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Fusarium langsethiae infection and mycotoxin production in oats

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TABLE OF CONTENTS

ABSTRACT

This investigation aimed to identify and understand the fungus responsible for the production of HT-2 and T-2 mycotoxins in UK oats; *Fusarium langsethiae* was found to be the causal species. A quantitative competitive PCR (QC-PCR) and a real-time PCR assay for quantifying *F. langsethiae* biomass in plant material were successfully developed. Real-time PCR was found to have a wider range of quantification than QC-PCR.

In glasshouse experiments, point inoculation technique and high spore load (10^6 spores ml^{-1}) were found to significantly increase (P = 0.036 and 0.016 respectively) the level of *F. langsethiae* infection in oat panicles. HT-2 and T-2 appeared to increase in line with the level of infection. For both glasshouse and field experiments, all inoculation methods failed to achieve high levels of infection and high levels of HT-2 and T-2 observed in some commercial fields.

Detached leaf assays showed some host preference of *F. langsethiae* towards oats than wheat. Lesion lengths were longest on leaves of an oat cultivar (Gerald) that has been reported to accumulate highest HT-2 and T-2 and shortest on leaves of the cultivar (Millennium) reported to accumulate the lowest levels of these mycotoxins.

Fusarium langsethiae was not found to be a seedling blight pathogen of oats and wheat in a controlled environment study comparing its pathogenicity with those of known *Fusarium* and *Microdochium* species. *Fusarium langsethiae* is therefore unlikely to reduce crop stand and yield where infected seeds are sown.

Fusarium langsethiae failed to produce visual symptoms in infected oat panicles or wheat ears in all experiments and in commercial oat fields surveyed. However, all evidence indicates that it is responsible for high concentrations of HT-2 and T-2 in oats, consequently the presence of this fungus in oats is important in human and animal health.

INTRODUCTION

Cereal crops have always played an important role in human nutrition as well as animal feed and therefore, both directly and indirectly, have been closely associated with the socio-cultural development of mankind. Depending on climatic conditions experienced in a given geographic region, different types of cereals are grown for different purposes. The six commonest cereal crops through out the world are wheat, maize, rice, barley, sorghum and oats. Whereas maize, rice and sorghum are mainly grown in the tropics, wheat, barley and oats are often grown in the cool temperate climate. The productivity of these cereal crops depends on many factors including the prevailing weather conditions (rainfall, relative humidity and temperature for example), cultivars used which can either be prone or resistant to diseases, and cultural field practices such as crop debris management which can influence incidence and severity of certain diseases such as fusarium head blight (FHB) and fusarium seedling blight (FSB). One of the most limiting factors in cereal, particularly small-grain cereal crops production is the attack by diseases. One such disease is FHB.

Fusarium head blight (fusarium panicle blight (FPB) in the case of oats) is also referred to as fusarium ear blight (FEB), scab or head fusariosis. It is a description of a disease of small grain cereals such as oats, wheat and barley that is caused by several fungal species of the genus *Fusarium* including *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *Microdochium nivale*. FHB was first described in 1884 in England and was considered a major threat to wheat and barley during the early years of the twentieth century. Since then, FHB has increased worldwide and recent outbreaks have been reported in many countries. FHB has been identified by CIMMYT as a major factor limiting wheat production in many parts of the world. The disease in wheat appears as blighted head and peduncle tissues which turn brown or tan and senesce prematurely. Similar disease symptoms are also found on barley and oats. FHB is a sporadic disease because *Fusarium* fungal growth is largely dependent on the prevailing weather conditions which also determine the disease severity, especially in the UK where the risk of infection is associated with warm and humid summers. These conditions are prevailing from cereal head emergence to grain ripening. Cereals are most susceptible at flowering although the infection can take place from head emergence and throughout the grain-filling time to harvest depending on the weather conditions. The incidence and severity of FHB differs from one small-grain cereal crop to another. For example, the disease is reported to be more common and severe in

wheat and barley than in oats. This may be the reason why most research on cereal head blight has focused on wheat and barley, with oats receiving less attention; hence there is limited published literature on FHB of oats. In some parts of the world, FPB has not been considered a disease of oat as this crop is reported to be more resistant to *Fusarium* than other small-grain cereal crops. It is important, however, to recognise this disease in oats as it can sometimes result in severe yield losses. For example, yield losses of between 12-48% caused by FPB of oats in Poland have been reported.

FHB is a unique disease with the ability to influence every aspect of the grain industry, causing hardship and economic losses to producers and grain industry alike. It is economically important because of the negative effects it has on cereal yield, quality and the possibility of contaminating cereals with mycotoxins. *Fusarium* infection causes shrivelling of the uninfected grains that do not ripen to their normal size, which, in addition to reducing the test weight, may be light enough during harvesting to be expelled from the combine with the chaff. Infected plants do not grow normally and therefore produce fewer grains due to floret abortion and seeds of reduced size which is a direct economic loss that can affect food security. The presence of *Fusarium*-damaged grains or kernels (FDG or FDK) down-grades grains making producers lose quality premiums, as well as the suffering of domestic and export markets as a result of lost sales because of low customer tolerances for FDG. Grain quality of cereals to be used for human food processing is greatly affected due to decreased nutritive value, discoloration and the development of off-flavours. Due to fungi causing FHB destroying grain starch granules, cell walls and endosperm proteins, milling, baking and pasta-making properties are affected. Use of seeds from crops infected with FHB is likely to lead to reduced germination and vigour and provide further inoculum for root, stem-base and head blight in the following crop. Previous research has shown that seeds sown from infected crops can result in seedling blight, therefore infected seeds are one means of fungal survival between crops and can be a source of primary inoculum.

FHB can result in cereal mycotoxin contamination. Mycotoxins are natural products produced by fungi that evoke a toxic response when introduced in low concentration to higher vertebrates and other animals by natural routes such as food or feed ingestion. They are secondary fungal metabolites produced by many plant pathogenic and food spoilage fungi of the genera *Fusarium*, *Aspergillus* and *Penicillium*. Mycotoxins are considered to be the most significant food contaminants with regard to

their negative impact on public health, food security and the national economy of many countries, especially the developing ones. Historically, mycotoxins have been called secondary metabolites because their biosynthesis is not required for the primary functions of growth and reproduction of the producing fungi. Fungi have been reported to produce these toxic metabolites under stressful conditions such as change in temperature, moisture and aeration. The presence of mycotoxins in cereals and cereal products can be of public concern because of the potential of processed foods and feeds to contain them as some of them have been associated with chronic or acute poisoning in livestock and humans. It is estimated that 10-30% of the harvested grain is lost due to fungal spoilage and the Food and Agriculture Organisation (FAO) of the United Nations estimates that about 25% of the world food crops are affected by mycotoxins. Economic loss caused by mycotoxins is immense and threatening to human and animal life. They cause unwanted increases in human and veterinary health care costs, reduce crop and animal production, increase the handling and processing requirements, and reduce food and feed nutritional quality. Other costs include disposing contaminated food and feed, and investment in research and applications to reduce mycotoxin contamination in food and feed products. *Fusarium* mycotoxins of significant public health and agri-economic importance include trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid. Trichothecenes, which are considered the main *Fusarium* mycotoxins, are a major contaminant of cereals in temperate climatic zones unlike aflatoxins produced by the genus *Aspergillus* which are common in the tropics. The two common trichothecene mycotoxins found in UK cereals are deoxynivalenol and combined HT-2 and T-2, of which deoxynivalenol is the most frequently encountered. Toxicologically, trichothecenes produce a number of common effects including skin irritation, haemorrhagic lesions and depression of immune response. In humans, the major responses to trichothecene ingestion have been reported to be vomiting, diarrhoea and skin reaction. Deoxynivalenol is one of the most studied *Fusarium* trichothecenes which, when ingested in high doses by farm animals, causes vomiting and diarrhoea; at lower doses, monogastric animals like pigs exhibit feed refusal and weight loss. For this reason, it is variously referred to as a feed refusal factor or vomitoxin. Although more studied and most prevalent in small-grain cereals, deoxynivalenol has been reported to be less toxic than other major trichothecenes (nivalenol, HT-2 and T-2).

Worldwide, *F. culmorum* and *F. graminearum* are considered the most important mycotoxin producers in small grain cereals. Of the two, recently, *F. graminearum* has

become the most commonly occurring *Fusarium* in the UK wheat. Both species are potent producers of deoxynivalenol and to a lesser extent zearalenone, and have been studied extensively in wheat. Wheat has also been shown to be more susceptible to FHB infection than oats and barley. In recent years in the UK and in some other Northern European countries including Norway, Finland and Sweden, high levels of HT-2 and T-2 have been reported in oats produced by a fungus of unconfirmed identity. *Fusarium culmorum* and *F. graminearum* are not producers of HT-2 and T-2. During a previous four year study in the UK, the incidence and concentration of HT-2 and T-2 in oats was found to be high with concentrations greater than 10 ppb in 92% of samples and a combined (HT-2 + T-2) median, mean and maximum concentrations of 213, 570 and 9,990 ppb respectively of all analysed samples. The incidence and concentration of HT-2 and T-2 in barley and wheat were similar, with approximately 1% of the samples exceeding 100 ppb. This clearly indicated that these mycotoxins were more of an issue on oats rather than on wheat and barley, and that oats were probably more susceptible to the fungus responsible for their production. The European Commission (EC) is considering setting maximum levels of HT-2 and T-2 in cereals and cereal products, particularly in oats and oat products, taking into consideration the progress of the scientific knowledge on these toxins in foods which is currently limited. The EC had previously set a combined limit for HT-2 and T-2 for discussion, which for oats was 500 ppb. Each year, between 20-50% of the UK oat crop (2002-2005) exceeded this proposed limit. Oat is an useful crop and the interest in it has continuously been shown since the 1980s when it was reported to be a functional food as it contains soluble fibres (β-glucan) that have been shown to reduce cholesterol promoting healthy lifestyle. Use of oats for human food production in the UK has been increasing steadily in the past 23 years and trend looks to continue in the foreseeable future possibly due to its enhanced image as a healthy life-style food. At least 40% of the UK oat harvest is utilised in the production of oat based foods and the remainder used in animal feed, as seed stock or exported. There is also increased demand for high quality oats in the 'race horse market' whose category includes oats fed to race, show and pleasure horses most of which are owned by affluent people who pay a high price for a high quality commodity believed to be good for their horses. Oats possess several characteristics which make them a useful field crop over other small grain cereals. They tolerate wet weather and acidic soils far better than wheat and barley; they are relatively resistant to foliar diseases and require less agro-chemical and fertilizer input. Oats are often cultivated on land deemed unsuitable for wheat and barley. Therefore, their economic growing cost can

be lower often with gross margins similar to wheat and barley. Oats have been used as a break crop, incorporated in a grain monoculture system because they are the only cereal crop that reduces the incidence of 'take all' (*Gaeumannomyces graminus* var. *tritici*), an important wheat disease. This, in addition to oats high reputation for weed control partly due to its high biomass production and the availability of new varieties has led to a renewed interest in the crop in recent years.

This study aimed to: To isolate, identify and characterise the fungus responsible for the production of HT-2 and T-2 mycotoxins in UK oats, develop assays for quantifying the biomass of this fungus in oat material, investigate possible sources of inoculum for infection and mycotoxin production in oats by this fungus, investigate potential pathogenicity and aggressiveness of this fungus, and determine the epidemiology of the fungus in UK oats.

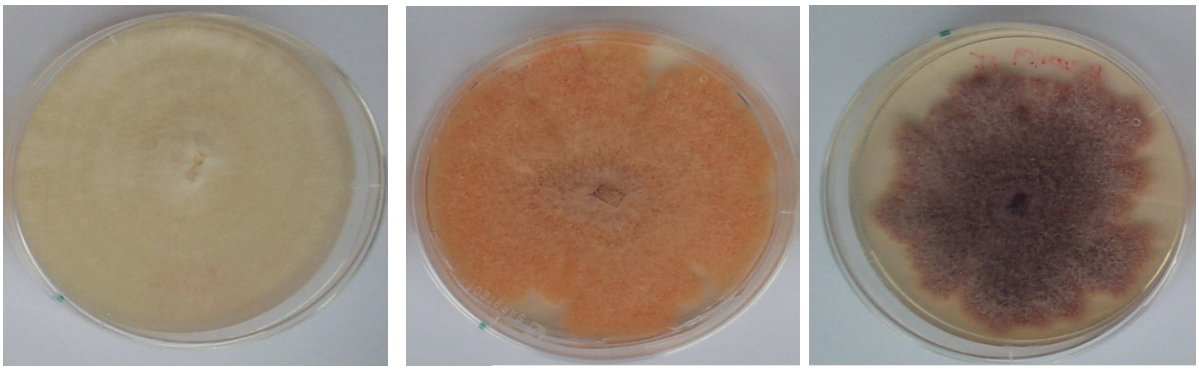
INVESTIGATIONS

Isolation, identification and characterisation of the fungus producing HT-2 and T-2 in UK oats

The first objective of the study was achieved by the use of a laboratory method of fungal isolation from infected grain samples. During isolation, it was borne in mind that the most common *Fusarium* species reported to produce high levels of HT-2 and T-2 in temperate climate was *Fusarium sporotrichioides. Fusarium acuminatum* and *F. armeniacum* have also been reported to produce these mycotoxins though not in as high amounts as *F. sporotrichioides*, they are also not very common. Isolations were carried out from oat samples containing more than 500 ppb and samples containing less than 10 ppb HT-2 and T-2. Isolations were also attempted from wheat samples containing more than 50 ppb HT-2 and T-2. From all the samples, none of the *Fusarium* species implicated in the production of HT-2 and T-2 was isolated except *F. langsethiae*. This is a recently identified species (given a species status in 2004) and which was initially referred to as 'Powdery *Fusarium* poae' due to its production of spores that resembled those of a common species called *F. poae* but with a rather profuse powdery appearance on the growth medium. The fungus was however, not isolated in abundance even in samples highly contaminated with HT-2 and T-2. From oat and wheat samples containing greater than 500 and 50 ppb HT-2 and T-2 respectively, *F. langsethiae* was isolated from 5-10% of the grain. No *F. langsethiae* was isolated from oat samples containing less than 10 ppb HT-2 and T-2. The fungus identity was confirmed by polymerase chain reaction (PCR) technique, a method that does not rely on the growth characteristics of the fungus on artificial media but on its DNA presence. On studying *in vitro* growth characteristics of *F. langsethiae*, it did seem to have a slow growth rate which may make it less competitive in an environment in which more aggressive *Fusarium* species exist. This may explain why its recovery from highly infected grain samples was low during isolation from grain. However, *F. langsethiae* isolates did share certain attributes with other common *Fusarium* species such as *F. sporotrichioides* and *F. poae*. These include different shades of colony pigmentation and an optimum growth rate temperature of about 24°C. An example of *F. langsethiae* colony growth characteristics and growth curve on an artificial medium is shown in **Figures 1** and **2** respectively. The study found that *F. langsethiae* isolates exhibits different colony colour shades which enabled categorisation into three groups; white, orange and purple colonies. All colonies were

characterised by 'powdery' appearance on the growth medium surface. The optimum growth rate temperature of around 24°C is common in summers when the UK cereal crops are in flower, indicating the temperature is ideal for the fungus when infection is likely to occur and contaminate cereals with HT-2 and T-2 mycotoxins.

(a) White-entire margin **(b)** Orange-lobed margin **(c)** Purple-lobed margin



Isolate FI 062/2 Isolate W2001/69(b) Isolate FI 2004/59

Figure 1. Three categories of oat and wheat isolates of *F. langsethiae* based on colony colour. These isolates were grown on potato dextrose agar at 25°C for 9 days.

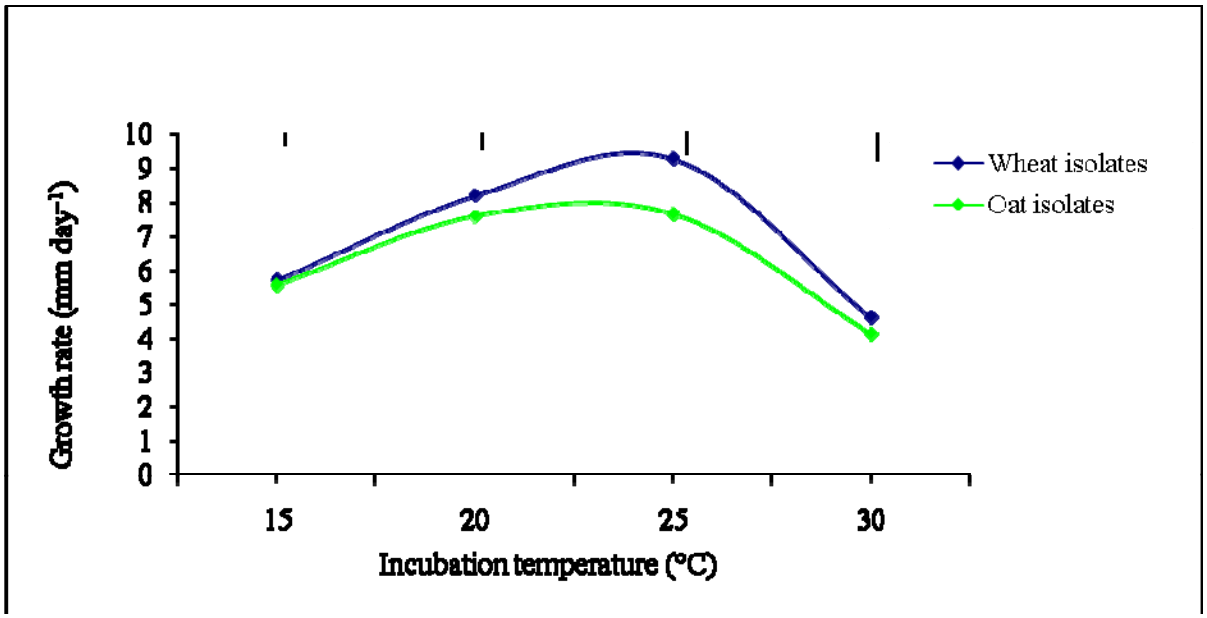


Figure 2. Effect of temperature on the growth rate of *F. langsethiae* isolates obtained from oat and wheat grains containing high HT-2 and T-2 mycotoxins. Values are the

means of four replicates. Bars represent least significant difference (LSD (5%)). Growth rate was calculated 6 days after incubation.

Development of assays for quantifying *F. langsethiae* biomass in plant material

Two quantitative assays for determining the level of infection by *F. langsethiae* in plant material were successfully developed fulfilling objective two of the study. Development of quantitative assays is important as many cereal diseases occur as a result of mixed infections, for example, infections of many *Fusarium* species have been reported together with other foot rot pathogens from *Oculimacula* and *Microdochium* species. As a result of this, visual diagnosis and relative quantification of a given species and its contribution in disease development is often hampered. Quantification of FHB in the field has depended on visual disease assessment for a long time but reliance on visual symptoms is difficult to distinguish FHB symptoms caused by a given fungus as well as quantify the level of the disease resulting from a particular pathogen where several pathogens are involved. In addition, some fungi may grow as saprophytes without causing any disease symptoms probably because they are weak pathogens, yet they may contaminate cereals with mycotoxins while others may cause severe disease symptoms but produce no mycotoxins e.g. *M. nivale*. Difficulties of obtaining accurate results with these traditional methods is also compounded by the fact that there is lack of selective media for most of the pathogens and as a result, accurate determination of the amount of each pathogen may not be possible as some species out-compete others on the medium. These factors may lead to problems of incorrectly identifying species present or a disease which may result in the adoption of incorrect and poorly timed disease prevention measures. To overcome these problems there was an urgent need for the development of rapid, sensitive and specific diagnostic and quantification assays to determine the extent of specific fungal colonisation, as measured by pathogen DNA concentration in plant material. The assays developed were quantitative competitive PCR (QC-PCR) and real-time PCR. QC-PCR involved the construction of a competitor oligonucleotide (internal standard (IS)). This IS contains the same primer binding sites as the target DNA but the two are distinguishable in size from each other on an agarose gel after electrophoresis. When a serial of fungal standard dilutions are amplified with a fixed concentration of the IS, ratios of their gel band intensities can be used to construct a standard curve whose equation can be used for estimating the fungal DNA in the unknown sample which is a measure of the level of infection or

colonisation by the fungus. An example of a standard curve obtained in one of the runs is shown in **Figure 3**.

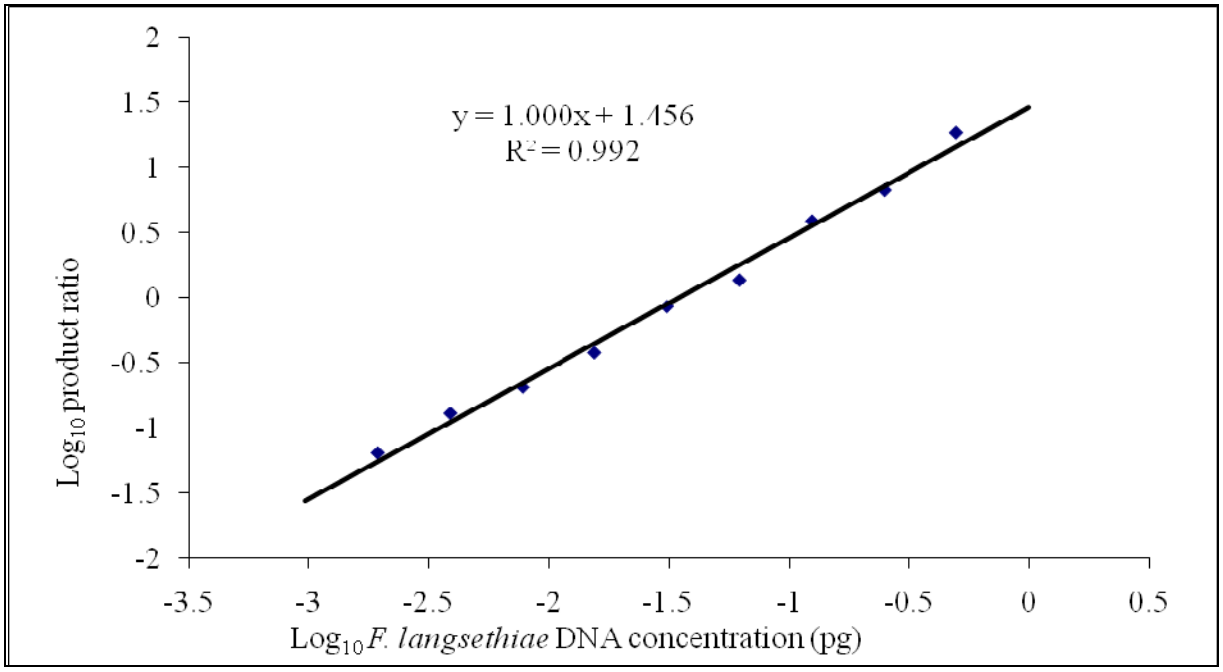


Figure 3. Relationship between the amount of *F. langsethiae* DNA and the resulting PCR product ratio (target/standard).

Real-time PCR is a quantification technique that allows each cycle of DNA amplification to be observed on a computer screen throughout the sequence of thermal cycling, hence the designation 'real-time'. Real-time PCR enables calculation of the amount of PCR product present in a sample at a point of the reaction in which strict exponentiality of DNA amplification is given. This is a point at which all reagents are fresh and available, where the kinetics of the reaction push it to favour doubling of PCR products, therefore there is no variability experienced compared with traditional PCR, which is an end-point technique. A standard curve is prepared using a series of amplifications with known concentrations of target fungal DNA from which a real-time PCR software automatically computes the fungal DNA concentration within the unknown samples. An example of a real-time PCR standard curve in one of the experimental runs is shown in **Figure 4**.

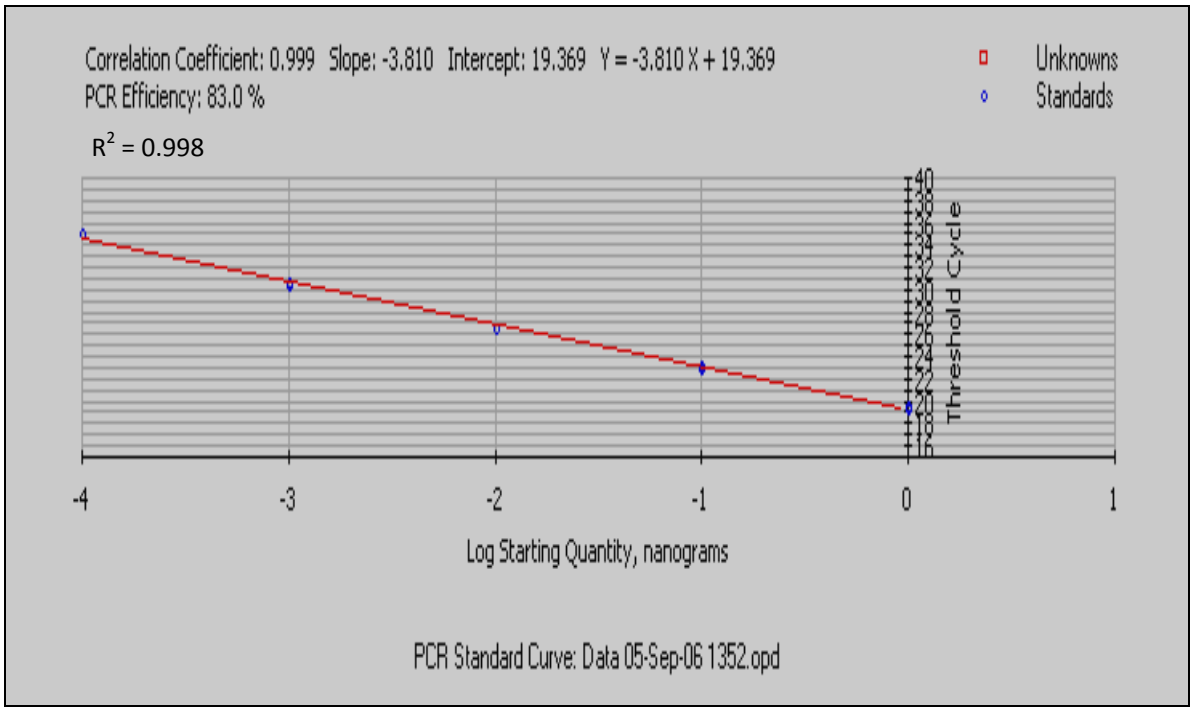


Figure 4. Logarithmic concentration of *F. langsethiae* DNA versus threshold cycle number.

Both QC-PCR and real-time PCR assays developed in this study offer comparable accuracy on quantification of *F. langsethiae* DNA in plant material as they gave similar coefficients of determination (r^2) (0.992 and 0.998 respectively). However, real-time PCR had a wider range of quantification than QC-PCR making it considerably more sensitive than the latter. In addition, elimination of post-PCR processes in real-time PCR reduces assay time making it the method of choice for analysis over QC-PCR. The assays were very specific for the quantification of *F. langsethiae* as there was no amplification of any other closely related *Fusarium* species in terms of spore morphology (*F. poae* and *F. sporotrichioides*) as well as in terms of mycotoxin profile (*F. sporotrichioides*, *F. acuminatum* subsp. a*rmeniacum* and *F. acuminatum* subsp. *acuminatum*). This means that these assays are robust and valid as tools for the identification of *F. langsethiae* species without any indication of amplification of any other related *Fusarium* species in plant material under the conditions employed during this study.

Determination of possible sources of *F. langsethiae* inoculum and their effects on the levels of HT-2 and T-2 in the harvested grain

Objective three was accomplished by carrying out glasshouse and field experiments using different types of inoculation techniques (glasshouse) and various types of

inoculum (field experiments) under different conditions. The glasshouse experiments were investigating; the effect of spray inoculation at different oat panicle growth stages, with and without bagging on the infection and mycotoxin production by *F. langsethiae,* the effect of point and spray inoculation techniques and spore concentrations on the infection and mycotoxin production by *F. langsethiae* in oats, and the effect of spray inoculation at different spore loads and bagging on infection and mycotoxin production by *F. langsethiae* in oats and wheat. In the glasshouse studies, either spray or point inoculation of a mixture of eleven isolates of *F. langsethiae* was used. Spray inoculation is considered less laborious for large-scale studies and involves spraying a mist of spore suspension on the cereal heads while point inoculation involves injecting a spore suspension into spikelets or florets. In all glasshouse experiments (oats and wheat), *F. langsethiae* infection did not produce FHB (FPB) symptoms. The reason for *F. langsethiae* not being able to cause head blight of oats and wheat is not known but may be because it is a weak pathogen as weak pathogens have been reported to cause little or no disease symptoms on cereals, particularly in oats which is reported to be more resistant to FHB compared with wheat. Infection of oats by less pathogenic species of *Fusarium* may not result in typical FPB symptoms. Apparently, *F. langsethiae* may be behaving as a symptomless endophyte upon infection. The most aggressive *Fusarium* species known to cause significant visual symptoms of FHB in wheat but rarely in oats are *F. culmorum* and *F. graminearum*; therefore, if *F. langsethiae* is less pathogenic than these two, which it seems to be, it is less likely to cause noticeable FHB symptoms in oats. No scientific reports of reaction of oats to *F. langsethiae* have been published to compare these results with. Symptomless infections on oats and wheat caused by *F. langsethiae* as demonstrated in these studies calls for novel measures of infection monitoring to be designed and implemented if correct diagnosis is to be made in the field rather than relying on visual disease symptoms (FHB) as carried out in wheat studies. All inoculation methods employed in the glasshouse studies worked as they all resulted in *F. langsethiae* infection. In general, inoculation and bagging of oats with plastic bags resulted in significantly more infection than inoculation without bagging probably due to increased humidity created by the bagging effect which is necessary for fungal spore germination and tissue colonisation. Mycotoxins HT-2 and T-2 were also generally, found to increase in line with these levels of infection. This demonstrates the importance of relative humidity in the oats infection by *F. langsethiae*. Oat growth stage timing at which the inoculum was applied was not found to have any significant influence on the infection and mycotoxin production implying that infection of oats by

F. langsethiae may occur at any panicle growth stage with none of the growth stages investigated being particularly critical to infection. This is unlike in wheat studies where most of the work carried out and reported in the literature suggests that greatest infection takes place mainly at the flowering stage. Level of infection by *F. langsethiae* can differ depending on the technique of inoculation used. Results obtained in the study clearly indicated that point inoculation was the most effective method for producing greater *F. langsethiae* infection in oats compared with spray inoculation technique. Greater infection caused by point inoculation may be explained by the fact that the inoculum in this method was directly placed in an environment which was more conducive i.e. inside the spikelet glume and close to the florets where moisture required for conidia germination and infection was not limited. On the other hand, spray inoculation would result in the spore mist landing anywhere on the panicle, and most of the conidia may not land on a suitable site for germination and infection particularly because oats panicles are drooping in nature and the spores are less likely to land inside the spikelets where conditions for infection are expected to more favourable. Low humidity, which may limit or inhibit spore germination, is likely to be experienced on rather than inside the spikelets. Point inoculation may mimic the role played by insects in the transfer of inoculum to infection sites in the infested field when they are moving about the plants. More HT-2 and T-2 mycotoxins were produced in the point inoculated panicles than in the spray-inoculated panicles which may be explained by the extent of infection by *F. langsethiae*. Generally, increased inoculum concentration led to an increase in *F. langsethiae* infection in both oats and wheat. This result can be explained by the fact that increasing spore concentration may not only increase the likelihood of a large number of spores landing on suitable infection sites in or on spikelets, but at the same time, it may also increase the number of viable spores required to cause infection. This observation means that, as in other plant infections by various micro-organisms, the extent of colonisation of oats or wheat head tissues by *F. langsethiae* can depend on the amount of naturally available inoculum particularly at the plant development stages when the infection is likely to occur. HT-2 and T-2 were found to increase in line with the level of infection. Overall, the glasshouse studies have demonstrated that the method of inoculum application as well as the inoculum load influences the level of infection and mycotoxin production by *F. langsethiae* in oats. However, glasshouse inoculation experiments failed to achieve the high level of infection and HT-2 and T-2 concentration commonly found in UK commercial oat fields. More research is required to understand how such

high levels of infection and mycotoxin concentration can occur under natural field conditions.

Field experiments employed three types of *F. langsethiae* inoculums; these were in the form of spore suspension, oat grain culture and oat straw culture. Three field experiments were carried out in the 2004/2005, 2005/2006 and 2006/2007 growing seasons. The experiments were investigating; the effect of spray inoculation and misting on oat infection by *F. langsethiae*, the effect of inoculated oat grain as a ground-level source of inoculum and misting on infection by *F. langsethiae* in oats, and the effect of inoculated oat straw and cultivation methods on infection and mycotoxin production by *F. langsethiae* in oats. The three field trials demonstrated that *F. langsethiae*, although it may be part of FHB complex, did not result in typical FHB disease symptoms in the infected oat panicles. A result which confirmed those observed in all the glasshouse experiments. Where reliance on field disease symptoms is depended on, these three trials have shown that it may overlook symptomless infections caused by *F. langsethiae* in oats. Reasons given for lack of disease symptoms in glasshouse experiments also apply to these field experiments. All forms of inoculum applied in the Gerald oat variety in the three field trials had no significant influence in the infection of oats by *F. langsethiae*. Misting was found not to play any significant role in the infection of oats by *F. langsethiae* unlike in wheat studies where it has been demonstrated to promote infection and FHB development caused by *F. culmorum* and *F. graminearum*. The fact that *F. langsethiae* infection did not increase in misted plots means that infection by this fungus is either not dependent on misting/moisture or it is inhibited by it, and that it behaves quite differently from most other FHB pathogens. *Fusarium langsethiae* DNA was however quantified in all the samples at different concentrations. It is not clear why there was no significant infection in the three field trials when types of inoculums commonly used successfully in the study of FHB of wheat (except oat straw culture) and that produces high levels of infection and severe disease only gave low infection in oats by *F. langsethiae*. The following observations though, may explain this scenario; oats and wheat are different plant species and the concentration of spore suspension inoculum capable of causing high infection in oats may necessarily not be able to produce the same level of infection in oats. By increasing inoculum load during oats inoculation, a high infection level in oats may be obtained. *Fusarium langsethiae* infection of wheat under field conditions has never been reported, therefore, it is inappropriate to compare results of *F. langsethiae*-inoculated oats with results of *F. culmorum* and *F. graminearum*-

inoculated wheat, pathogenicity and aggressiveness of the latter two has been thoroughly studied while that of the former is unknown. Low infection where oat grain and oat straw cultures were used may be explained by the fact that around and during the time of flowering and most grain-filling period of the crop, little rainfall was experienced. *Fusarium langsethiae* produces asexual spores which are reported to be best dispersed by rain-splash. Lack of or reduced rainfall around this time meant the ground-level inoculum was not effectively transferred to the infection site during the time of plant susceptibility. There is a possibility that if a high amount of rainfall is experienced during the most critical time of plant susceptibility, more infection by *F. langsethiae* may be observed. The importance of cultural field practices was evident in the field trial 2006/2007 where significant differences were observed among four agronomic practices (minimum tillage with straw incorporated, minimum tillage with straw removed, ploughed with straw removed, and ploughed with straw incorporated). Minimum tilled plots with straw incorporated had significantly more *F. langsethiae* DNA in harvested oat grains than in the other three cultural field practices demonstrating the importance of cultural field practices such as management of previous crop debris and soil tillage in *Fusarium* infection of cereals. This also shows that oats can be affected in the same way as infection of wheat by cultural field practices when it comes to infection by *Fusarium* species. The differences in the cultural practices in this study can be mainly attributed to the method of cultivation. Crop residue can be a source of inoculum for FHB where it can act as a substrate on which the pathogens can survive as saprophytes and be able to infect the crop at its susceptible stage. The way the field is managed determines whether the natural inoculum present in the residue is available for infection or not. Ploughing served to bury the residue, significantly reducing the amount of inoculum available for causing an infection. Minimum tillage on the other hand would result to a considerable amount of debris lying on the soil, which, if it contains natural inoculum may cause greater infection. HT-2 + T-2 mycotoxin concentration was only analysed for 2006/2007 trial. HT-2 and T-2 was not quantified from 2004/2005 and 2005/2006 trials because analysis of random samples of oats from inoculated and uninoculated plots from these trials resulted in levels that were below the quantification limit of the assay (3.5 ppb). Results obtained after mycotoxin analysis from 2006/2007 trial showed that neither inoculation nor agronomy influenced HT-2 and T-2 significantly. However, a trend of mycotoxin contamination level was evident which depended on method of cultivation employed. The trends in HT-2 and T-2 concentration were similar to that reported in this trial concerning infection as determined by *F. langsethiae* DNA concentration. Oat

grains from ploughed plots were less contaminated with HT-2 and T-2 compared with those from minimum tilled plots. This suggests that crop debris is important in the epidemiology of the HT-2 and T-2 producing *Fusarium* species. It appears that factors predisposing oats to infection by *F. langsethiae* also influence mycotoxin contamination in the grains in the same way and that the higher the level of infection, the higher the likelihood of quantifying high levels of mycotoxins in the grains. The three oat field trials have shown that all methods of inoculation which on wheat give severe FHB and high levels of mycotoxins by *F. culmorum* and *F. graminearum* failed to produce high infection of oats by *F. langsethiae* and its HT-2 and T-2 mycotoxins. They also failed to produce FHB disease symptoms in oats. It is unclear as to why methods of inoculation which result in high levels of DON in wheat when inoculated with *F. graminearum* and *F. culmorum* failed to achieve high levels of HT-2 and T-2 in oats when inoculated with *F. langsethiae* when high levels (HT-2 + T-2 greater than 1,000 ppb) regularly occur in commercial crops.

Determination of potential pathogenicity and aggressiveness of *F. langsethiae* on detached leaf assays and seedlings of oats and wheat

The pathogenicity and aggressiveness of *F. langsethiae* was determined by carrying out a detached leaf pathogenicity assay and a seedling blight study. The objectives of the detached leaf pathogenicity assay were; to investigate the pathogenicity and aggressiveness of oat and wheat isolates of *F. langsethiae* on detached leaves of oat and wheat relative to isolates of known *Fusarium* and *Microdochium* species, to find out whether host preference existed between oat and wheat isolates of *F. langsethiae*, and to determine the aggressiveness of *F. langsethiae* isolates on detached leaves of different oat and wheat varieties. To achieve this, four experiments were carried out. Two of these experiments were carried out using a mixture of isolates while the other two were carried out using single isolates of *F. langsethiae* on wounded or unwounded oats and wheat leaves. All fungal isolates used as a mixture on wounded oats and wheat leaves were found to be pathogenic but differed in their aggressiveness on the leaves upon infection. Oats and wheat isolates of *F. langsethiae* had the same degree of aggressiveness on both oats and wheat wounded leaves. They were also found to be more aggressive on oat than on wheat leaves. On the unwounded oats and wheat leaves, *F. langsethiae* isolates from both oats and wheat were found to be pathogenic to oat leaves only. There was no significant difference observed in aggressiveness between composite mixtures *F. langsethiae* isolated from oat and wheat in the experiments, implying that they are probably from the same population of *F.*

langsethiae. For both wounded and unwounded leaves, representative lesion characteristics are shown in **Figures 5a** and **5b**.

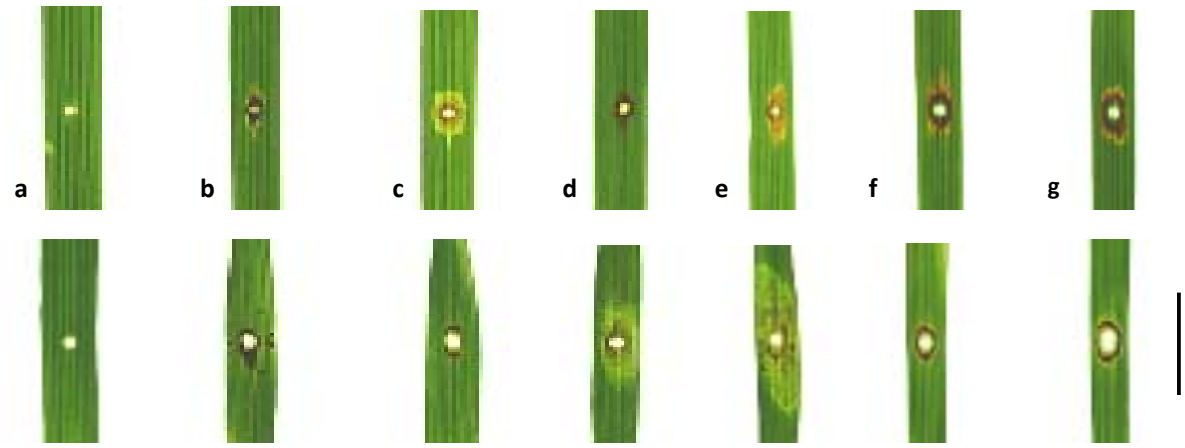


Figure 5a. Wounded detached leaves 6 days post-inoculation at 20°C, a representation of replicates assessed; a, b, c, d, e, f and g are oat leaves treated with sterile distilled water (SDW), *F. culmorum*, *F. poae*, *M. majus*, *M. nivale*, *F. langsethiae* (oat isolates) and *F. langsethiae* (wheat isolates) respectively. Corresponding wheat leaves are below. Bar = 20 mm.

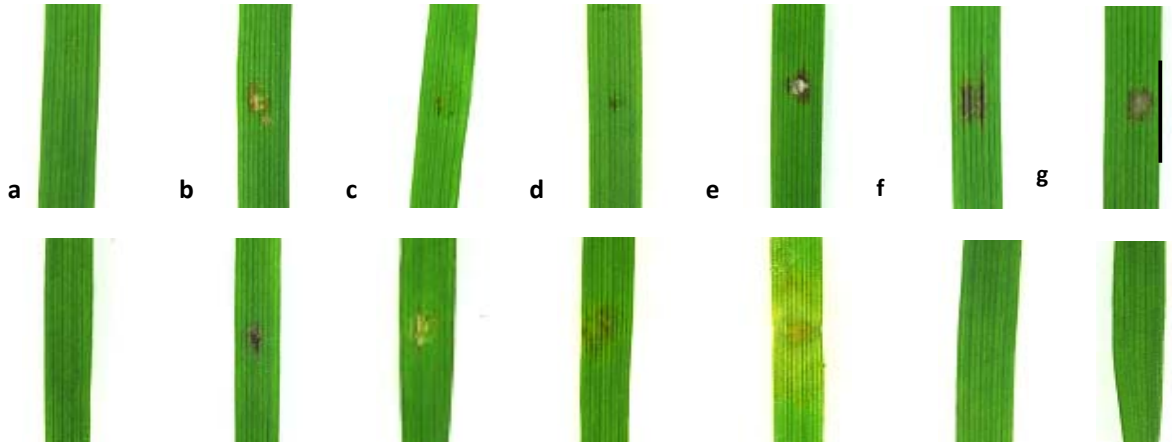


Figure 5b. Unwounded detached leaves 7 days post-inoculation at 20°C, a representation of replicates assessed; a, b, c, d, e, f and g are oat leaves treated with SDW, *F. culmorum*, *F. poae*, *M. majus*, *M. nivale*, *F. langsethiae* (oat isolates) and *F. langsethiae* (wheat isolates) respectively. Corresponding wheat leaves are below. Bar = 20 mm.

Gerald and Claire were used in the initial experiments as these were the two most popular winter oat and wheat varieties in the UK at the time of the study. When differences were highlighted between these two cereals it was necessary to determine if

this difference was consistent across a number of wheat and oat varieties. Three wheat varieties were selected based on the range of head blight resistance available in UK varieties for a further study using *F. langsethiae* (oats and wheat) isolates singly. Claire, Malacca and Ambrosia have a resistance rating of 7, 5 and 3 respectively; scale 1-9, 9 being resistant. There are currently no head blight resistance ratings for oat varieties although HT-2 and T-2 content has been quantified from winter oat variety trials. Gerald, Kinross and Millennium were selected as they represented varieties with high, moderate and low HT-2 and T-2 concentrations within these trials respectively. Lesion sizes on wheat leaves were not consistent with the wheat varieties resistance scores in the wounded detached leaf assay in that Claire with a FHB resistance rating of 7 developed longer lesions than Malacca which has a resistance rating of 5. However, the trends in lesion lengths obtained from this study for the three oat varieties were consistent with their reported differences in HT-2 and T-2 accumulation. The results from detached leaf assays suggest that *F. langsethiae* may be a pathogen of oats and wheat. The results indicate that *F. langsethiae* could be a greater problem on oats than on wheat which agrees with HT-2 and T-2 concentrations found in commercial crops of wheat and oats in the UK. However, it must be remembered that other plant characteristics, such as the large morphological differences in oat and wheat head structure, will also have a major impact on their susceptibility to head blight pathogens. The lack of symptoms on heads of cereals would indicate that either head blight resistance results in superficial saprophytic growth of *F. langsethiae* or that symptom expression does not occur resulting in a 'hidden' infection in the field. Clear symptoms in the detached leaf assay may correlate to varietal resistance in oats to *F. langsethiae*. More studies with increased number of oat varieties are required to confirm that varietal resistance of oats to *F. langsethiae* is correlated to lesion size in an *in vitro* assay, and if this is the case, these assays could be useful in predicting the susceptibility of oat varieties to infection by this fungal species. This in turn would help chose varieties less susceptible to *F. langsethiae* which can help limit HT-2 and T-2 mycotoxins in the harvested grain.

The potential pathogenicity of *F. langsethiae* was evaluated in an oats and wheat seedling blight study. The objectives of this study were; to investigate whether *F. langsethiae* was pathogenic to oats and wheat seedlings, to investigate whether isolates of *F. langsethiae* from oats and wheat differed in their pathogenicity towards wheat and oats seedlings, and to determine whether *F. langsethiae* pathogenicity in oats and wheat differed from that of other fusarium seedling blight pathogens. The results

obtained showed that oats and wheat isolates of *F. langsethiae* were not pathogenic to both oats (variety Gerald) and wheat (variety Claire) seedlings. This is because oat and wheat *F. langsethiae*-inoculated seeds gave rise to seedlings that were healthy-looking, growing with vigour and that did not differ visually from those of uninoculated controls at the temperatures used in the study (both 5 and 15°C). They consequently resulted in normal healthy-looking seedlings growing with vigour. This observation was similar to that obtained from both oat and wheat seedlings resulting from the uninoculated control seeds and applied to results obtained at both 5 and 15°C. Of the fungal species that resulted in seedling blight disease, *F. culmorum* was the most aggressive followed by *M. nivale*. Representative seedling characteristics at the time of disease assessment are shown in **Figure 6**.

(a)

(b)

(c)

(d)

(e)

(f)

Figure 6. Seedling blight disease manifestation by isolates of *Fusarium* and *Microdochium* spp. on oat and wheat seedlings grown at 15°C and assessed at Zadoks GS 12. *(a)* and *(b)* are oat and wheat seedlings respectively resulting from *F. langsethiae*-inoculated seeds, *(c)* and *(d)* are oat and wheat seedlings respectively resulting from *M. nivale*-inoculated seeds, *(e)* and *(f)* are oat and wheat seedlings respectively resulting from *F. culmorum*-inoculated seeds. Bar = 40 mm.

Symptoms of seedling blight disease observed caused by the pathogenic fungi were pre-emergence death, post-emergence death and development of lesions along seedlings mesocotyl and coleoptile regions which appeared to differ greatly with the type of fungal species in question. These results indicates that *F. langsethiae* may not be a seedling pathogen of oats and wheat as there was no significant seedling blight disease observed when oats and wheat seedlings resulting from *F. langsethiae*-inoculated seeds were compared with uninoculated controls. These results have also shown that both oat and wheat isolates of *F. langsethiae* had no host preference, as their percentage disease indices were statistically similar at each of the temperatures employed for both hosts. This study has demonstrated that since *F. langsethiae* may not be an important seedling blight disease-causing fungus of oats and wheat, it is unlikely to lead to reduced crop stand in the field (through pre- and post-emergence death of seedlings), and therefore, yield if there is no subsequent foot-rot and/or head blight in case infected seeds are sown. The results have also identified that seedling blight may not be a part of the disease cycle for *F. langsethiae*. It is therefore important to identify the source of *F. langsethiae* inoculum for wheat ears and oats panicles. Though *F. langsethiae* was not found to be pathogenic towards oat and wheat seedlings, more studies need to be carried out under varied environmental conditions using artificially and naturally infected grain of a wide range of oats and wheat varieties to confirm these results.

Epidemiological study investigating occurrence of fusarium panicle blight of oats, *F. langsethiae* infection and its related mycotoxins in ten commercial oat fields

Epidemiological study investigating the occurrence of fusarium panicle blight, *Fusarium langsethiae* infection and its related mycotoxins HT-2 and T-2 was carried out in ten commercial fields in Shropshire and Staffordshire counties during the 2006/2007 growing season. The fields were assessed for natural disease prevalence and *F. langsethiae* infection between 24th April and 2nd August 2007 when samples were taken from a pre-determined location within each field at a given growth stage. For all the fields, samples were taken at Zadoks growth stages (GS) 32/33, 69, 77-85 and 90-92 (second node detectable/third node detectable, anthesis complete, late milk-soft dough, and caryopsis hard respectively) for laboratory analyses. In addition to quantification of *F. langsethiae* biomass within sample material, panicle samples collected at GS 69, 77-85 and 90-92 were also tested and quantified for HT-2 and T-2 mycotoxins. For all the fields, at all the four sampling times, no FHB disease symptoms were observed but *F. langsethiae* was detected and quantified from various plant parts (dead and live leaves and panicles) at different levels. This confirmed the earlier studies (glasshouse and field experiments) where the fungus was not found to cause any disease symptoms upon infection. *Fusarium langsethiae* was also detected and quantified in weeds originating from only one field which shows the potential of weeds acting as a source of the inoculum reservoir for this fungus. Panicle material from all the ten fields sampled at the three growth stages (GS 69, 77-85 and 90-92) produced different levels of *F. langsethiae* DNA. Generally, across all fields, oats infection by *F. langsethiae* seemed greatest at GS 77-85 and least at GS 69 (**Figure 7**). Lower *F. langsethiae* biomass at GS 69 (anthesis complete) may be as a result of insufficient time for the fungus to grow and spread within the spikelets, assuming *F. langsethiae* infection in oats takes place at anthesis as reported for *Fusarium* species in wheat, and that natural inoculum is available at that time. On the other hand, at GS 77-85, the fungus is expected to have had time to grow and multiply (if infection took place at anthesis) increasing its biomass within the plant material. It may also be that there is an extended infection period by *F. langsethiae* where infection peaks after anthesis. No clear pattern of infection was apparent with the method of cultivation or previous crop.

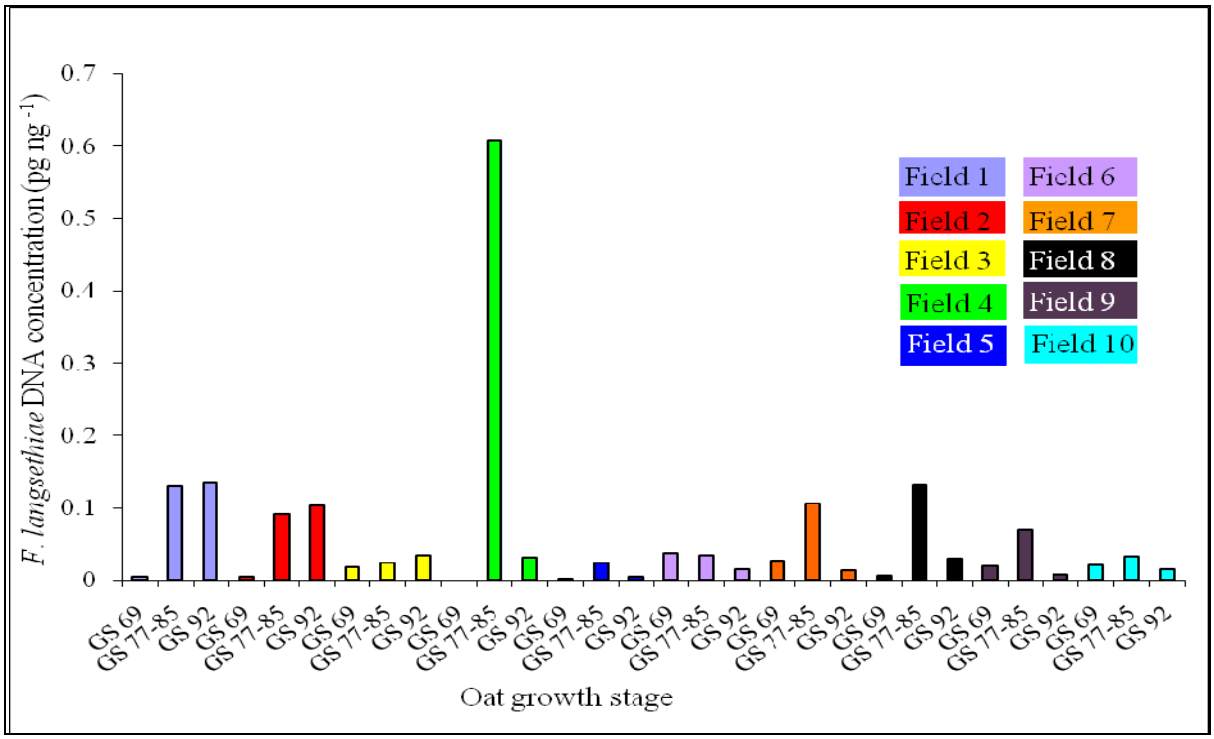


Figure 7. *Fusarium langsethiae* DNA concentration of oat spikelets at three growth stages for ten commercial oat fields.

In all the fields and at all growth stages, HT-2 and T-2 mycotoxins were quantifiable in spikelet samples but at different levels (**Figure 8**). HT-2 and T-2 concentration seemed to follow the same trend as that which was observed with *F. langsethiae* DNA concentration. In six out of ten fields (2, 4, 6, 7, 8 and 9), these mycotoxins were observed to be at higher concentrations at GS 77-85 than at any other growth stage. They were least concentrated in GS 69 samples, peaked at GS 77-85 and decreased again at harvest. At harvest, seven out of ten samples were found to be contaminated by HT-2 and T-2 at a concentration greater than 500 ppb while four out of the ten samples accumulated levels greater than 1,000 ppb. With a potential European legal limit of 500 ppb in oats, this would indicate that a large proportion of UK oats could not enter the market as the level of contamination would be unacceptable.

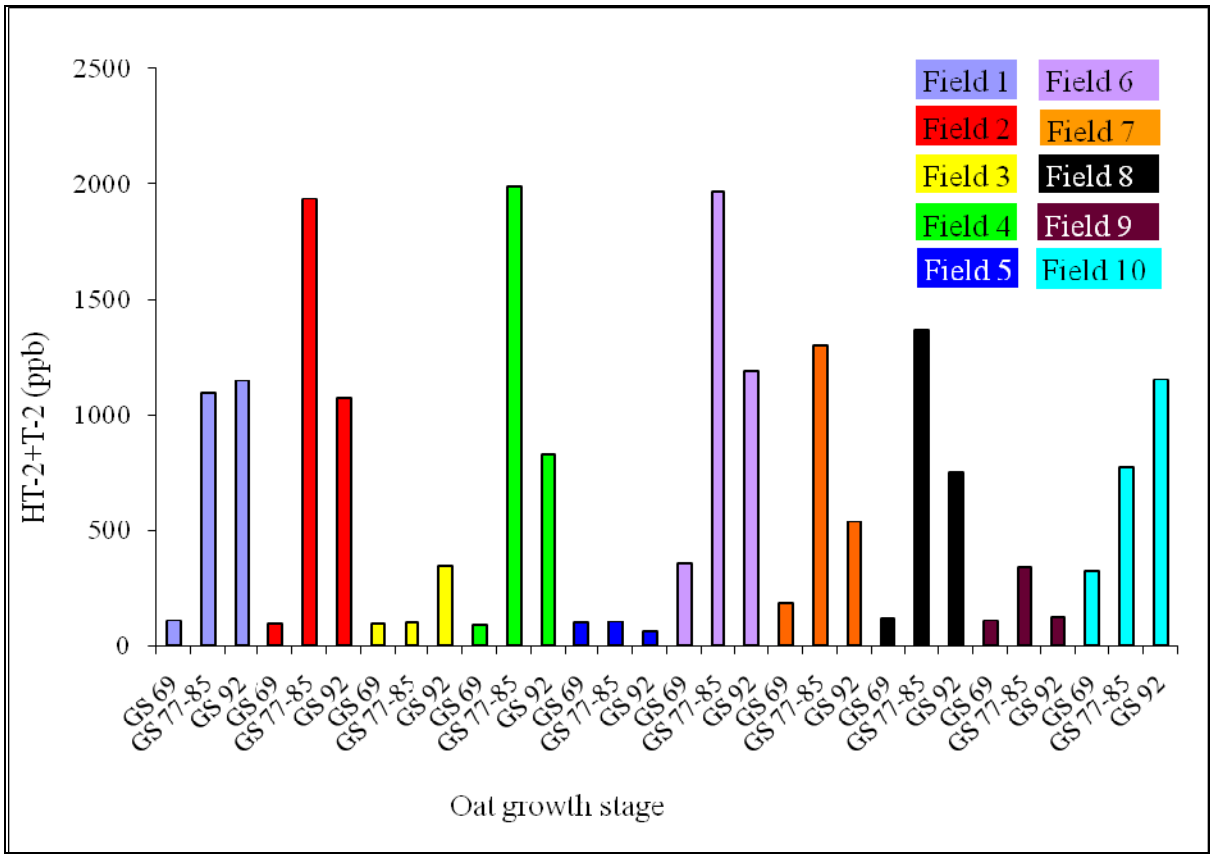


Figure 8. HT-2 and T-2 concentration of spikelet samples at three oat growth stages for the ten commercial oat fields.

A relationship between *F. langsethiae* DNA and HT-2 and T-2 mycotoxin concentrations was observed upon carrying out a regression analysis. The overall correlation between HT-2 + T-2 and *F. langsethiae* DNA concentration was reasonably good and the regression was highly significant ($p<0.001$) (**Figure 9**). A coefficient of determination (r^2) of 0.546 was obtained which signified that 54.6% of the total variation in mycotoxin concentration could be explained by the amount of *F. langsethiae* DNA quantified. The regression also shows that prediction of HT-2 and T-2 mycotoxin contamination in oats can be made with reasonable accuracy when *F. langsethiae* DNA concentration is known. This is important as it can help reduce costs of carrying out both mycotoxin and fungal DNA analyses.

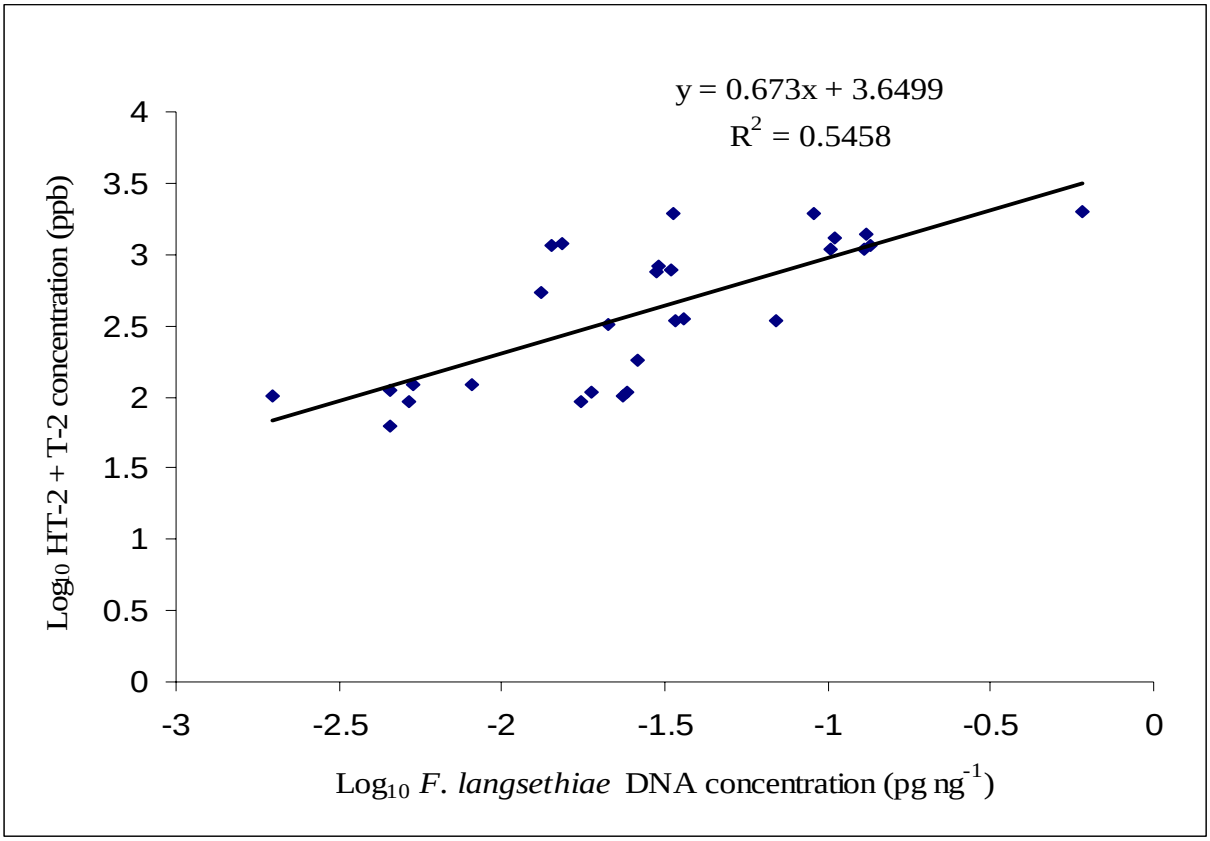


Figure. 9. Regression of HT-2 + T-2 on *F. langsethiae* DNA concentration.

CONCLUSIONS

This study has demonstrated that *F. langsethiae* may be a widespread fungus in UK oats that can infect the crop and go unnoticed as it causes no disease symptoms on infected spikelets. *Fusarium langsethiae* may not interfere with oat yield but may result in indirect economic losses where the grain is rejected or downgraded at marketing as a result of high levels of contamination with HT-2 and T-2 which may be a human food and animal feed safety hazard. The likely source of natural inoculum of *F. langsethiae* and the route it takes to infect the oat panicles is currently not clear and requires an urgent investigation to improve on the understanding of this fungus epidemiology to help limit its metabolites in harvested grain. This would also ensure that the UK oats meet the legal limit for HT-2 and T-2 once this is set by the EC and ensure food and feed safety of processed oat products. Without concern for HT-2 and T-2 contamination in cereals, then *F. langsethiae* appears not to be an issue in oat production.