) between fungicide treatments were seen for seed infection with *M. nivale*. The greatest control compared with the untreated was the azoxystrobin / metconazole mixture closely followed by azoxystrobin alone with no significant difference between the two fungicide treatments. Metconazole alone gave no significant control over the untreated. Treatment means for percentage *M. nivale* grain infection are presented in Table 3.13. Low levels of *F. culmorum* were found in the seed, although it was not an inoculated pathogen. Control over the untreated was

shown by all fungicide treatments but there was no significant ($P < 0.05$) difference however between individual fungicide treatments. Treatment means for the percentage *F. culmorum* grain infection are shown in Table 3.13.

Laboratory germination.

The use of a fungicide on the ear significantly ($P < 0.05$) improved germination in the laboratory when compared with the untreated. The highest percentage germination was from seed that received an azoxystrobin ear treatment followed by metconazole and the azoxystrobin / metconazole mixture. Treatment means for Laboratory germination are given in Table 3.13.

Individual grain weight

Individual grain weight ranged from 62.1 – 64.7 mg which is comparable to the TGW measured at harvest (Table 3.12). As with the TGW no fungicide treatment gave an improvement over the untreated plots. The mean weights for individual grains are given in Table 3.14, and seed weight distribution tables are given in Appendix 5.

Viability.

In all cases the application of a fungicide improved field seed viability, and ranged from 65.3% (untreated) to 82.3% azoxystrobin / metconazole mixture. There was no significant ($P < 0.05$) difference between the azoxystrobin treatment (76.2%) and the metconazole treatment with the azoxystrobin / metconazole mixture being significantly better than either fungicide applied alone. Results are detailed in Table 3.14 and shown in graph form in Appendix 6.

Table 3.12. The effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate, grain yield, thousand grain weight and specific weight for winter wheat field trial (Experiment 3) inoculated with *F. culmorum* and/or *M. nivale* on the incidence and severity of FEB 21 and 28 days after inoculation.

	Disease assessments				**Yield data**		
	21 days after inoculation		28 days after inoculation				
Treatments (g a.i ha^{-1})	Incidence of Diseased ears %	% Spikelet infection	Incidence of Diseased ears %	% Spikelet infection	Grain yield @ 15% M.C. (t ha^{-1})	TGW (g)	Specific weight (kg hl^{-1})
1) Untreated	91.8	48.6	100.0	64.9	8.4	61.5	58.4
2) Azoxystrobin (250)	75.2	12.2	96.7	30.2	8.7	64.2	64.5
3) Metconazole (90)	89.5	27.5	100.0	49.6	8.4	63.2	61.6
4) Az. (125) + Met. (45)	90.7	16.6	99.3	31.9	8.60	65.5	64.3
P values	0.002	<0.001	0.119	<0.001	0.83	0.81	0.004
LSD	8.2	7.8	3.2	9.1	0.40	4.1	1.4
SED	2.7	2.6	1.0	3.0	0.13	1.4	0.5
CV	7.7	24.1	2.6	16.8	3.70	5.2	1.9

Az. = Azoxystrobin Met. = Metconazol

Table 3.13. The effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate on the percentage grain infection and percentage laboratory germination for experiment 3, fungicide winter wheat field trial inoculated with *M. nivale*.

Treatments		**% grain Infection**		**% Germination**
(g a.i ha^{-1})		*M.n*	*F.c*	
1) Untreated		51.3	9.7	49.3
2) Azoxystrobin (250)		28.7	1.8	75.0
3) Metconazole (90)		49.5	0.2	70.0
4) Az. (125) + Met. (45)		26.8	0.5	63.3
	P values	0.03	0.01	0.04
	LSD	7.4	1.8	2.9
	SED	2.5	0.6	1.0
	CV	15.5	49.3	3.7

Az. = Azoxystrobin Met. = Metconazole

Table 3.14. Individual grain weight and percentage field seed viability. Mean grain weight of 100 seeds per plot taken from harvested field plots of experiment 3, fungicide winter wheat field trial inoculated with *M. nivale*.

Treatments (g a.i ha^{-1})		**Individual grain weight (mg)**	**% Seed viability**
1) Untreated		62.1	65.3
2) Azoxystrobin (250)		64.7	76.2
3) Metconazole (90)		61.8	76.0
4) Az. (125) + Met. (45)		64.1	82.3
	P values	0.06	0.05
	LSD	3.0	4.6
	SEM	1.0	1.5
	CV	3.9	5.0

Az. = azoxystrobin Met. = metconazole

Experiment 4: The effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate on the development of ear blight caused by *Fusarium culmorum* and the subsequent infection of grain.

Incidence of diseased ears.

Diseased ears were seen in all plots at each assessment date. At 21 DAI both the metconazole and the metconazole / azoxystrobin mixture gave a significant ($P <$

0.001) reduction in the incidence of diseased ears over the untreated At 28 DAI no treatment gave significant (P = 0.195) control over the untreated plots which had 100% diseased ears. Individual treatment means are presented in Table 3.15.

Spikelet infection per ear.

Significant (P < 0.001) control of spikelet infection over the untreated was shown by all three fungicide treatments at 21 and 28 DAI (Table 3.15). At 21 DAI significant (P < 0.001) differences were seen between metconazole and azoxystrobin and the azoxystrobin / metconazole mixture. This result was repeated at 28 DAI. Treatment means for spikelet infection per ear for both assessment dates are given in Table 3.15.

Grain yield.

No statistically significant differences (P = 0.769) between treatments were shown for grain yield (8.48 – 8.75 t ha^{-1}), (Table 3.15).

Thousand grain weight.

Thousand grain weights were significantly (P < 0.02) increased by the use of all fungicide treatments when compared with the untreated. There was no significant difference between the treatments that contained azoxystrobin either alone or in the mixture, but the use of metconazole alone gave a significant improvement of TGW over all other ear treatments.

Specific weight.

The only fungicide treatment that significantly (P < 0.05) increased specific weight when compared with the untreated was the azoxystrobin. Azoxystrobin also gave a

significant improvement over both the metconazole and the azoxystrobin / metconazole mixture treatments. All grain yield, thousand grain weight and specific weight results are presented in Table 3.15.

Table 3.15. The effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate on the incidence and severity of FEB 21 and 28 days after inoculation and grain yield, thousand grain weight and specific weight for winter wheat field trial (Experiment 4) inoculated with *F. culmorum.*

	Disease assessments				Yield data		
	21 days after inoculation		28 days after inoculation				
Treatments (g a.i. ha^{-1})	Incidence of Diseased ears %	% Spikelet infection	Incidence of Diseased ears %	% Spikelet infection	Grain yield @ 15% M.C. ($t\ ha^{-1}$)	TGW (g)	Specific weight ($kg\ hl^{-1}$)
1) Untreated	100.0	44.7	100.0	72.0	8.48	58.9	60.2
2) Azoxystrobin (250)	98.0	35.6	100.0	54.2	8.43	61.6	64.8
3) Metconazole (90)	86.7	16.9	99.3	33.7	8.70	63.6	60.6
4) Az. (125) + Met. (45)	88.7	18.7	98.7	35.1	8.75	60.9	62.4
P value	<0.001	<0.001	0.195	<0.001	0.769	0.02	0.002
LSD	3.2	4.7	1.4	7.1	0.25	0.9	2.3
SEM	1.6	1.6	0.5	2.3	0.78	2.7	1.1
CV	1.7	13.2	0.8	11.8	7.30	1.2	3.0

Az. = azoxystrobin Met. = metconazole

Seed infection.

Significant differences (P = 0.04) between treatments were seen for seed infection with *F. culmorum*. The best control compared with the untreated was the metconazole closely followed by the azoxystrobin / metconazole mixture Azoxystrobin alone gave significant control over the untreated, but significantly less control than the other two fungicides. No *M. nivale* was found in any seed lot. Treatment means for percentage grain infection are given in Table 3.16. The regression graphs are shown in Appendix 8.

Laboratory germination.

The application of metconazole to the ear significantly (P = 0.02) improved germination in the laboratory when compared with the untreated. Neither azoxystrobin nor the azoxystrobin / metconazole mixture gave a significant improvement in germination over the untreated. Treatment means for Laboratory germination are given in Table 3.16.

Individual seed weight.

The mean seed weight of individual seeds for each treatment ranged from 55.2 to 66.0 mg, which is comparable to the TGW. A significant (P < 0.05) increase in seed weight was seen when the azoxystrobin and the azoxystrobin / metconazole mixture was compared to the untreated; this was not reflected in the TGW. However, there was no significant increase when the metconazole only was compared with the untreated; this was not seen in the TGW results. Mean individual grain weights are given in Table 3.17. Seed weight distribution frequency tables are shown in Appendix 7.

Grain viability.

Grain viability tested in the laboratory was significantly ($P < 0.05$) improved by the application of either metconazole or the azoxystrobin / metconazole mixture when compared with the untreated plots. The application of azoxystrobin only did not give a significant ($P < 0.05$) improvement over the untreated. When compared with each other there was no significant ($P < 0.05$) difference between the fungicide treatments. The treatments means for viability are shown in Table 3.17. The regression graphs are shown in Appendix 8.

Table 3.16. The effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate on the percentage grain infection and percentage laboratory germination for experiment 4, fungicide winter wheat field trial inoculated with *F. culmorum*.

Treatments (g a.i ha^{-1})		**% grain Infection** *M.n*	*F.c*	**% Germination**
1) Untreated		0.0	63.7	54.8
2) Azoxystrobin (250)		0.0	49.3	58.5
3) Metconazole (90)		0.0	37.0	60.0
4) Az. (125) + Met. (45)		0.0	38.3	58.5
	P value	-	0.04	0.02
	LSD	-	2.8	4.2
	SEM	-	0.9	1.4
	CV	-	4.9	5.9

Az. = azoxystrobin Met. = metconazole

Table 3.17. The effect of azoxystrobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate on the individual grain weight and percentage laboratory seed viability. Mean grain weight of 100 seeds per plot taken from harvested field plots of experiment 4, fungicide winter wheat field trial inoculated with *F. culmorum*.

Treatments (g a.i ha^{-1})		**Individual grain Weight (mg)**	**% seed viability**
1) Untreated		55.2	55.8
2) Azoxystrobin (250)		66.0	63.7
3) Metconazole (90)		61.5	66.7
4) Az. (125) + Met. (45)		63.0	67.2
	P value	0.02	0.02
	LSD	6.4	8.4
	SEM	3.0	3.9
	CV	8.5	10.8

Az. = azoxystrobin Met. = metconazole

Experiment 5: The effect of azoxystobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate at GS 59 and fludioxonil seed treatment on the emergence of winter wheat (cv. Equinox) after inoculation of parent crop with *Microdochium nivale* at GS 65.

Seedling emergence.

Analysis of the data revealed a significant interaction ($P < 0.001$) between the factors fungicide ear treatment and seed treatment. The use of fludioxonil as a seed treatment significantly ($P < 0.05$) improved emergence in the field from 22.6% to 54.2% when compared with seed that did not receive a treatment before drilling. Individual treatment means are presented in Table 3.18.

For seed that received no treatment before drilling the only significant ($P < 0.05$) improvement in emergence was when an ear treatment of the azoxystrobin / metconazole mixture was applied.

When the seed received a treatment of fludioxonil emergence ranged from 33.5% (untreated) to 54.2% (azoxystrobin 125 g ha^{-1} + metconazole 45 g ha^{-1}). Seed from ears sprayed with azoxystrobin (40.5%) only significantly improved the seedling emergence when compared with the untreated. Seed from ears sprayed with metconazole only (35.8%) did not significantly improve emergence when compared with the untreated. The ear spray of azoxystrobin and metconazole gave a significant ($P < 0.05$) improvement when compared to any other ear treatment.

Table 3.18. The effect of azoxystobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate on the percentage emergence for experiment 5, fungicide winter wheat field trial inoculated with *M. nivale*.

Treatments ($g\ a.i.\ ha^{-1}$)	**% Emergence**	
	Seed with no treatment	**Seed with fludioxonil**
Untreated	25.9	33.5
Azoxystrobin (250)	29.2	40.5
Metconazole (90)	22.6	35.8
Az (125) + Met (45)	34.4	54.2
P value	<0.001	
LSD	11.0	
SEM	3.73	
CV	21.6	

Az. = azoxystrobin Met. = metconazole

Experiment 6: The effect of azoxystobin and metconazole applied individually at manufacturers' full rate and in a mixture of azoxystobin at full rate and metconazole half rate at GS 59 and fludioxonil seed treatment on the emergence of winter wheat (cv. Equinox) after inoculation of parent crop with *Fusarium culmorum* at GS 65.

Seedling emergence.

Analysis showed no significant ($P < 0.05$) differences in emergence between the fungicide treatments applied as ear sprays to the ear blight trial when either the untreated seed or seed treated with fludioxonil was drilled. Results for the fludioxonil treated seed showed greater variation in emergence between ear treatments. The ear treatment of azoxystrobin alone gave an improvement in emergence over all other treatments but this was not significant. Treatment factor means are shown in Table 3.19.

Table 3.19. Percentage emergence in the 2000 emergence trial, after ear sprays of either azoxystrobin and or metconazole and inoculation with *F. culmorum.* Experiment 6.

Treatments (g a.i/ha)	Seed Treatments		Factor Means
	Untreated	Fludioxonil	
Untreated	14.9	16.4	15.6
Azoxystrobin	17.9	21.5	19.7
Metconazole	14.9	17.7	16.3
Az + Met	16.6	18.4	17.5
Factor Means	16.1	18.5	
P Value	0.01		
LSD	3.0		
SEM	1.0		
CV	11.8		

Discussion.

Ear Blight trials (Experiments 1, 3 & 4).

Liggitt (1997) reported the control of *Fusarium* in the field to be usually poor and inconsistent. The reason it is limited may be in part to the seed infection data for *F. culmorum* and *M. nivale*, which were used in this study, this agrees with Liggitt (1997). Where both *F. culmorum* and *M. nivale* were present the azoxystrobin/metconazole mixture proved the most effective. The increase in yield for the *F. culmorum* inoculated plots sprayed with azoxystrobin may be due to the known greening effects of strobilurins, where the leaf stays green longer during grain filling and ripening so increasing yield (Godwin *et al.*, 1992 and Gooding *et al.*, 1988), the fungicide increased yield but did not lower the infection of the seed and as GLAD was not measured the greening effect was not accounted for in these experiments.

When *M. nivale* seed infection was reduced by treatment with azoxystrobin an increase in *F. culmorum* was observed (Table 3.8). Control of the disease symptoms caused by *M. nivale* may have occurred in the field and been masked by an increase in those caused by *F. culmorum* as no difference in ear disease was observed. Azoxystrobin is reported (Godwin *et al.*, 1992) to be more active against *M. nivale* than *F. culmorum* so the control observed was not unexpected. However, the increase in *F. culmorum* seed infection shown by the azoxystrobin treatment is interesting and may have resulted in a change in the ear microflora following the fungicide treatment as demonstrated by Liggitt (1997 & 1998). There is evidence from experiment 1 that the balance between the saprophytes and pathogens present on the ear may affect the severity of Fusarium ear blight symptoms. Azoxystrobin may remove many

saprophytes and some pathogens (i.e. *M. nivale*) from the ear leaving *F. culmorum* to colonise the ear and infect the seed with little competitive opposition. This agrees with work by Liggitt (1997) and Pirgozliev (2002) that applications of certain fungicides could have an effect on the interaction between fungal communities due to their differing activity towards individual species found on the ear.

In experiment 3, grain infection by *M. nivale* was significantly reduced by the use of azoxystrobin and azoxystrobin in mixture with metconazole (Table 3.13) but the use of metconazole alone did not have a significant effect. Seed infection tests showed that the seed contained low levels of *F. culmorum.* This was expected due to the high levels of natural infection in the field in this year seen across all trials at the site. Grain yield differences were not significant in experiment 3 or experiment 4; this may be due to the crop exhibiting a closed flowering period. Strange and Smith (1971) produced evidence that anthers provide an important role in the infection of wheat ears by *F. graminearum.* Following inoculation they observed significant infection in non-emasculated spikelets while emasculated spikelets rarely became infected. Jenkinson (1994) demonstrated that when four species of *Fusarium* including *M. nivale* and *F. culmorum* were grown in the presence of pollen 70-79% of conidia germinated in twenty four hours this was reduced to 40 – 50 % in the absence of pollen. With no anthers visible it was very difficult to inoculate the crop at exactly GS 65. Due to this inoculation may have been applied late and as a result the grain set had already taken place. In experiment 4 all fungicide treatments significantly improved TGW when compared with the untreated, with no one treatment being significantly better than the others. These results do not wholly agree with work by Hare *et al.*, (1999) who showed that TGW is reduced by infection with *F. culmorum* but infection

with *M. nivale* appears not to reduce the TGW of grain. Azoxystrobin was the only treatment that gave a significant improvement in specific weight compared with the untreated, this agrees with work by Ruske *et al.*, (2003) on the effects of azoxystrobin on disease control, leaf senescence, grain yield and quality of winter wheat. Specific weight was also significantly heavier than the other fungicide treatments, and again, this could be due to the greening effect of the fungicide as stated by Godwin *et al.*, (1994). Overall in this trial (experiment 4), where the inoculated pathogen was *F. culmorum* an ear spray of metconazole alone gave significantly better control of FEB on the ear and in the seed than an ear spray of azoxystrobin alone. In general there was no significant difference between the performance of metconazole only and that of the azoxystrobin / metconazole mixture. This was not unexpected as azoxystrobin is known to be less effective against *F. culmorum* than *M. nivale* and agrees with previous work by Dardis & Walsh (2000).

Homdork (2000) demonstrated that it is possible to give effective control of FEB using a two-spray programme with fungicides applied before and after inoculation. This however, is unlikely to be practiced in commercial situations due to restraints on time and cost. Overall, for the ear blight trials, fungicide treatment was inconsistent but treatment with azoxystrobin alone gave the best results. In experiment 1 field trial plots inoculated with *M. nivale* and sprayed with azoxystrobin showed an increased infection with *F. culmorum*, this was not seen in experiment 3. The disease pressure from sooty moulds was greatly reduced in experiment 3 and this may have enabled the azoxystrobin to show some control of *F. culmorum* at low infection rates.

Emergence trials. (Experiments 2, 5 & 6).

Infected grain can provide a primary source of inoculum for the subsequent development of *Fusarium* seedling blight (Hewett, 1983). Seedling blight can also be caused by soil-borne inoculum (Bateman, 1977); however, under UK field conditions soil- borne inoculum is thought to be of little importance (Paveley *et al.,* 1996) and was not considered to be a factor in these experiments.

During harvesting of the ear blight trials it is expected that a certain amount of grain (usually cracked or very small grains) will be lost over the back of the combine. Hare *et al.* (1999) reported that grain infected with *F. culmorum* is smaller, lighter and shrivelled in appearance so during harvest of the trials a proportion of *F. culmorum* infected grain may have been lost during combining. The decision to harvest with a combine rather than to hand thresh each ear was made on the strength that the sample needed to be as close as possible in physical quality to that obtained and used by farmers as certified or home saved seed. The seed was cleaned before re-drilling to remove chaff, crop debris and broken grains; again this removed possible sources of inoculum that may have had an effect on the emergence trial but would not be present in a commercially treated seed sample. Seedling emergence can be seen as a measure of seed quality as it was known that the seed contained the pathogens *M. nivale* and *F. culmorum* in various quantities depending on the ear treatment.

Weather conditions varied greatly between the two years of the emergence trials. It is well documented that autumn drilling across the UK was delayed in 2000 due to the exceptionally wet autumn (experiments 5 and 6). At Harper Adams University College drilling was postponed until January 2001 but seedbed conditions were

extremely unsuitable and the soil waterlogged. This can be used in part to explain the lower than expected emergence in the trial and agrees with previous work on seedling blight that states that seed bed conditions will affect emergence (Colhoun (1964), Hare *et al.* (1999) and Haigh (2004).

In both years the use of a fludioxonil seed treatment significantly improved the emergence of the infected seed when compared with seed that received no treatment prior to drilling. Prior to all emergence trials seed infection was calculated using laboratory tests for *M. nivale* on PDA treated with MBC and for *F. culmorum* using the moist blotter test. It is not known how many seeds contained both pathogens, as they were isolated separately. No work has been published on the specific effects that mixed infections have on seedling blight, and it is impossible to learn more from this study.

In experiment 2 (emergence trial 1999) for the plots drilled with seed that received no seed treatment, the ones where the grain came from plots sprayed with azoxystrobin or the azoxystrobin/ metconazole mixture as the ear spray showed a higher percentage emergence than those sprayed with metconazole only, regardless of the pathogen applied to the ear in the fungicide trial. This may be due the ear treatment containing azoxystrobin controlling *M. nivale* which will have a negative effect on emergence of seedlings.

For the *M. nivale* inoculated trial (experiment 5) the greatest number of seedlings emerged from the seed that had the lowest seed infection, this agrees with work by Gilbert *et al.,* (2003) who found that seedling emergence decreased significantly with

rising infection. The seed with the lowest infection was that which had received an ear spray of azoxystrobin alone or in a mixture with metconazole. Where no fungicide ear spray was applied, seedling emergence was low even following seed treatment due to the presence of the pathogen in the seed. In the *F. culmorum* inoculated trial (experiment 6) the greatest number of emerged seedlings was not from seed with the lowest infection levels, which suggests that the use of the seed treatment had an effect on the emergence of seedlings. Where seed had received an ear spay containing metconazole to control the *F. culmorum* grain infection the emergence was poor and was not significantly improved even following the application of fludioxonil seed treatment.

These results show overall that the use of an effective ear spray to control Fusarium ear blight and the subsequent infection of grain combined with a seed treatment of fludioxonil before drilling is the most effective protection against seedling blight. The amount of inoculum used in the ear blight trials was far in excess of that found naturally in field conditions. As these trials were designed to be as close to commercially produced seed as possible the decision was taken to reduce the amount of inoculum applied to the ear in the following years' trials to simulate a more realistic natural inoculum potential in the field. Chapter 4 discusses the work on inoculum load.

Chapter 4

An investigation into the effect of spore concentration on the development of *Fusarium* ear and seedling blight caused by *Microdochium nivale* and *Fusarium culmorum*.

Introduction.

There are numerous records that identify *Fusarium* ear blight (FEB), as an economically important disease worldwide. *Fusarium* ear blight has been widespread throughout UK cereal crops and is recognised as a sporadic threat to wheat production (Parry *et al.*, 1995). Over the last 30 years grain infection has increased in the UK; a survey in 1992/3 showed all samples tested to be infected with *M. nivale*. From these results it is known that in commercially grown crops FEB is not being completely controlled. In addition, the infection of grain can be an important source of inoculum for *Fusarium* seedling blight if infected seed is sown in conditions favourable for the disease (Hare *et al.*, 1995). More recently, Jennings *et al.* (2000) took grain samples from 53 severely infected wheat fields (6 in Wales and 47 in England). They found the main head blight pathogen was *M. nivale*, present in 96% of samples. The percentages of other ear blight pathogens found in the samples were F. *avenaceum 43%, F. graminearum 40%, F. poae 34% and F. culmorum 21%.*

Despite the publication of research showing that infection of grain has increased in recent years, no work has been published on the relationship between spore concentration on the ear and the percentage infection of harvested grain. It is well known that it is not always necessary to observe visible symptoms for the grain to be infected; infection can only be confirmed by isolating and identifying the pathogen in laboratory tests (Jenkins *et al.*, 1998).

Aims.

A series of experiments (No's 7-11) (Table 4.1) were designed with the aim of investigating the effect of spore concentration, applied at anthesis, on the severity of FEB symptoms and subsequent grain infection. Two glasshouse experiments (experiments 7 & 8) were conducted in 2000 to study the effect of fungicides on different spore concentrations. Three field-based experiments (9, 10, and 11) were designed and executed in the harvest year of 2000.

Three further field-based experiments (12, 13 and 14) (Table 4.1) were designed and executed in the harvest year of 2000 with the aim of investigating the relationship between seed infection and subsequent seedling blight and its control by chemical seed treatment. These three experiments used seed produced in experiments 9, 10 and 11 respectively, enabling any relationship between spore concentration applied to the ear and subsequent seedling blight to be investigated.

In the following year (2001) a field-based experiment (15) (Table 4.1) was performed with the aim of investigating the effect of lower spore concentrations on ear blight severity.

Objectives.

1. To evaluate the effect of different spore concentrations on the incidence and severity of FEB symptoms caused by *M. nivale* or *F. culmorum*.
2. To evaluate the effect of azoxystrobin, tebuconazole and metconazole on the incidence and severity of ear blight symptoms caused by *M. nivale* and *F. culmorum* at different spore concentrations.
3. To determine the effect of different spore concentrations on grain yield, the infection of harvested grain and grain infection.
4. To evaluate the effect of a fludioxonil seed treatment when applied to infected grain harvested from the ear blight experiments on emergence under field conditions.

Null Hypotheses.

1. The amount of inoculum present on the ear has no effect on the symptom severity of ear blight caused by *M. nivale* and or *F. culmorum* without the application of fungicides when grown in the glasshouse.
2. The amount of inoculum present on the ear has no effect on the symptom severity of ear blight caused by *M. nivale* and or *F. culmorum* with the application of fungicides under field conditions.
3. The amount of inoculum present on the ear has no effect on the subsequent development of seedling blight with or without the application of a fludioxonil seed treatment.

Methods.

Experiments 7 and 8 were carried out on winter wheat (cv. Cadenza) in the glasshouse at Harper Adams University College. The plants were cultivated from treated seed as discussed in Chapter 2.

Five replicates for each treatment were placed in a completely randomised block design. The plants were sprayed according to the treatment list (Table 4.2) with Folicur (tebuconazole 250g l^{-1}, SC. (Bayer Crop protection. Eastern Way, Bury St Edmunds, UK)), Amistar (azoxystrobin 250g l^{-1}, SC. (Syngenta Crop Protection. Whittlesford, Cambridge, UK)) or Caramba (metconazole 60g l^{-1}, SL. (BASF Agriculture Division, Cheadle Hulme, Cheshire, UK).

Spore suspensions of *M. nivale* (experiment 7) and *F. culmorum* (experiment 8) (concentrations: $1x10^4$, $2x10^4$, $5x10^4$, $1x10^5$, $2x10^5$, and $5x10^5$ spores ml^{-1}) were prepared as described in Chapter 2. Inoculation took place at GS 65; the pathogen was sprayed to run-off onto the ears using a hand-held atomiser. After inoculation a self-sealing clear polythene bag was placed over each ear for 48 hours to provide conditions conducive for ear colonisation by the pathogen isolates.

At GS 83 all ears were assessed for severity of *Fusarium* ear blight. In experiment 8 the total number of spikelets and the number of spikelets showing necrosis or bleaching was counted on one hundred randomly selected ears. The percentage of visibly infected spikelets was then calculated.

Table 4.1 Overall outline of the experiments discussed in Chapter 4 - effect of spore concentration on ear blight and emergence experiments carried out under glasshouse and field conditions

Experiment Number	Hypothesis tested	Location	Year	Pathogen	Target
7	2	Glasshouse	2000	*M. n*	Ear Blight
8	2	Glasshouse	2000	*F. c*	Ear Blight
9	1	Field	2000	*M. n*	Ear Blight
10	1	Field	2000	*F. c*	Ear Blight
11	1	Field	2000	*M. n & F. c*	Ear Blight
12	3	Field	2000	*M. n*	Seedling Blight
13	3	Field	2000	*F. c*	Seedling Blight
14	3	Field	2000	*M. n & F. c*	Seedling Blight
15	2	Field	2001	*M. n & F. c*	Ear Blight

M. n - M. nivale, F. c – F. culmorum

After the plants were harvested the number of grains per ear was counted for each treatment. The effect of the spore concentrations and the treatments on seed weight was assessed by individually weighing 100 seeds selected at random from each treatment.

Data were analysed using ANOVA (Genstat 5.1. Lawes Agricultural Trust. IACR, Rothamsted, UK) following arc sine transformation where required.

Three field experiments (Experiments 9, 10 and 11) were conducted in the harvest year 2000 at Harper Adams University College in plots of winter wheat cv. Equinox. The crop husbandry and operations carried out are given in Appendix 2. The methods used are discussed in detail in Chapter 2. Each experiment contained six treatments (Table 4.3) that were laid out in a randomised block design with treatments allocated to plots at random. Three replicates of each treatment were used giving three blocks and 18 plots.

The plots were inoculated at GS 65 (anthesis half way) with a water-based suspension of either *F. culmorum* only, *M. nivale* only or a mixture of both pathogens at six different inoculation loads ranging from 2 x10^4 spores ml^{-1} to 1 x10^6 spores ml^{-1} (Table 4.3). The spore suspensions were applied with a hand-held plot sprayer, at 33ml m^{-2}. The *F. culmorum* spore suspensions contained five isolates of *F. culmorum* in equal proportions. The *M. nivale* only suspension contained five isolates of *M. nivale* var. *majus* and five isolates of *M. nivale* var. *nivale* in equal proportions. The mixture of the two pathogens contained 50% of each of the mixtures above. After inoculation the plots were mist irrigated for 21 days as described in Chapter 2.

After the grain was harvested a bulk sample of seed for each field experiment treatment was produced, by taking a sample of seed harvested from field plots and combining the samples from individual plots. These grain samples were used to drill emergence experiments (Experiments 12, 13 and 14). Each emergence experiment had eight treatments. Four replicates of each treatment were drilled at a rate of 350 seeds m^{-2}, giving five blocks and 32 plots.

Table 4.2. Fungicides, rates and pathogen spore concentration applied in experiments 7 (*M. nivale*) and 8 (*F. culmorum*) winter wheat experiments carried out under glasshouse conditions.

Fungicide	Rate (g a.i ha^{-1})	Spore concentration (spores ml^{-1})
Untreated	-	1×10^4
tebuconazole	250	1×10^4
azoxystrobin	250	1×10^4
metconazole	90	1×10^4
Untreated	-	2×10^4
tebuconazole	250	2×10^4
azoxystrobin	250	2×10^4
metconazole	90	2×10^4
Untreated	-	5×10^4
tebuconazole	250	5×10^4
azoxystrobin	250	5×10^4
metconazole	90	5×10^4
Untreated	-	1×10^5
tebuconazole	250	1×10^5
azoxystrobin	250	1×10^5
metconazole	90	1×10^5
Untreated	-	2×10^5
tebuconazole	250	2×10^5
azoxystrobin	250	2×10^5
metconazole	90	2×10^5
Untreated	-	5×10^5
tebuconazole	250	5×10^5
azoxystrobin	250	5×10^5
metconazole	90	5×10^5

Note: Two experiments were conducted on *M. nivale* and one on *F. culmorum* using the same spore concentrations.

Fungicides applied at GS 59. Pathogen applied at GS 65.

Assessments of emergence were performed by counting the number of plants that emerged between two static markers 0.5m apart every two days from the first emerged coleoptile until emergence was complete, this was then used to calculate the percentage field emergence as described in Chapter 2.

Table 4.3. Spore concentrations for the winter wheat field experiments (9, 10 & 11).

Spore concentration (spores ml^{-1})
2×10^4
5×10^4
1×10^5
2×10^5
5×10^5
1×10^6

All data were then analysed using the statistical software package Genstat 5.1 (Lawes Agricultural Trust. IACR. Rothamsted, UK). Factorial ANOVA was performed and percentages were either arc sine or log transformed, where data was not normally distributed. Regression analysis was also used where appropriate.

Results.

Experiment 7: Determination of optimum spore concentration of *M. nivale* for control with tebuconazole, azoxystrobin or metconazole fungicides at manufacturer's maximum rates.

Spikelet infection and incidence of diseased ears.

When the plants were at GS 83 there were unusually no visible symptoms present on the ears to assess.

Grain infection.

Analysis of the data revealed a significant interaction (P = 0.03) between the factors fungicide and spore concentration. Grain infection increased with increasing spore concentration in untreated plots and in plots treated with fungicides, but to a lesser extent. Azoxystrobin however, the increase in grain infection was not always significant. The response from the fungicides to increasing inoculum load was different for the individual treatments. Azoxystrobin appeared to be less effective than metconazole or tebuconazole although there was little evidence of a statistically significant difference at the majority of spore loads tested. Metconazole significantly reduced grain infection at all but one spore concentration when compared to the untreated and tebuconazole significantly reduced grain infection at four out of six spore concentrations. Individual treatment means are presented in Table 4.4 and the relationship between spore concentration and grain infection for each fungicide treatment is show graphically in Appendix 9.

Table 4.4. The effect of *M. nivale* spore concentration and fungicide ear treatment on the subsequent percentage infection of winter wheat grain under glasshouse conditions (Experiment 7).

	Spore concentration (spores ml^{-1})					
fungicide treatment	1×10^4	2×10^4	5×10^4	1×10^5	2×10^5	5×10^5
Untreated	23.30	18.88	26.78	33.30	34.07	51.20
Tebuconazole	15.61	21.45	18.26	32.55	26.08	31.58
Azoxystrobin	22.80	20.46	24.35	28.17	32.90	23.00
Metconazole	16.03	14.25	21.89	26.81	25.73	33.11

P value: <0.05
LSD: 6.57
SEM: 2.12
CV: 20.20

Experiment 8: Determination of optimum spore concentration of *F. culmorum* for control with three fungicides at manufacturer's standard rates.

Grain infection.

No visible symptoms of FEB were seen at GS 83 however, grain infection was seen in all treatments for each spore load at harvest. Factorial analysis of the data revealed a significant interaction ($P < 0.001$) between the fungicide and spore concentration. As expected grain infection increased with increasing spore concentration in untreated plots. A similar effect was seen in plots treated with azoxystrobin and at the two highest spore concentrations grain infection was higher than the untreated. However, tebuconazole and metconazole both significantly reduced grain infection at each spore concentration except

the highest, when compared with the untreated and at concentrations but the highest metconazole significantly reduced grain infection when compared with tebuconazole. For the lowest two spore loads significant ($P < 0.05$) differences were seen for grain infection between the untreated and each fungicide, with metconazole being significantly lower than either azoxystrobin or tebuconazole. Metconazole significantly reduced grain infection when compared to the untreated at all spore loads except the highest.

No fungicide gave a significant reduction in grain infection at the highest spore concentration.

Table 4.5. The effect of *F. culmorum* spore concentration and fungicide ear treatment on grain infection of winter wheat under glasshouse conditions (Experiment 8).

	Spore concentration (spores ml^{-1})					
Treatment	1×10^4	2×10^4	5×10^4	1×10^5	2×10^5	5×10^5
Untreated	71.8	74.0	79.6	87.8	93.8	99.6
Tebuconazole	60.6	69.4	77.6	82.0	87.6	100.0
Azoxystrobin	61.0	73.4	74.2	83.2	99.0	100.0
Metconazole	43.0	57.0	68.8	76.2	83.4	99.0
P value	<0.001					
LSD	4.0					
SEM	2.6					
CV	4.0					

Thousand grain weight.

Analysis of the thousand grain weights revealed a significant interaction (P = 0.03) between the fungicide and spore concentration. Thousand grain weights decreased with increasing spore concentration in untreated plots as would be expected. The application of a fungicide significantly (P < 0.05) improved thousand grain weight in two cases, azoxystrobin at the lowest spore concentration (1 x 10^4) and metconazole at 5 x 10^4, no other significant improvements were seen. At all spore loads the application of tebuconazole reduced the thousand grain weight to below that of the untreated although it was only significant at 5 x 10^4 spores ml^{-1}. Metconazole significantly improved thousand-grain weight at only one spore load (5 x 10^4 spores ml^{-1}). Treatment means for thousand grain weight are presented in Table 4.6.

Table 4.6. The effect of *F. culmorum* spore concentration and fungicide ear treatment on thousand grain weight of winter wheat under glasshouse conditions (Experiment 8).

Treatment (g a.i ha^{-1})	Spore concentration (Spores ml^{-1})					
	1 x 10^4	2 x 10^4	5 x 10^4	1 x 10^5	2 x 10^5	5 x 10^5
Untreated	53.74	54.96	53.16	46.88	46.86	44.78
Azoxystrobin (250)	59.36	54.64	51.56	45.04	45.58	45.30
Metconazole (90)	55.48	55.48	56.34	46.40	45.10	45.14
Tebuconazole (250)	52.94	53.00	48.40	46.50	46.30	44.56
P value	0.03					
LSD	2.27					
SEM	0.08					
CV	3.6					

Grains per ear.

Analysis of grains per ear revealed a significant interaction (P = 0.017) between the factors fungicide and spore concentration for azoxystrobin and metconazole used alone although it was not at all spore concentrations.

In some cases the application of a fungicide significantly (P = 0.017) increased the number of grains per ear. At 1 x 10^5 spores m^{-1} the application of azoxystrobin gave a significant increase in the grains per ear over all other treatments and the untreated. Tebuconazole gave a significant increase at 5 x 10^5 spores ml^{-1}. The mean grains per ear results are shown in Table 4.7.

Table 4.7. The effect of *F. culmorum* spore concentration and fungicide ear treatment on the mean grains per ear of winter wheat grain under glasshouse conditions (Experiment 8).

Treatment (g a.i ha^{-1})	Spore concentration (Spores ml^{-1})					
	1 x 10^4	2 x 10^4	5 x 10^4	1 x 10^5	2 x 10^5	5 x 10^5
Untreated	22.60	23.20	23.20	23.20	23.40	22.40
Azoxystrobin (250)	23.20	23.00	25.20	25.80	21.20	22.40
Metconazole (90)	25.00	24.40	22.20	23.20	24.40	21.60
Tebuconazole (250)	22.80	24.60	23.40	23.60	23.80	25.20
P value	0.017					
LSD	2.55					
SEM	0.91					
CV	8.6					

Seedling emergence.

Factorial analysis of seedling emergence data showed a significant interaction (P = 0.033) between some fungicides and spore concentration. For each fungicide treatment the trend was decreasing seedling emergence as the spore concentration increased though this was not always statistically significant. For ears that received a spray of tebuconazole seedling emergence was significantly reduced as the spore concentration increased.

In some cases percentage seedling emergence was significantly (P = 0.033) improved by the application of a fungicide. At rates of 1 x 10^5 spores ml^{-1} for metconazole only and azoxystrobin at 5 x 10^5 spores ml^{-1} significant improvements over the untreated were seen. At 2 x 10^4 spores ml^{-1} all fungicides gave a significant improvement over the control. At 5 x 10^4 spores ml^{-1} all treatments but tebuconazole gave significant control over the untreated. Metconazole only gave significant control over the untreated and at 5 x 10^5 spores ml^{-1} azoxystrobin only gave significant control. Treatment means are presented in Table 4.8.

Table 4.8. The effect of *F. culmorum* spore concentration and fungicide ear treatment on the mean seedling emergence of winter wheat grain under glasshouse conditions (Experiment 8).

Treatment (g a.i ha^{-1})	Spore concentration (Spores ml^{-1})					
	1 x 10^4	2 x 10^4	5 x 10^4	1 x 10^5	2 x 10^5	5 x 10^5
Untreated	85.40	84.90	82.60	54.20	68.60	53.00
Azoxystrobin (250)	87.60	95.00	66.20	49.10	72.70	74.90
Metconazole (90)	84.40	95.00	83.70	75.70	70.30	65.50
Tebuconazole (250)	82.40	78.60	75.40	64.20	59.90	56.80
P value	0.033					
LSD	15.69					
SEM	5.59					
CV	17.0					

Experiment 9: Effect of spore concentration on the severity of *Fusarium* ear blight and the infection of harvested grain caused by *M. nivale*.

Incidence of diseased ears.

Analysis of the data revealed a significant (P = 0.005) increase in the incidence and symptom severity with increasing spore concentrations at both assessment dates (21 days and 28 days after inoculation). Treatment means are presented in Table 4.9. At 21 days after inoculation (DAI) the percentage incidence ranged from 68.0 - 100% and by 28 DAI

this had increased to 74.3 - 100%. At the first assessment significant ($P < 0.05$) differences were observed between treatments except between 2×10^4 spores ml^{-1} and 5×10^4 spores ml^{-1}, 1×10^5 and 2×10^5spores ml^{-1}, and 2×10^5 and 5×10^5 spores ml^{-1}. At the second assessment the percentage incidence between 2×10^4 spores ml^{-1} and 5×10^4 spores ml^{-1} had become significant ($P < 0.05$). There were no significant ($P < 0.05$) differences between 1×10^5 and 2×10^5 spores ml^{-1} (both 96%) and 2×10^5 and 5×10^5 spores ml^{-1} (both 100%). Means for percentage incidence of diseased ears are given in Table 4.9.

Spikelet infection.

Spikelet infection increased with increasing spore concentration at both 21 and 28 DAI. At 21 DAI no significant ($P < 0.05$) differences were seen between 2×10^4, 5×10^4, and 1×10^5 spores ml^{-1} $\times 10^4$ or 1×10^5, 2×10^5 and 5×10^5 spores ml^{-1}. At 28 DAI the spikelet infection ranged from 15.5 to 46.8%. Significant ($P < 0.001$) differences were seen between some treatments. All data for percentage spikelet infection are given in Table 4.9.

Grain yield.

No significant ($P = 0.414$) differences in grain yield at harvest were seen between any treatments in the experiment.

Specific weight.

Specific weight decreased from 64.5 hl kg^{-1} to 56.47 hl kg^{-1} as spore load increased. There were also significant ($P = 0.02$) differences between some individual spore concentrations. The lowest spore concentration 2x 10^4 spores ml^{-1} was significantly ($P < 0.05$) higher than any other treatment and the highest spore concentration 1 x 10^6 spores ml^{-1} was significantly lower than the others.

Thousand grain weight.

The thousand grain weight (TGW) at harvest ranged from 60.7 to 67.3g. Although there were significant ($P = 0.001$) differences between some spore concentrations, the TGW did not always fall in line with the increase in spore load. Data for mean TGW values are given in Table 4.9.

Table 4.9. Treatment means for the effect of spore concentration on the incidence and severity of FEB 21 and 28 days after inoculation, grain yield, thousand grain weight and specific weight for winter wheat spore load field experiment inoculated with *M. nivale*. Numbers in parentheses are back transformed means.

		Disease assessments				Yield data		
		21 days after inoculation		28 days after inoculation				
Treatments (spores ml^{-1})	Inoculum	% Incidence of Diseased ears	Arc sine % Spikelet infection	% Incidence of Diseased ears	Arc sine % Spikelet infection	Grain yield @ 15% M.C. (t ha^{-1})	TGW (g)	Specific Weight (Kg hl^{-1})
2×10^4	*M.n*	68.0	12.9 (5.1)	74.3	15.5 (2.3)	5.33	65.8	64.53
5×10^4	*M.n*	73.3	14.3 (6.1)	90.7	19.7 (11.5)	5.04	60.7	62.27
1×10^5	*M.n*	83.7	18.6 (10.3)	96.0	25.5 (18.5)	5.23	62.6	61.03
2×10^5	*M.n*	89.3	20.4 (12.3)	96.0	26.1 (19.6)	5.71	67.3	60.13
5×10^5	*M.n*	85.3	24.0 (16.7)	100.0	33.2 (30.1)	5.48	65.3	59.03
1×10^6	*M.n*	100.0	33.7 (31.1)	100.0	46.8 (52.9)	5.11	65.4	56.47
	P value	0.03	0.02	0.04	<0.001	0.414	0.001	0.002
	LSD	7.8	7.1	5.9	9.0	0.7	3.9	1.4
	SEM	2.4	3.2	1.8	4.4	0.2	1.7	0.4
	CV	52	18.8	3.5	17.7	7.6	3.3	1.2

M. n- M. nivale

Grain infection.

Percentage grain infection with *M. nivale* increased with increasing spore concentration. Significant ($P = 0.01$) differences were seen between some treatments. Infection with *M. nivale* increased from 27.7 to 53.3%. Two treatments 1×10^5 and 2×10^5 spores ml^{-1} were infected with *F. culmorum* (1.0 and 4.3% respectively) although low rates of infection of both these were significantly ($P < 0.05$) different to the other treatments with zero infection. Means for percentage grain infection are shown in Table 4.10.

Laboratory germination.

Laboratory germination decreased with the increase in spore load no significant ($P < 0.05$) differences were seen in laboratory germination between the lowest three spore loads when compared to each other. Significant ($P < 0.05$) differences were seen between the highest three spore loads (5×10^5 and 2×10^5 and 1×10^6 spores ml^{-1}) and the lowest one (2×10^4 spores ml^{-1}). The laboratory germination means are shown in Table 4.10.

Individual grain weight

Significant ($P = 0.02$) differences were seen between some treatments as the spore load increased, the individual grain weight ranged from 70.2 to 56.7mg. The mean values for individual grain weights are given in Table 4.10 and mean individual grain weight distribution graphs are shown in Appendix 10.

Viability.

Viability as determined in the laboratory by the tetrazolium test (ISTA, 1985), decreased from 73.7% for 2 x 10^4 spores ml^{-1} to 44.7% for 1 x 10^6 spores ml^{-1}. Significant ($P < 0.05$) differences were seen between each of the treatments except for 1 x 10^5 spores ml^{-1} and 2 x 10^5 spores ml^{-1}. Mean values for grain viability are given in Table 4.10. See also Appendix 10 for seed weight distribution graphs.

Table 4.10. The effect of spore concentration on percentage grain infection, percentage laboratory germination, individual grain weight and grain viability for winter wheat inoculated with *M. nivale* at GS65 under field conditions (Experiment 9).

Treatments (spores ml^{-1})	**Inoculum**	**% grain Infection**		**% laboratory germination**	**Individual grain weight (mg)**	**% Grain Viability**
		F.c	***M.n***			
2 x 10^4	*M. n*	0.0	27.7	72.0	70.2	73.7
5 x 10^4	*M. n*	0.0	30.7	66.7	58.0	69.7
1 x 10^5	*M. n*	1.0	38.0	65.0	59.0	66.3
2 x 10^5	*M. n*	4.3	40.7	52.0	63.0	64.3
5 x 10^5	*M. n*	0.0	50.0	45.7	65.0	53.3
1 x 10^6	*M. n*	0.0	53.3	44.3	56.7	44.7
P value		0.02	0.01	0.04	0.012	0.04
LSD		0.9	4.3	9.9	7.1	3.2
SED		0.4	1.9	4.4	3.1	1.4
CV		53.0	5.9	9.2	6.7	2.9

M. n M. nivale

Experiment 10: Effect of spore concentration on the severity of *Fusarium* ear blight and the infection of harvested grain caused by *F. culmorum.*

Incidence of diseased ears.

Analysis of the data revealed that increasing the spore concentration increased the percentage incidence of diseased ears in untreated plots at both dates. By 28 DAI plots with the higher spore concentrations were exhibiting 100% incidence of diseased ears.

Significant ($P < 0.05$) differences were observed between 2×10^4 spores ml^{-1} and 1×10^5 and 2×10^5 spores ml^{-1} and 2×10^5, 5×10^4 and 5×10^5 spores ml^{-1} and 1×10^6 spores ml^{-1}. All means for percentage incidence of diseased ears are presented in Table 4.11.

Spikelet infection.

Spikelet infection increased with increasing spore concentration at both 21 and 28 DAI. There were some significant ($P < 0.001$) differences between spore concentrations at both 21 and 28 DAI. There was no significant ($P < 0.05$) difference between 2×10^4 spores ml^{-1} and 5×10^4 spores ml^{-1} and 1×10^5 and 2×10^5 spores ml^{-1} at either of the assessment dates. All means for percentage spikelet infection are presented in Table 4.11.

Grain yield.

For spore concentrations ranging between 2×10^4 and 5×10^5 spores ml^{-1} there were no significant ($P < 0.05$) difference in grain yields. Grain yield for 1×10^6 spores ml^{-1} was significantly lower than any of the other spore loads except 5×10^5 spores ml^{-1}.

Thousand grain weight.

Thousand-grain weights ranged from 39.2 to 60.2 g and significant ($P < 0.001$) differences were seen between some spore concentrations. The trend was that as spore concentration increased the TGW decreased. The reduction in TGW was significant between 5×10^4 spores ml^{-1}, 1×10^5 and 2×10^5, 5×10^5 1×10^6 and spores ml^{-1}. The mean values for TGW are shown in Table 4.11.

Table 4.11. The effect of spore concentration on the incidence and severity of FEB 21 and 28 days after inoculation, grain yield, thousand grain weight and specific weight for winter wheat spore load field trial inoculated with *F. culmorum*. (Experiment 10).

		Disease assessments				Yield data		
		21 days after inoculation		28 days after inoculation				
Treatments (spores ml^{-1})	Inoculum	% Incidence of Diseased ears	% Spikelet infection	% Incidence of Diseased ears	% Spikelet infection	Grain yield @ 15% m.c($t\ ha^{-1}$)	TGW (g)	Specific Weight ($Kg\ hl^{-1}$)
2×10^4	*F. c*	73.3	5.7	92.0	15.1	3.42	58.8	64.5
5×10^4	*F. c*	69.3	7.2	94.7	21.7	3.44	60.2	62.3
1×10^5	*F. c*	88.0	13.7	100.0	34.3	3.47	51.6	61.0
2×10^5	*F.c*	94.7	11.3	100.0	37.0	3.39	55.5	60.1
5×10^5	*F. c*	98.7	19.3	100.0	44.3	3.24	45.1	59.0
1×10^6	*F. c*	98.7	39.4	100.0	68.7	2.90	39.2	56.5
	P values	<0.001	<0.001	<0.001	<0.001	0.137	<0.001	<0.001
	LSD	7.0	6.6	5.3	7.8	0.46	5.1	1.3
	SED	3.1	3.0	2.4	3.5	0.21	2.3	0.6
	CV	4.4	22.7	3.0	11.6	7.70	5.4	1.2

F. c – F. culmorum

Specific weight.

Specific weights decreased from 64.5 hl kg^{-1} to 56.4 hl kg^{-1} in line with the increase in spore concentrations. Significant ($P < 0.05$) differences were seen between 2 x 10^4 spores $ml^{-1}$and 5 x 10^4 spores $ml^{-1}$and 1 x 10^5 spores $ml^{-1}$and 2 x 10^5 spores ml^{-1}. The mean values for specific weights are given in Table 4.11.

Grain infection.

All grain samples were infected with both *M. nivale* and *F. culmorum*; the *M. nivale* infection was due to 'natural background infection' in the field. *Microdochium nivale* infection ranged from 6.7 to 32.4%; the lowest *M. nivale* infection occurred at the highest but one spore concentration of *F.culmorum* inoculation and the lowest but one spore concentration had the highest *M. nivale* infection levels. There were significant ($P < 0.05$) differences in seed infection between some treatments. The *F. culmorum* infection increased with spore concentration, there were no significant ($P < 0.05$) differences between the lowest four spore concentrations but significant differences were seen between 2 x 10^5, 5 x 10^5 and 1 x 10^6 spores ml^{-1}. Means for percentage grain infection for both *F. culmorum* and *M. nivale* are given in Table 4.12. See also Appendix 11 for seed weight distribution and regression graphs.

Laboratory germination.

Only with the highest two spore loads did laboratory germination decrease with increasing spore load. The only significant ($P < 0.05$) reductions were seen between 2 x 10^4 and 5 x 10^5 and 1 x 10 spores ml^{-1}, and 2 x 10^5 spores ml^{-1}compared with 5 x 10^5 and

1 x 10^6 spores ml^{-1}. Treatment means for laboratory germination are given in Table 4.12; see also Appendix 11 for seed weight distribution and regression graphs.

Individual grain weight.

Overall, individual grain weight significantly ($P < 0.05$) decreased with increased spore concentration. However, individual grain weight for 2 x 10^5 spores ml^{-1} was lower than might have been expected. The individual grain weight ranged from 40.9 to 59.4 mg, which is comparable to the TGW, measured at harvest. Mean values for individual grain weight are shown in Table 4.12. Mean individual grain weight graphs are shown in Appendix 11.

Table 4.12. The effect of spore concentration on percentage grain infection, percentage laboratory germination, individual grain weight and grain viability for winter wheat (cv. Equinox) spore concentration field experiment inoculated with *F. culmorum* (Experiment 10).

Treatments (spores ml^{-1})	**Inoculum**	**% Seed Infection**		**% Germination**	**Individual Grain Weight (mg)**	**% Seed viability**
		F. c	*M. n*			
2 x 10^4	*F. c*	30.7	17.0	65.3	58.8	65.0
5 x 10^4	*F. c*	32.3	32.4	72.3	59.4	63.3
1 x 10^5	*F. c*	33.7	24.7	65.7	49.3	58.3
2 x 10^5	*F. c*	36.0	10.7	66.0	58.4	57.4
5 x 10^5	*F. c*	61.0	6.7	40.0	46.1	57.2
1 x 10^6	*F. c*	69.7	10.0	25.3	40.9	55.6
	P values	<0.001	<0.001	<0.001	<0.001	<0.001
	LSD	3.7	9.2	13.1	1.8	4.2
	SED	1.7	4.1	5.9	4.1	1.9
	CV	4.6	29.8	12.9	4.3	3.9

F. c – F. culmorum, M. n – M. nivale

Seed viability.

Seed viability, measured in the laboratory by the tetrazolium test (ISTA, 1985), decreased from 65.0% to 55.6% with increased spore concentration. Significant ($P < 0.001$) differences were seen between treatments. Treatment means for seed viability are given in Table 4.12 and in Appendix 11 for seed weight distribution and regression graphs.

Experiment 11: Effect of spore concentration on the severity of *Fusarium* ear blight and the infection of harvested grain caused by a mixture of *M. nivale* and *F. culmorum*.

Incidence of diseased ears.

Analysis of the data revealed that increasing the spore concentration significantly ($P < 0.001$) increased the percentage incidence of diseased ears in untreated plots at both dates. At 21 days after inoculation (DAI) the percentage incidence ranged from 66.7 - 93.3% and by 28 DAI this had increased to 76.0 - 100%.

At the first assessment (21 DAI) significant ($P < 0.05$) differences were observed between treatments 2×10^4 spores ml^{-1} and 5×10^4 spores ml^{-1}, 5×10^4 spores ml^{-1} and 1×10^5 spores ml^{-1}. No differences were seen between 2×10^5 spores ml^{-1} , 5×10^5 spores $ml^{-1}$and 1×10^6 spores ml^{-1} when compared with the inoculum concentration either preceding or following it.

At the second assessment (28 DAI) the significant ($P < 0.05$) differences for percentage incidence remained the same as before, except for differences between 5×10^5 spores ml^{-1} and 1×10^6 spores ml^{-1} that were now significant ($P < 0.05$). All means for percentage incidence of diseased ears are given in Table 4.13.

Spikelet infection.

Spikelet infection increased with increasing spore concentration at both 21 and 28 DAI. At 21 DAI no significant ($P < 0.05$) differences were seen between 2×10^4 spores ml^{-1} and 5×10^4 spores ml^{-1} and 1×10^5 spores ml^{-1} and 2×10^5 spores ml^{-1}. At 28 DAI significant ($P < 0.001$) differences were seen between some treatments. All data for percentage spikelet infection are given in Table 4.13.

Grain yield.

No significant ($P < 0.05$) differences in grain yield at harvest were seen between any treatments in the trial. Means for grain yield measured at harvest are given in Table 4.13.

Table 4.13. The effect of spore concentration on incidence and severity of FEB 21 and 28 days after inoculation, grain yield, thousand grain weight and specific weight for winter wheat (cv. Equinox) spore load experiment inoculated with a mixture of *F. culmorum* and *M. nivale*. (Experiment 11)

		Disease assessments				**Yield data**		
		21 days after inoculation		28 days after inoculation				
Treatments (spores ml^{-1})	Inoculum	% Incidence of Diseased ears	% Spikelet infection	% Incidence of Diseased ears	% Spikelet infection	Grain yield @ 15% M.C. ($t\ ha^{-1}$)	TGW (g)	Specific Weight ($Kg\ hl^{-1}$)
2×10^4	*F. c & M. n*	66.7	7.0	76.0	11.1	3.17	61.8	64.5
5×10^4	*F. c & M. n*	74.7	8.0	89.3	14.5	3.19	56.0	62.2
1×10^5	*F. c & M. n*	82.7	19.0	96.0	24.3	3.07	63.0	61.0
2×10^5	*F. c & M. n*	85.3	17.0	96.0	25.1	2.98	58.1	60.1
5×10^5	*F. c & M. n*	90.7	24.7	98.7	42.0	2.92	63.8	59.0
1×10^6	*F. c & M. n*	93.3	38.2	100.0	59.7	2.88	54.0	56.5
	P values	<0.001	<0.001	<0.001	<0.001	0.35	<0.001	<0.001
	LSD	7.5	4.5	4.0	7.4	0.4	3.7	1.3
	SEM	3.4	2.0	1.9	2.3	0.2	1.7	0.4
	CV	5.0	12.9	2.4	13.8	1.8	0.8	1.0

M. n = *M. nivale* *F. c* = *F. culmorum*

Specific weight.

Specific weight decreased as expected from 64.5 hl kg^{-1} to 56.5 hl kg^{-1} as the spore concentration increased. There were significant ($P < 0.001$) differences between spore concentrations. The lowest spore concentration 2 x 10^4 spores ml^{-1} was significantly ($P < 0.05$) higher than any other treatment and the highest spore concentration 1 x 10^6 spores ml^{-1} was significantly lower than the others. Means for specific weight are given in Table 4.13.

Thousand grain weight.

The thousand grain weight (TGW) at harvest ranged from 63.8 to 54.0 g. Although there were significant ($P < 0.001$) differences between spore concentrations, the decrease in TGW did not always follow the increase in spore load, the highest TGW came from the treatment 5 x 10^5 spores ml^{-1}but this was not significantly higher than the 2 x 10^4 spores ml^{-1}. Means for thousand grain weight measured at harvest are given in Table 4.13.

Grain infection.

All grain samples were infected with both *M. nivale* and *F. culmorum.* The amounts of *F. culmorum* in grain increased in all but one case (2 x 10^5 spores ml^{-1}) with increasing spore concentration from 16.0 to 56.0% but the amount of *M. nivale* infection in grain remained almost constant (20.0 to 30.3%) with no significant ($P < 0.05$) difference between any spore concentrations. All means for grain infection are shown in Table 4.14 and in the seed weight distribution graphs are shown in Appendix 12.

Laboratory germination.

Germination under laboratory conditions ranged from 38.3 to 79.3% depending on spore concentration giving some significant ($P < 0.05$) differences. Germination declined with increasing spore concentration in all but one case, although there was a rise in germination for 5 x 10^5 spores ml^{-1}, but this was not significant ($P < 0.05$). Means for percentage laboratory germination are given in Table 4.14 and the seed weight distribution graphs are shown in Appendix 12.

Table 4.14. The effect of spore concentration on percentage grain infection, percentage laboratory germination, individual grain weight and grain viability for winter wheat (cv. Equinox) spore concentration field experiment inoculated with *F. culmorum* (Experiment 11).

Treatments (spores ml^{-1})	Inoculum	% Seed Infection		Germination (%)	Individual Grain Weight (mg)	Grain Viability (%)
		F.c	*M.n*			
2 x 10^4	*F. c & M. n*	16.0	28.0	79.3	59.8	67.8
5 x 10^4	*F. c & M. n*	23.0	30.3	68.7	57.0	62.7
1 x 10^5	*F. c & M. n*	32.0	20.0	60.3	56.0	63.0
2 x 10^5	*F. c & M. n*	23.3	26.7	59.7	55.3	60.0
5 x 10^5	*F. c & M. n*	51.3	20.7	64.7	54.4	56.3
1 x 10^6	*F. c & M. n*	56.0	26.0	38.3	52.8	49.0
	P values	<0.001	<0.001	<0.001	<0.001	<0.001
	LSD	10.5	9.2	6.4	12.8	6.7
	SED	4.7	4.1	2.9	5.8	6.1
	CV	16.1	20.1	5.7	12.6	2.1

F. c – F. culmorum, M. n – M. nivale

Individual grain weight.

Individual grain weight decreased with increased spore concentration, but there was no significant ($P < 0.05$) difference between any of the treatments. Treatment means for individual grain weight are given in Table 4.14.

Seed viability.

Seed viability measured in the laboratory by the tetrazolium test decreased from 67.8 to 49.0 % with increased spore concentration. Significant ($P < 0.05$) differences were seen between treatments. Treatment means for seed viability are given in Table 4.14. Frequency distribution by weight graphs and viability verses spore concentration regression graphs are shown in Appendix 12.

Experiment 12. Effect of spore concentration and seed treatment with fludioxonil on the emergence of seed from ears inoculated with *M. nivale*.

Seedling emergence.

Factorial analysis of the data revealed a significant interaction ($P = 0.03$) between the factors spore concentration and seed treatment. Emergence decreased with increasing spore concentration in plots with no seed treatment. The use of fludioxonil significantly increased seedling emergence at each spore concentration. Individual treatment means are

presented in Table 4.15 and the relationship between inoculum load and percentage emergence is show in Appendix 13.

The seed treatment appears to be more effective at increasing seedling emergence where there was lower seed infection at the lower inoculum rates, so disease pressure was also lower.

Table 4.15. The effect of spore concentration on the effect of seed treatment with fludioxonil on subsequent seedling emergence following ear inoculation with *M. nivale* (experiment 12).

Spore Concentration (spores ml^{-1})	**% Emergence**	
	Grain with no seed treatment	Grain treated with Fludioxonil
2×10^4	37.9	58.4
5×10^4	29.8	46.6
1×10^5	28.5	40.6
2×10^5	24.5	38.1
5×10^5	23.0	33.0
1×10^6	23.6	30.7
P value	0.03	
LSD	8.4	
SEM	2.9	
CV	17.0	

F. c – F. culmorum, M. n – M. nivale

Experiment 13: Effect of spore concentration and seed treatment with fludioxonil on the emergence of seed from ears inoculated with *F. culmorum*.

Seedling emergence.

Analysis of the data revealed a significant interaction (P = 0.02) between the spore concentration and seed treatment. Emergence decreased with increasing spore concentration in plots with no seed treatment. The use of fludioxonil significantly (P < 0.05) increased seedling emergence at each spore concentration, individual treatment means are presented in Table 4.16 and the relationship between emergence and inoculum load is shown in Appendix 14.

Table 4.16. The effect of spore concentration on percentage the effect of seed treatment with fludioxonil on subsequent seedling emergence following ear inoculation with *F. culmorum* (Experiment 13).

Spore Concentration (spores ml^{-1})	**% Emergence**	
	Grain with no seed Treatment	Grain treated with Fludioxonil
2×10^4	24.9	49.0
5×10^4	25.4	46.2
1×10^5	23.5	36.7
2×10^5	20.7	35.8
5×10^5	16.9	34.4
1×10^6	14.6	25.4
P value	0.02	
LSD	6.8	
SEM	2.3	
CV	16.1	

Experiment 14: Effect of spore concentration and seed treatment with fludioxonil on the emergence of seed from ears inoculated with *M. nivale* and *F. culmorum*.

Seedling emergence.

Analysis of the data revealed a significant interaction ($P = 0.02$) between the spore concentration and seed treatment. Emergence decreased with increasing spore concentration in plots with no seed treatment. The use of fludioxonil significantly ($P < 0.05$) increased seedling emergence at each spore concentration. With the exception of the highest and lowest inoculation load there was a significant ($P < 0.05$) improvement in emergence for each spore concentration when untreated seed was compared to treated seed. Individual treatment means are presented in Table 4.17 and the relationship between emergence and spore load is shown in Appendix 15.

Table 4.17. The effect of spore concentration on the effect of seed treatment with fludioxonil on subsequent seedling emergence following ear inoculation with a mixture of *M. nivale* and *F. culmorum* (Experiment 14).

Spore Concentration (spores ml^{-1})	% Emergence	
	Grain with no seed treatment	Grain treated with Fludioxonil
2×10^4	44.3	47.3
5×10^4	34.4	45.7
1×10^5	33.7	44.3
2×10^5	26.9	41.2
5×10^5	19.8	35.8
1×10^6	17.4	23.6
P values	0.02	
LSD	7.72	
SEM	2.68	
CV	22.0	

F. c – F. culmorum, M. n – M. nivale

Experiment 15: The effect of varying spore concentrations of *F. culmorum, M. nivale* or a mixture of both pathogens on the control from three fungicides at manufacturers' maximum rates on winter wheat in the field 2001.

Incidence of diseased ears.

At the first assessment the only evidence of disease was where the inoculum was *F. culmorum.* Analysis of these data revealed significant ($P < 0.001$) interactions between fungicides and spore load.

As expected an ear spray of azoxystrobin alone gave no reduction in the incidence of diseased ears at any spore load when compared to the untreated. Metconazole alone significantly reduced the incidence of diseased ears at 1 x 10^4 spores ml^{-1} when compared to the untreated and azoxystrobin treatments. The azoxystrobin / metconazole mixture gave a significant reduction in incidence of diseased ears.

By 28 DAI diseased ears were seen in all plots for all pathogens and inoculum loads, and as expected factorial analysis showed significant ($P < 0.001$) interactions between the factors pathogen fungicide, pathogen and spore concentration. When the inoculum was *F. culmorum* alone applications of metconazole either alone or in the fungicide mixture gave a significant reduction, this however, was not seen at the lowest spore concentration where a slight but not significant increase in numbers were seen.

For plots inoculated with M. nivale at the lowest and highest rates all fungicide treatments gave a significant reduction in incidence of diseased ears. At 1 x 10^4 spores ml^{-1} only the azoxystrobin / metconazole mixture gave a significant reduction.

When the inoculum contained both *M. nivale* and *F. culmorum* at the highest spore concentration only fungicide treatments containing metconazole either alone or in the fungicide mixture gave a significant reduction, at the lowest spore concentration all fungicides gave a significant reduction. Individual treatment means for percentage incidence of diseased ears are shown in Table 4.18.

Spikelet infection.

At the first assessment (21 DAI) spikelet infection was only seen where the inoculum was *F. culmorum* and not for either *M. nivale* or the mixture of the two pathogens. Analysis of the data revealed a significant interaction ($P < 0.05$) between the fungicide and spore concentration. Spikelet infection increased with increasing spore concentration in untreated plots and in some treated plots. Application metconazole significantly reduced the spikelets infected at 1×10^3 spores ml^{-1} and 1×10^4 spores ml^{-1}. At 5×10^4 spores ml^{-1} no fungicide treatment gave a significant reduction in spikelets infected and there were no significant differences between fungicide treatments. Azoxystrobin alone gave no significant ($P < 0.05$) reduction in the numbers of spikelets infected at any of the spore loads tested. Individual treatment means are presented in Table 4.18.

At the second assessment (28 DAI) factorial analysis showed significant interactions ($P < 0.001$) between pathogen, fungicide and spore concentration. Spikelet infection increased for plots inoculated with *F. culmorum* or the pathogen mixture but not for *M. nivale*, this supports the previous experiments. Fungicide treatments of azoxystrobin alone gave no reduction in spikelet infection when *F. culmorum* was present on the ear.

Table 4.18 The effect of spore concentration and fungicide ear treatment on incidence and severity of FEB 21 and 28 days after inoculation, for winter wheat (cv. Equinox) spore load experiment (2001) inoculated with *F. culmorum* and *M. nivale*. (Experiment 15)

		1st ASSESSMENT (21 DAI)						2nd ASSESSMENT (28 DAI)					
		% incidence (Log Transformed)			% spikelets infected (Log Transformed)			% incidence (Log Transformed)			% spikelet infection (Log Transformed)		
Fungicide	Rate Inoc	1×10^3	1×10^4	5×10^4	1×10^3	1×10^4	5×10^4	1×10^3	1×10^4	5×10^4	1×10^3	1×10^4	5×10^4
Untreated	*F. c*	1.63	1.13	0.55	0.82	0.75	0.41	1.79	1.87	1.88	1.08	1.42	1.46
Az	*F. c*	1.77	1.18	0.69	0.91	0.78	0.31	1.88	1.87	1.85	1.09	1.40	1.05
Met	*F. c*	1.64	0.69	0.67	1.04	0.12	0.30	1.80	1.74	1.79	0.79	0.83	0.88
Az & Met	*F. c*	1.04	0.81	0.75	0.47	0.22	0.47	1.80	1.74	1.79	0.81	0.93	1.11
Untreated	*M. n*	-	-	-	-	-	-	1.80	1.79	1.88	0.93	0.88	0.97
Az	*M. n*	-	-	-	-	-	-	1.67	1.80	1.78	0.62	0.62	0.58
Met	*M. n*	-	-	-	-	-	-	1.67	1.82	1.76	0.78	0.83	0.56
Az & Met	*M. n*	-	-	-	-	-	-	1.65	1.68	1.64	0.53	0.63	0.65
Untreated	*Mix*	-	-	-	-	-	-	1.82	1.88	1.81	0.92	0.97	0.83
Az	*Mix*	-	-	-	-	-	-	1.70	1.82	1.80	0.54	0.77	0.82
Met	*Mix*	-	-	-	-	-	-	1.62	1.86	1.71	0.56	0.83	0.65
Az & Met	*Mix*	-	-	-	-	-	-	1.64	1.84	1.72	0.52	0.57	0.49
	P value		<0.001			<0.001			<0.001			<0.001	
	LSD		0.29			0.20			0.08			0.26	
	SEM		0.10			0.07			0.03			0.09	
	CV		19.6			27.1			3.10			19.3	

Az – azoxystrobin *M. n – M. nivale* Met - metconazole *F. c – F. culmorum* *Mix - M. nivale & culmorum*

Yield

There were no significant differences between grain yield for any fungicide, pathogen nor spore concentration. Grain yield ranged from 7.9 to 8.5 t ha^{-1} at 15% MC.

Grain infection.

The grain infection data for the experiment are shown in Table 4.19. A significant ($P < 0.001$) interaction was seen between fungicide, pathogen and spore concentration. The reason for this significant three way interaction is not easy to explain due to the nature of the data set (Table 4.19). However, when the inoculated pathogen was *F. culmorum* no significant reductions in infection were seen at the lowest two spore concentrations, however at the highest spore concentration all three fungicide treatments significantly reduced *F. culmorum* in the grain. When the inoculated pathogen was *M. nivale* the only significant reductions in *F. culmorum* infection were seen at the highest inoculum concentration where an ear spray of metconazole or the azoxystrobin / metconazole mixture had been applied. For the spore mixture F. *culmorum* grain infection was not significantly reduced at the lowest spore or middle concentration by the use of any fungicide treatment when compared to the untreated. However, at the highest spore concentration application of azoxystrobin to the ear increased the *F. culmorum* grain infection when compared with the untreated although it was not significant. However, the application of metconazole alone significantly reduced infection when compared to the untreated.

For grain infection with *M. nivale* when *F. culmorum* only and *M. nivale* only were applied as inoculum significant ($P < 0.05$) reductions in infection were seen when azoxystrobin or the azoxystrobin / metconazole mixture were applied to the ear. Metconazole alone did not always give a significant reduction. For both of these grain infection increased with increasing spore concentration. When the inoculum was the pathogen mixture infection increased with spore concentration but was only reduced by the use azoxystrobin on the ear.

Table 4.19 The effect of spore concentration and fungicide ear treatment on grain infection for winter wheat (cv. Equinox) spore load experiment (2001) inoculated with *F. culmorum* and *M. nivale*. (Experiment 15).

		% grain Infection *F. culmorum*			% grain Infection *M. nivale*		
Fungicide	Rate Inoc	1×10^3	1×10^4	5×10^4	1×10^3	1×10^4	5×10^4
Untreated	*F. c*	10.50	11.50	46.00	7.00	6.00	25.50
Az	*F. c*	9.50	15.50	33.50	1.00	8.50	19.50
Met	*F. c*	9.00	13.50	24.00	6.50	15.00	28.50
Az & Met	*F. c*	4.00	11.00	27.50	3.00	10.00	17.50
Untreated	*M. n*	9.00	10.50	20.00	10.75	46.00	57.75
Az	*M. n*	9.00	18.00	18.50	5.00	25.00	25.50
Met	*M. n*	5.00	16.50	12.29	10.50	40.00	44.50
Az & Met	*M. n*	5.50	10.50	14.25	8.00	30.00	30.50
Untreated	*Mix*	10.50	16.00	19.50	7.00	22.50	25.50
Az	*Mix*	9.50	15.50	25.50	5.00	16.00	18.50
Met	*Mix*	9.00	13.50	16.50	7.50	12.50	25.50
Az & Met	*Mix*	6.50	11.00	21.50	5.00	13.50	26.50
	LSD		6.04			8.72	
	SEM		2.12			3.08	
	CV		25.40			31.2	

Az – azoxystrobin, Met – metconazole, *M. n – M. nivale*, *F. c – F. culmorum*, *Mix* – mixture of *M. nivale* and *F. culmorum* in equal parts.

Discussion.

Field based experiments were performed at Harper Adams University College in the harvest year of 2000 (9, 10 and 11) in plots of winter wheat (cv. Equinox) to investigate the effect of increasing spore concentration on the visible symptoms of *Fusarium* ear

blight and the subsequent infection of grain. As expected in all three experiments as the spore concentration increased the amount of visible symptoms increased, and in some cases visible symptoms reached 100% by the second assessment. This result is not unexpected as relationships between spore concentration and disease severity have been established for a number of diseases, and the relationship for seedling blight is well documented (Colhoun, 1968). However, there is no published data on the specific relationship between spore concentration and ear blight severity. Work has demonstrated the importance of the presence of conidia for infection under natural conditions but the concentration of spores was not investigated fully. Rossi *et al.* (2002) studied the dynamics of airborne fusarium macroconidia in wheat fields naturally affected by head blight. They counted the number of spores per m^3 and related it to the meteorological conditions. It was found that there was an association between rainfall and peaks of the macroconidia sampled. No or very few conidia were sampled before rainfall, but the numbers progressively increased during rainfall; in the presence of high humidity, conidia continued to be sampled at high densities for some hours after rainfall had ceased, and they reached their peak under these conditions. The density of the airborne conidia rapidly decreased when the relative humidity dropped. This study provides the first evidence of a direct linear relationship between spore concentration (number of conidia) and ear blight severity.

In 2000 the crop exhibited a closed flowering period with no anthers visible, this may have affected the infection and colonisation of ears by the inoculated pathogens meaning the relationships observed between spore concentration and disease may not be representative of most situations – when open flowering occurs. Strange and Smith

(1971) produced evidence that anthers provide an important role in the infection of wheat ears by *F. graminearum*. Following inoculation, they observed significant infection in non-emasculated spikelets while emasculated spikelets rarely became infected. Jenkinson and Parry (1994) demonstrated that when four species of Fusarium including *M. nivale* and *F. culmorum* were grown in the presence of pollen, 70-79% of conidia germinated within twenty-four hours. However, conidial germination was reduced to 40 – 50 % in the absence of pollen. Therefore, it could be suggested that a given spore concentration could have a greater effect under usual conditions of open flowering.

Thousand-grain weight as expected decreased with increasing spore concentration, where *F. culmorum* was the inoculated pathogen or applied in the mixture, which agrees with work by Hare *et al.* (1999). Hare *et al.* (1999) found that thousand-grain weight is reduced by *F. culmorum*, but *M. nivale* infection had no effect on thousand-grain weight of grain. Where *M. nivale* was applied the TGW did not always fall concomitantly with an increase in inoculum, but when this did occur it was never significant, which again agrees with Hare *et al.* (1999).

In each experiment all grain samples were infected with the inoculated pathogen. In the experiments where the pathogens were applied alone some plots were infected with the other pathogen; this was due to the high levels of natural infection found in the field that year. The results provide some evidence that as *F. culmorum* decreased *M. nivale* may have increased, this agrees with work by Liggitt (1997) who investigated the interaction between fungi on ears of wheat. A similar effect was seen with respect to grain infection

when a mixture of the two pathogens was applied. The amount of *F. culmorum* in the grain increased in line with the increasing spore concentration; however, this was not the case with *M. nivale* where grain infection levels remained constant regardless of the spore concentration applied. In all three experiments results for percentage laboratory germination and percentage seed viability (established by the tetrazolium test) showed that increasing spore concentration reduced germination and viability.

Overall the results obtained from the field experiments (Experiments 9. 10, and 11) showed that when spore concentration increased the symptoms of *Fusarium* ear blight and the subsequent infection of harvested grain increased also.

The decrease in TGW with increasing spore concentration (*F. culmorum* alone and in Mixture) is to be expected given the known relationship between ear blight severity and TGW for *F. culmorum* (Snijders and Perkowski, 1990). There is some debate in the literature regarding the effect of *M. nivale* on TGW. The results of this study support the findings of Hare *et al.* (1999) who did not observe reductions in TGW with increasing seed infection. In the same study Hare *et al.* (1999) observed a good relationship between the percentage of grains infected by *F. culmorum* and TGW, again supporting the work of Snijders and Perkowski (1990).

The results for the last field experiment (Experiment 15) were disappointing and this may be due to the position of the experiment within the field. As previously discussed results from Rossi *et al.*, (2002) would suggest that with plot misting and the other experiments

in the field the amount of airborne pathogen present was extremely high. The Null hypothesis could not be tested fully due to the disease pressure from other ear blight experiments in the field. Although guard plots were used, they were not large enough to prevent the spread of ear blight pathogens from the surrounding experiments. This may have allowed inoculum from these to spread into this experiment, affecting the results, but as no evidence of disease spread was collected during the field work it could be due to natural infection. To establish which of the explanations was correct the experiment would need to be repeated in an area remote from other ear blight work.

Results from both years' seedling emergence experiments agree with previous work by Richardson (1974) who found a good relationship in field experiments with oats, between seed infection with *M. nivale* and seedling emergence, and also yields. The effect on yield was thought to be due to a reduction in ear numbers resulting from a low seedling population. Colhoun (1970) states that losses of this kind are unlikely to occur often in commercial crops. More recently work by Gilbert *et al.* (2003) found that seedling emergence and number of seedlings per row significantly decreased with rising proportions of infected seed. Prior to all emergence experiments seed infection was calculated using laboratory tests for *M. nivale* on PDA treated with MBC and for *F. culmorum* using the moist blotter test. It is not known how many seeds contained both pathogens, as they were only isolated separately. No work has been published on mixed inoculations so the effect it has on the emergence experiment as seed containing both pathogens is not known.

Owing to the exceptionally wet autumn of 2000, autumn drilling across the UK was severely delayed and this affected the seedling emergence experiments (experiments 12, 13 and14). At Harper Adams University College drilling was postponed until January 2001 but seedbed conditions were still extremely unsuitable. This can be used in part to explain the lower than expected emergence in the experiments and agrees with previous work on seedling blight that states soil water and temperature conditions will affect emergence (Colhoun, 1964., Hare *et al.,* 1999 and Haigh, 2004).

Chapter 5

An Investigation into the relationship between point of inoculation of wheat ears and the infection of grain by *Fusarium culmorum* and *Microdochium nivale.*

Introduction.

Both *F. culmorum* and *M. nivale* are significant seed-borne pathogens causing seedling blight in small grain cereals. Infection of seed could arise through the infection of ears and the development of FEB. The extent of ear and grain infection by ear blight pathogens could therefore provide an indication of subsequent seed health and vigour (Parry *et al.*, 1995). Several authors have identified in their work factors affecting resistance to FEB. For example Hilton (1999) demonstrated that the disease characteristically spreads from the point of infection to neighbouring spikelets and that the spread may continue until many or all are infected.

Environmental factors such as weather may help to accelerate the spread and death of infected tissues. Adams (1921) reported that under conditions in Pennsylvania infection was usually restricted to one or two spikelets per ear. However, in some cases the upper half or the complete ear may be infected. Pugh *et al.* (1933) sectioned wheat ears infected with *Gibberella zeae* and reported evidence of hyphal invasion up and down the rachis, but no evidence of invasion into adjoining spikelets.

Doohan *et al.* (2003) stated in their review of FEB that climatic conditions will influence competition between, and the predominance of, different fungi within the ear blight complex. More specifically Brennan *et al.* (2005) found that varying temperature (16°C and 20°C) affected the visual symptoms and thousand-grain weight of eight wheat varieties when inoculated with *F. graminearum* or *F. culmorum*. *Fusarium culmorum* gave greater visual symptoms at 20°C than 16°C while *F. graminearum* gave greater symptoms at 16°C and both pathogens caused a greater loss in yield at 20°C. In addition, Argyris *et al.* (2005) investigated infection

of wheat ears following point inoculation in glasshouse experiments. They stated that spikelet infection by *F. graminearum* was poorly associated with infection in grain and other floral components from the same spikelets. Pathogen movement was localised around the point of inoculation in resistant varieties or moved basally from the point of inoculation in susceptible cultivars.

The movement of the pathogen on the ear following inoculation was investigated by Kang & Buchenauer (2000) who examined the infection of wheat ears by *F. culmorum* using microscopy and found that 6 – 12 hours after inoculation conidia germinated but did not infect the host tissues immediately, but gave rise to hyphae that grew on the host surfaces. Hyphal networks were usually formed on the inner surfaces of the lemma, palea and glume two days after inoculation. The pathogen reached the rachis 4 – 5 days after inoculation and hyphae grew upwards and downwards inter and intra-cellularly in vascular bundles and cortical parenchyma tissue of the rachis. They also stated that during colonisation of the wheat spikelet changes occurred in the host tissues including degeneration of host cytoplasm and organelles and collapse of parenchyma cells. Work on maize by Reid *et al.* (2002) found differences between infection and disease severity for three *Fusarium* species (*F. graminearum, F. subglutins* and *F. verticillioides*).

Several types of cultivar resistance to FEB have been identified. In 1963 Schroeder and Christenson suggested that ear blight resistance consisted of at least two types: Type I resistance, which is resistance to initial infection and Type II resistance, which is resistance to fungal colonisation within the spikelet. Since this initial research further work has resulted in the suggestion of two further types of resistance: Type III,

which is based on the ability of the host cultivar to degrade DON (Miller and Arnison, 1986) and Type IV resistance which is tolerance of the host to high DON concentrations (Wang and Miller, 1988).

In this chapter a glasshouse experiment was designed to investigate the movement of pathogens from the point of inoculation of wheat ears, and the subsequent infection of grain by *M. nivale* and *F. culmorum.* At harvest, ears were studied under a light and a scanning electron microscope (SEM) for evidence of fungal colonisation of the ears.

Aims.

1. To investigate the movement of *M. nivale* and *F. culmorum* within ears of winter wheat following artificial inoculation with conidia at a given point.
2. To determine the effect of such colonisation on grain infection and weight of individual grains within the ear.

Objectives.

1. To determine the effect of point inoculation on the development of visible *Fusarium* ear blight symptoms.
2. To determine where the pathogen moves within wheat ears.
3. To determine how the pathogen affects individual grain weight and grain infection with respect to grain position on the ear.

Null Hypotheses.

1. The pathogens do not move from point of inoculation on ear.
2. The pathogens have no effect on the individual grain weight of infected ears.

Materials and Methods.

Wheat plants (cv. Cadenza) were grown in the glasshouse and ears (100 of each treatment) were individually inoculated with conidia of either *F. culmorum* or *M. nivale* at decimal growth stage 65. Inoculation was achieved by placing 25 μl of spore suspension (100,000 spores ml^{-1}), using a pipette, between the lemma and palea of spikelet ten according to the procedure used by Hilton (1999) (Figure 2.1, Chapter 2). Ten spikelets were inoculated with distilled water to act as a control. Four weeks after inoculation, the number of spikelets showing symptoms e.g. necrosis or bleaching in both the distal and proximal parts of the ear was recorded. When ripe, all ears were studied under a light microscope (Olympus CH compound microscope, manufactured by Olympus Corporation, Tokyo, Japan) and assessed for evidence of external colonisation of the rachis. If the colonisation was dense, grain position on the ear was noted, the grain was removed and the rachis was sectioned and examined for internal colonisation. For each ear, grain position in relation to the point of inoculation was noted and then all grains were individually weighed, before being plated out on to PDA (see Chapter 2 for details). The SEM (Cambridge S200, manufactured by Cambridge Instruments, Cambridge, UK.) was used to view external hyphae growth on the stem, rachis and grains.

Results.

At GS 83 the number of spikelets exhibiting visible symptoms on ears inoculated with *F. culmorum* ranged from occurring on an individual spikelet to the whole ear (Figure 5.1). Only 1% of ears inoculated with *M. nivale* showed necrosis or bleaching and that consisted of symptoms on only one spikelet at the point of inoculation.

On examination under a light microscope external hyphal growth was found on 55% of ears inoculated with *F. culmorum* and 28% of ears inoculated with *M. nivale*. Evidence of hyphal growth within the rachis was seen in 8% of ears inoculated with *F. culmorum*, however, no hyphae were found following inoculation with *M. nivale*. See Figure 5.2 for SEM pictures of hyphal growth on grain.

For both *F. culmorum* and *M. nivale*, a difference in the percentage of grains infected from individual spikelets was observed. Grain infection ranged from 3 to 83 % for *F. culmorum* and 0 to 75 % for *M. nivale*. However, on analysis there was no statistical significant difference in grain infection for corresponding spikelets between the two species (Figure 5.3). Both pathogens, once they had colonised the ear, appeared to move downwards more than upwards.

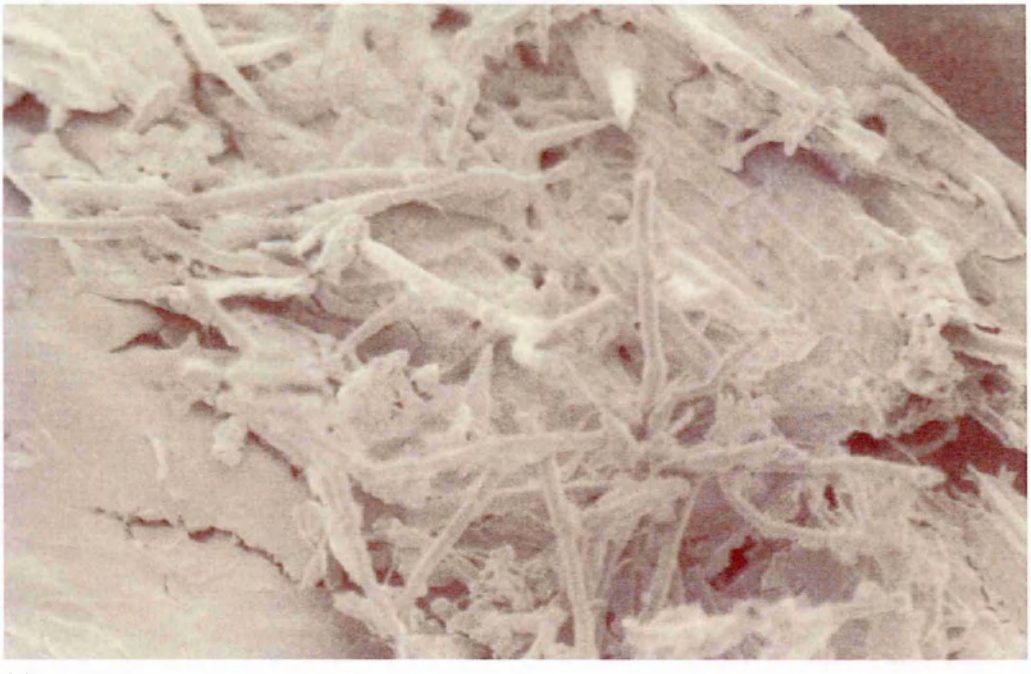
Percentage infection of grain by *F. culmorum* (Figure 5.4) significantly ($P< 0.05$) reduced individual grain weight from 50.5mg to 40.0mg. However, a significant relationship was not evident for grain infection by *M. nivale* (Figure 5.5). On ears inoculated with distilled water there was no difference in individual grain weight.

Figure 5.1. A wheat ear point inoculated with *F. culmorum* conidia at GS 83. Showing visible bleaching of spikelets.

a.

20 μm

b.



20 μm

Figure 5.2 Electron micrograph of *F. culmorum* hyphae growing on winter wheat grain surface (a) and stem surface (b).

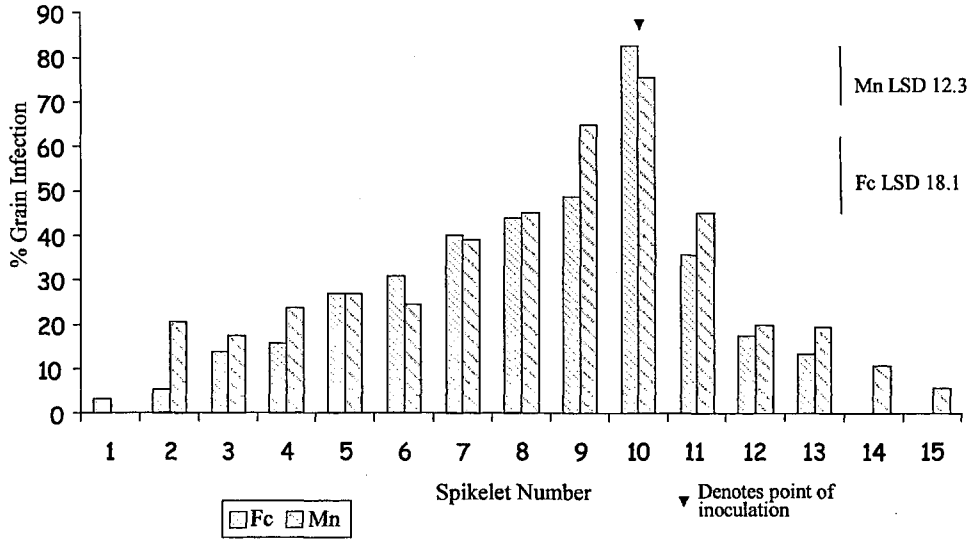
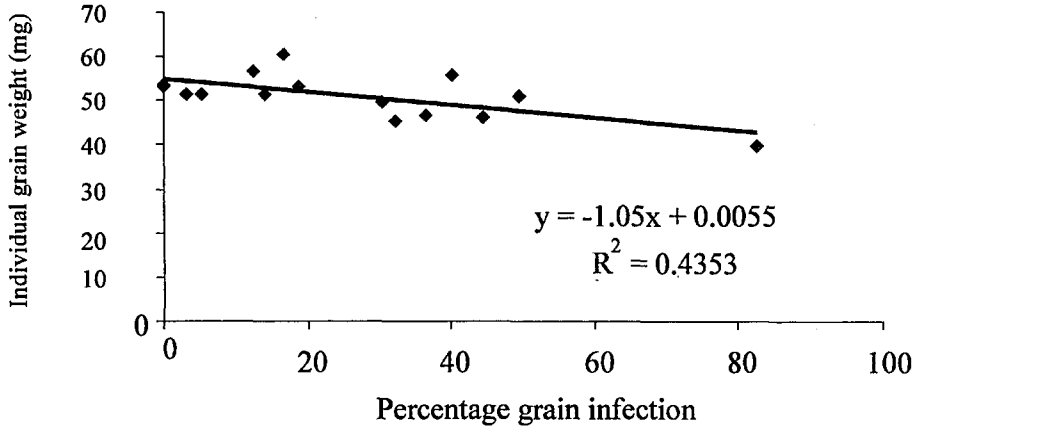


Figure 5.3. Percentage grain infection per spikelet for winter wheat ears grown in the glasshouse and inoculated at spikelet ten.



P < 0.05

Figure 5.4. The relationship between percentage grain infection and individual grain weight for winter wheat ears inoculated at spikelet 10 with *F. culmorum* conidia.

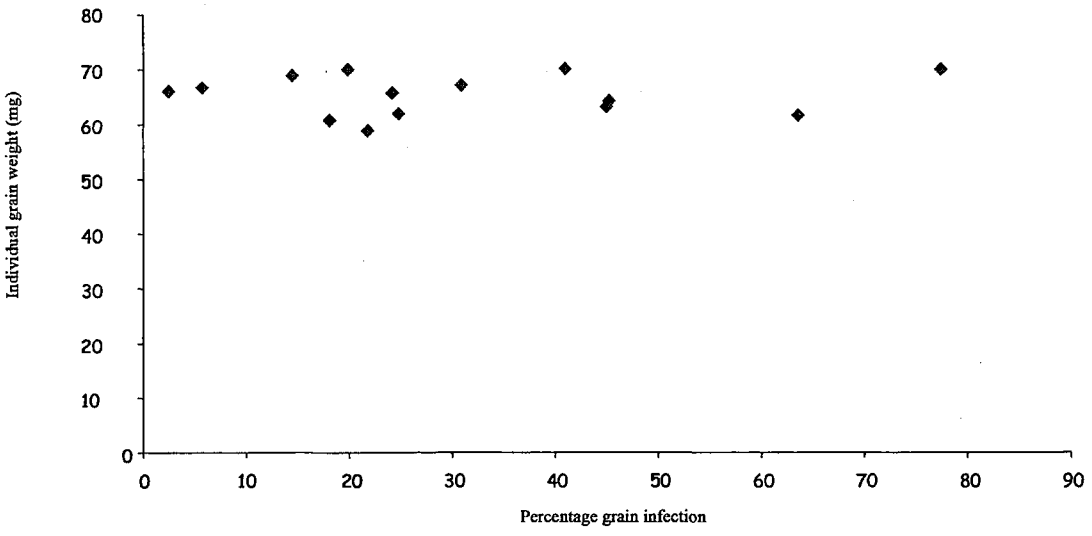


Figure 5.5. The relationship between percentage grain infection and individual grain weight for winter wheat inoculated with *M. nivale* conidia at spikelet ten.

Discussion.

Results from this experiment show that movement of the pathogens up and/or down the ear does not have to be solely within the rachis, as was suggested by Pugh *et al.* (1933) as mycelium corresponding to the extent of the visible symptoms where they occurred was visible on the surface of the rachis. There was evidence of external pathogen growth on the rachis and spikelets, which agrees with the work by Zang & Buchenauer (2000) who examined the infection of wheat spikelets using *F. culmorum*. Evidence from the present study showed that both *M. nivale* and *F. culmorum* can also travel up through the rachis to the spikelets above the point of inoculation, again this was found for *F. culmorum* by Kang & Buchenauer (2000) who observed that *F. culmorum* hyphae grew upwards and downwards inter and intracellularly in vascular bundles and cortical parenchyma tissue of the rachis.

However, this movement of pathogens within vascular bundles of wheat ears disagrees with work by Whingwiri *et al.* (1981). They suggested that spikelets in the distal region of the ear are supplied by smaller bundles and that the assimilate movement to the distal region is reduced when compared with the basal spikelets. Assimilate movement within the plant could be used to explain why in this experiment there was more downward movement of the pathogens.

There are conflicting reports on the appearance of *M. nivale* infected grain. Millar & Colhoun (1962b) described *M. nivale* infected seed as shrivelled and discoloured in appearance whilst Hare (1997) and Hare *et al.* (1999) found no difference in the weight or visual appearance of *M. nivale* infected and non-infected grain. However, (Hare *et al.*, 1999) stated that there is a consensus in the literature with respect to the effect of *F. culmorum* on grain weight and appearance. *Fusarium culmorum* reduces the weight of individual grains and can cause a red discolouration. Results from this experiment agree with the work of Hare and Hare *et al.* that grain infection with *M. nivale* has no effect on grain weight (Figure 5.5), while infection with *F. culmorum* does cause a reduction in grain weight (Figure 5.4).

Results from this experiment show that there is a significant relationship for *F. culmorum* between grain infection and individual grain weight, but no relationship was evident for *M. nivale*. These results wholly agree with work by (Hare *et al.*, 1999) who showed that TGW is reduced by infection with *F. culmorum* but not with *M. nivale*. Thus, grain weight cannot be used as a measure of infection for *M. nivale*, but reduced grain weight may be an indication of the presence of *F. culmorum*.

Chapter 6

General Discussion and Proposals for further work.

General Discussion.

Ear blight caused by *Fusarium* species is of economic importance because it can reduce yield (Wong *et al.,* 1992), reduce seed quality (Reeves & Wray, 1994) and cause mycotoxin contamination of grain for feed stuffs (Joffe, 1978). As detailed in Chapter 1 the majority of disease can be attributed to one of five species, *F. avenaceum, F. culmorum, F. graminearum, F. poae* and *Microdochium nivale.* Yield reductions of between 10 and 70% have been reported by Martin & Johnston, (1982). In 2000, Jennings *et al.* took grain samples from 53 severely infected wheat fields (6 in Wales and 47 in England). They found the main head blight pathogen was *M. nivale*, present in 96% of samples. Other ear blight pathogens were found in 43% *F. avenaceum, F. graminearum* 40%, *F. poae* 34% and *F. culmorum* 21% of samples.

Previous studies by the likes of Mesterhazy and Bartock (1996) and Jones (2000) have reported fungicidal control of FEB to be successful while studies by Martin and Johnston (1982) and Milus and Parsons (1994) reported only partial or no control of the disease. More recent studies by Jennings *et al.* (2000) using azoxystrobin, tebuconazole, metconazole and carbendazim applied to the ear demonstrated that applications of tebuconazole, metconazole and carbendazim resulted in a significant reduction in the extent of grain colonisation by *Fusarium* species and DON concentration. Conversely, applications of the same fungicides resulted in an increase in the extent of grain colonisation by *M. nivale.* In the first year of the study the application of azoxystrobin caused a reduction in competition between *Fusarium* spp. and *M. nivale* and as a result greater colonisation by *Fusarium* spp. was observed. Mesterhazy (2003) states that

attempts to control FEB with fungicides have been highly variable. This is caused by cultivar resistance, fungicide efficacy, fungicide coverage, timing and pathogen aggressiveness. He studied the effect of different fungicides on wheat varieties of different resistances using isolates of *F. culmorum* and *F. graminearum.* It was found that in most cases that the application of a fungicide reduced visual symptoms, yield loss and DON concentration. Mesterhazy concluded that research was required into developing more resistant cultivars, better spraying technology and more effective fungicides.

The findings of Jennings *et al.* (2000) in general agree with the results of experiment 3 where the application of azoxystrobin and metconazole to ears inoculated with *M. nivale* reduced the incidence of diseased ears and spikelet infection per ear when compared to the untreated at the first assessment (21 DAI), but by 28 DAI only plots treated with azoxystrobin showed a significant reduction in the incidence of diseased ears when compared to the untreated. Unfortunately this was not true across all work presented in this thesis.

The discrepancies between observations following applications of fungicides in experiments 1, 3 and 4 may be due in part to a number of different reasons. Work published by Dardis and Walsh in 2000 stated that azoxystrobin is widely known to be less effective against strains of *F. culmorum* than *M. nivale* and this was demonstrated in each of the experiments (1, 3 and 4), one explanation could be that it was due to the interaction between FEB causing pathogens and saprophytic ear microflora. Much work

has been published (Liggitt, 1997; Jennings *et al.*, 2000; Simpson *et al,*. 2001 and Pirgozliev, 2002) on pathogen / ear microflora interactions and they all concluded that the application of certain fungicides could have an effect on the interaction between fungal communities due to their differing activity towards individual species found on the ear.

The effectiveness of selected fungicides at full and half rate were tested in the field against *F. graminearum* by Korptis and Skorda (2002). They found that full rate applications were effective against the pathogen and green leaf area (GLA) was extended in all cases by the application of the fungicides. These findings on GLA agree with work by Godwin *et al.* (1992) who stated that ear sprays of strobilurin kept the crop greener for longer and so increased yield. Unfortunately GLA was not taken into account in experiment 4 so these findings can not be used to explain the results where an increase in yield was seen for plots inoculated with *F. culmorum* and sprayed with azoxystrobin.

In both experiments 1 and 3 fungicide treatments containing azoxystrobin lowered grain infection with *M. nivale* but had no effect on the levels of infection caused by *F. culmorum*. In all field experiments disease pressure was high in due to the natural inoculum and or close proximity of the other ear blight trials at the college and the use of plot misting equipment. Previous work by Rossi *et al.* (2002) identified the role of humidity in the levels of natural air-borne inoculum present stating that the density of air-borne conidia rapidly deceased when the relative humidity dropped. The disease pressure from sooty moulds was very high in experiment 1, in the following years field trials (experiments 3 and 4) this was reduced and so was not considered as a factor.

In experiment 1 analysis showed significant difference in thousand grain weight between inoculums only, and no fungicide produced a significant improvement. *F. culmorum* significantly reduced thousand grain weight when compared with *M. nivale*, and this agrees with work by Hare *et al.* (1999) on thousand grain weight and seed infection.

Infected grain can provide a primary source of inoculum for the subsequent development of *Fusarium* seedling blight (Hewett, 1983). Seedling blight can also be caused by soil-borne inoculum (Bateman, 1977); however, under UK field conditions soil-borne inoculum is thought to be of little importance. If severe, *Fusarium* seedling blight can result in low seedling establishment, and yield reductions of up to 40% in the field have been observed in the UK (Noon and Jackson, 1992). Seedling blight is usually controlled by the use of fungicide seed treatments and an advisory threshold for treatment set by the NIAB of 10% infected seeds exists in the UK. In 1993/4 over 90% of wheat seed samples were infected by *M. nivale* and of those more than 90% exceeded the 5% advisory limit (Reeves and Wray, 1994). It is clear that in seed crops the control of FEB and grain infection is of great importance.

To date there has been no published work on the link between controlling ear blight with fungicide applications and the use of this grain as a seed crop with or without a seed dressing and the emergence of infected seed. Korptis and Skorda (2002), investigated the effectiveness of fungicides against *F. culmorum* on the ear and against subsequent seedling blight. Grain was collected from ears at GS 85 and at harvest and drilled to measure emergence. Significant differences were found in emergence from healthy and

FEB infected spikes, seedlings from infected grains were characterized by reduced coleoptile elongation and seedling death. They conclude that fungicide treatments may reduce inoculum and increase yield but are not sufficient to reduce FEB contamination in the grain of wheat. An objective of both Chapters 3 and 4 was to determine the effect of a seed treatment of fludioxonil on the incidence of seedling blight when applied to infected grain harvested from the ear blight trials.

During harvesting of the ear blight trials (experiments 1, 3, 4, 9, 10, 11 & 15) it is expected that a certain amount of grain (usually cracked or very small grains) would have been lost over the back of the combine. Hare *et al.* (1999) reported that grain infected with *F. culmorum* is smaller, lighter and shrivelled in appearance so during harvest of the trials a high proportion of the lost grains may have been infected with *F. culmorum.* The only way to prevent this would have been to collect the ears and thresh them by hand but due to the large numbers of plots involved this was considered to be impractical. The decision to harvest with a combine rather than to hand thresh each ear was made on the strength that the grain sample needed to be as close as possible in physical quality to that used by farmers as home saved seed. The seed was cleaned before re-drilling to remove chaff, crop debris and broken grains, and again this removed a possible source of inoculum that may have had an effect on the emergence trial but also it would not be present in a commercially treated farm saved seed sample.

One variable that could not be overcome was the weather; this varied greatly between the two years' emergence trials (experiments 2, 5, 6, 12, 13 & 14). It is well documented that

autumn drilling across the UK was delayed in 2000 (experiments 5 and 6) due to the exceptionally wet autumn. At Harper Adams University College drilling was postponed until January 2001 but seedbed conditions remained extremely unsuitable and the soil waterlogged. This can be used in part to explain the lower than expected emergence in the trial and agrees with previous work on the effects of seedbed conditions on emergence of infected seed by Colhoun (1964), Hare (1999) and Haigh (2004).

It is known from Hare *et al.* (1999) that *M. nivale* may be present in the seed lot with the absence of visible symptoms on the seed and this was evident in all field trials. In both years the use of a fludioxonil seed dressing significantly improved the emergence of the infected seed regardless of the ear spray when compared with seed that received no treatment prior to drilling. This agrees with work by Haigh (2003) who states that seed treatments gave robust control of *M. nivale* seedling blight under a range of seedbed conditions. These field trial results show overall that the use of an effective ear spray to control FEB and the subsequent infection of grain, combined with a seed treatment of fludioxonil before drilling is the most effective protection against seedling blight and will improve emergence in the subsequent crop.

The amount of inoculum used in the ear blight trials (experiments 1, 3 and 4) was thought to be far in excess of that found naturally in UK field conditions although the levels of natural inoculum were not measured during the field trials. As the trial was designed to be as close to farm saved seed as possible it was decided to vary the amount of inoculum

on the ear in the following trials (experiments 9, 10, 11 and 15) to give a more realistic approach to the problem.

Results of these experiments showed that as the amount of inoculum increased so did the amount of visible symptoms, in some cases reaching 100% by the second assessment. A possible reason no differences in grain yield were seen in these experiments may be due to the crop exhibiting a closed flowering period. The lack of visible anthers made it hard to judge when flowering was taking place and so inoculation may have been applied after the grain in the ear had set. On reflection a measurement of number of grains per ear may have given a more reliable indication of disease severity and this should be incorporated into any further work.

For these experiments the thousand-grain weight results compliment the previous work by Hare *et al.*(1999) on the relationship between seed weight, infection by *F. culmorum* or *M. nivale*, germination and seedling disease. Again due to the high disease pressure from other FEB trials in the field and the presence of natural inoculum it is highly likely cross infection occurred between plots, but this was not assessed so further work would be required to establish if there was a link.

Results from experiments 7, 8, 9, 10, 11 and 15 concur with previous work by Hewitt (1983) who stated that infected grain could act as a primary source of inoculum for the

subsequent development of *Fusarium* seedling blight, by demonstrating emergence and viability are reduced with increasing inoculum load in the seed.

Overall the results obtained from the field trials show that by increasing the inoculum load the symptoms of FEB and the subsequent infection of harvested grain are also increased. This is likely to be due to the amount of inoculum present on the ear. If there are greater numbers present the environmental conditions are not so crucial for infection.

In general the percentage emergence for all seedling blight trials was very poor and this was due to the exceptionally wet autumn, which delayed drilling until mid January 2001, and then the poor seedbed conditions and water logged soil. Even with the delay in drilling as expected in all three trials the percentage seedling emergence decreased with an increase in spore load on the ear, and the use of fludioxonil as a seed treatment pre-drilling significantly improved emergence as in the previous year's emergence trial. Results from both years' emergence trials agree with previous work by Richardson (1974) who found a good relationship in field trials with oats, between seed infection with *M. nivale* and seedling emergence and yields. The effect on yield was thought to be due to a reduction in ear numbers resulting from a low seedling population. Colhoun (1970) stated that losses of this kind are unlikely to occur often in commercial crops. These current experiments showed that when conditions are unfavourable for seedling disease (this included the use of treated seed) little or no loss of yield occurred. The trend from all trials show that even when the ear received no ear blight sprays and the grain

infection is high the use of a seed treatment before drilling can be an effective protection against seedling blight in the field.

The results for the last field trial (experiment 15) were disappointing and this may be due the position of the trial within the field. It was surrounded by other *Fusarium* ear blight trials. Although guard plots were used round the trial, they were not large enough to prevent the spread of ear blight pathogens from the surrounding trials. This allowed both naturally occurring airborne inoculum and inoculum from the other trials to spread into this trial, affecting the results. As previously discussed results from Rossi *et al.* (2002) would suggest that with plot misting and the other trials in the field the amount of airborne pathogen present was extremely high.

The point inoculation experiment studied the spread of pathogen infection as it characteristically spreads from the point of inoculation to neighbouring spikelets on the ear. The results from this experiment agree with results from the inoculation load experiments and Hare *et al.* (1999) that there is a relationship between grain infection and thousand-grain weight for *F. culmorum* but none was evident for *M. nivale.* Also results showed grain infection for *M. nivale* could be symptomless and show no reduction in thousand-grain weight. The variety of wheat used in this experiment (Cadenza) is not rated by the National Institute Agricultural Botany (NIAB) as resistant to ear blight and so this was not taken into consideration when studying the movement of the pathogen on the ears.

This work aimed to establish a link between ear blight, grain infection, quality and yield and the subsequent development of seedling blight when infected seed was sown. The findings have confirmed the link between *F. culmorum* infection and reductions in thousand grain weights and the fact that this is not evident for *M. nivale*. This means that thousand-grain weight cannot be used as a measure of infection when assessing the seed quality and health on farms for home saved seed.

Proposed further studies.

In this study the, the effectiveness of fungicides against *Fusarium* ear and seedling blight in wheat under glasshouse and field conditions has been demonstrated. The work has shown that for effective fungicide control under field conditions the type of pathogen must be known at the time of spraying. Results also agreed with work by Liggitt (1997) and Pirgozliev (2002) that there is a relationship between *Fusarium* pathogens and other fungal species present on the ear. However, it is proposed that in order to further our knowledge and understanding of the relationship between chemical control of symptoms on the ear and the link with seedling blight further studies are required.

The further studies include:

1. Field studies of fungicide efficacy using different low infection levels of the pathogens to examine the effect on ear blight symptoms, grain quality and yield.

The harvested grain could then be used to drill a more realistic emergence trial using a seed treatment.

2. The relationship between differing inoculum loads of Fusarium pathogens and other naturally occurring fungal species present on wheat ears in the glasshouse. This would be extremely hard do carryout in the field due to the presence of naturally occurring inoculum.

3. Point inoculating spikelets of different varieties with different levels of resistance and different types of resistance to study the differences in pathogen colonisation of the ears.

4. Previous work by Mesterhazy and Bartok (1996) demonstrated that a combination of fungicides and resistant wheat varieties could provide more effective control of ear blight. The application rate of fungicides and inoculum levels of the pathogens could be varied on a range of wheat varieties which differ in levels or types resistance to ear blight.

5. Hilton (1999) identified a link between straw height of wheat and severity of Fusarium ear blight; this work could be furthered by the application of fungicides and the comparison of grain quality and yield.

6. Grain harvested from all of the above trial could be cleaned and drilled under field conditions to study the effect of a seed treatment on the occurrence of Fusarium seedling blight caused by drilling infected grain.

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Appendices

Appendix 1 - Agronomic Diary for 1999 Ear blight trial (trial 1).

Date	Operation
26/10/98	Seed cv Equinox treated with Panoctine (guazatine) drilled at 375 seeds m^2 and ring rolled
23/03/99	Nitram (35.4% N) @ 42 kg N ha^{-1} applied
24/03/99	Herbicide applied - Panther (diflufenican + isoproturon) 0.6 l ha^{-1} Isoguard (isoproturon) 2.0 l ha^{-1} Ally (metsulfuron-methyl) 0.015 kg ha^{-1}
06/04/99	Nitram (35.4% N) @ 103.5 kg N ha^{-1} applied
28/04/99	Fungicide applied - Rover 500 (chlorothanil) 1.0 l ha^{-1}
28/04/99	Plant growth regulator applied - New 5C Cycocel (chlormequat + chloline chloride) 2.5 l ha^{-1}
26/05/99	Fungicide applied - Rover 500 (chlorothanil) 2.0 l ha^{-1} + Opus (epoxiconazole) 0.7 l ha^{-1} + Fortresss (quinoxyfen) 0.2 l ha^{-1}
26/05/99	Plant growth regulator applied - Terpal (2-chloroethylphoshonic acid + metiquat chloride) 2.0 l ha^{-1}
31/08/99	Plots harvested

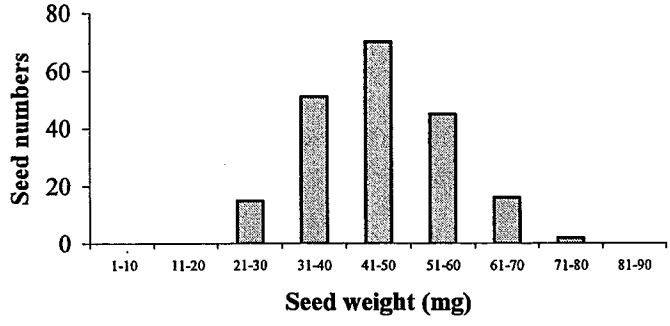
Appendix 2 - Agronomic Diary for 2000 Ear blight trials (trials 3 & 4).

Date	Operation
29/10/99	Seed cv Equinox treated with Panoctine (guazatine) drilled at 375 seeds m^2 and ring rolled
11/03/00	Fungicide applied - Fortresss (quinoxyfen) 0.3 l ha^{-1}
17/03/00	Granular Sulphur applied 75 kg ha^{-1}
10/04/00	Nitram (35.4% N) @ 100 kg N ha^{-1} applied
09/05/00	Herbicide applied - Starane (fluroxypyr) 1.0 l ha^{-1} + Ally (metsulfuron-methyl) 0.015 kg ha^{-1}
09/05/00	Manifol (manganese) 0.5 l ha^{-1} applied
18/05/00	Plant growth regulator applied - Terpal (2-chloroethylphoshonic acid + metiquat chloride) 2.0 l ha^{-1}
20/05/00	Fungicide applied - Opus (epoxiconazole) 0.7 l ha^{-1}
24/05/00	Nitram (35.4% N) @40 kg N ha^{-1} applied
31/08/00	Plots harvested

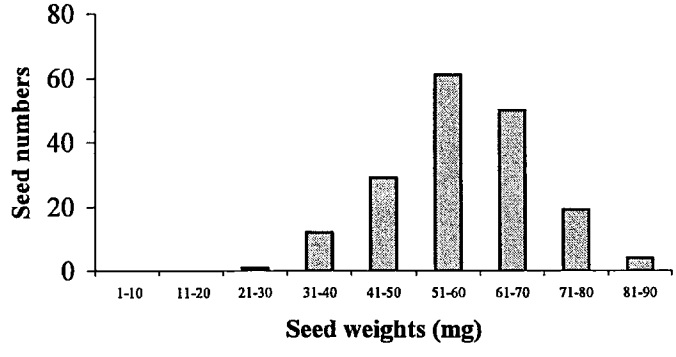
Appendix 3

Seed weight distributions for Experiment 1.

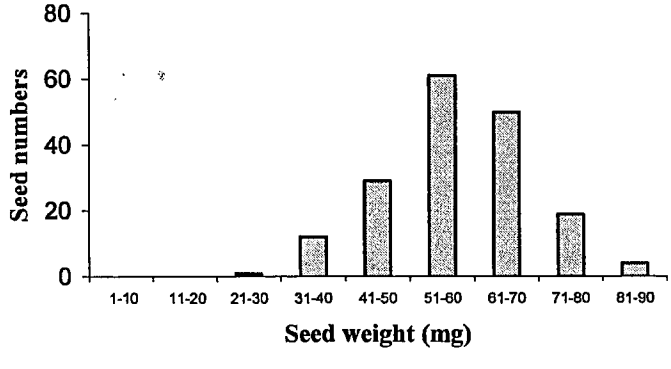
Treatment 1



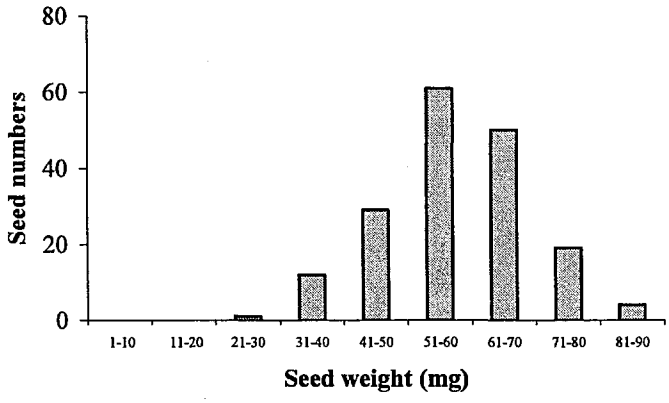
Treatment 2



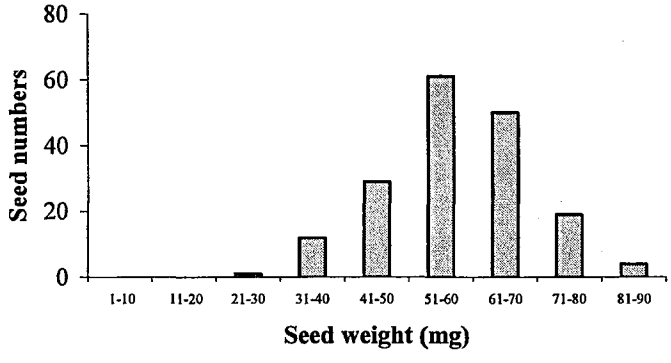
Treatment 3



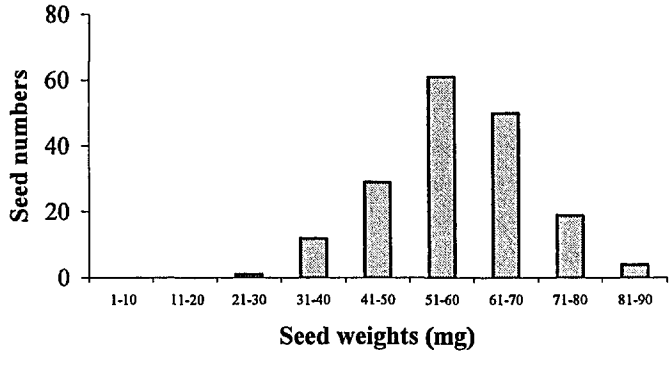
Treatment 4



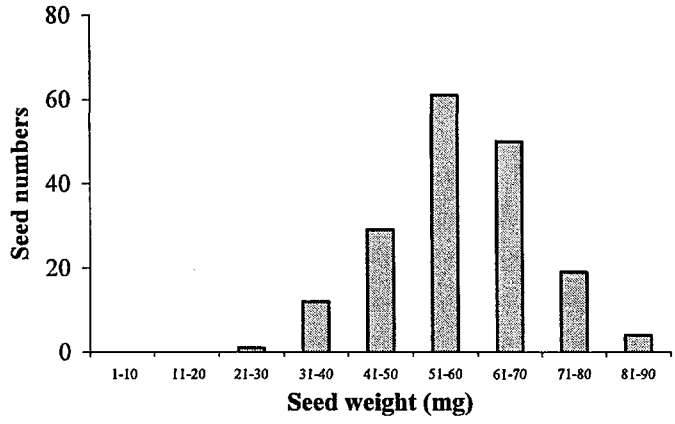
Treatment 5



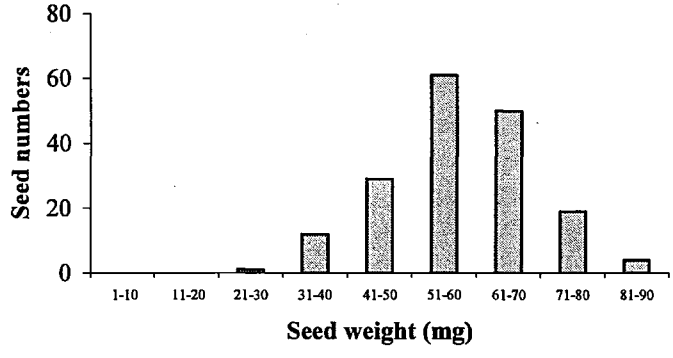
Treatment 6



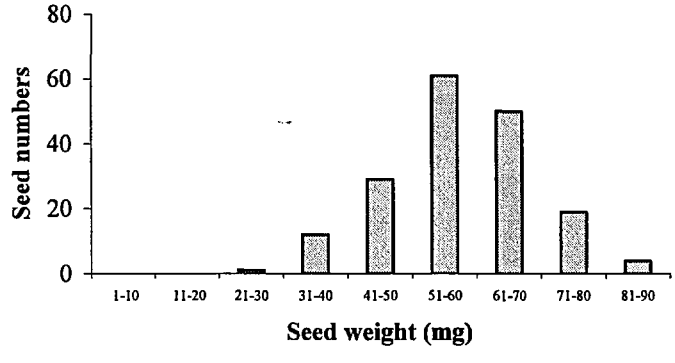
Treatment 7



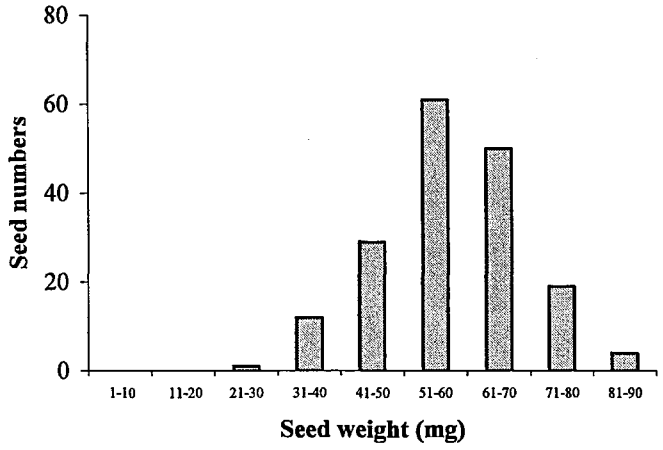
Treatment 8



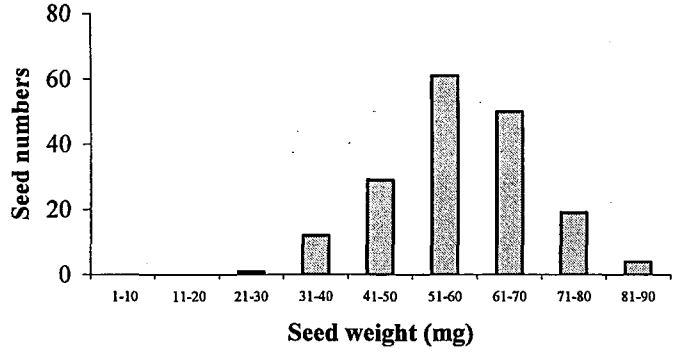
Treatment 9



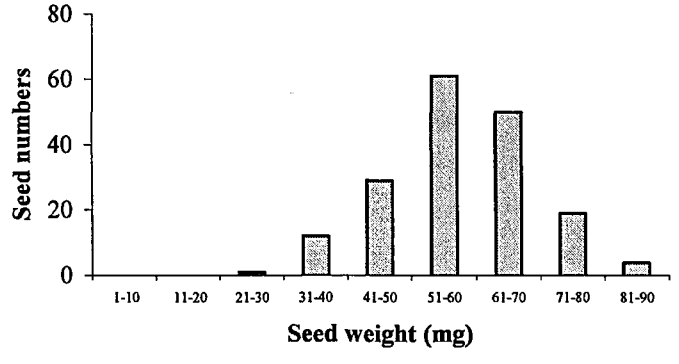
Treatment 10



Treatment 11



Treatment 12



Appendix 4

Photographs of the 1999 seedling blight trial (Trial 2)

Azoxystrobin ear spray. Fludioxonil seed treatment

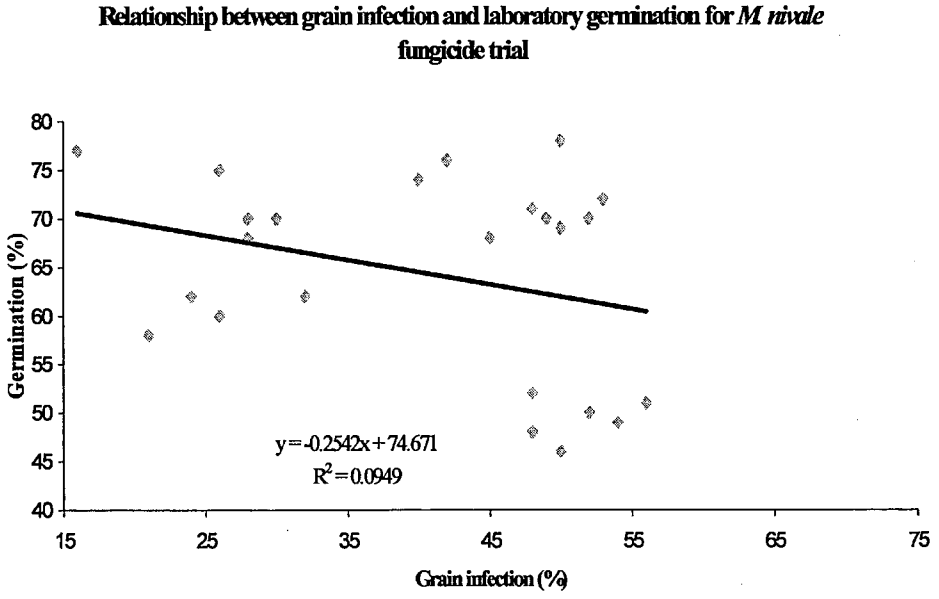
No ear spray. No seed treatment

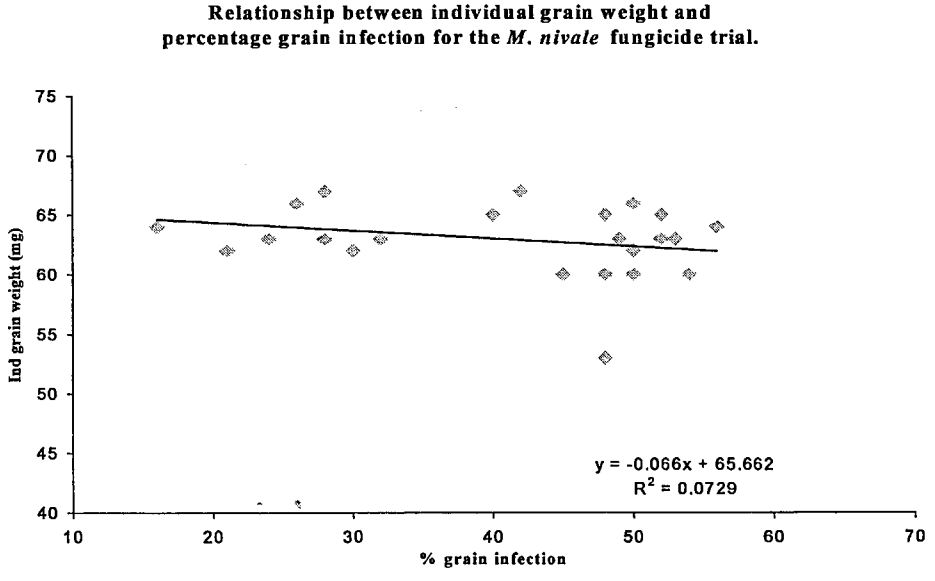
No ear spray. Fludioxonil seed treatment

Azoxystrobin ear spray. No seed treatment.

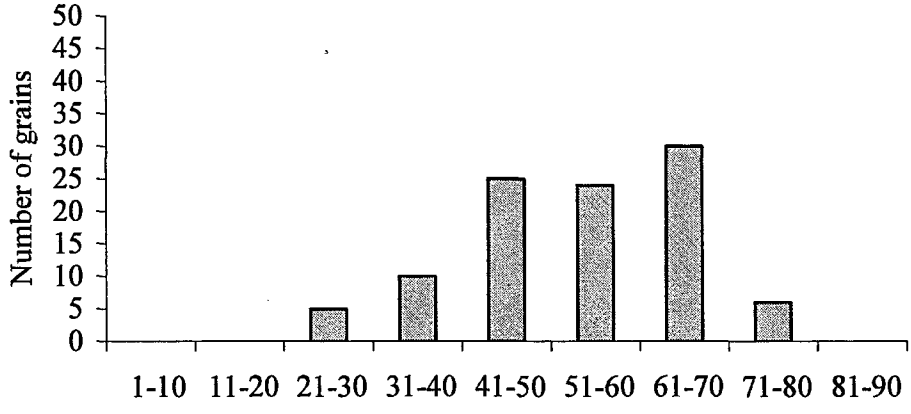
Appendix 5.

Seed weight distributions for 2000 *M. nivale* field trial (Experiment 3).

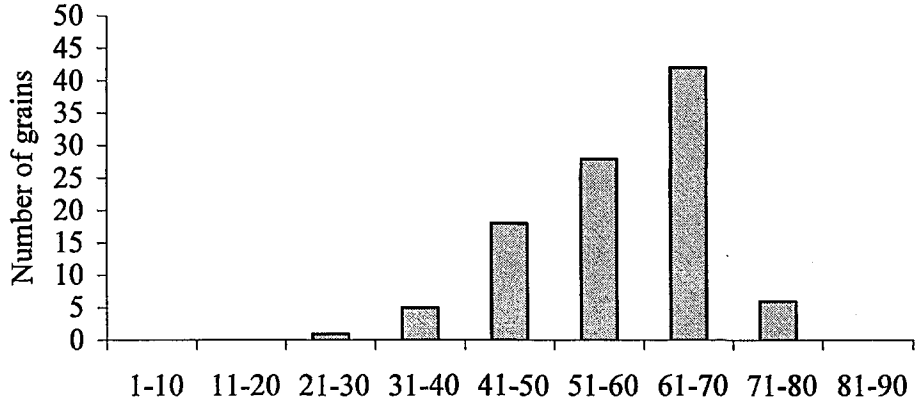




Treatment 3



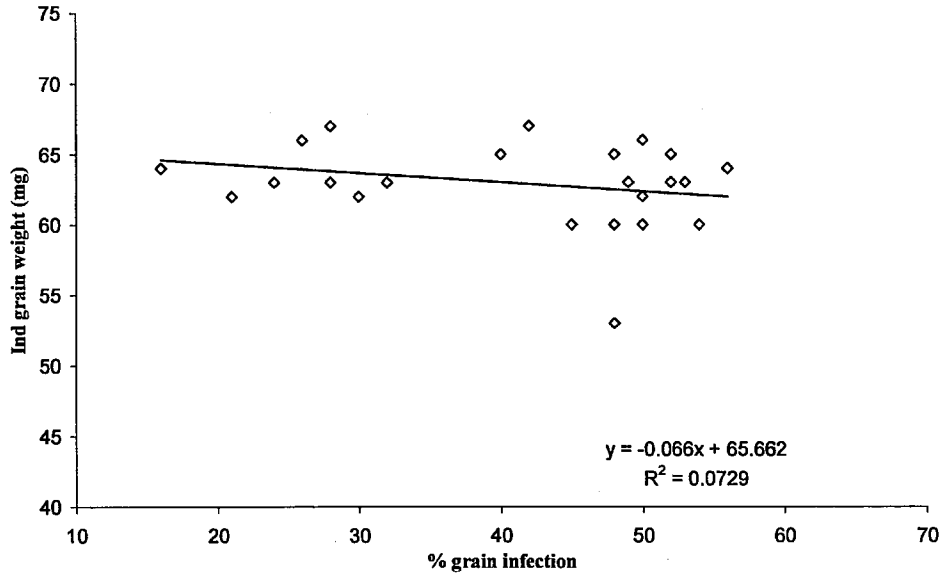
Treatment 4



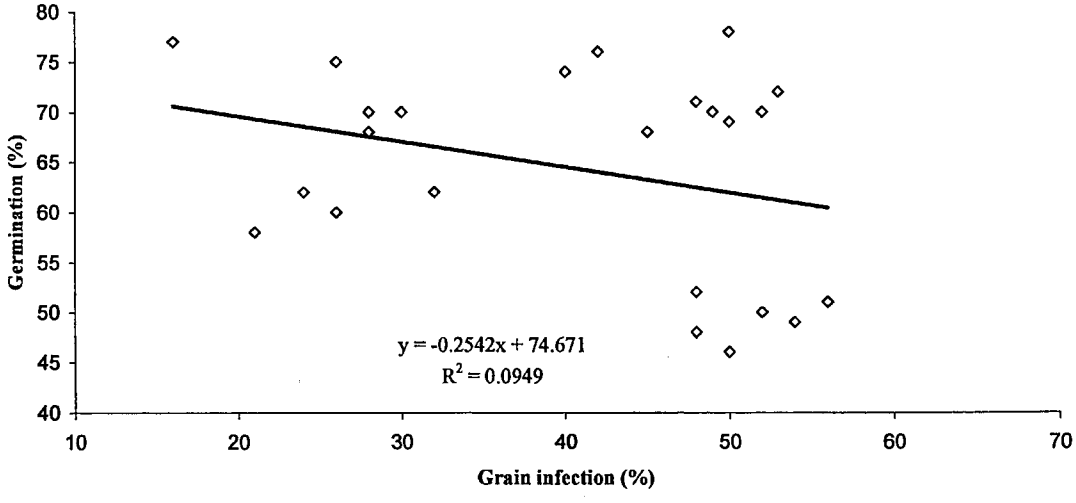
Appendix 6.

Regression graphs for Experiment 3.

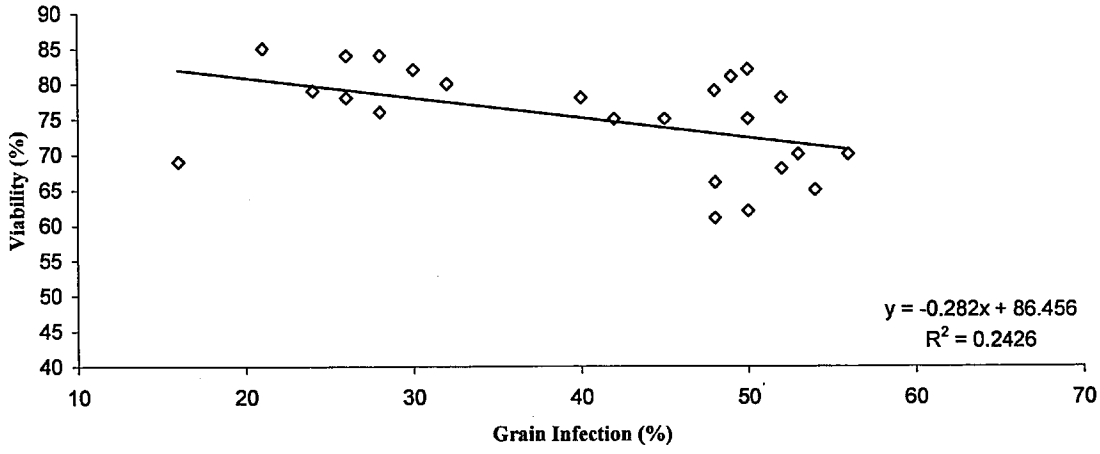
Relationship between individual grain weight and percentage grain infection for the *M. nivale* fungicide trial.



Relationship between grain infection and laboratory germination for *M. nivale* fungicide trial



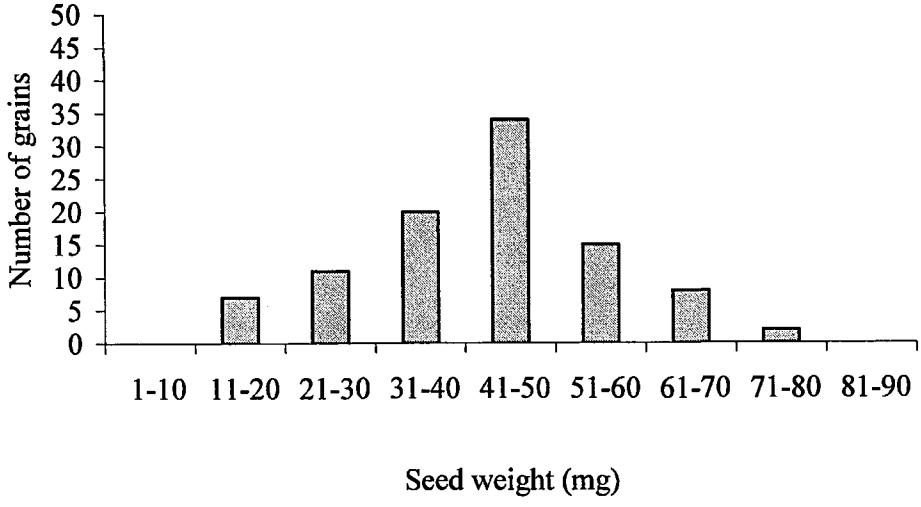
Relationship between grain infection and seed viability for *M. nivale* fungicide trial



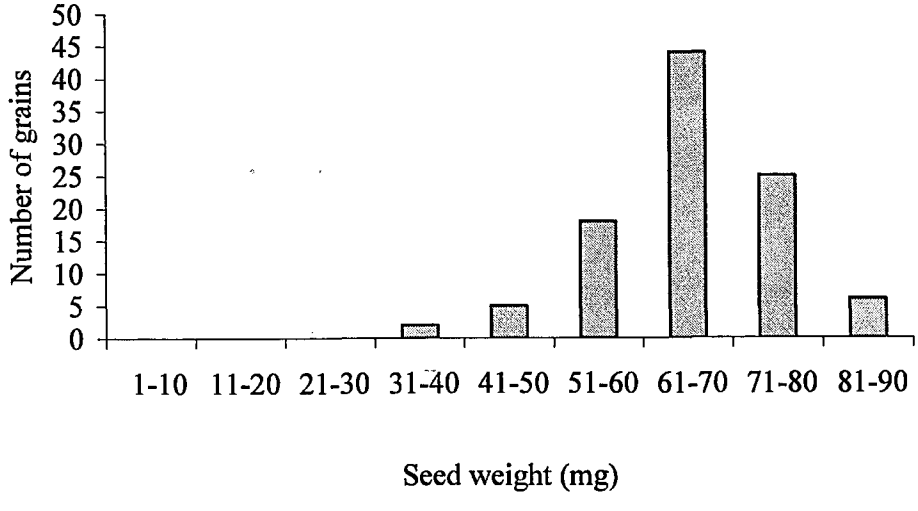
Appendix 7.

Seed weight distributions for 2000 *F. culmorum* field trial (Experiment 4).

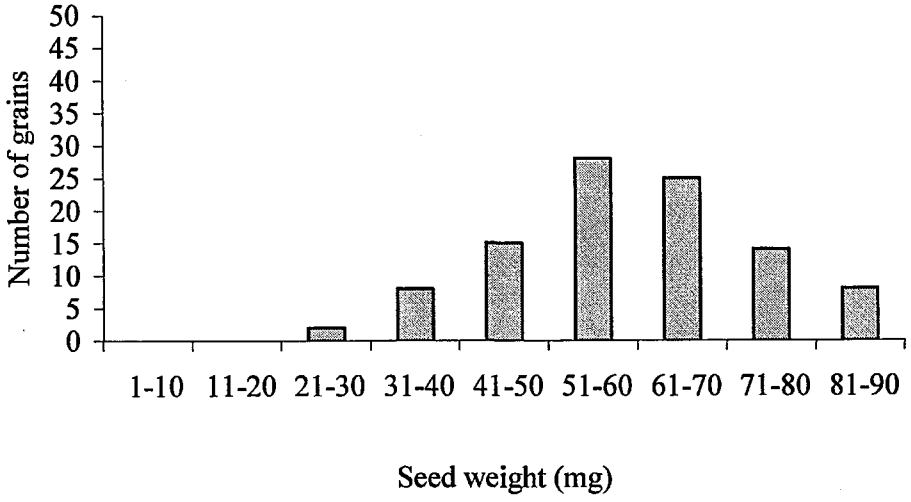
Treatment 1



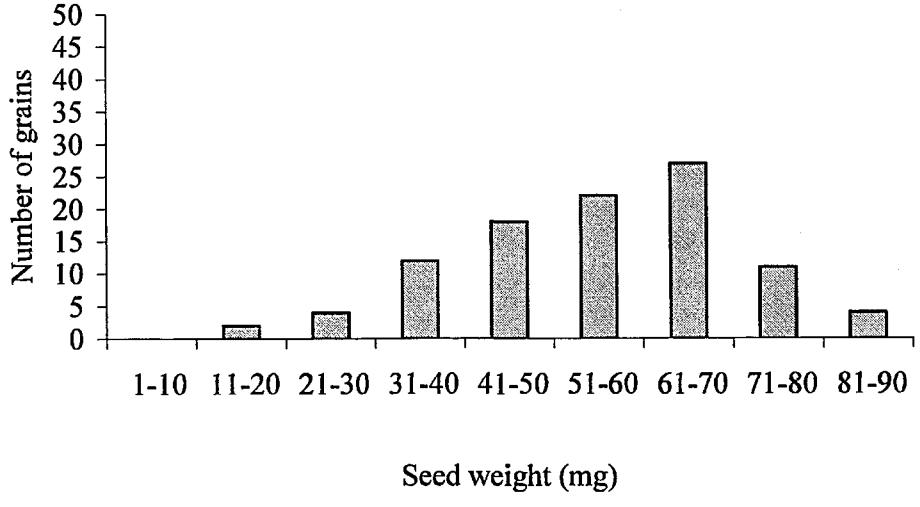
Treatment 2



Treatment 3



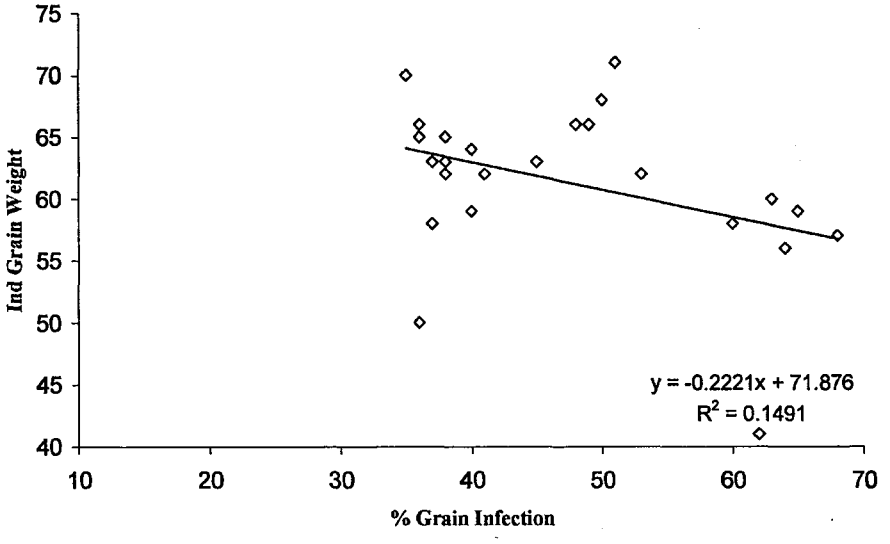
Treatment 4



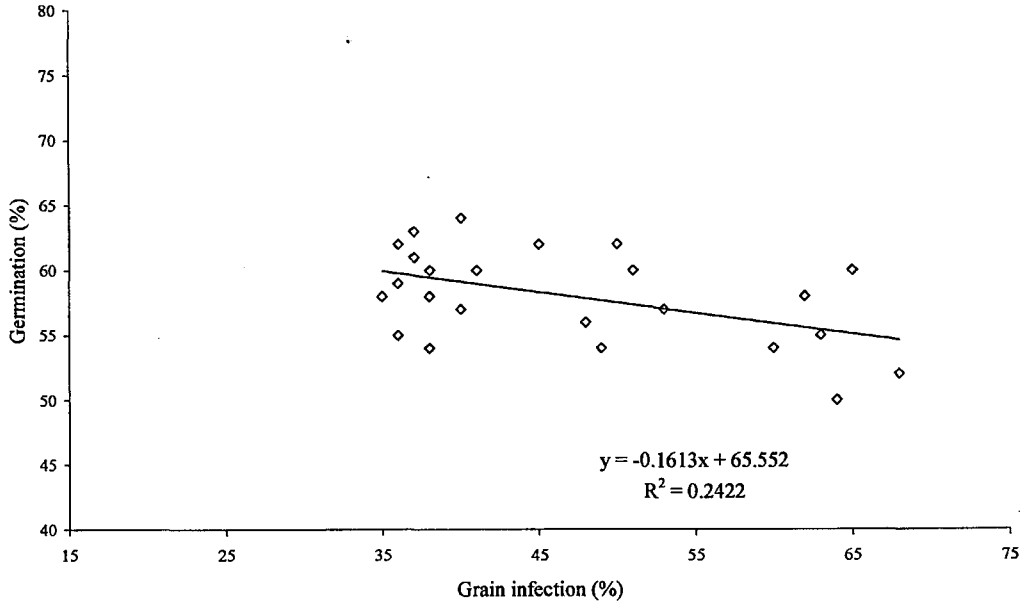
Appendix 8.

Regression graphs for Experiment 4

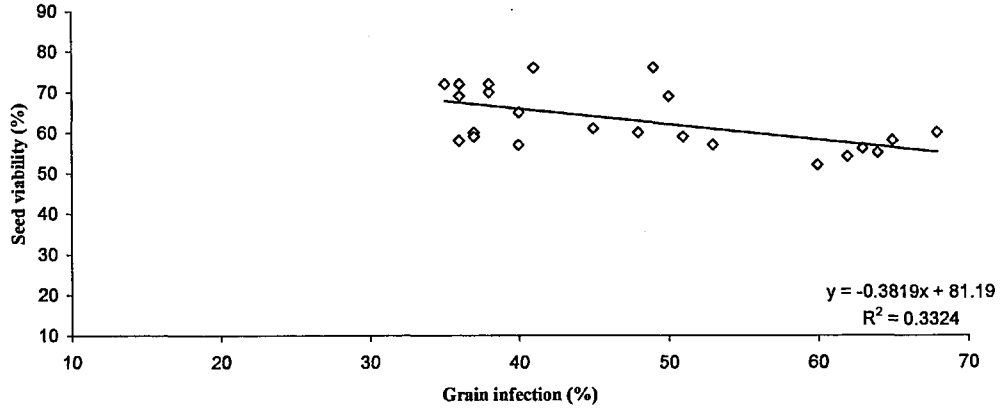
Relationship between individual grain weight and percentage grain infection for the *F. culmorum* fungicide trial.



Relationship between individual grain weight and grain infection for *F. culmorum* fungicide trial



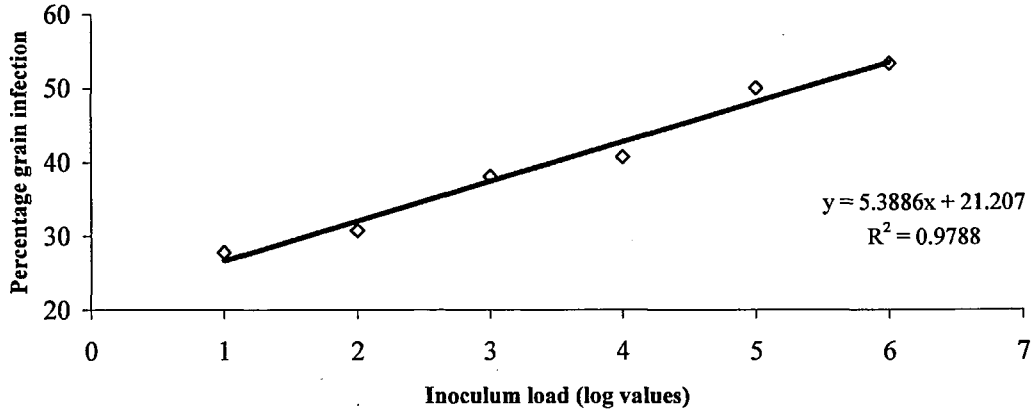
Relationship between grain infection and seed viability for *F. culmorum* fungicide trial.



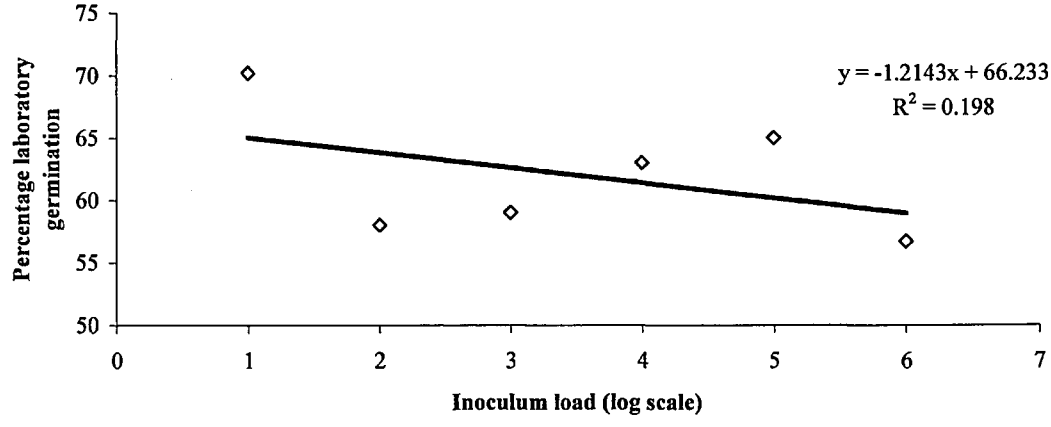
Appendix 9

Regression graphs for the Inoculum load trial for *M. nivale* (Experiment 7)

Relationship between Inoculum load and percentage grain infection for *M. nivale* trial.



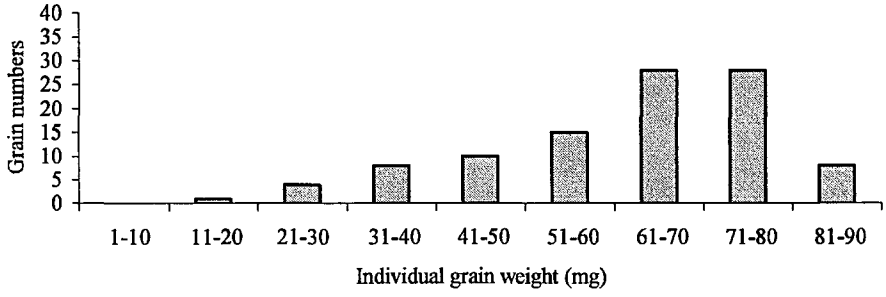
Relationship between Inoculum load and percentage laboratory germination for *M. nivale* trial.



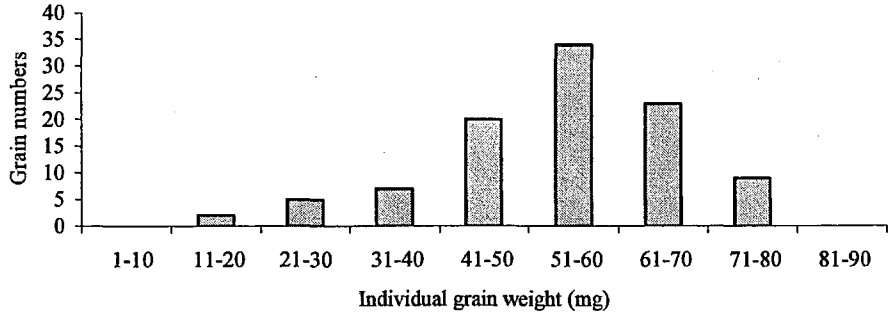
Appendix 10.

Seed weight distribution graphs for *M. nivale* inoculum load field trial (Experiment 9).

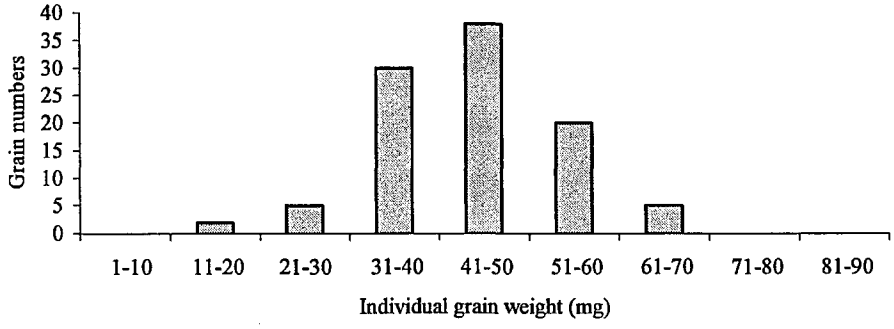
Inoculum load 2 x 10^4 spores ml^{-1}



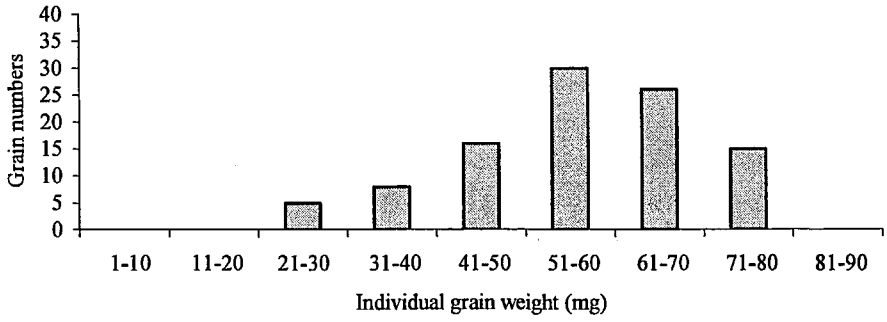
Inoculum load 5 x 10^4 spores ml^{-1}



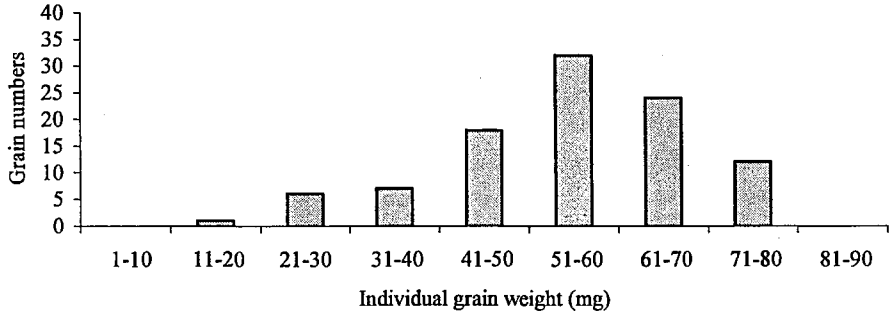
Inoculum load 1 x 10^5 spores ml^{-1}



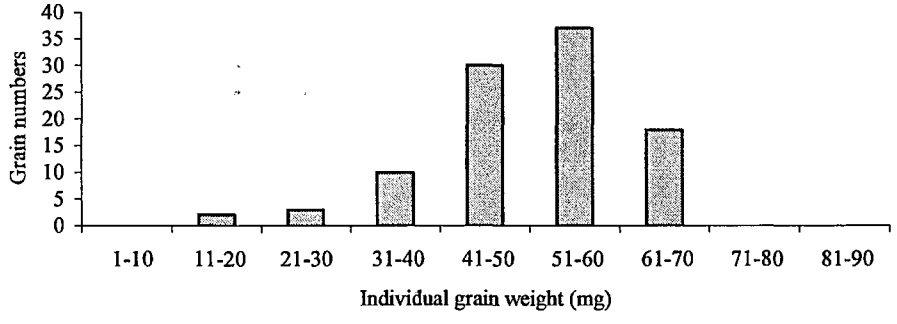
Inoculum load 2 x 10^5 spores ml^{-1}



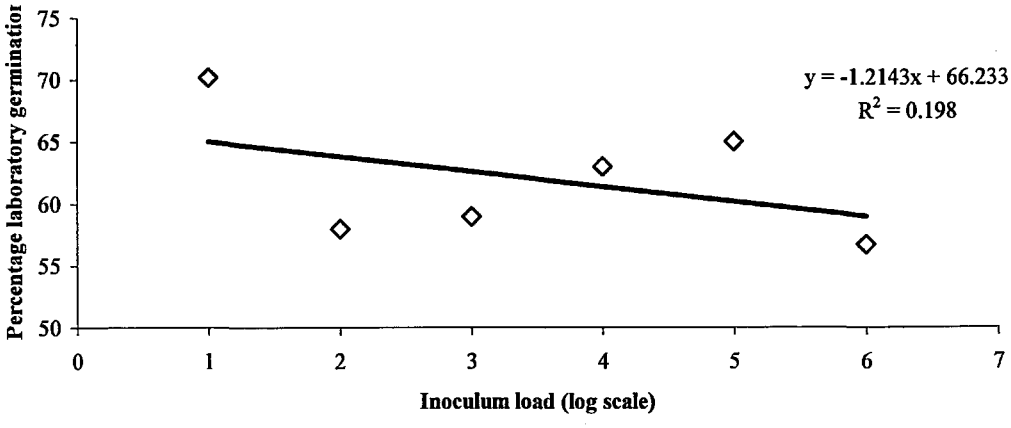
Inoculum load 5 x 10^5 spores ml^{-1}



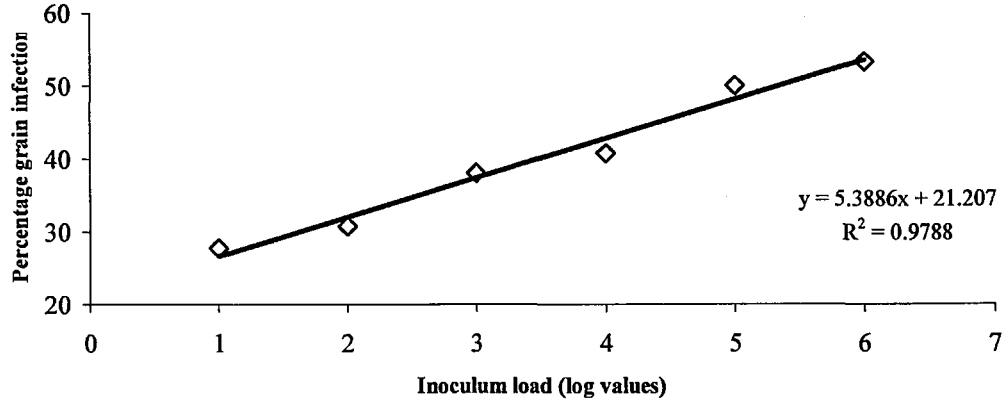
Inoculum load 1 x 10^6 spores ml^{-1}



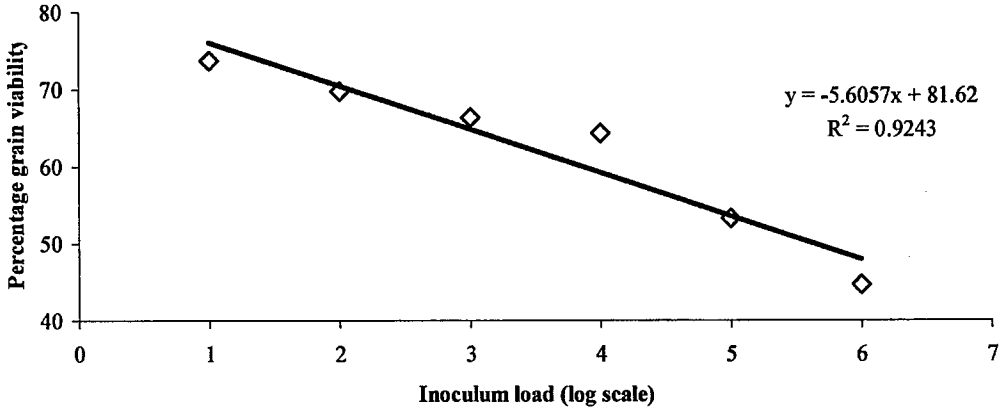
Relationship between inoculum load and percentage laboratory germination for *M. nivale* trial.



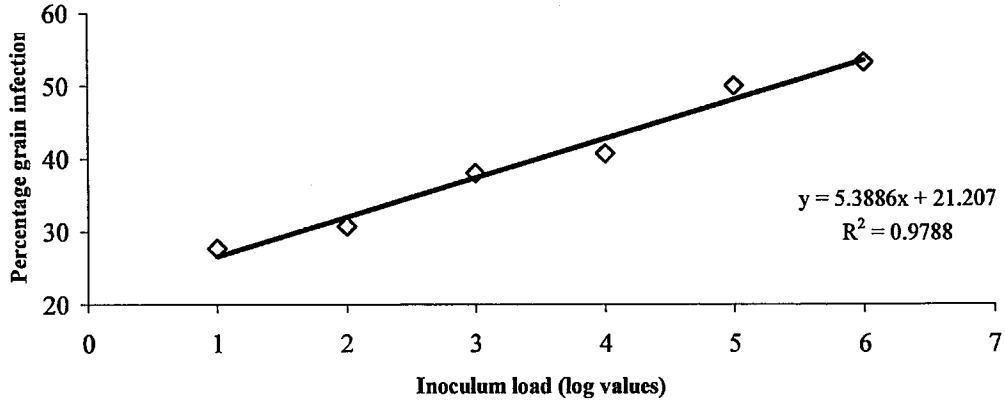
Relationship between inoculum load and percentage grain infection for *M. nivale* trial.



Relationship between inoculum load and percentage grain viability for *M. nivale* trial.



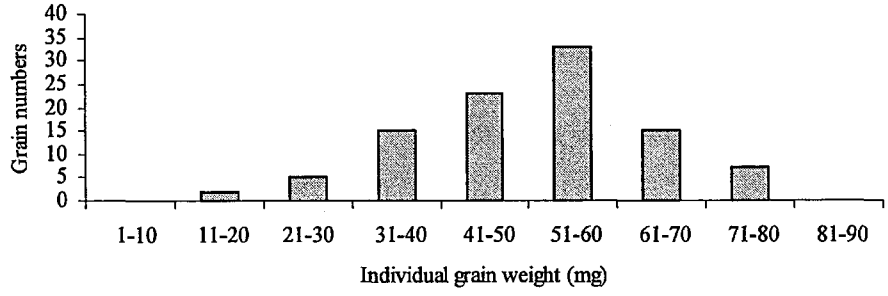
Relationship between inoculum load and percentage grain infection for *M. nivale* trial.



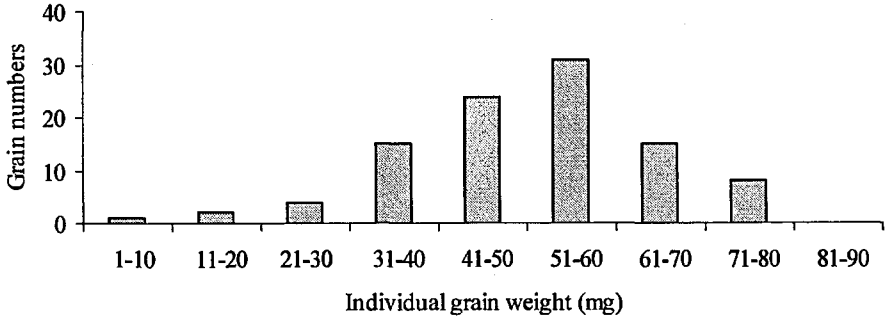
Appendix 11:

Seed weight distribution and regression graphs for *F. culmorum* inoculum load trial (Experiment 10)

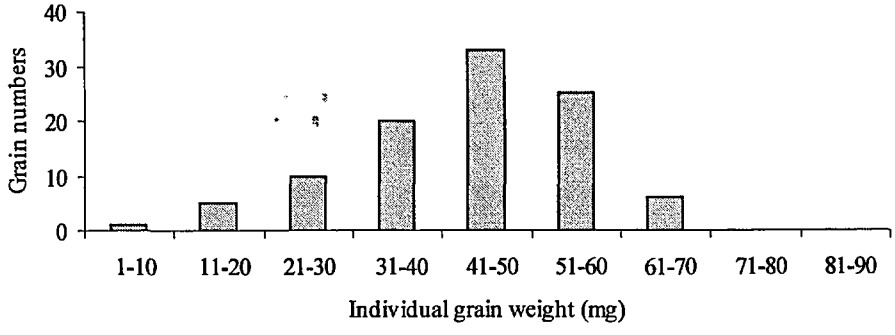
Inoculum load 2 x 10^4 spores ml^{-1}



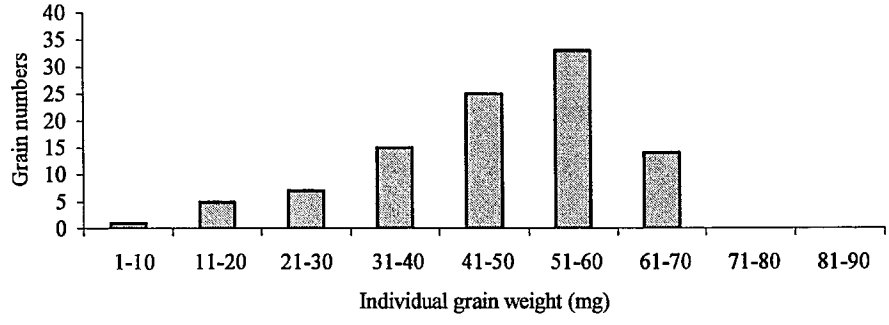
Inoculum load 5 x 10^4 spores ml^{-1}



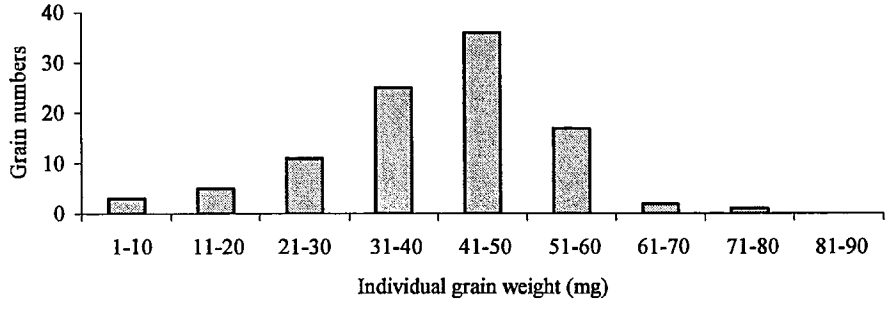
Inoculum load 1 x 10^5 spores ml^{-1}



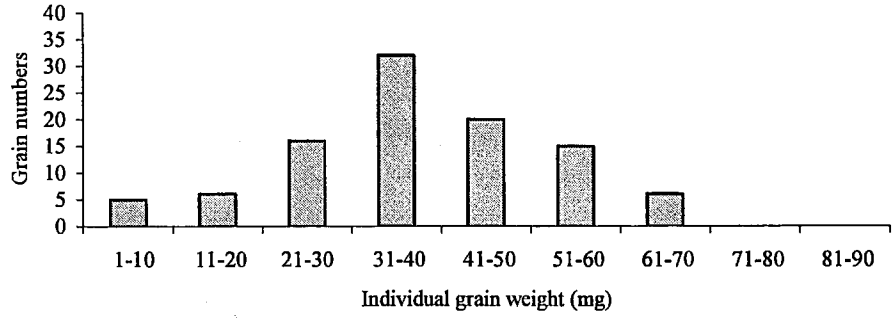
Inoculum load 2 x 10^5 spores ml^{-1}



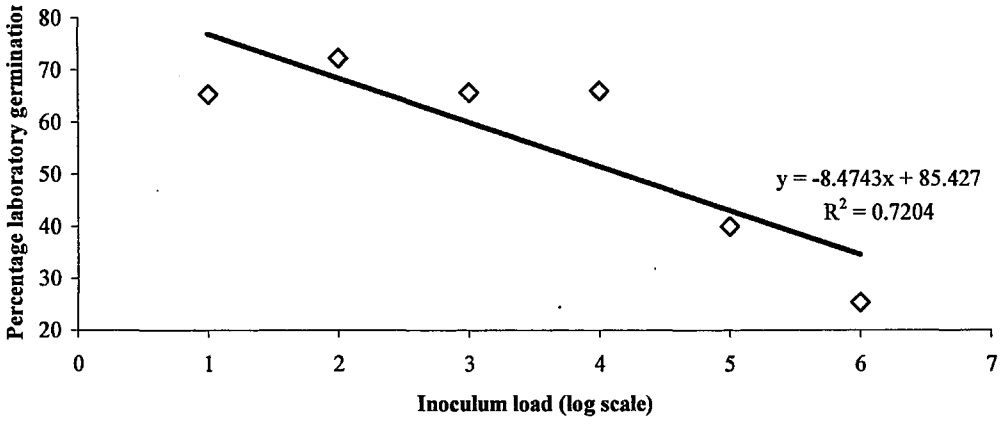
Inoculum load 5 x 10^5 spores ml^{-1}



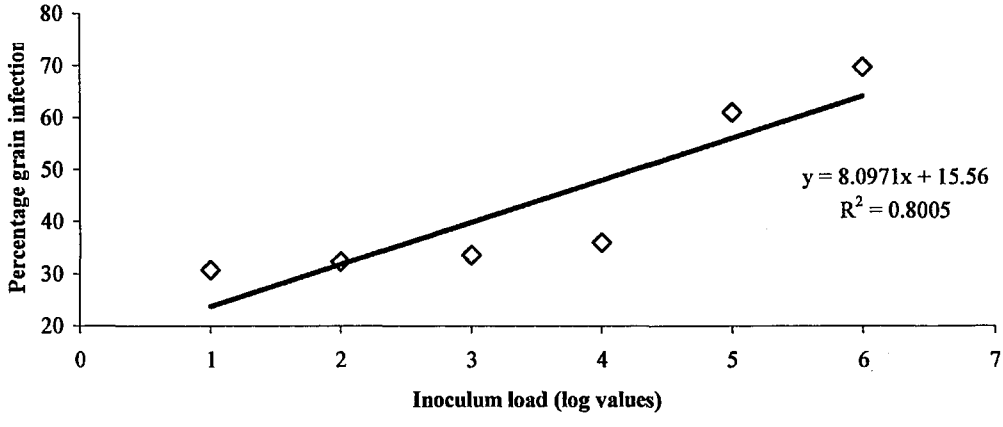
Inoculum load 1 x 10^6 spores ml^{-1}



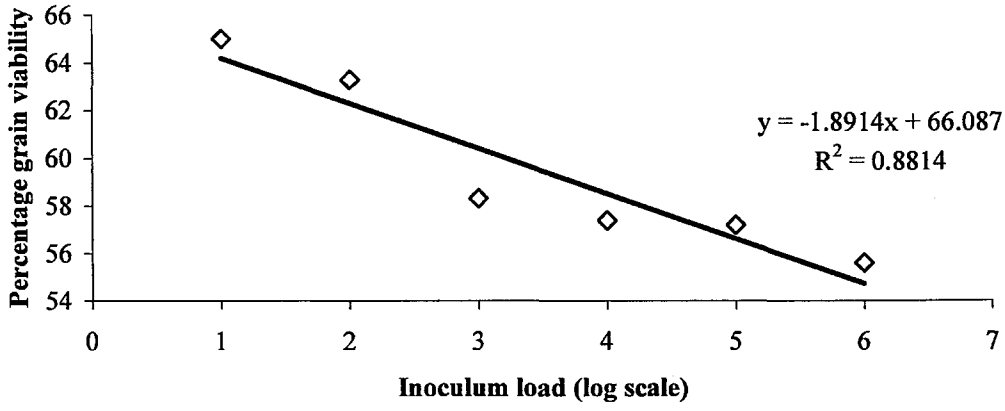
Relationship between inoculum load and percentage laboratory germination for *F. culmorum* trial.



Relationship between inoculum load and percentage grain infection for *F. culmorum* trial.



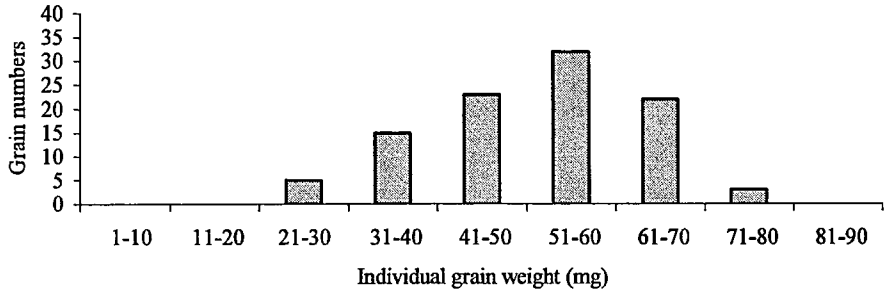
Relationship between inoculum load and percentage grain viability for *F. culmorum* trial.



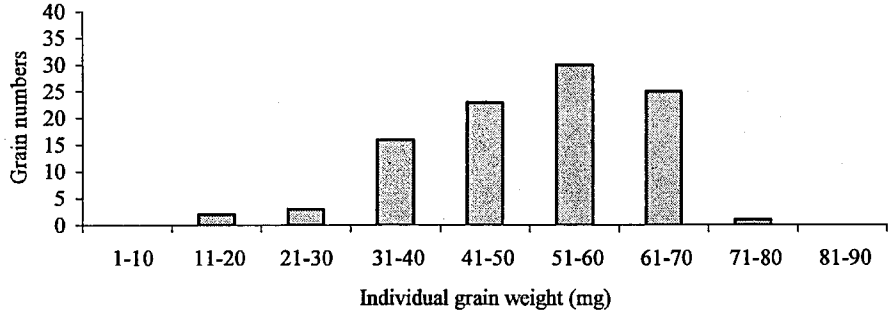
Appendix 12:

Seed weight distribution graphs for spore mixture inoculum load field trial.(Experiment 12)

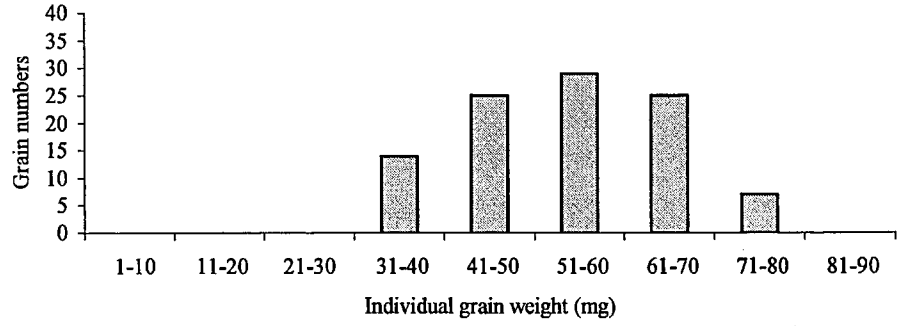
Inoculum load 2 x 10^4 spores ml^{-1}



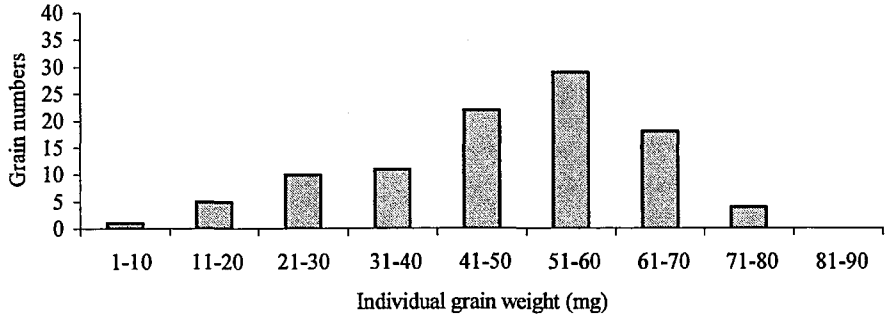
Inoculum load 5 x 10^4 spores ml^{-1}



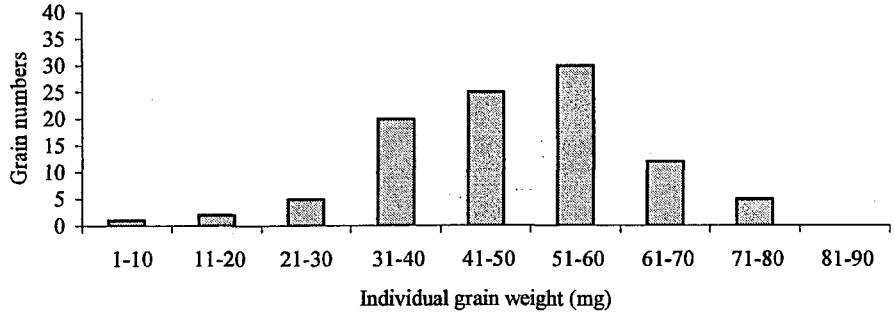
Inoculum load 1 x 10^5 spores ml^{-1}



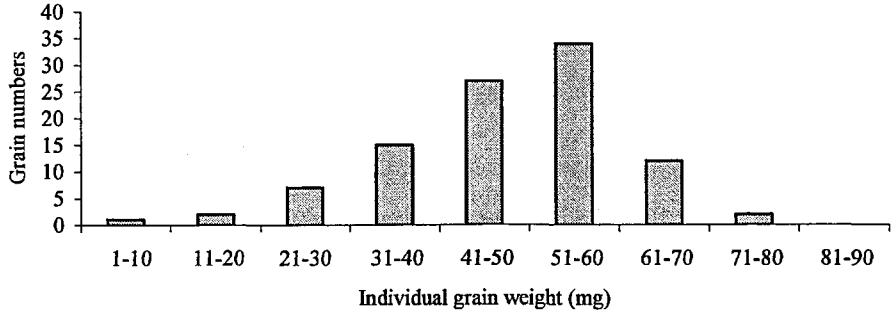
Inoculum load 2 x 10^5 spores ml^{-1}



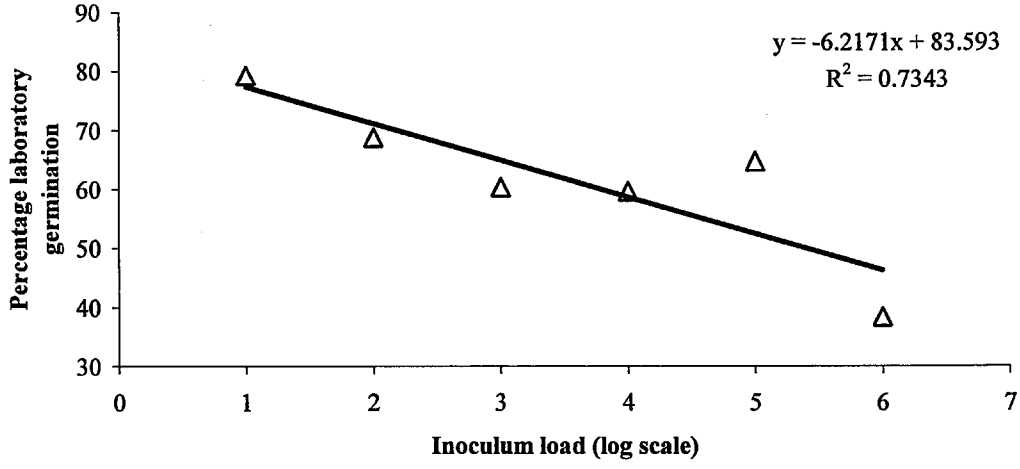
Inoculum load 5 x 10^5 spores ml^{-1}



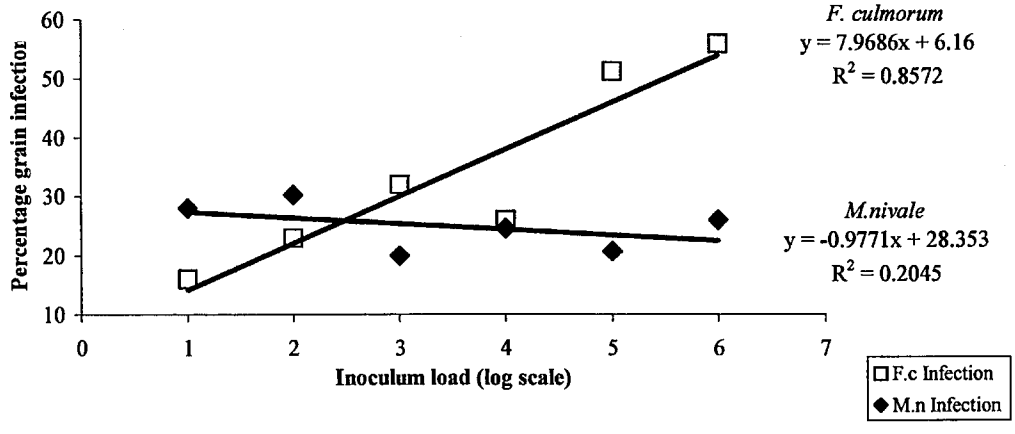
Inoculum load 1 x 10^6 spores ml^{-1}



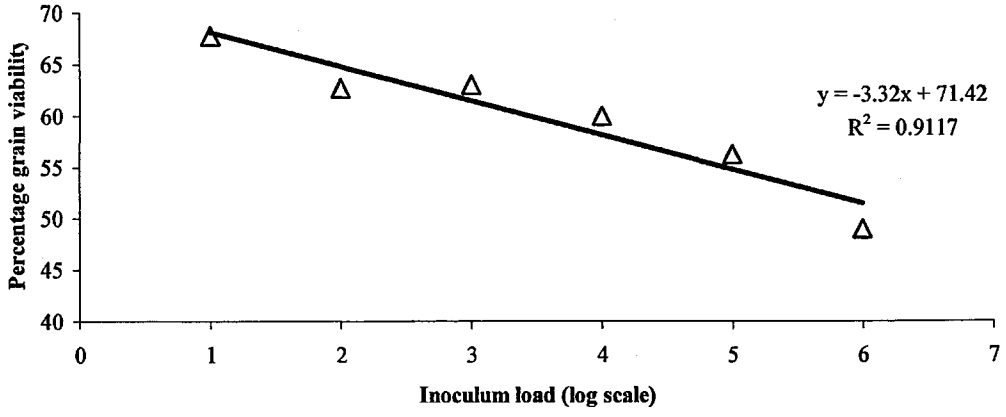
Relationship between inoculum load and percentage laboratory germination for *F. culmorum and M. nivale* trial.



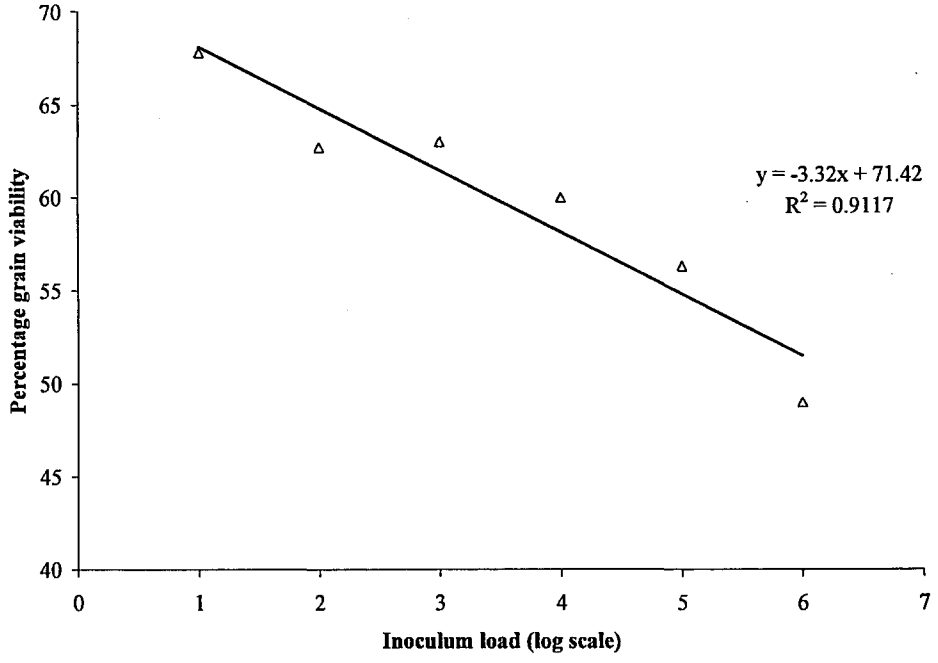
Relationship between inoculum load and grain infection for *F. culmorum and M. nivale* trial



Relationship between inoculum load and percentage grain viability for *F. culmorum* and *M. nivale* trial.



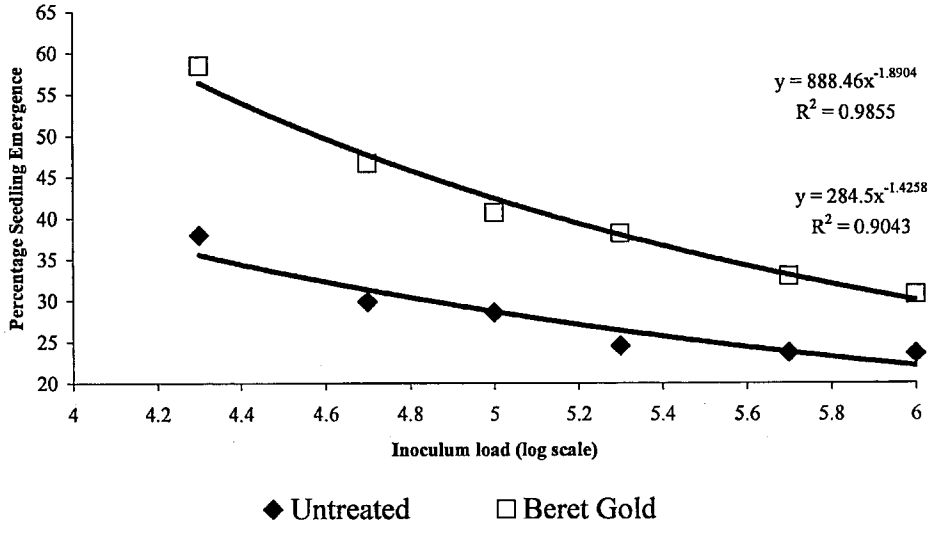
Relationship between Inoculum load and percentage grain viability for *F. culmorum* and *M. nivale* trial.



Appendix 13

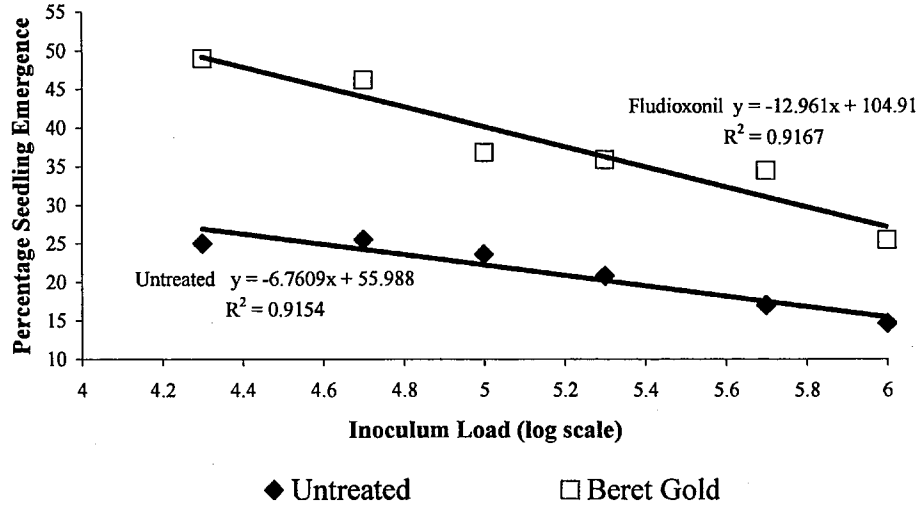
Relationship between percentage emergence for *M .nivale* inoculum load trial

(Experiment 12)



Appendix 14

Relationship between percentage emergence for *F. culmorum* inoculum load trial (experiment 10)



Appendix 15

Relationship between percentage emergence for *M. nivale* and *F. culmorum* inoculum load trial (experiment 14)