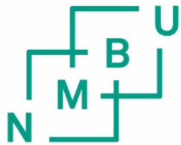

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Fusarium poae and *Fusarium langsethiae* in an oat field- time point of infection and possible inoculum sources

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Abstract

Fusarium head blight in cereals is a global issue, threatening food production and food security. Most *Fusarium* head blight organisms are able to produce a wide range of mycotoxins, posing human- and animal health risks related to consumption. However, over the past decades the prevalence of the two trichothecene producing *Fusarium* species *F. poae* and *F. langsethiae* has increased in small grain cereals in European and Nordic countries. Despite the increased prevalence of these problematic *Fusarium* species contaminating cereal grains, little is known about their biology.

Oat plants collected during two consecutive growth seasons, 2017 and 2018, from a Norwegian oat field were analysed for the presence of *F. poae, F. langsethiae, F. avenaceum, F. graminearum, F. culmorum* and *F. sporotrichioides*. Oat plants were collected weekly to examine the time point of initial infection of *F. poae* and *F. langsethiae* in the field. In accordance with other studies, our observations indicate that initial infection of oat plants by *F. poae* and *F. langsethiae* occur prior to flowering, supporting the hypothesis of their biology differing from other *Fusarium* head blight pathogens, infecting cereals around flowering. To our knowledge, this is the first field study analysing the relationship between abnormal oat plants and infection of *F. poae* and *F. langsethiae*. Abnormal oat plants were significantly more infected with *F. poae* and *F. langsethiae* than normal oat plants.

Oat grains harvested both years, were morphologically- and molecularly analysed for presence of *Fusarium* DNA, in addition to mycotoxin analyses.

The gramineous weed *P. annua* was investigated as possible source of *F. poae* and *F. langsethiae* inoculum. *Fusarium* DNA in *P. annua* plants was quantitatively assessed by running species-specific qPCR analysis. Low levels of *F. poae* DNA and negligible levels of *F. langsethiae* DNA were observed in *P. annua* plants throughout the growth season.

Analysis of *Fusarium* DNA in air samples provided new information of high levels of *F. poae* and *F. langsethiae* DNA in air samples, around ripening, which emphasizes the possibility of inoculum being dispersed by air.

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Introduction

The ever-expanding world population, projected to reach 9 billion people by 2050, pressures global food production which have to increase in order to meet the future demand (Godfray et al., 2010). To meet this demand, the global production of crop products must increase by 70-110% within 2050 (Godfray et al., 2010; Tilman et al., 2011). Also, in Norway the cereal production must increase with 20% by 2050 in order to maintain the current self-sufficiency rate, ranging between 40-50% (Løvberget, Rognstad and Steinset, 2016; Stabbetorp, 2018). To increase global and local food production to meet the future demand is an immense challenge, jeopardized by factors beyond our control. The most important- weather and climate change.

In Norway about three percent of the land area is used for agricultural purposes, and 90% of this area is used for feed production (Løvberget et al., 2016). Over the past decades, oats (*Avena sativa*) has been the third most important food and feed crop in Norway after wheat and barley. Human consumption of oat products is recommended because of the beneficial health effects related to oat consumption (Andon and Anderson, 2008), nevertheless the vast majority of Norwegian oats is used as animal feed. The Swedish, late maturing, oat cultivar Belinda has for several years been the most cultivated oat variety in Norway (Russenes et al., 2018). A decade ago Belinda owned over 60% of the Norwegian market share in the period from 2005-2009 (Åssveen, 2016), however the market shares of Belinda have decreased, owning <20% in 2018 (Russenes et al., 2018). One reason for the drop in Belinda cultivation might be attributed to its low resistance towards the persistent fungal plant pathogen *Fusarium* (Åssveen, 2016).

The *Fusarium* genus and the *Fusarium* disease cycle

The *Fusarium* genus covers a large span of plant pathogenic fungi, able to cause severe epidemics on a wide range of crop plants worldwide (Parry, Jenkinson and Mcleod, 1995). In 2008, Leslie and Summerell reviewed The American Phytopathology Society's list of plant pathogens associated with the economically important plants and revealed that 81 out of the 101 listed plants were susceptible to at least one *Fusarium* disease. For instance, all members of the Poaceae (or Gramineae) family, which include all cereals, are susceptible to *Fusarium* infection (Parry et al. 1995). Small grain cereals are frequently associated with the damaging *Fusarium* diseases: *Fusarium* seedling blight, foot rot and the highly problematic *Fusarium* head blight (also named *Fusarium* ear blight or "scab") (Ruckenbauer, Buerstmayr and Lemmens, 2001). *Fusarium* head blight disease in small grain cereals can be caused by at least

17 different organisms (Parry et al., 1995). However, *Fusarium* head blight disease in small grain cereals is mainly associated with four *Fusarium* species: *F. graminearum, F. culmorum* and *F. avenaceum, F. poae* (Parry et al., 1995). In addition to these *Fusarium* species, *F. langsethiae* is commonly occurring in oats in the Nordic countries (Kosiak et al., 2003; Parikka et al., 2008; Fredlund et al., 2010; Nielsen et al., 2011; Hofgaard et al., 2016a; Karlsson et al., 2017).

Fusarium species are plant pathogenic fungi of global importance. A survey ranking the Top 10 most important plant pathogenic fungi based on economic and scientific impact carried out for the *molecular plant pathology* journal mentioned two *Fusarium* species as the most important (Dean et al., 2012). These two *Fusarium* species were *F. graminearum* and *F. oxysporum* respectively, ranked as the 4th and 5th most important fungal plant pathogens globally. *F. graminearum*, ranked as the 4th most important fungal plant pathogen, is the main *Fusarium* head bight causing organism in small grain cereals in many parts of the world (Goswami and Kistler, 2004). For this reason, *F. graminearum* is one of the most extensively studied fungal plant pathogens (Goswami and Kistler, 2004). Because of the extensive research on the biology and epidemiology of *F. graminearum*, its lifecycle is well understood making it the model species explaining the disease cycle of the *Fusarium* genus (Fig. 1).

Most *Fusarium* pathogens are anamorph, only producing asexual spores (conidia), while some *Fusarium* species also reproduce sexually, producing sexual ascospores. This is true for the widespread *F. graminearum*, which rely on both conidia and ascospores for spore dissemination and inoculum production. Spore dispersal pattern of conidia and ascospores varies with conidia predominantly being splash- or water dispersed and ascospores mainly dispersed by wind (Jenkinson and Parry, 1994b; Hörberg, 2002). Furthermore, the initial source of inoculum varies between *Fusarium* species with inoculum being resting chlamydospores in the soil, spores and saprophytic mycelium on plant debris or *Fusarium* infested seeds (Parry et al., 1995; Goswami and Kistler, 2004). Furthermore, *Fusarium* species has been isolated from a wide range of insects which suggests that they might play an important role in transmission of *Fusarium* inoculum (Parry et al., 1995; Drakulic et al., 2017).

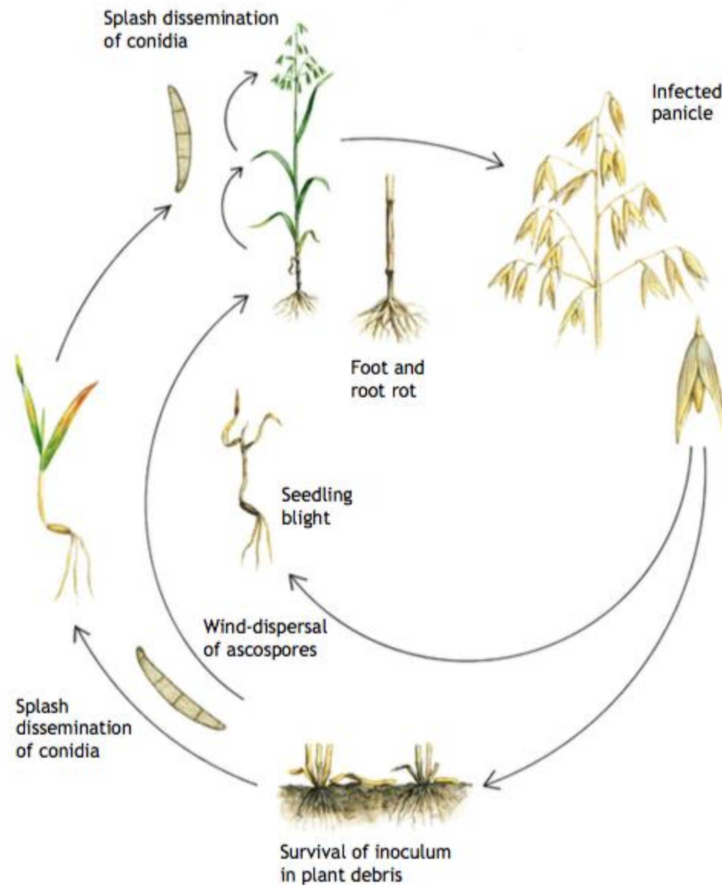

Figure 1. Life cycle of Fusarium in oats. (Brodal et al., 2009)

The saprophytic abilities of *Fusarium* species varies. For instance, *F. avenaceum*, *F. graminearum* and *F. culmorum* can survive on plant debris for two to three years, or until the plant material is fully decomposed (Pereyra and Dill-Macky, 2008). Oppositely, the saprophytic ability of *F. langsethiae* is not well known and subject to further research (Imathiu et al., 2013b).

There is little evidence that sowing of *Fusarium* infected seeds lead to major primary inoculum production causing head blight infection, however it might contribute to introducing *Fusarium* species to new areas (Leonard and Bushnell, 2003 p.92).

Seedlings that survive *Fusarium* infection are more exposed to develop foot- and root rot which weakens the straw and may result in subsequent lodging. *Fusarium* spores might then be formed at the stem- and node base of the lodged plants, which further can be spread to cereal heads by rain splash and cause *Fusarium* head bight (Fig. 1).

The general *Fusarium* head blight symptoms are similar for all small grain cereals. Initial infection can often be seen as small brown or water-soaked spots on the glume and rachis (Parry

et al., 1995; Osborne and Stein, 2007). Furthermore, bleaching of infected cereal heads are quite common, giving an early-ripe appearance. At late infection stage, towards grain maturity, masses of conidia (sporodochia) may be produced on the cereal heads giving the spikelets a distinctive salmon-pink colour. Shrinking of the grains is also a common symptom of *Fusarium* head blight infection. Expression of *Fusarium* head blight symptoms are less severe in oats than for wheat and barley. However, heavily infected oat heads might show symptoms like bleaching and formation of salmon-pink sporodochia at the base of the spikelet (Tekle et al., 2012).

Mycotoxin contamination of cereals caused by *Fusarium* species

In addition to causing plant disease, the majority of *Fusarium* head blight causing organisms are associated with mycotoxin production (Mcmullen, Jones and Gallenberg, 1997). Unfortunately, two thirds of the world cereal production are highly affected by *Fusarium* mycotoxin contamination, posing a risk to human and animal health (Miller 1994; Botallico and Perrone, 2002). As a consequence, mycotoxin contamination of cereal grains has become one of the biggest and most challenging problems plant pathologists are facing today (Edwards 2004; Savary et al., 2012).

The *Fusarium* head blight causing species produce a wide range of mycotoxins and each fungal species possesses different mycotoxin profiles (Botallico and Perrone, 2002). The most commonly found mycotoxins associated with *Fusarium* head blight pathogens belongs to the large group of compounds called trichothecenes (Anon, 2002; Stenglein, 2009). Trichothecenes are divided into type A- or type B trichothecenes because of their structural differences, where type A mycotoxins are more toxigenic than type B (Langseth and Rundberget, 1999; Edwards, 2004; Van der Fels-Klerx and Stratakou, 2010). In addition to thrichothecenes, *Fusarium* head blight pathogens are associated with production of a wide range of mycotoxins belonging to different chemical groups including Zearalenone (ZEN), Fumonisins (FUM), Enniatins (ENN), Beauvericin (BEA) and Moniliformin (MON) (D'mello et al., 1999; Bottalico, 1998). The most frequently found trichothecenes in Norwegian small grain cereals are Deoxynivalenol (DON), Nivalenol (NIV), T-2 and HT-2 (Bernhoft et al., 2012; Hofgaard et al., 2016a).

DON and its associated derivatives belong to the type B trichothecenes and is the most prevalent mycotoxin contaminating cereals in Norway (Sundheim et al., 2017) and worldwide (JECFA, 2001). Two *Fusarium* species are predominantly associated with DON production; *F.*

graminearum and *F. culmorum* respectively (Parry et al., 1995; JECFA, 2001). Another frequently found mycotoxin contaminating cereal grains is NIV, a type- B trichothecenes (JECFA, 2001). Several *Fusarium* species are known NIV producers, but *F. poae* is often pointed out as the main NIV producer (Langseth and Rundberget, 1999; Bottalico and Perrone, 2002; Thrane et al., 2004). In addition to NIV, *F. poae* is known to produce a wide range of other mycotoxins including BEA, ENNs (Bottalico and Perrone, 2002) and the type A trichothecenes T-2 and HT-2 (Thrane et al., 2004; van der Fels-Klerx and Stratkou, 2010). Production of the type A trichothecenes T-2 and HT-2 is often associated with *F. sporotrichioides* (Bottalico and Perrone, 2002) and *F. langsethiae* (Imathiu et al., 2013a). In Norway, *F. langsethiae* is the main T-2 and HT-2 producing species (Hofgaard et al., 2016a).

There are several adverse health effects related to consumption of mycotoxin contaminated cereal grains. The health complications associated with mycotoxin consumption varies depending on the type of mycotoxin. The frequently found mycotoxin DON, associated with *F. graminearum* and *F. culmorum*, is known to cause emesis (vomiting), feed refusal, depressed feed intake and growth- and weight reduction in farm animals (D'Mello et al., 1999) and laboratory rodents (JECFA, 2001). In Asia outbreaks of acute vomiting, nausea, diarrhoea, headache and gastrointestinal upset in humans has been attributed to consumption of *Fusarium* contaminated grains with high DON levels (JECFA, 2001 p.37). The toxicological effects of the type A trichothecenes are more prominent than the effect of the type B mycotoxins. After consumption, the T-2 toxin is quickly metabolised into HT-2 *in vivo*, therefore these two mycotoxins are often assessed combined in terms of toxicity and concentration (Van der Fels-Klerx and Stratakou, 2010). At low concentrations T-2 toxin is a potent inhibitor of the protein synthesis *in vivo* and *in vitro* (JECFA, 2001, p.43). Furthermore, consumption of T-2 and HT-2 contaminated grains is linked to oral lesions in poultry, reduced feed intake and immunological effects in pigs, reproductive disorders in livestock and reduced weight gain in ducks and rabbits (D'Mello et al., 1999).

Because of the human and animal health risks associated with mycotoxin contamination, legislative regulations are required in order to maintain food safety. Restrictions regulating the mycotoxin content in cereals intended for human and animal consumption has been evaluated by The European Food Safety Authority (EFSA). In 2006, EFSA set a legal limit of DON concentration in oats intended for food to 1750 $\mu g\ kg^{-1}$ (European Commission, 2006), and a legislative limit on the combined concentration of T-2 and HT-2 content is under evaluation

(Edwards, 2009). In 2013, EFSA proposed an indicative maximum level of T-2 and HT-2 toxins in unprocessed oats to 1000 µg kg^{-1} (Edwards, 2018) and a tolerable daily intake of 0.06 µg kg^{-1} body weight per day (Edwards, 2009). Such restrictions to mycotoxin content in grains intended for food- and feed consumption are important since concentrations as high as 25 000 µg kg^{-1} DON has been recorded in commercially harvested oats in Norway (Bjørnstad and Skinnes, 2008), and T-2 and HT-2 concentrations in oats up to 9990 µg kg^{-1} in the UK Edwards (2009).

In addition to the obvious yield and quality reduction aspects related to *Fusarium* head blight infection of small grain cereals, mycotoxin contamination constitutes the largest risk factor as it threatens food and feed security. Presence of *Fusarium* species does not necessarily lead to mycotoxin production and degree of *Fusarium* infection is not strictly correlated with mycotoxin contamination (Xu and Nicholson, 2009; Hofgaard et al., 2016a). *Fusarium* infection and subsequent mycotoxin production is influenced by several external factors including weather conditions, fungicide treatment and competition with other fungal species (Xu and Nicholson, 2009). In order to predict the risk and possibly mitigate mycotoxin contamination, it is of great importance to identify the factors which influence *Fusarium* infection and subsequent mycotoxin production in cereals.

Factors influencing *Fusarium* development and mycotoxin production

Influence of weather conditions on *Fusarium* infection

Several factors are known to influence *Fusarium* head blight infection and subsequent mycotoxin production in cereal fields. Indubitably, the presence of viable inoculum and a susceptible host is the most important, but when these requirements are fulfilled the climatic conditions during the growth season is the major factor influencing *Fusarium* infection and mycotoxin production (Osborne and Stein, 2007). The predominating *Fusarium* species in cereal fields vary between continents, countries, geographic location and years because of different weather conditions favoured by different *Fusarium* species (Parry et al., 1995). The weather conditions during the most susceptible stage of plant development is important for disease development and mycotoxin production. The optimal weather conditions for a successful fungal infection of plants varies between species, hence climatic observations can serve as a tool in forecasting *Fusarium* species establishment, infection and mycotoxin production. Particularly the optimal temperature and humidity requirements varies between *Fusarium* species (Doohan et al., 2003)

The highly problematic *Fusarium* head blight organism, *F. graminearum,* predominates in temperate and warmer climatic zones (Xu et al., 2005; Xu et al., 2008; Osborne and Stein, 2007). *F. graminearum* infection is uttermost associated with high temperatures, favourably around 25-30ºC, and high relative humidity (≈ 100% RH) over longer periods (Champeil et al., 2004). In periods of frequent rainfall during flowering and temperatures above 15ºC, *F. graminearum* can potentially cause major epidemics if no preventative- or control measures are implemented. In countries with cooler temperatures, for example in Northern Europe, *F. culmorum* has been the dominating *Fusarium* head blight pathogen followed by *F. avenaceum, F. graminearum, F. poae, F. langsethiae* and *F. tricinctum* (Parry et al., 1995; Brennan et al., 2003; Osborne and Stein, 2007). The temperature optima for growth and infection of *F. avenaceum*, *F. poae* and *F. culmorum* is around 20-25ºC, slightly lower than for *F. graminearum* (Parry et al., 1995; Doohan et al. 2003; Champeil et al., 2004). *F. poae* is one of the most frequently isolated *Fusarium* head blight pathogens in Norway (Kosiak et al., 2003; Hofgaard et al., 2016a), Hungary, Ireland, the UK (Xu et al., 2005) and Canada (Tekauz et al., 2004). In addition to temperature, humidity is an important factor influencing *Fusarium* field infection. Bernhoft et al. (2012) found strong correlation between precipitation in July and total amount of *Fusarium* in wheat, barley and oat grains harvested from farmers' fields in Norway.

Similar to *Fusarium* growth, mycotoxin production is also weather dependent however, the weather conditions promoting *Fusarium* growth does not necessarily promote mycotoxin production (Bernhoft et al., 2012). The risk of DON and T-2/HT-2 contamination of cereal grains increases with low temperatures and high humidity before harvest (Bernhoft et al., 2012). In oats, risk of DON contamination increases with rain, long periods of humidity and warm temperatures during flowering (Hjelkrem et al., 2017). Furthermore, humidity and cool temperatures during heading, and humid and warm temperatures after flowering, are factors which increases the risk for T-2 and HT-2 production in oats (Hjelkrem et al., 2018).

Apart from weather conditions which are uncontrollable, there are factors influencing *Fusarium* infection and mycotoxin production which can be controlled. These factors include choice of cereal variety, previous crop, tillage and fungicide treatment (VKM, 2013).

Host resistance towards plant pathogens is an important trait which can reduce the extent of damage upon infection. Important host factors to consider are genetic pathogen resistance and physiological host factors influencing susceptibility, these include age, hydration and nutrition (Osborne and Stein, 2007). Breeding for *Fusarium*- and mycotoxin resistant cereal varieties is difficult since *Fusarium* resistance is complex and dependent on many genes

(Leonard and Bushnell, 2003 p.224-225). Complete gene-for-gene resistance towards *Fusarium* head blight have not been found in cereals, however, less susceptible cereal varieties exists (Leonard and Bushnell, 2003 p.224-225; Beyer et al., 2006).

Oats has been regarded as less susceptible to *Fusarium* head blight infection than other small grain cereals because of the lack of visible symptoms and damage upon infection (Tekauz et al., 2004), However, high concentrations of accumulated mycotoxins are often found in oats despite the lack of visible disease symptoms (Imathiu et al., 2013b). The suggested resistance of oats to *Fusarium* head blight infection can debatably be attributed to its distinctive panicle structure consisting of individually and dispersed spikelets (Bjørnstad and Skinnes, 2008). This resistance to *Fusarium* infection is expressed by type II resistance which impede spread of the pathogen within the host (Parry et al., 1995). Oats putative type II resistance might debatably be one of the reasons why little effort in breeding *Fusarium* resistant oat cultivars has been made (Ohm and Shaner, 1992 and Rodinova et al., 1994, cited in Gagkaeva et al., 2013). Despite the lack of visible *Fusarium* symptoms, considerable damage from trichothecene contamination of the oat grains occurs.

The choice of cultivar is an effective method for *Fusarium* control. For instance, naked (hull-less) oat cultivars is documented to be more resistant to *Fusarium* head blight damage and mycotoxin contamination (Tekauz et al., 2004; Gavrilova et al., 2008).

Tillage and previous crop

Tillage is an important factor in reducing *Fusarium* infection and subsequent mycotoxin contamination in cereals. Ploughing is commonly regarded as the most effective soil cultivation method to reduce *Fusarium* infestation (Dill-Macky and Jones, 2000; Hofgaard et al., 2016b). Likewise, Hofgaard et al. (2016b) found ploughing (spring or autumn) more effective in reducing *Fusarium* inoculum potential in oats compared to harrowing (no-till). Beyer et al. (2006) found ploughing to reduce DON contamination with 66% in wheat compared to no tillage management. These findings are in agreement with Maiorano et al. (2008) who found removal of *Fusarium* infested crop debris to be the most important factor in reducing the risk of *Fusarium* infection and mycotoxin accumulation.

Previous crop also plays an important role in *Fusarium* head blight development as it can serve as a source of inoculum (Champeil et al., 2004). Wheat in rotation with another *Fusarium* head blight host increases risk of *Fusarium* infection. *Fusarium* infection and mycotoxin

contamination (DON) in wheat is found high in rotation with wheat, but even higher in rotation with maize (Dill-Macky and Jones, 2000). Parikka et al. (2008) reported increased risk of T-2 and HT-2 accumulation in oat fields without crop rotation, especially in combination with conservation tillage.

Fungicides

The use of fungicides for controlling *Fusarium* infection and reduce mycotoxin contamination have had limited efficacy (Parry et al., 1995). Triazole fungicides (except fenbuconazole) are the most effective fungicides in control of *Fusarium* head blight as its application has shown to reduce DON contamination of wheat grains with up to 50% compared to untreated wheat (Beyer et al., 2006). Edwards et al. (2001) also found tebuconazole and metconazole to be effective in reduction of *F. graminearum* and *F. culmorum* infection, and subsequent DON contamination in winter wheat. The effect of fungicide application in control of *F. langsethiae* and T-2 and HT-2 production have had little success (Mateo et al., 2011; Elen et al., 2008; Pettersson et al., 2008; Edwards and Anderson, 2011). Edwards and Anderson (2011) did not find any significant difference in T-2 and HT-2 content of oat grains, from spring- and winter oat varieties, between fungicide treated and non-fungicide treated plots. They tested several of the most commonly used fungicides including tebuconazole which is found effective against *Fusarium* and DON contamination of winter wheat. Elen et al. (2008) found similar results in Norway where fungicide treatment with Proline reduced total *F. graminearum* DNA in wheat and an average DON reduction of about 70%. Similar effects on DON and *Fusarium* content were observed in oats. However, Proline application did not reduce *F. langsethiae*/*F. sporotrichioides* DNA or T-2/HT-2 content in oat grains.

Fusarium and mycotoxins in Norway

The *Fusarium* species complex present in Norwegian oat fields varies between regions and years, mainly because of different seasonal weather conditions. Earlier studies on the prevalence of *Fusarium* species in Norwegian cereals pointed out *F. avenaceum* as the most commonly detected *Fusarium* species in Norwegian (Kosiak et al., 2003) and Scandinavian cereal fields (Nielsen et al., 2011; Karlsson et al., 2017). Norwegian cereals have been reported to be highly contaminated with DON (Langseth and Elen, 1997) and Hofgaard et al. (2016a) identified *F. culmorum* to be the main DON producing *Fusarium* species in Norway. However, the increased prevalence of *F. graminearum*, another DON producer, during the past decade

has led to a shift from *F. culmorum* being the main DON producer in Norway to *F. graminearum* (Hofgaard et al., 2016a). Similar trends have also been seen in other Scandinavian- and European countries including Sweden (Fredlund et al., 2013), Denmark (Nielsen et al., 2011), Italy, Ireland, Hungary and the UK (Xu et al., 2005). The increased abundance of *F. graminearum*, in temperate to cool climates, can debatably be explained by climate change, increasing the seasonal temperatures and favouring growth of *F. graminearum* (Bernhoft et al., 2012). Furthermore, since *F. graminearum* is a teleomorph (sexual reproduction), new ecotypes which are better adapted to cooler climates might have evolved (Kosiak et al., 2003; Bernhoft et al., 2012).

For the past decade, in addition to increased presence of *F. graminearum*, the prevalence of *F. poae* and *F. langsethiae* has increased in cereal fields in Scandinavia and Europe (Kosiak et al., 2003; Xu et al., 2005; Schöneberg et al., 2018a). More attention has been diverted towards *F. poae* and *F. langsethiae* because of their ability to produce mycotoxins and cause human and animal toxicosis (Stenglein, 2009). Never the less, little is known about the life cycle of these frequently isolated and mycotoxin producing *Fusarium* species. Knowledge about the biology and epidemiology *F. poae* and *F. langsethiae* is needed in order to develop effective measures of disease control and prevention.

Fusarium poae

The importance of *F. poae* as a *Fusarium* head blight causing species is increasingly recognized globally (Stenglein, 2009). The temperature requirements for growth of *F. poae* is above 15 ºC, with an optimal temperature around 25 ºC (Doohan et al., 2003; Stenglein, 2009; Nazari et al., 2018). Xu et al. (2008) found *F. poae* to thrive better in relatively dry and warm climatic conditions compared to *F. graminearum* which prefers humid and warm conditions for optimal growth. The pathogenicity of *F. poae* is influenced by temperature and it is most pathogenic within the 15-20 ºC temperature range (Brennan et al., 2003).

All strains of *F. poae* produce globose or napiform microconidia with 0 or 1- septate (Leslie and Summerell, 2008 p. 220). Some strains also produce short and slender macroconidia which usually have 3- septate (sometimes 4-5 septate). Opposed to *F. graminearum* which is a teleomorph, *F. poae* is an anamorph and the asexual conidia spores are the main mode of dispersal (Jenkinson and Parry, 1994a; Stenglein, 2009). On Potato Dextrose Agar (PDA) *F.*

poae produce compact aerial mycelium with colour varying from withe to pinkish. The mycelial colour might darken with age and turn rust red. The colouration of the agar also varies from white, yellow and burgundy red (Leslie and Summerell, 2008 p. 220; Stenglein, 2009).

Fusarium langsethiae

Fusarium langsethiae was first described in 1999 as a new *Fusarium* species called "powdery *Fusarium* poae". Despite a morphology similar to that of *F. poae*, the mycotoxin profile of the newly described variety did not match the mycotoxin profile of *F. poae* (Torp and Langseth, 1999). In 2004 "powdery *Fusarium* poae" was accepted as a new *Fusarium* species called *F. langsethiae* (Torp and Nirenberg, 2004). The disease cycle of *F. langsethiae* is still a mystery (Fig. 2) (Imathiu, 2008; Van der Fels-Klerx, 2010; Divon et al., 2012).

Figure 2. Illustration of the known part of the disease life cycle of F. langsethiae. (Aamot et al., 2018)

Optimal temperature conditions for growth of *F. langsethiae* on artificial media is between 20-30ºC, with high water activity raging between 0.98-0.995 a_w (Torp and Nirenberg, 2004; Medina and Magan, 2010). No growth of *F. langsethiae* occurs at water activity levels of 0.9a_w or below (Medina and Magan, 2010). Despite high optimal temperature for growth on artificial media, investigations on *F. langsethiae* growth *in planta* has revealed growth to occur at temperatures from 10ºC which is way below the suggested optimum (Schöneberg et al., 2018b).

This indicates that strains of *F. langsethiae* have the ability to adapt to different sub-optimal climatic conditions which explains the frequent isolation of *F. langsethiae* in cooler climates.

Morphologically *F. langsethiae* resembles *F. poae*. The differences in morphological characters between *F. langsethiae* and *F. poae* is the inability of *F. langsethiae* to produce macroconidia and some isolates of *F. langsethiae* produce polyphiladies while *F. poae* only possesses monophyalides (Torp and Nirenberg, 2004). The microconidia of *F. langsethiae* are globose or napiform, the same shape as the microconidia of *F. poae*, but their size is smaller. Furthermore, *F. langsethiae* differs from *F. poae* with slower growth on artificial media (PDA) at optimal temperatures 20-25°C, and is lacking the sweet characteristic odor often found for *F. poae* grown on artificial medium.

Different from other FHB causing pathogens, like *F. graminearum* and *F. avenaceum*, *F. langsethiae* is regarded as a weak plant pathogen, debatably possessing endophytic abilities as it does not cause visible disease symptoms upon infection (Imathiu, 2008; Divon et al., 2012; Imathiu et al., 2013b). There are no clear symptoms of infection associated with *F. langsethiae* in small grain cereals. The problems associated with *F. langsethiae* infection occurs post-harvest due to the possible accumulation of T-2 and HT-2 toxins in the grains, which might lead to economic losses because of quality degradation and grains unsuited for food and feed consumption. Oats has several times been described as the small grain cereal most susceptible to *F. langsethiae* infection and subsequent mycotoxin accumulation in Norway and Europe respectively (Langseth and Stabbetorp, 1996; Torp and Nirenberg, 2004; Bernhoft et al., 2010; Fredlund et al., 2010; Imathiu et al., 2013a). The morphological traits of oats are believed to be one of the reasons for the susceptibility of *F. langsethiae*. The spikes are individually spaced on the oat panicle which hampers possible secondary *Fusarium* infection between the spikes (Divon et al., 2012).

Aim of study

The aim of the study is to gain insight into the biology of *Fusarium poae* and *Fusarium langsethiae*. Their biology seems to differ from the biology of the other common *Fusarium* head blight causing species; *F. graminearum*, *F. avenaceum* and *F. culmorum*. One clear indication of the biology of *F. poae* and *F. langsethiae* being different from that of for example *F. graminearum* is their deviating response to fungicides. Application of fungicides known to reduce the content of *F. graminearum, F culmorum* and DON in oats have not been found to influence the content of *F. langsethiae* or T-2 and HT-2 production in oats (Elen et al., 2008; Edwards and Anderson, 2011). Another reason for this discrepancy could be the timing of the infection, which has been suggested to occur at earlier growth stages than for *F. graminearum* (Xu et al., 2013; Hjelkrem et al., 2018).

Furthermore, important parts of the biology of *F. graminearum* is successful colonization of plants during the growth season and thus establishment of fungal inoculum in straw residues that may influence the next years' crop (Pereyra and Dill-Macky, 2008). *Fusarium graminearum* is also well known for its dispersal of ascospores/conidia by wind and rain splash (Parry et al., 1995; Maldonado-Ramirez et al., 2005; Osborne and Stein, 2007). As for *F. poae* and *F. langsethiae,* little evidence is pointing in the direction of straw residues serving as a main source of inoculum (Pereyra and Dill-Macky, 2004; 2008; Hofgaard et al. 2016b). At the same time, only small amounts of *F. poae* and *F. langsethiae* DNA are found in air samples (Hofgaard et al., 2016b; Schöneberg et al., 2018b) which weakens the hypothesis that spores produced by these *Fusarium* species are dispersed over long distances by wind or rain splash. Knowledge about the biology of *Fusarium* species is crucial for understanding their epidemiology which is key in the development of effective methods for disease control and prevent mycotoxin contamination. Therefore, further research on the inoculum sources of *F. poae* and *F. langsethiae* and their mode of dispersal is highly necessary.

The main objective of this investigation is to gain insight into the biology of *Fusarium poae* and *Fusarium langsethiae*. The main objective is divided into two secondary objectives:

1. Investigate the role of the gramineous weed *P. annua* as a possible source of *Fusarium poae* and *Fusarium langsethiae* inoculum in an oat field.
2. Identify the growth stage of main shoots of oats at the time of initial *Fusarium poae* and *Fusarium langsethiae* infection.

3. Investigate whether the degree of *F. poae* and *F. langsethiae* infection differs between normal and abnormal plants, and between different plant parts.

Materials and Methods

Field Trial

The field trial was located at the border of the experimental fields of the Norwegian Institute of Bioeconomy Research (NIBIO), next to a road, at Kirkejordet Nord, Ås. The oat field measured 2.5x52 m and for the past five years there have been cereals in rotation on the field, with only oats in rotation for the past three years (Table 1).

Table 1. Crop rotation in the field trial for the past six growth seasons.

	2018	**2017**	**2016**	**2015**	**2014**	**2013**
Cereal crop	Oats	Oats	Oats	Barley	Wheat	Peas

Oats was planted in the field the 2nd of May in 2017 and 7th of May in 2018 and harvested 31st of August in 2017 and 22nd of August in 2018, respectively. The Swedish oat variety Belinda was sown both years and the seeds were bought at Felleskjøpet. There was no application of pesticide-, herbicide- or insecticides on the field, only mineral fertilizers were applied as recommended. The field was harrowed in spring/autumn both years.

Plant material and air samples collected from the field

Collection of plant material

During the 2017 and 2018 growth seasons, oat plants were weekly collected from the field from the two-leaf stage (Zadoks growth stage 12; Lancashire et al. 1991) and onwards. Each week, a total of 20 oat plants were collected, 10 normal- and 10 abnormal plants. The abnormal plants differed from normal plants with for example early tillering due to reduced development of the main shoot, strongly reduced growth, shoots stagnating in the boot or plants with aborted grains (Appendix IX). In 2017, the oat plant material was collected by employees at NIBIO, whereas in 2018 the plant material was collected by me .
In 2017, 10 plants from each group (normal or abnormal) were randomly collected from within the whole field. In 2018, the field was divided into five grids of 2,5x10m (25 m^2) and two normal- and abnormal plants were sampled from each grid. The field was divided into grids to assure random sampling of plants representative for the whole field. Samples of the annual monocot weed *Poa annua* were monthly collected in 2018. Two *P. annua* plants were sampled from each of the five grids during the first week of the month from May to August.

For all oat plants, the main shoot was separated from the tillers and only the main shoots were analysed in our investigation, the separated tillers were stored in the freezer at -20°C. The sampled oat plants were dissected and divided into five groups of plant organs: the panicle, flag leaf, other leaves, stem and remaining plant parts including dead leaves and root. All the plant material from each group of plant organs, from each point of sampling, for instance all the panicles, were merged, however, normal and abnormal plants were kept separated. The plant material was stored in the freezer at -20°C until further analysis. In 2017, the collected oat plants were directly placed in the freezer (-20°C) after sampling, and the plants were later dissected and separated into the five different groups of plant organs in a cold working lab (4°C) to avoid rapid defrosting. The plant parts were then replaced in the freezer until further analysis. In 2018, the plants were dissected and separated into the different organ groups immediately after sampling, and stored in the freezer at -20°C until further analysis. Dissecting fresh plant material proved more efficient than dissecting frozen plant material which were fragile and difficult to separate. The panicle was cut underneath the panicle node, the flag leaf was separated from the stem and cut at the node, the same for the other leaves. At late developmental stages (GS35-GS89), it was easy to separate the plant organs even though the plants were frozen. But, at early developmental stages when the plants were small (<GS30) they were difficult to divide into the different organ groups. Therefore, these plants were instead separated into upper- and lower parts. The lower part contained stem, panicle and leaves, whereas the upper part mainly consisted of leaves. These plants (<GS30) were not included into the *Fusarium* DNA analysis, but stored in the freezer at -20°C.

The collected plant material was freeze-dried using the Labconco FreeZone -105°C 4.5 L Cascade Benchtop Freeze Dry System. The plants had been stored in the freezer at -20°C from collection until freeze-drying. When using the freeze dryer, the plant material should be completely frozen. Plant material was put in suited containers (120ml or 900ml) which were connected to a stainless steel drying chamber. Only one third of the container's volume were filled to ensure complete and equal freeze-drying of the material. The plant material was dried for 24h. The dry weight of the plant material was weighed and put back into zip-lock bags and stored in the freezer at -20°C, to keep the material dry.The freeze-dried plant material was ground, using an Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany) at 12000 rpm. A 0.5 mm screen was used to grind the plant material (panicle, flag leaf and stem). The ground plant material was collected into 15 ml plastic tubes and stored in the freezer at -20°C until DNA extraction.

The collected *P. annua* plants were freeze-dried following the same procedure as for plant material. However, the whole *P. annua* plants were milled.

Collection of air samples

During both growth seasons, two Automatic Multi-Vial Cyclone Samplers (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) spore-traps were placed in each end of the field to collect air samples throughout the growth season. One of the two spore-traps failed, consequently only air samples from one of the traps were included in the analysis. The spore-traps actively collected air samples through an air intake situated approximately 1 m above the ground with an air movement of 16.5 l/min. Air samples were collected into 1.5 ml Eppendorf tubes which were automatically changed every 24 hours, at 00:00 h. Insects such as flies, beetles and aphids amongst others, were occasionally sucked into the tubes. After seven days, the Eppendorf tubes were collected and replaced with new clean tubes. During one week, eight tubes were collected since the first and last tube only collected air samples for half a day. The tubes were stored in the freezer at -20°C until DNA extraction. In 2017 air samples were collected during 13 weeks, from early leaf development (week 23) to harvest (week 35).

Due to damage and service on the spore-traps in 2018, they were first placed in the field in week 28, at end-flowering, and air samples were collected until senescence (week 34). The air samples from 2018 were not analysed.

Oat grain harvesting and sample preparation

Oat grains were harvested at maturity (GS 89) in 2017 and at senescence (GS 99) in 2018. After harvest the oat grains were dried for 3-5 days in a drying chamber till the oat grains reached about 16% water content. Out of the total amount of harvested oat grains, a representative sub-sample stored in dry conditions at room temperature was used for further morphological analysis of the *Fusarium* infestation rate and analysis of *Fusarium* DNA by qPCR. To run the qPCR analysis, a thoroughly mixed sample of harvested oat grains (200 g) were milled following the same procedure as for plant material except, however a 1 mm sieve was used. Grains were added to the centrifugal mill in small amounts to avoid overheating of the mill. The ground, homogenized material was collected into a zip-lock bag and stored in the freezer at -20°C until DNA extraction.

Analysis of *Fusarium* in plant material sampled at different growth stages

Extraction of DNA from plant material

The content of *Fusarium* DNA in the oat plant material and *P. annua* plants was analysed using species-specific qPCR. Of the five groups of oats plant organs collected, only the panicles, flag leaves and stems were analysed for DNA content of selected *Fusarium* species.

The FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) was used to extract DNA from freeze-dried and ground plant material according to manufacturer's protocol. First, 150 mg of sample were added to the Lysing Matrix E tube, followed by addition of 978 µl of Sodium Phosphate Buffer. Since the sample material is voluminous the buffer solution must carefully be added to the tube, so that the ground plant material can absorb the buffer solution. Next, 122 µl MT buffer were added to the Lysing Matrix E tube, then homogenized in the FastPrep instrument at speed six m/s for 40 seconds followed by 10 minutes in the centrifuge at 14 000 x g. The supernatant was transferred to a clean 1.5 ml Eppendorf tube and 250 µl Protein Precipitation Solution were added. The tubes were inverted 10 times following five minutes centrifuging. The supernatant was transferred to a clean 15 ml tube and 1 ml of Binding Matrix Suspension was added. The tubes were inverted for two minutes to promote binding of DNA, then placed to rest for three minutes. Then, 700 µl of the supernatant was removed and discarded. The Binding Matrix was carefully mixed with remaining supernatant with the pipette. 600 µl of the mixture was transferred to a Spin Filter. The spin filter was centrifuged for one minute. The catch tube was emptied, and remaining supernatant was added to the Spin Filter, centrifuged and catch tube emptied again. 500 µl of SEWS-M was added and the pellet was carefully mixed into the SEWS-M solution with the pipet tip. The Spin Filter was centrifuged for one minute and catch tube emptied and centrifuged again for two minutes in order to remove all of the remaining solution. The Spin Filter was transferred to a clean tube and air dried for five minutes, with open lid, at room temperature. Then, 100 µl DNase/Pyrogen-free Water were added to the Spin Filter and the Binding Matrix was re-suspended. Finally, the tube was centrifuged for one minute at 14 000 x g. The Spin Filter was discarded and the final solution in the tube containing the spore DNA was stored in the freezer at -20ºC.

To test if the quality and presence of DNA in the DNA eluate was sufficiently good for running a quantitative Polymerase Chain Reaction (qPCR) analyses, a qualitative test on a 1% agarose gel was performed on DNA from a selection of the samples (Appendix VIII). The agarose gel was prepared by weighing 0.5 g of Agarose which was mixed with 50 ml of 1xTBE

electrophoresis buffer (Appendix I) in a 50 ml Erlenmeyer flask. The solution was heated in the microwave for approximately five minutes, until the agarose was totally dissolved. The solution was cooled for two minutes at the benchtop, then cooled down to approximately 60°C in cold water. One drop of ethidiumbromide was added to the cooled solution and carefully mixed. The solution was then poured into a tray with an eight wells comb and left to rest for 20 minutes until solidified. Then, the gel was placed in an electrophoresis tray and 3µl of 1 kb ladder was added to the first well. In the preceding wells, 5 µl of sample DNA was added. The DNA sample was a pre-prepared mixture of 2 µl DNA eluate, 1µl loading dye (FastDNA® SPIN Kit for Soil) and 2µl dH_2O. The gel was run at 80V for 40 minutes and visually analysed with the Gel Doc™ EQ (Bio-Rad Laboratories inc.).

Analysis of *Fusarium* DNA in plant material by qPCR

A quantitative analysis of the presence and amount of *Fusarium* DNA in the plant material of oats and *P. annua* plants was carried out running qPCR. The samples were analysed for presence of six *Fusarium* species: *F. poae, F. langsethiae, F. avenaceum, F. graminearum, F. culmorum* and *F. sporotrichioides* DNA using the C1000 Touch Term Cycler with the CFX Real-Time system from Bio-Rad Laboratories Inc. The qPCR plate consisted of 96 wells (Low profile, thin-walled, Hard-Shell® 96-Well PCR Plate, Bio-Rad Laboratories, Inc.). The wells contained 25 µl solution: 4 µl diluted sample DNA and 21 µl of a pre-prepared mastermix specific for each of the *Fusarium* species. The extracted sample DNA was diluted 10× by mixing 10 µl extracted DNA and 90 µl Milli-Q water. The mastermix contained 300 nM pre-pared forward- and reverse primers and 100 nM prepared probe solution. The primer- and probe solutions were prepared as follows: A 500 µl of stock solution consisting of specific primers and probes were prepared for each *Fusarium* species (Appendix III). Primers were diluted from a stock concentration of 100 pmol/µl to 7,5 pmol/µl. Probes were diluted from a concentration of 100 pmol/µl to 2,5 pmol/µl. These primers and probes were stored at -20ºC until use. The qPCR plate was thoroughly sealed with a plastic film (Microseal® 'B' seal, Bio-Rad Laboratories inc.), and centrifuged for approximately 30 seconds prior to analysis. The amplification program was set to run three minutes at 95 °C, followed by, 45 cycles of 95°C for 10 Seconds, then 60°C for 30 Seconds.

To quantify the amount of fungal DNA in the plant material, the amount of DNA in the samples was compared to a known amount of template DNA using a standard curve. The standard curve consisted of a serial dilution curve of a standard DNA already available at NIBIO, that was

originally obtained from mycelia of pure cultures of the respective *Fusarium* species, with known initial DNA concentration. The standard DNA was diluted to concentrations of 4000 pg, 400 pg, 40 pg, 4 pg and 1 pg.

The results were presented as amount of *Fusarium* DNA per amount of dried plant material that was the basis for the total DNA in the qPCR reaction (pg/mg). Since DNA from 150 mg plant material was diluted in a volume of 100 µl in the DNA extraction, 4 µl of 10× diluted genomic DNA is equivalent to an amount of plant material per reaction of 0.6 mg.

Analysis of *Fusarium* in air samples

Extraction of DNA from air samples

The amount of *Fusarium* DNA in the air samples were analysed based on weekly samplings, therefore all spores from eight Eppendorf tubes were merged into one sample (from one week) through a wash-through procedure. In some tubes flies, beetles and spiders were sucked into the Eppendorf tubes by the air sampler and these were counted and included into the DNA extraction. All tubes were centrifuged at 14,000 x g for two minutes to spin down the content before the tubes were opened. The FastDNA® SPIN Kit for Soil was used to extract DNA from air samples according to manufacturer's protocol, except the first step which was a wash-through step. The total amount of Sodium Phosphate Buffer (996 µl) was added in two rounds. First, 498 µl solution was added the first of eight tubes belonging to one week and vortexed. Then the air sample-Sodium Phosphate Buffer mix from the first tube was transferred to the second tube and vortexed. This procedure was followed for all eight tubes belonging to one week. The air sample-buffer solution washed through all eight tubes was transferred to a Lysing Matrix E tube. The whole wash-through step was repeated by adding the remaining 498 µl Sodium Phosphate Buffer to the first tube (of eight) and it were transferred through all tubes and the air sample-buffer solution was added to the same Lysing Matrix E tube. Finally, after the second wash-through the air sample tubes were centrifuged for one minute at 14,000 x g and the remaining solution were added to the Lysing Matrix E tube. Next, the protocol for The FastDNA® SPIN Kit for Soil, as described for DNA extraction of plant material, was followed until the last step where 50µl DNase/Pyrogen-free Water (instead of 100µl) was added to the Spin Filter. The Binding Matrix was re-suspended and centrifuged for one minute at 14000 x g. Then, the spin filter was transferred to a new clean tube and the remaining 50µl DNase/Pyrogen-free Water was added and the procedure repeated. Hence, the DNA extracted

from the air samples were divided into two eluates and stored in the freezer at -20ºC until further analysis.

The concentration of the total DNA in the air samples were measured with the Qubit® 2.0 Fluorometer together with the Qubit HS assay (Appendix X). A statistical analysis of variance between the two eluates were carried out to evaluate whether the DNA eluates should be kept separated or mixed. A paired t-test, at 95% confidence level, showed a significantly higher DNA concentration in the 1st eluate compared to the 2nd eluate (P=0.0004). The eluates were therefore kept separated to avoid diluting the DNA.

Analysis of *Fusarium* DNA in air samples by qPCR

A quantitative analysis of amount of fungal DNA present in the air samples were performed by running qPCR according to the same procedure as described for the plant material except that different standards and DNA were used. The sample DNA were run non-diluted (1st eluate). The standards were based on DNA extracted from spore suspensions of known concentration (instead of standards based on mycelia from pure cultures as used in analysis of plant material). The DNA concentrations in standard one varied between the *Fusarium* species and was based on the DNA concentration in the spore solutions of each species (Appendix VII).

The spore solutions with known amounts of spores of the different Fusarium species were prepared as follows: pure cultures of *F. poae, F. langsethiae, F. graminearum* and *F. avenaceum* spores were plated out on five Mung Bean Agar (MBA) Petri dishes under sterile conditions. The plates were incubated in a light chamber with NUV- and white light at 20ºC, under 12 hours shifting light/dark periods for 14 days to promote sporulation. Then, after 14 days' incubation, spore solutions were made by adding 5 ml distilled water to each plate. A sterile glass rod was used to mix the spores with water by carefully gliding the rod over the agar. For each isolate, spore solution from all five Petri dishes were transferred to a 50 ml plastic tube with a 5 ml pipette and mixed by shaking. Then, <10 µl spore suspension was applied to a KOVA® Glasstic Slide with a pipette. The spore concentration was registered by counting spores within nine small grids on the slide according to manufacturer description. The average spore number of the nine grids was used to calculate the concentration. Then, 700 µl of the spore solution was added to 10 new MBA plates and incubated in a light chamber with 20ºC temperature and 12 hours shifting light/dark periods for three weeks. After 3 weeks' incubation the plates were washed with five ml of distilled water as described above. The number of spores

in the spore solution was calculated based on the average spore count found with the KOVA® Glasstic Slide. The average spore number was found by counting spores within 12 squares in five individual grids. After mixing the spore solution from all 10 Petri dishes the final amount of spore solution is approximately 30-40 ml with known concentration. Finally, 6 ml of the solution was pipetted into small plastic tubes of 15 ml and stored in the freezer at -20ºC until DNA extraction.

A given amount (ml) of spore solution was put into the DNA extraction process. The amounts were based on the spore count and desired spore concentration (Appendix VII). The amount of spore solution put into the DNA extraction process was different for each *Fusarium* species. The calculated amount of spore solution was pipetted into a clean 15 or 50 ml tube, depending on the required amount (ml) of solution. The tubes were centrifuged for 15 minutes to spin down all spores. The supernatant was carefully removed with a pipette to avoid removing settled spores. Then, 978 µl of Sodium Phosphate Buffer were added to the tube and mixed with the spores using the pipette. The spore-buffer solution was transferred to the lysing matrix E tube, and The FastDNA® SPIN Kit for Soil protocol was followed.

The final DNA concentration in the spore solutions were measured using a Qubit® dsDNA HS Assay together with the Qubit® 2.0 Fluorometer. First, a work-solution was prepared by mixing 10 µl Qubit® dsDNA Reagent with 1980 µl Qubit® dsDNA HS Buffer in a 2 ml Eppendorf tube. This equals work-solution for 10 samples: work-solution for one sample consist of 1µl Qubit® dsDNA Reagent mixed with 199 µl Qubit® dsDNA HS Buffer. Then, DNA standard one and two were prepared by adding 190µl of the work-solution and 10 µl of Qubit® standard one and two into clean PCR tubes which were vortexed for 2-3 seconds. For the spore solutions, 2 µl extracted spore DNA was added to 198µl working solution in a PCR tube and vortexed for three seconds. The prepared solutions were placed on the working bench for two minutes to rest. The Qubit® 2.0 Fluorometer was used to measure DNA concentration in the samples. The fluorometer was calibrated by reading the two DNA standards before running analysis of the spore solutions. The DNA concentrations were measured in ng/µl.

Analysis of *Fusarium* in harvested oat grains

Investigation of the ratio of *Fusarium* infested grains and the level of *Fusarium* DNA from different *Fusarium* species from the 2017 harvested were carried visually by incubation on *Fusarium* selective medium, on filter paper and quantitatively by qPCR. The oat grains harvested in 2018 were only analysed by qPCR. Two plating methods were carried out for visual and morphological identification of *Fusarium* species, these were the freezer blotter method and plating onto *Fusarium* selective medium (CZPD). Three replicates for each method were carried out.

Morphological characterization of *Fusarium* species using a selective medium

For each replicate, 100 Surface sterilized and 100 non-surface sterilized seeds were plated out on CZPD medium (Appendix I) in petri dishes. Seeds were surface sterilized by 10 minutes immersing in a 1% active chlorine solution, followed by three minutes air-drying on filter paper. On each Petri dish, nine randomly selected oat seeds plated out with an even distance. A total of 11 Petri dishes were prepared for each group (surface sterilized or non-surface sterilized). All Petri dishes were marked with date, as surface- or non-surface sterilized and with individual numbers on the plates and grains so that each grain could be followed throughout the process. The Petri dishes were placed in a light chamber for 14 days with shifting 12 hours' near ultraviolet (NUV) light and 12 hours' darkness at 20°C. Near-UV light is reported to stimulate sporulation of *Fusarium* (Torp and Nirenberg 2004).

After one week, the grains on each plate were registered as *Fusarium* infested or non-infested based on mycelial growth and fungal colonisation on the seeds and agar then replaced in the light chamber. Morphological determination of *Fusarium* and *Fusarium* species were based on the thorough description of *Fusarium* species in the *Fusarium* laboratory manual by Leslie and Summerell (2008, p. 117-274).

Then, after 14 days' incubation the *Fusarium* infested seeds were sorted into groups based on mycelial characteristics and agar coloration (Appendix II). Samples of mycelium from different seeds and groups were transferred to petri diches containing SNA (Appendix I) and PDA medium, using a sterilized metal smear loop, and the dishes incubated in the same light chamber with the same light- and temperature conditions for 14 days. Two pieces of sterilized filter paper (1x2 cm) were placed on the SNA agar prior to mycelium transfer to promote sporulation upon incubation. Then, after 14 days, the colonies growing on SNA were identified to species based on spore morphology observed with a light microscope. A small square of agar

containing spores was cut using a sterilized scalpel and placed on a microscopic slide. Agar pieces were cut close to the filter paper. The aerial mycelium with phialides, macro- and microconidia characteristic for different *Fusarium* species were observed with the microscope and served as a tool for *Fusarium* species identification. Adding one drop of lactic acid on top of the agar piece, and covering it with a coverslip, releases spores into the lactic acid and facilitates spore observation, hence species identification. The clean *Fusarium* isolates (little bacterial contamination) from the SNA dishes, were again transferred to new SNA plates to make single spore isolates.

The morphology of the mycelium formed on the PDA dishes served as an extra tool for *Fusarium* species identification as the mycelium formed on PDA express species-specific characteristics.

Morphological characterization of *Fusarium* species by freezing blotter method

First, two layers of squared filter paper were cut to fit into transparent incubation dishes measuring 245 mm x 245 mm x 2 mm (Nunc Bio-assay dish). Two incubation dishes were prepared for each replicate: one for surface sterilized seeds and one for non-surface sterilized seeds. The incubation dishes were marked with date, as surface- or non-surface sterilized, and with grains numbers. The filter paper in the incubation dishes was moistened with 25 ml of distilled water and straightened out with a sterilized tweezer. It is important that the filter paper is straightened out to prevent the seeds from displacing within the incubation dish when moved. Then, 100 surface- and non-surface sterilized seeds were plated out in each dish with approximately 2 cm distance between the seeds. The same surface sterilization procedure as used in the *Fusarium* selective medium method. The dishes were then incubated at room temperature for 24 hours (≈20°C) followed by 24 hours in the freezer at -20°C. After freezing, the plates were incubated in a light chamber as described above. After five days' incubation, the seeds were registered as *Fusarium* infested or non-infested. Seeds registered as *Fusarium* infested had mycelial characteristics fitting into the descriptions of aerial mycelium formed by different *Fusarium* species descried in the *Fusarium* laboratory manual.

The plates were then returned to the light chamber for another seven days' incubation. Moisture of the filter paper was monitored during the whole incubation period, and distilled water was added when needed in order to avoid drying of the filter paper. After 14 days' incubation the infested seeds separated into five groups based on different characteristics of the visible mycelium (Appendix II). Mycelium from the different groups were transferred to Petri dishes containing SNA medium with small pieces of sterilized filter paper (1x2cm), and incubated for

14 days in the NUV-light chamber. After 14 days, a small square of agar containing spores were cut using a sterilized scalpel and placed on a microscopic slide. Agar pieces were cut close to the filter paper. The *Fusarium* species were identified based on spore morphology under the microscope as described for the CZPD medium

Molecular identification of *Fusarium* species from single spore isolates

To confirm the identification of *Fusarium* colonies according to the morphological assessment, single spore isolates were made for a selection of the colonies, and their species identity were assessed by species-specific PCR analyses and in one case also ITS sequencing. Single spore isolates were made by cutting small pieces of agar from the incubated SNA plates and transferred to a 50 ml tube. The tube was filled with approximately 50 ml of distilled water and shaken to thoroughly mix the spores in water. Then, 5 ml of the spore-water mix was transferred to a new 50 ml tube and filled with 50 ml distilled water. This was repeated in a third tube. In this manner, three concentrations of spore suspensions were made. The three spore mixtures, with different concentrations, were poured onto individual water agar plates. Excess water on the plates was removed, and the plates were placed in the sterile cabinet overnight (between 12-18 hours) in an upright position balanced against the wall. The agar plates were observed under an inverse microscope and it should now be possible to find single spores on the agar plates. Single spores were easiest to detect on the agar plates with the lowest spore concentration. One single spore from each isolate was transferred to new PDA plates, using a small round cutter, and the agar piece was placed in the middle of the plate. The PDA dishes were placed in room temperature on the benchtop, upside-down to avoid condensation and promote mycelial growth. The plates were stored on the benchtop for five days, until DNA extraction.

DNA from the single spore isolates were extracted from mycelium from the PDA dishes using the DNeasy® Plant Mini Kit from Qiagen. First, 400 µl Buffer AP1 and 4 µl RNase A stock solution was mixed in a clean 1.5 ml Eppendorf tube. Then, mycelium from the PDA dishes were scraped off of the agar with a clean scalpel and placed in a mortar. Liquid nitrogen was added to the mortar and the mycelium was crushed thoroughly into a fine fraction. Approximately a spatula with crushed mycelium powder were added to the Eppendorf tube containing Buffer AP1 and RNase A stock solution. The tubes were incubated for 10 minutes at 65°C on a heat rotator. Then, 130 µl Buffer P3 was added, vortexed and placed on ice for five minutes. The tubes were centrifuged at 20 000 x g for five minutes to spin down precipitate. The supernatant was transferred to a QIAshredder column with a 2 ml collection tube. The

tubes with the spin column were centrifuged for two minutes at 20 000 x g. The flow-through was transferred into a new clean tube without disturbing the precipitate, and 675 µl Buffer AW1 was added and mixed by gently inverting the tubes two times. Then, 650 µl of the solution was pipetted into a DNeasy Mini spin column and spun down for one minute at 6000 x g. The flow-through was discarded. This step was repeated with the remaining liquid. The spin column was transferred to a new 2 ml Eppendorf tube and 500 µl Buffer AW2 was added and centrifuged for one minute at 6000 x g. The flow-through was discarded. Another 500 µl Buffer AW2 was added and centrifuged for two minutes at 20.000 x g, and the flow-through was discarded. Contact between the spin column and flow-through was avoided. The spin column was transferred to a new clean 1.5 ml Eppendorf tube and 100 µl Buffer AE for elution was added and followed by incubation at room temperature for five minutes. Finally, the tubes were centrifuged at 6000 x g for one minute to bring down the DNA elution. The DNA eluates were stored at -20°C until further analysis.

To confirm the identity of the single spore isolates identified morphologically, species-specific qPCR analyses were performed the same way as for plant material. The standard DNA used in the qPCR reactions were standard DNA already available at NIBIO.

One of the single spore isolates which was identified as *F. sporotrichioides,* based on spore morphology, turned out negative in the species-specific qPCR analysis. The isolate was therefore sent to Eurofins for ITS sequencing to correctly identify the *Fusarium* species. Eurofins require at least 20 µl of PCR product for running the genomic analysis. The PCR product of the single spore isolate was prepared in a clear 0.2 ml 8-tube PCR strip with domed cap (Bio-Rad Laboratories, Inc.) and amplified using the T-100™ Thermal Cycler from Bio-Rad Laboratories, Inc. The PCR reaction was run on both undiluted DNA and a 10× dilution, in addition to including a positive control containing plant DNA and a negative (non-template) control containing MiliQ water. The total reaction volume was 25 µl, consisting of 22 µl pre-prepared mastermix and 3 µl of either fungal DNA, plant DNA (positive control) or MiliQ water (negative control) were added to the wells. The mastermix for one sample was prepared by mixing 2,5 µl of PCR Buffer (Thermo Fisher Scientific), 2 µl dNTPs (2,5mM), 0.75 µl $MgCl_2$, 1 µl ITS4 primer (10µM), 1 µl ITS5 primer (10µM), 0.1 µl Platinum Taq polymerase and 14,65 µl MiliQ water. The PCR amplification program was set to run for five minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 33 seconds at 55°C and one minute at 72°C,

then finishing with seven minutes at 72°C.

An 1% Agarose gel with eight wells were prepared to test if the obtained PCR product was of good quality before it was sent to Eurofins. In each well, 5 µl of solution were added: 4 µl of PCR product and 1 µl loading buffer, and the gel was run for 40 minutes at 80 V against a 100 base pair ladder.

The raw genome sequence obtained from Eurofins was trimmed and assembled using CLC Main Workbench software. The sequence was then used to support identification of the isolate based on searches in the public databases GenBank and Bold. This procedure was carried out by an employee at NIBIO.

Molecular quantification of *Fusarium* species in harvested oat grains

The presence of *F. poae, F. langsethiae, F. avenaceum*, *F. graminearum, F. culmorum* and *F. sporotrichioides* DNA was analysed by species-specific qPCR. DNA was extracted from 150 mg of milled oat grains using the FastDNA® SPIN Kit for Soil protocol as described for the ground plant material. Analysis of amount of fungal DNA present in the harvested oat grain by qPCR was carried out following the same procedure as described for plant material.

Analysis of *Fusarium* in oat seed

The Belinda oat seeds sown in the oat field both in 2017 and 2018 were analysed for *Fusarium* DNA in 2018. A 200 g sub-sample of oat seed which had been kept in storage for two years under dry conditions, was milled following the same procedure as for the harvested oat grains. The DNA extraction of qPCR analysis also followed the same procedure as for harvested oat grains.

Mycotoxin analysis

Harvested oat grains from 2017 and 2018 were ground (as previously described), and 1 g of oat flour were sent to BOKU (University of Natural Resources and Life Sciences, Vienna) for mycotoxin analysis. The mycotoxin analysis was carried out following the LC-MS/MS multi-analyte method (Malachová et al. 2014). For a complete overview of the mycotoxin content, see Appendix V.

Statistics

All statistical calculations were performed using two statistics software programs: R Commander (NMBU settings) and Minitab 18 Inc. All figures were designed in Microsoft Excel (for Mac) version 15.26. A Chi-Square test was performed to test if the difference in *Fusarium* infestation rate between the two plating methods was significant.

Data of the *Fusarium* DNA concentration in the plant material was not normally distributed and was transformed using the Johnson distribution system in Minitab to obtain normality. A General Linear Model (LM) was used to test for significant difference between plant parts (stem, flag leaf and panicle) and normal versus abnormal plants.

The data from the two consecutive growth seasons were analysed separately and only data from the three last weeks (until harvest) were analysed. All tests were run at 95% confidence level ($\propto$=0.05). If statistical difference was found, a Tukey's post hoc test was performed to find which groups that significantly differed.

Results

In 2017, all plant material collected from week 25 to 33 were analysed for the DNA content of four different *Fusarium* species. In 2018, plant material from eight weeks, out of the in total 14 weeks in which plant material was collected, was analysed for *Fusarium* DNA. These eight sampling time points were selected because the plants were at specific growth stages (heading, flowering and ripening) and due to the results of the *Fusarium* DNA analysis of plant material from 2017 which indicated high levels of Fusarium DNA at the point past ripening.

The oat plants developed faster in 2018 than in 2017 which can be attributed to the different weather conditions between the two years with much higher temperatures in 2018 (Fig. 14). In 2018, both flowering and ripening of the oat plants occurred three weeks earlier than in 2017, measured as weeks from sowing.

The levels of *Fusarium* DNA in the panicles, flag leaves and stems of normal and abnormal oat plants at different growth stages were analysed by qPCR. The results indicated higher concentrations of *Fusarium* DNA in the abnormal plant parts for all four *Fusarium* species.

The average DNA content of *F. poae, F. langsethiae, F. avenaceum* and *F. graminearum*, in the plant material collected from abnormal oat plants were highest towards the end of the growth season, at ripening (GS 80-89) and senescence (90-99) respectively, both in 2017 and

in 2018 (Fig. 3-4). Analysis of the presence of *F. culmorum* and *F. sporotrichioides* were also carried out on several samples of plant material, but because of no DNA in any of the samples analysed they were not included in the study. The level of *Fusarium* DNA in the plant material was measured in pg *Fusarium* DNA per mg plant material and will from now on be referred to as pg DNA/mg.

In 2017, *F. poae* and *F. langsethiae* stood out as the most prevalent *Fusarium* species in the plant material, with remarkably high DNA concentrations of 142 533 pg DNA/mg and 93 666 pg DNA/mg in the abnormal panicle at ripening compared to *F. avenaceum* (478 pg DNA/mg) and *F. graminearum* (33.7 pg DNA/mg) (Fig. 3-4).

In 2018, *F. poae* and *F. langsethiae* were the most abundant in the oat plant material with the highest levels of *Fusarium* DNA observed in the abnormal panicles, with levels of 19 429 and 4024 pg DNA/mg respectively (Fig. 3 B-D).

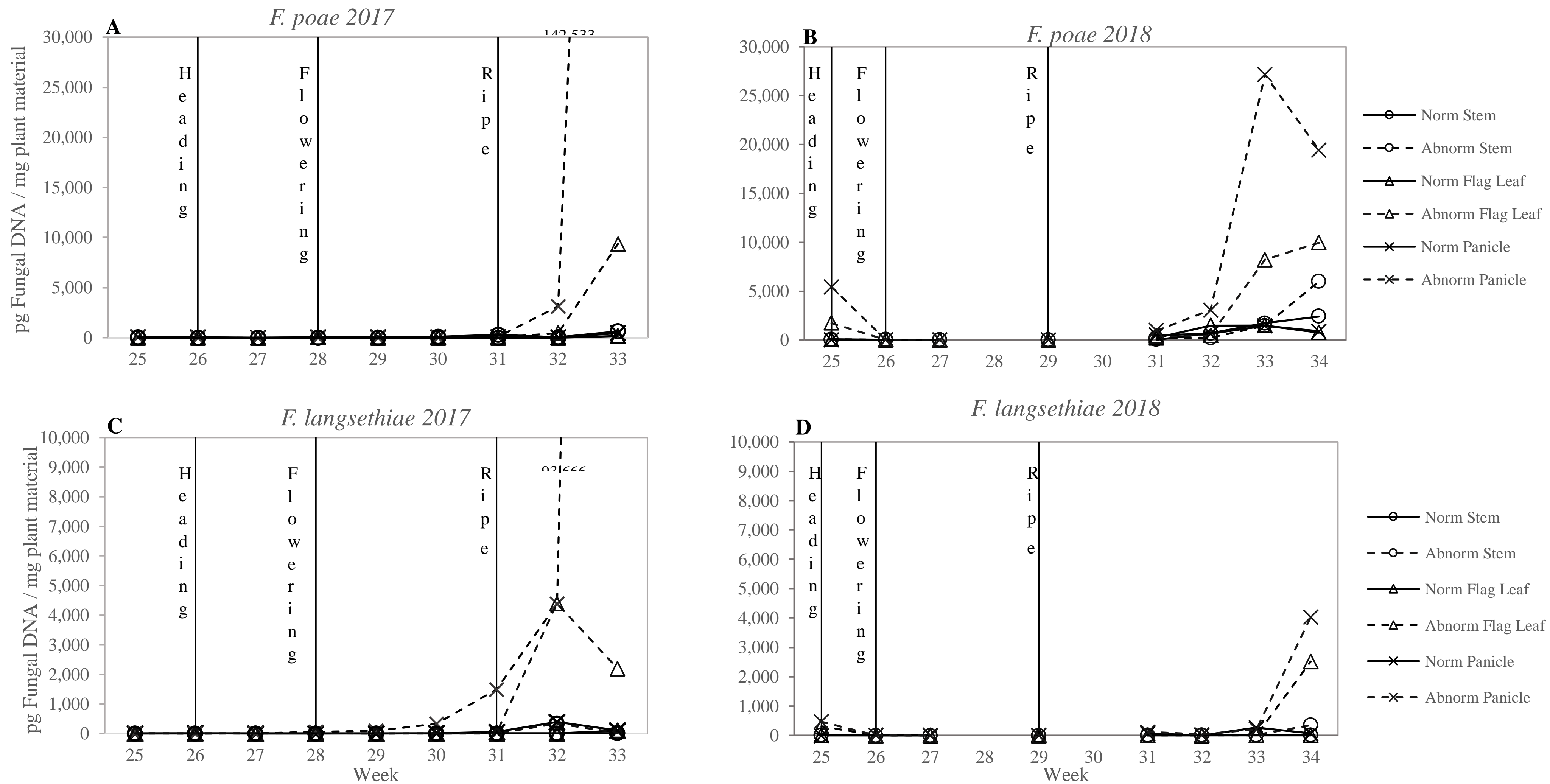

Figure 3. The content of Fusarium DNA (pg DNA/mg plant material) in different parts of plants collected in 2017 and 2018. The plant material was separated into stems, flag leaves and panicles, and material collected from normally developing plants was separated from plant material collected from abnormally developed plants. The Y-axis range up to 30 000 for Fusarium poae (A-B), whereas the y-axis for Fusarium langsethiae (C-D) range up to 10000. In figure A and C the fungal DNA content of plants collected in week 33 exceeded the scale and the value is listed above the figure. Heading = GS 50-59, Flowering = GS 60-69 and ripe= GS 80-89 (Lancashire et al. 1991).

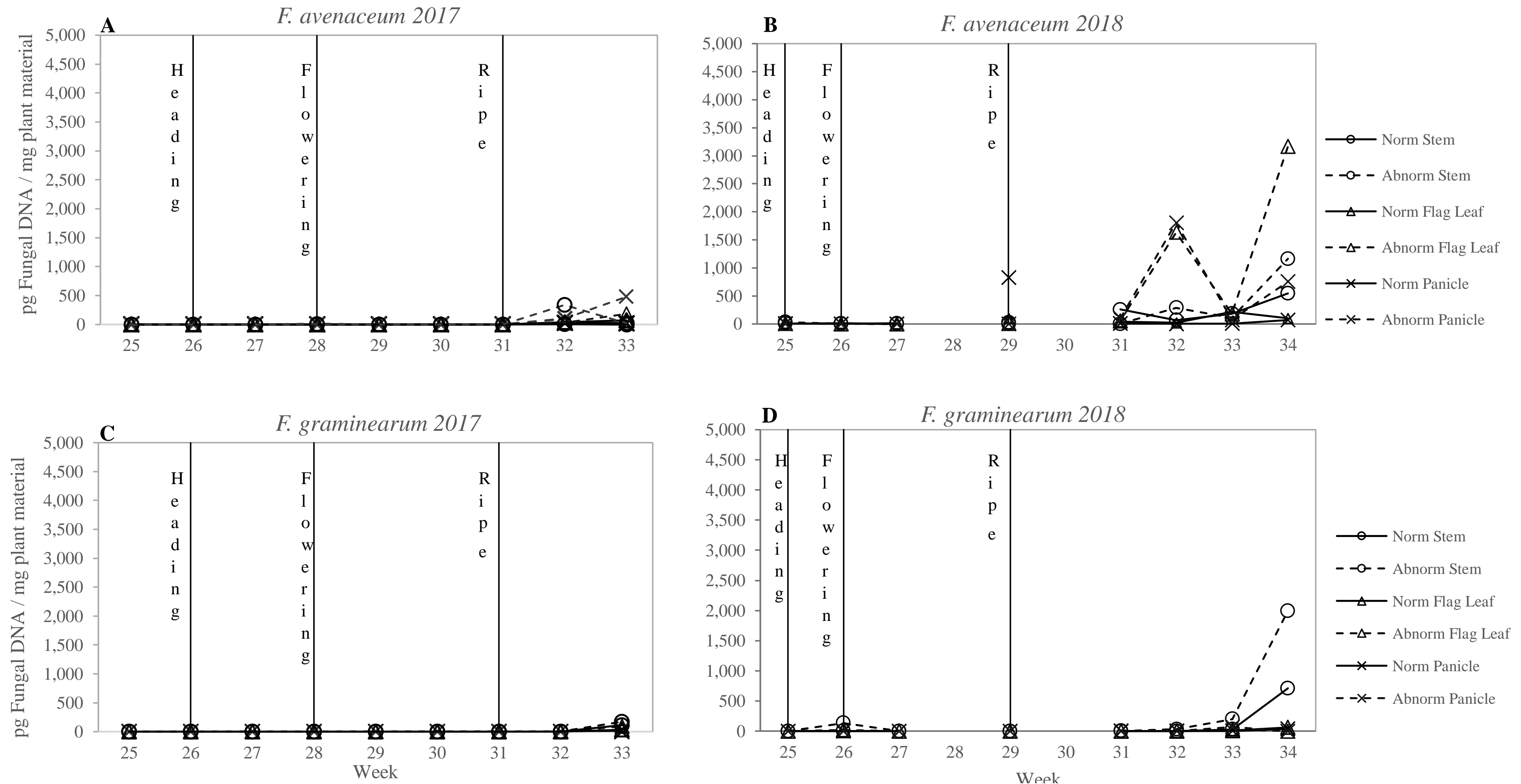

Figure 4. The content of Fusarium DNA (pg DNA/mg plant material) in different parts of plants collected in 2017 and 2018. The plant material was separated into stems, flag leaves and panicles, and material collected from normally developing plants was separated from plant material collected from abnormally developed plants. The Y-axis range up to 5000 both for Fusarium avenaceum (A-B) and Fusarium graminearum (C-D).. Heading = GS 50-59, Flowering = GS 60-69 and ripe= GS 80-89 (Lancashire et al. 1991).

Content of *Fusarium poae* DNA in oat plant material

In 2017 and 2018, *F. poae* was the most abundant *Fusarium* species in the plant material with maximum levels of 142 533 and 19 429 pg DNA/mg observed in the abnormal panicles (Fig. 3 A-B). Total DNA content of *F. poae* in the plant material varied between plant parts and growth stages both years.

In 2017, the concentration of *F. poae* DNA in normal stems, was negligible at all growth stages with a seasonal mean DNA concentration of 1.9 pg DNA/mg. No *F. poae* DNA was detected in abnormal stems collected in the period from stem elongation to end of flowering (week 25-29), whereas, in the period from grain development until harvest (week 30-33) the DNA concentration increased with plant development, exceeding 600 pg DNA/mg in the abnormal stem in week 33 (end of ripening). The level of DNA concentration in the flag leaf followed the same tendency as seen in the stem. Throughout the season, low levels of *F. poae* DNA were detected in the normal flag leaves (season mean 23 pg DNA/mg). In the abnormal flag leaves, the level of *F. poae* DNA were low (around 60 pg DNA/mg) during the early development stages, but at end of ripening the level of DNA increased to 470 and 9400 pg DNA/mg plant material in week 32 and 33 respectively. The highest *F. poae* DNA levels in 2017 were observed in the panicle. In normal panicles, the DNA concentration varied between 40–70 pg DNA/mg plant material from booting (week 25) until ripening (week 31) and increased to 450 pg DNA/mg the last week (33). In the abnormal panicles the DNA concentration was lower than in the normal plants until end flowering/ripening (week 31-33) where an extreme increase in *F. poae* DNA concentration occurred during ripening which reached >140 000 pg DNA/mg in week 33 (Fig. 3 A).

Similar to 2017, *F. poae* was the most abundant *Fusarium* species in the plant material in 2018. The amount of *F. poae* DNA in the plant material seemed to follow the same tendency in 2018 as in the previous season whit higher *F. poae* DNA concentrations in abnormal plants compared to in normal plants (Fig. 3 A-B). At oat ripening (week 31) a steep increase in *F. poae* DNA occurred in abnormal plant parts. An increase in *F. poae* DNA in the normal plant parts was also registered during this period, but at much lower concentrations. The DNA levels of *F. poae* were higher in the stems of both normal- and abnormal plants in 2018 compared to 2017. In normal- and abnormal plants, low levels of DNA in the stems were registered from heading until ripening, but from ripening (week 33) the DNA concentration increased (Fig. 3 B), most in abnormal stems. There were low levels of *F. poae* DNA in flag leaves of normal and abnormal plants from week 25-31, and the DNA levels were higher in abnormal plants. From ripening to end of ripening (week 32-34), a steep increase in *F.* poae DNA especially

occurred in abnormal flag leaves, reaching >9900 pg DNA/mg. The abnormal panicles had accumulated high levels of *F. poae* DNA at all developmental stages, with highest DNA concentrations post-ripening (from week 31-34). The DNA concentration in the normal panicles were lower than in the abnormal panicles, but the same tendency was observed in normal and abnormal plants, with an increase in *F. poae* DNA at late stages. In the abnormal panicles, the *F. poae* DNA level reached >19 000 pg DNA/mg in week 34 which were over 20 times higher than in the normal panicles the same week.

The results from the two seasons showed an increase in *F. poae* DNA in plant material late season, especially in the upper plant parts (flag leaves and panicles) of abnormal plants. Both in 2017 and 2018, DNA of *F. poae* was first detected in the plant material around heading (week 26 and 28 respectively), though at low levels.

Content of *Fusarium langsethiae* DNA in oat plant material

In 2017, the amount of *F. langsethiae* DNA in both normal and abnormal stems were low (<50 pg DNA/mg) throughout the season (Fig. 3 C). In normal flag leaves the amount of *F. langsethiae* DNA never exceeded 100 pg DNA/mg. In abnormal flag leaves, the DNA concentration was below 100 pg DNA/mg until week 31, when the DNA concentration increased reaching a maximum of 4392 pg DNA/mg at ripening (31-32). The concentration of *F. langsethiae* DNA in normal panicles were low. In abnormal panicles, increasing quantities of *F. langsethiae* DNA was recorded around flowering and onwards, reaching a DNA concentration of 93 667 pg DNA/mg two weeks post-ripening. Compared to the amount of *F. langsethiae* DNA in the normal panicle, the DNA amount in the abnormal panicle was >90% higher (week 31-33).

In 2018, the quantity of *F. langsethiae* DNA in the normal stems were low (<13 pg DNA/mg), this also applied to abnormal stems (Fig. 3 D). The amount of *F. langsethiae* DNA in normal and abnormal flag leaves were low, until full senescence (week 34) where the DNA concentration increased in the abnormal flag leaf (>2500 pg DNA/mg). The amounts of *F. langsethiae* DNA in the normal panicles were low, having a seasonal mean of 50.6 pg DNA/mg. The highest *F. langsethiae* DNA concentrations in the oat plant material of were observed in the abnormal panicles at senescence with a DNA concentration of >4000 pg DNA/mg in week 34. The level of *F. langsethiae* DNA in the abnormal panicle was higher around heading than at flowering, and the DNA concentration increased steadily from ripening to harvest.

Both years the level of *F. langsethiae* DNA in the plant material peaked late season between ripening and senescence. There were more *F. langsethiae* DNA in abnormal plants

than in normal plants, with the upper plant parts including flag leaf and panicle being most infected. In 2017 *F. langsethiae* were first detected in the plant material from flowering and onwards, whereas in 2018 *F. langsethiae* DNA was first detected in the plant material at heading.

Content of *Fusarium avenaceum* DNA in oat plant material

The amounts of *F. avenaceum* DNA in the plant material were low both years compared to the amounts of *F. poae* and *F. langsethiae* in the plant material.
In 2017, the amount of *F. avenaceum* DNA in normal plant parts never exceeded 100 pg DNA/mg (Fig. 4 A). The concentration of *F. avenaceum* DNA in abnormal plant parts was higher than in normal parts. From week 25 to 31, from stem elongation to ripening, the levels of *F. avenaceum* DNA in the stem, flag leaf and panicle, irrespective of normal or abnormal plants, were negligible (Fig. 4 A). From end-ripening, the amount of *F. avenaceum* DNA in the abnormal panicle was 478 pg DNA/mg which was the highest levels of *F. avenaceum* observed in 2017.

The concentration of *F. avenaceum* in the plant material in 2018 was higher than in 2017 (Fig. 4 A-B). The amount of DNA increased after ripening in all plant parts of normal and abnormal plants, with the highest increase in abnormal plants (Fig. 4 B). From heading to ripening there were little *F. avenaceum* DNA in normal- and abnormal plant parts, but at end-ripening the amount increased in all plant parts. The highest levels of *F. avenaceum* DNA were observed in the abnormal flag leaves (3167 pg DNA/mg) and stem (1163 pg DNA/mg) in week 34.

Content of *Fusarium graminearum* DNA in oat plant material

The content of *F. graminearum* DNA was minimal in normal and abnormal plants both years (Fig. 4 C-D). In 2017, the levels of *F. graminearum* DNA in the different plant parts was negligible at all developmental stages in both normal and abnormal plants (Fig. 4 C). In 2018, the amount of *F. graminearum* DNA never exceeded 70 pg DNA/mg plant material in the flag leaf or panicle of neither normal or abnormal plants (Fig. 4 D). However, higher concentrations of *F. graminearum* DNA were observed in the stem. The *F. graminearum* DNA concentration in the normal stems at end of ripening (week 34) was 710 pg DNA/mg whereas the levels in the abnormal stems were 1994 pg DNA/mg.

Fusarium DNA in the different plant organs of normal and abnormal plants

When assessing the results in Figure 3 and 4 one can observe a trend of higher *Fusarium* DNA concentration in abnormal plants compared to normal plants. The data from the three latest points of plant collection were included into the statistical analysis. Because of the low concentrations of *F. avenaceum* and *F. graminearum* DNA in the plant material in 2017 and 2018, these data could not be transformed and were excluded in the statistical analysis. The transformed data were used in the statistical analysis, however the mean values presented in Table 2 and 3 are the original *Fusarium* DNA concentrations (pg DNA/mg).

A significant difference between level of *Fusarium* DNA in normal and abnormal plants were only observed for *F. poae* in 2017 (Table 2). The concentration of *F. poae* and *F. langsethiae* DNA in the abnormal panicles in 2017 were significantly higher than the DNA concentration in the oat stems (Table 3). No significant difference was observed between the *Fusarium* DNA in normal or abnormal plants or between the different plant organs in 2018.

Table 2. The mean Fusarium DNA content (pg DNA/mg) from 10 abnormal and normal plants collected at the three last points of sampling in 2017 and 2018.

	2017		**2018**	
	F. poae#	*F. langsethiae*#	*F. poae*#	*F. langsethiae*#
Normal plants	81 a	74 a	1285 a	41 a
Abnormal plants	15662 b	11802 a	8446 a	794 a
P-value*	0.001	0.132	0.215	0.433

 # The mean DNA content of Fusarium poae and *F. langsethiae* in the normal and abnormal plants

**The P- value obtained from the General Linear model. The Tukey Pairwise Comparison (Post-Hoc test) were carried out to investigate the significant difference in means between the groups of normal and abnormal plants. Groups that do not share letters are significantly different.*

Tabell 3. The mean Fusarium DNA content (pg DNA/mg) in the different groups of plant organs (stem, flag leaves, panicles) from 10 abnormal oat plants.

	2017		**2018**	
	F. poae#	*F. langsethiae*#	*F. poae*#	*F. langsethiae*#
Stem	333 a	15 a	2562 a	121 a
Flag leaves	3290 ab	2206 ab	62245 a	840 a
Panicle	48583 b	33183 b	16550 a	1422 a
P-value*	0.046	0.006	0.522	15.2

 # The mean DNA content of Fusarium poae and F. langsethiae in the stems, flag leaves and panicles of abnormal plants

**The P- value obtained from the General Linear model. The Tukey Pairwise Comparison (Post-Hoc test) were carried out to investigate the significant difference in means between the groups of normal and abnormal plants. Groups that do not share letters are significantly different.*

The DNA content of *Fusarium* in *Poa annua* plants

In 2018, ten *P. annua* plants were monthly collected, from May to August, within the oat field. DNA of *F. poae, F. langsethiae, F. avenaceum* and *F. culmorum* were detected in this plant material, however the amount of *Fusarium* DNA varied between the different fungal species (Fig. 5). Increasing levels of *F. poae* DNA were detected in the plant material of *P. annua* throughout the growth season from May to August. DNA of *F. langsethiae* DNA were detected in the *P. annua* plants at all sampling points, however the DNA concentration was negligible (<1 pg DNA/mg). The highest concentration of *Fusarium* DNA was observed for *F. avenaceum,* where the DNA concentration in the plant material increased throughout the season. The amount of *F. graminearum* DNA reached its peak in July (69 pg DNA/mg), before an abrupt decrease in August (< 3 pg DNA/mg). There was a slow increase in *F. poae, F. avenaceum* and *F. graminearum* DNA in the *P. annua* plants from oat heading through flowering and ripening.

No action of chemical or mechanical weed control were carried out on the oat field during the two years and the presence of weeds within and around the oat field were high. The following weed species were present in the oat field in 2018 were: *Achillea millefoluim, Chenopodium album, Lamium purpureum, Plantago major, Poa annua, Ranunculus repens, Spergula arvensis, Stellaria media, Taraxacum officinale, Trifolium pratense* and *Tripleurospermum inodorum*.

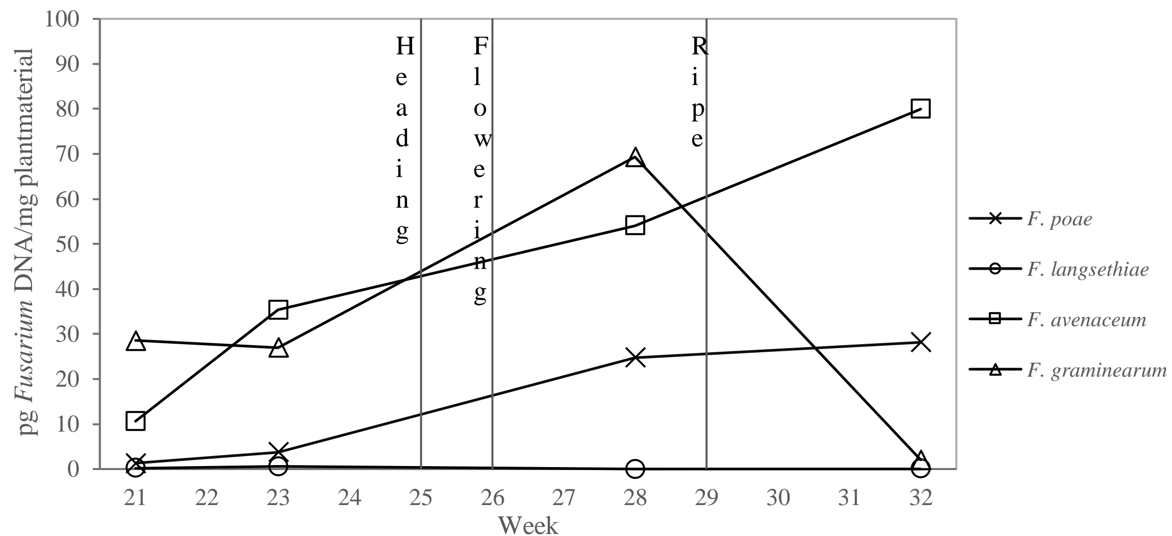

Figure 5. Concentration of Fusarium DNA (pg DNA/mg) in 10 whole plants of P. annua monthly collected within the oat field in 2018.

The DNA content of Fusarium in Air samples

Only air samples collected in 2017 were analysed and these samples were collected from June to End-August (13 weeks). The air samples were analysed for the DNA content of *F. poae, F. langsethiae, F. avenaceum* and *F. graminearum* on a weekly basis. The total number of spores of the respective *Fusarium* species collected during one week was calculated based on the DNA content recorded in the qPCR analysis (Appendix VII).

The insects which were sucked into the Eppendorf tubes were counted and included in the DNA extraction. As insects are assumed to contain large amounts of DNA, their presence might influence total DNA amount in the samples. The Pearson's correlation coefficient stated high correlation between total DNA in the first DNA eluate and number of insects (R^2=0.60) (Appendix X).

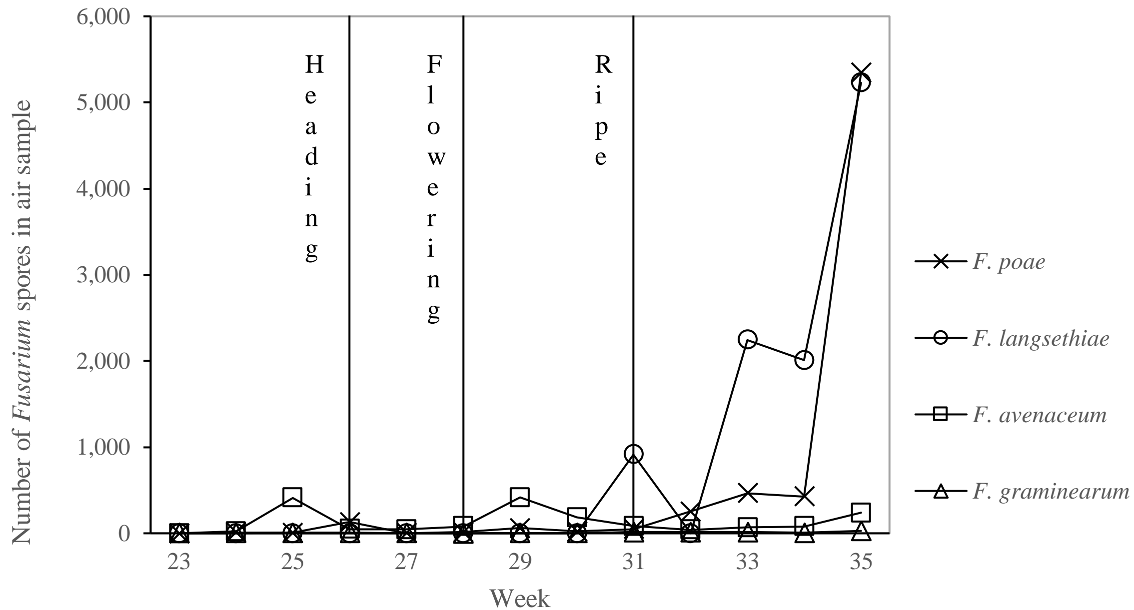

Figure 6. The weekly number of Fusarium spores in air samples collected in 2017. The number of spores is calculated based on a standard spore solutions and Fusarium DNA (calculation in Appendix VII). The growth stage (Lancashire et al. 1991) of normal oat plants is indicated in the figure.

Fusarium poae and *F. langsethiae* were the most abundant *Fusarium* species in the collected air samples (Fig. 6). The amount of *F. poae* and *F. langsethiae* spores in the air samples drastically increased late season, from post-ripening until harvest. The highest number of *Fusarium* spores were calculated for *F. poae* and *F. langsethiae* tin air samples collected he week after harvest (week 35). From week 34 to 35 there was a steep increase in *F. poae* DNA in the air sample, reaching an estimated number of 5200spores. There were no spores of *F. langsethiae* in the air sample from week 23 to 31, however from week 31 to 35 there was a stepwise increase in DNA reaching an estimated number of >5200 spores the week of harvest (week 35). The amount of *F. poae* and *F. langsethiae* spores in the air samples increased late season, from ripening until harvest. The DNA content of *F. avenaceum* and *F. graminearum* were low throughout the sampling period.

Morphological identification of *Fusarium* species in oat grains

Oat grains harvested in 2017 were visually analysed for infestation of *Fusarium* species using two different methods in which seeds were incubated on either moist filter paper (freezer blotter method) or on *Fusarium* selective agar (CZPD). The aims of these experiments were 1) to investigate which *Fusarium* species that infested the oat grains, and 2) investigate if the number of *Fusarium* species detected, and the average number of *Fusarium*-colonized grains, significantly differed between the two methods.

A significantly higher *Fusarium* infestation rate was observed on grains incubated on *Fusarium* selective medium, compared to incubation on filter paper (Chi-square test, P=0.04, Fig. 7). Furthermore, non-surface sterilized grains had an average *Fusarium* infestation rate of 53% and 65% after incubation on moist filter paper and *Fusarium* selective medium respectively (Fig. 7), and these levels were statistically higher than the *Fusarium* infestation rates observed for surface sterilized grains within the respective methods (Chi-square test, $P<0.05$).

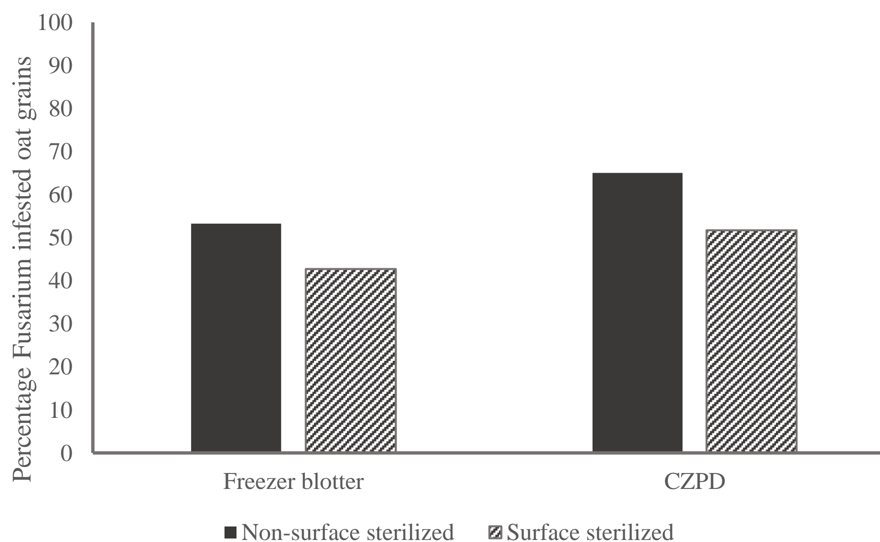

Figure 7. Average Fusarium infestation of oat grains (%) after incubation on Fusarium selective agar (CZPD) or on moist filter paper (freezer blotter). A total of 600 randomly selected seeds were analysed with each method, 300 surface sterilized and 300 non-surface sterilized.

The degree of mycelial growth on the grains differed between the two methods. On *Fusarium* selective medium the fungal colonies were between 1 and 3 cm in diameter after 14 days of incubation, and there was great variation in morphological characteristics between the fungal colonies (Fig. 8A). On filter paper, only sparse white- or pinkish mycelium could be observed on some of the grains, however most grains were colonized by other fungal species which made it difficult to distinguish *Fusarium* species from other fungal species (Fig. 8B).

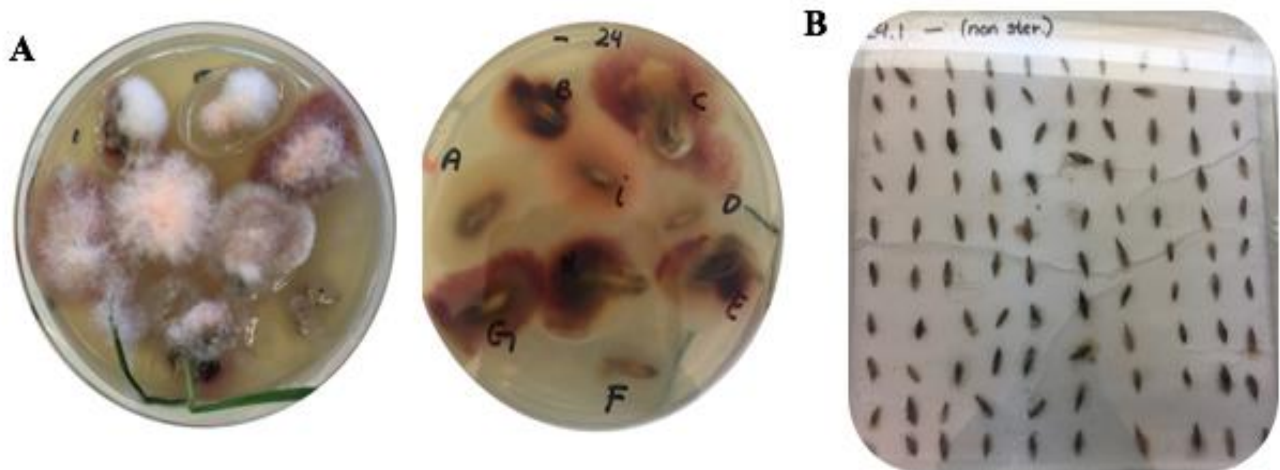

Figure 8. A) Picture illustrating the morphological variation of Fusarium colonies on a Petri dish with non-surface sterilized grains incubated on FUSARIUM SELECTIVE MEDIUM medium. Picture taken from above (left) and below (right) and B) grains after two weeks incubation on moist filter paper.

The mycelial growth on oat grains incubated on *Fusarium* selective medium expressed great variation in morphological characteristics between grains within and between each Petri dish (Fig. 8A). The differences were seen as variation in mycelium- colour, density, shape and texture, and variation in agar discoloration. *Fusarium* colonies formed on each grain were categorised into different groups based on morphological characteristics (Appendix II). Mycelium from each morphological group was transferred to SNA medium and incubated in a light chamber to promote sporulation.

Spores from the sporulating mycelium, from each morphological group, were assessed under the microscope and the *Fusarium* species were visually identified based on spore morphology (Fig. 9). The *Fusarium* species were identified based on their spore morphology thoroughly descirped in the *Fusarium* laboratory manual (Leslie and Summerell, 2008), except *F. langsethiae* which is described by Torp and Nirenberg (2004).

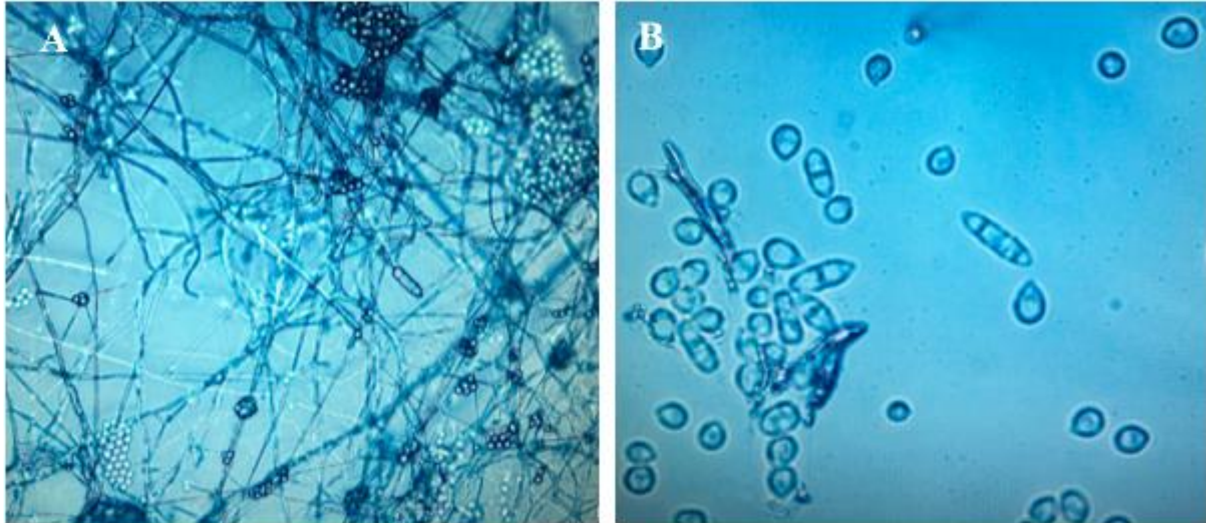

Figure 9. Aerial mycelium and spores of F. poae seen through the microscope. A) Aerial mycelium of F. poae with visible clusters of microconidia and one macroconidia. B) Characteristic microconidia and macroconidia of F. poae. Microconidia of F. poae are either globose or napiform, with 0 or sometimes 1 septa. Macroconidia are usually short with a falcate or lunar shape with 3-5 septa, normally 3 septa (Leslie and Summerell, 2008).

On *Fusarium* selective medium a wider range of *Fusarium* species were identified compared to on filter paper where only *F. poae* and *F. gramniearum* were identified (Fig. 10).
The majority of all *Fusarium* isolates on grains incubated on both *Fusarium* selective medium and moist filter paper were identified as *F. poae* (Fig. 10). On *Fusarium* selective medium, 52% of the non-surface sterilized grains were infested with *F. poae* compared to 32% of the non-surface sterilized (Fig. 10 A). The same trend was seen on the grains incubated on filter paper. Most grains incubated on filter paper were infested with *F. poae*, 53% of the non-surface sterilized grains and 42% of the surface sterilized grains (Fig. 10 B).

Cultures of *F. poae* grown on artificial media often have a distinctive sweet and peachy-like odor (Leslie and Summerell, 2008) and this characteristic odor was registered in 58 out of all 66 Petri dishes. This can only serve as an additional sensory tool to indicate presence of *F. poae* and is not a valid proof.

Other fungal and bacterial colonies were also present on the oat grains, these accounted for 57% of all 600 seeds incubated on *Fusarium* selective medium and 52% of all seeds incubated on filter paper (not included in Fig. 10).

A selection of isolates, identified to be *F. poae*, *F. avenaceum, F. graminearum* and *F. sporotrichioides* based on the morphological methods, were additionally tested by species-specific qPCR. The species-specific qPCR confirmed the species for all the isolates, except for the one identified as *F. sporotrichioides* which turned out negative in the species-specific qPCR. Because of this, the species identity of this isolate was additionally assessed by ITS sequencing. The ITS sequence analysis for this isolate resulted in a 545 bp sequence (Appendix XI), which had highest affinity (>99% match) to a number of *Fusarium acuminatum* acquisitions in both databases (GenBank and Bold), suggesting the isolate to be *F. acuminatum*.

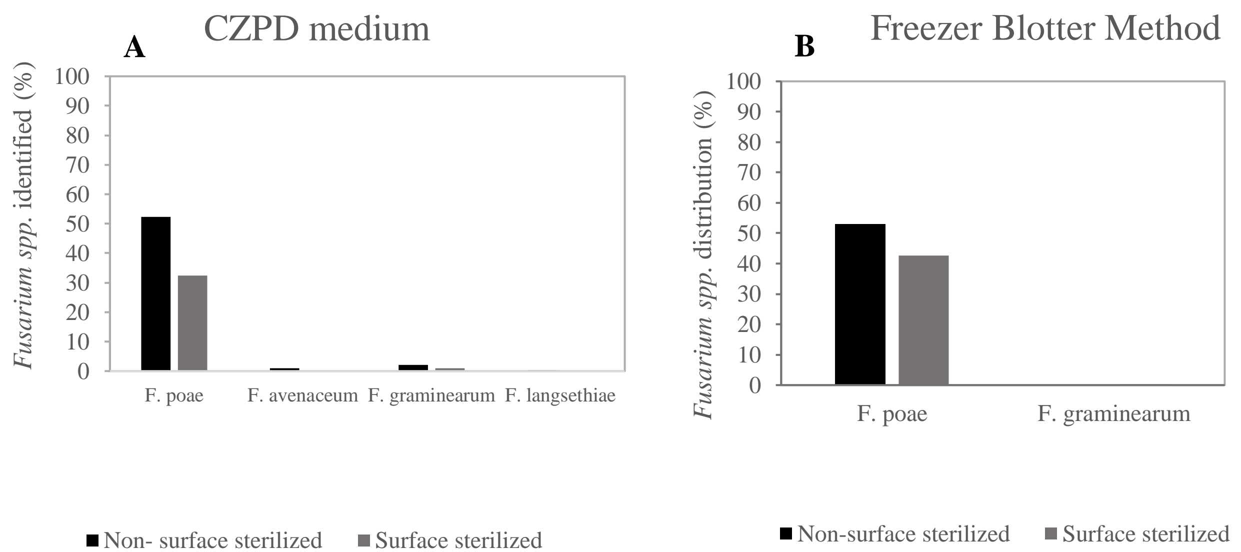

Figure 10. Distribution of Fusarium species identified on the harvested oat grains after incubation on A) Fusarium selective medium and B) blotted filter paper, given in percentage of all surface sterilized grains (300) and all non-surface sterilized grains (300).

The DNA content of Fusarium in oat seeds and harvested oat grains

The same seeds (cv. Belinda) were planted both years. Infested seeds might be a possible source of *Fusarium* inoculum, therefore qPCR analysis of *Fusarium* DNA was performed on this seed lot. The highest levels of *Fusarium* DNA in the oat seed were observed for *F. langsethiae* (490 pg DNA/mg) followed by *F. avenaceum* (44 pg DNA/mg), *F. poae* (26 pg DNA/mg) and *F. graminearum* DNA (0.35 pg DNA/mg) (Fig. 11).

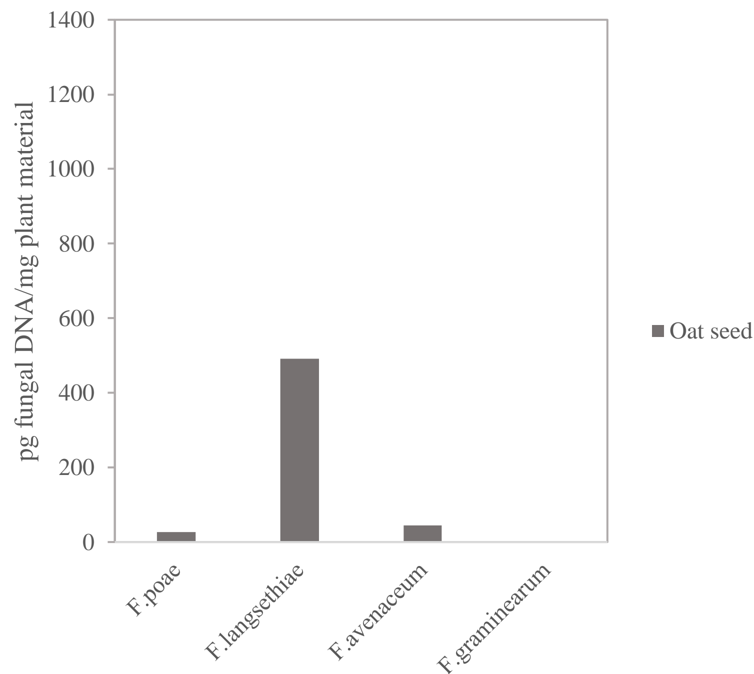

Figure 11. The DNA content of selected Fusarium species in oat seed planted both in 2017 and 2018.

In the harvested oat grains, the *Fusarium* DNA concentration was higher in 2018 than in 2017 for all the four species analysed (Fig. 12). The highest levels of *Fusarium* DNA in the harvested oat grains were observed in 2018 for *F. poae* with 1318 pg DNA/mg (Fig. 12). In 2017, the concentration of *F. poae* DNA was considerably lower (68 pg DNA/mg) compared to in 2018. Low levels of *F. langsethiae* DNA were detected in the harvested grains both years, but there were more *F. langsethiae* in the grains from 2018 than 2017 with 159 pg DNA/mg and 13 pg DNA/mg respectively. The same tendency of higher levels of DNA in 2018 compared to 2017 also applied to the amount of *F. avenaceum* DNA in the oat grains, with a DNA level of 98 pg DNA/mg in 2018 and 2 pg DNA/mg in 2017. Concentration of *F. graminearum* DNA in the samples was negligible (<1 pg DNA/mg plant material) both years (Fig. 12).

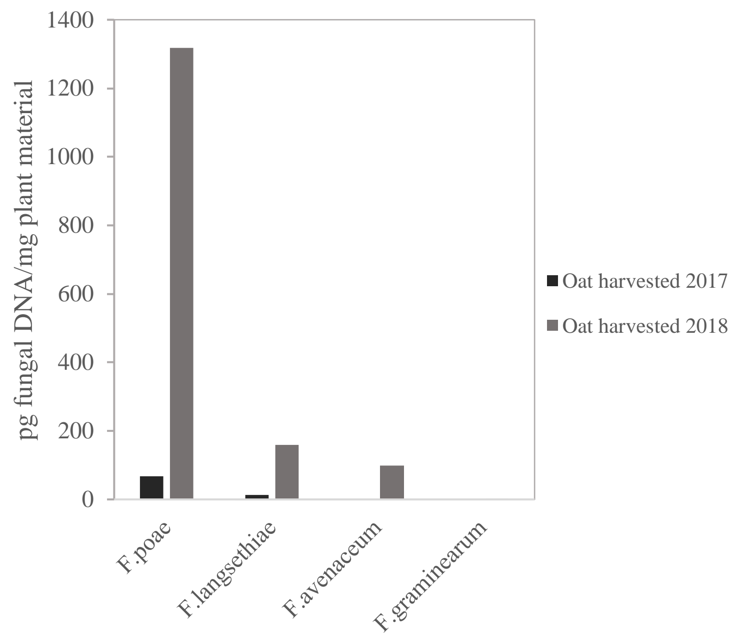

Figure 12. The DNA content of selected Fusarium spp. in oat grains harvested from a field in 2017 and 2018 as well as the Fusarium DNA-content of the oat seeds used for planting both years.

Because of the remarkably higher *Fusarium* DNA levels detected in 2018 (*F. poae*) compared to in 2017, the qPCR analysis of the oat grains harvested in 2018 was repeated. However, the results did not deviate from the results obtained in the first run.

Mycotoxin content in the oat grains

The mycotoxin content in the harvested oat grains showed great variation between the three seasons with the highest levels of DON and T-2/HT-2 observed in the oat grains harvested in 2016, and the highest levels of NIV and AUF observed in the oat grains harvested in 2017 (Fig. 13). The DON concentration in the oat grains harvested in 2016 were remarkably high, >9200 µg kg^{-1}, and also the combined concentration of T-2/HT-2 was much higher in 2016 than the than in 2017 and 2018, with levels of 360>74>3 µg kg^{-1} respectively. The levels of NIV was lower in 2016 than in the two following years.

The concentration of NIV in harvested oat grains was high both in 2017 and 2018, with higher contamination in 2017 (713 µg kg^{-1}) than in 2018 (462,2 µg kg^{-1}). Also, the levels of AUF were much higher in 2017 (696 µg kg^{-1}) than in 2018 (66 µg kg^{-1}). In 2017 and 2018, the levels of the two types of A-trichothecenes, T-2 and HT-2, was low with a combined T-2+HT-2 concentration of 74 µg kg^{-1} and 3,4 µg kg^{-1} (Fig. 13).

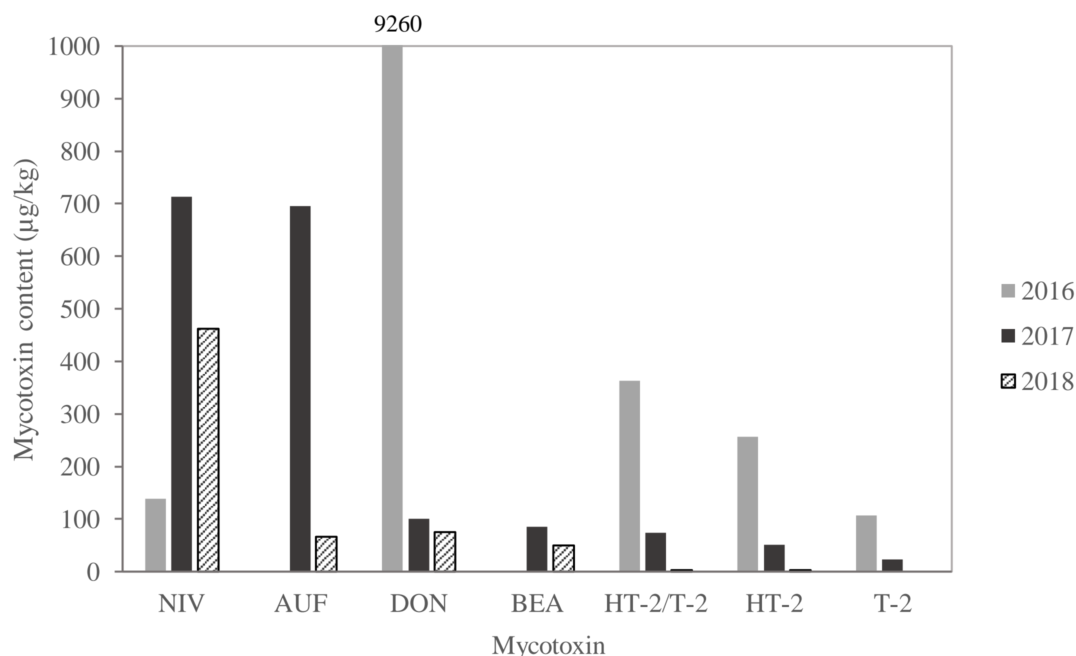

Figure 13. The content mycotoxins in oat grains harvested from a field trial in 2016, 2017 and 2018. The content of additional mycotoxins is listen in Appendix V. The analysis of mycotoxins was performed by University of Natural Resources and Life Sciences, Vienna (BOKU). NIV= Nivalenol, AUF= Aurofusarin, DON= Deoxynivalenol, BEA= Beauviricin.

Weather data

The weather conditions during the 2017 and 2018 growth seasons turned out very different. The season of 2017 was characterised as a good growth seasons with relatively high temperatures and evenly distributed periods of rainfall (Fig. 13). Oppositely, the 2018 season could be characterised by abnormally high temperatures and minimal precipitation (Fig. 13).

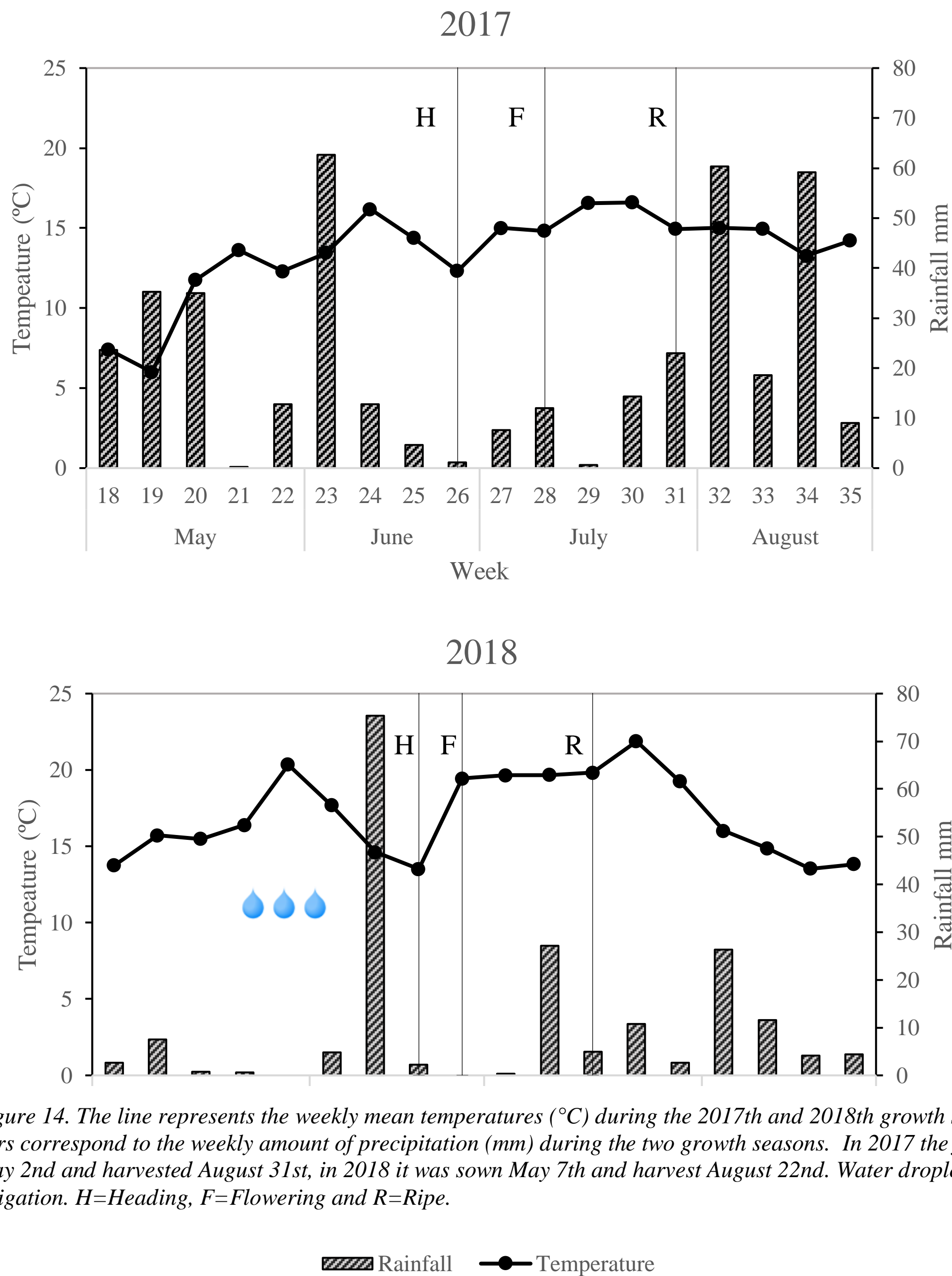

Figure 14. The line represents the weekly mean temperatures (°C) during the 2017th and 2018th growth season. The bars correspond to the weekly amount of precipitation (mm) during the two growth seasons. In 2017 the field was sown May 2nd and harvested August 31st, in 2018 it was sown May 7th and harvest August 22nd. Water droplet indicates irrigation. H=Heading, F=Flowering and R=Ripe.

During flowering in 2017, there were little precipitation (<2 mm) and the mean temperature was around 15 ℃. However, one week past flowering, there were weakly events of precipitation and the temperatures was stable around 15 ℃. In 2018, there were no precipitation during the week of flowering or the following weeks and the mean temperatures were around 20 ℃. The amount of precipitation in 2018 was scarce and the oat plants experienced drought stress.
The total amount of precipitation in 2018 were more than halved compared to 2017, and the mean temperatures were higher, compared to 2017 (Table. 4). Because of the scarce amount of precipitation in 2018 and high temperatures, the oat plants experienced drought stress. Due to the warm weather early season the oat plants matured earlier in 2018 than 2017, however the oat plants in 2018 turned out short and frail looking, possibly due to weather related abiotic stress (Appendix IX).

Table 4. Mean monthly temperatures (°C) and rainfall (mm) in 2017 and 2018 and the difference in weather conditions between the two seasons. The prepositions either indicates a decrease (-) or an increase (+) in monthly temperature or precipitation from 2017 to 2018.

	2017		**2018**		**Difference 2017/2018**	
	Temperature	*Rain*	*Temperature*	*Rain*	*Temperature*	*Rain*
May	10.2	21.4	15.3	2.9	+5.1	-18.5
June	14.1	20.2	16.3	20.6	+2.2	+0.3
July	15.6	11.5	20.0	9.2	+4.5	-2.3
August	14.4	36.8	14.6	11.6	+0.2	-25.2

Discussion

The main objective of this study was to gain insight into the biology of *F. poae* and *F. langsethiae*. Knowledge about the inoculum source and inoculum dispersal pattern of *Fusarium* species is important in understanding their biology. Weed (*P. annua*) was examined as a potential source of *F. poae* and *F. langsethiae* inoculum. Over two growth seasons, normal and abnormal oat plants were weekly collected from a minimum till field with continuous oats. The time point of *F. poae* and *F. langsethiae* infection in the main shoot of oats in this field was investigated by quantifying *Fusarium* DNA in different plant parts at different growth stages of normal and abnormal oat plants. The harvested oat grains were analysed for *Fusarium* DNA and mycotoxin content.

Inoculum sources of *F. poae* and *F. langsethiae*

Low to moderate DNA-levels of *F. poae*, *F. avenaceum* and *F. graminearum* were observed in the *P. annua* plants. Our results coincide with the findings of Inch and Gilbert (2003) who depreciated *P. annua* as a common host of *F. poae, F. avenaceum, F. graminearum* and *F. culmorum*. Jenkinson and Parry (1994a) and Champeil et al. (2004) successfully isolated several *Fusarium* species, including *F. poae, F. avenaceum* and *F. graminearum*, from a wide range of common weeds found within cereal fields.
On the contrary, Landschoot et al. (2011) investigated the presence of *F. poae* DNA in 10 of the most common gramineous weeds in Belgium and disclaimed gramineous weeds a probable source of *F. poae* inoculum. In the light of our and others observations presenting no- or low amounts of *F. poae* DNA in weeds, one can hypothesise that the role of the investigated weeds as alternative hosts of *F. poae* is not of great importance. However, further research, on a wider range of weed species with large sample sizes is needed, to pin out their role as alternative hosts of *F. poae*.

Low or negligible levels of *F. langsethiae* DNA were detected in the *P. annua* plants from this field survey. Opoku et al. (2013) failed to quantify DNA of *F. langsethiae* in Italian ryegrass. However, Imathiu et al. (2013b) detected low quantities of *F. langsethiae* DNA in Italian ryegrass. Because of no- or low levels of *F. langsethiae* DNA detected in Italian ryegrass, its role as an alternative host is rather unlikely. Seen that DNA of *F. langsethiae* was quantified in *P. annua* plants in our study, and Imathiu et al. (2013b) quantified *F. langsethiae* DNA in Italian ryegrass, these weed species cannot be excluded as alternative hosts of *F. langsethiae*. However, the small sample size of Opoku et al. (2013), Imathiu et al. (2013b) and our study emphasize the importance of further

investigations, on a wide range of weed species with large sample sizes, to pin out their role as alternative hosts for *F. langsethiae*.

Both *F. poae* and *F. langsethiae* were the most abundant of the four *Fusarium* species in the oat plant material and harvested oat grains in 2018. The high DNA-level of these *Fusarium* species in the plant material and harvested oat grains, combined with their low levels of DNA observed in *P. annua* plants, further underlines the unlikelihood of *P. annua* being an alternative host of *F. poae* and *F. langsethiae* (rather than their absence in the field). It is therefore unlikely that *P. annua* plays an important role in the *Fusarium* head blight disease cycle of these species.

High concentration of *F. langsethiae* DNA was detected in the oat seeds planted both years. The level of *Fusarium* DNA of the other three *Fusarium* species in the oat seeds were low. Because of the high *F. langsethiae* DNA concentrations in the oat seed one can speculate if this might have contributed to the high levels of *F. langsethiae* DNA observed in the plant material and oat grains in 2017 and 2018. However, as qPCR analysis of *Fusarium* DNA cannot differentiate between dead or living fungal cells, we can therefore only speculate if the *F. langsethiae* DNA was viable, contributing to *F. langsethiae* infection in the field in 2017 and 2018. No analysis of the viability of the *F. langsethiae* DNA in the oat seed was performed, therefore we cannot exclude the possibility of the infected oat seed contributing to infection in the field. However, the infection- and survival rate of *Fusarium* is found to decrease over time during storage in oat- and barley grains (Kim, 2014). The oat seeds planted in 2018 had been stored for two years (from 2016) which weakens the probability of the *F. langsethiae* DNA in the oat seed being viable, capable of causing field infection in 2018.

Minimal levels of *F. poae* DNA were found in the oat seed planted in the current field study. This undermine the probability of oat seed serving as a source of *F. poae* inoculum in our field experiment. It is therefore more likely that the high levels of *F. poae* DNA seen in the oat plant material and harvested oat grains comes from a source other than infected oat seed.

The morphological analysis of *Fusarium* infestation of the harvested oat grains revealed that over 50% of the oat grains harvested in 2017 were *Fusarium* infested. Furthermore, the majority of the *Fusarium* isolates were identified as *F. poae*. All *Fusarium* colonies established on the incubated oat grains were viable *Fusarium*. With over 50% of the

harvested oat grains being *Fusarium* infested, mainly by *F. poae*, one can speculate whether grains spilled upon harvest in 2017 served as a source of *F. poae* inoculum the following season.

In 2017, there were high levels of *F. poae* and *F. langsethiae* DNA in plant material late season, and infected plant material could have contributed to early infection of plant material in 2018. In 2018, moderate levels of *F. poae* and *F. langsethiae* DNA were detected in the plant material at booting (GS 40-49), which was the earliest stage of DNA analysis. One can speculate whether DNA of *F. poae* and *F. langsethiae* were present in the plant material prior to booting, considering the moderate levels at booting. Early infection of *F. poae* and *F. langsethiae* could indicate that the inoculum was locally dispersed within the field, for instance from infected plant material deposited on the soil surface upon harvest the previous year.

High concentrations of *F. poae* DNA was present in all plant organs at ripening in 2017 and senescence in 2018. *Fusarium poae* DNA was detected in the panicles, flag leaves and stems, in descending order (pg DNA/mg). Hence, if plant residues of the different plant organs serve as *F. poae* inoculum sources, the residues originating from different plant organs could contain different amounts of inoculum. Straw residues has been investigated as a potential source of *F. poae* inoculum, and low levels of *F. poae* DNA has been observed in straw residues from a wide range of cultural plants including wheat, durum wheat, barley, oats, flax, lentil, pea, chickpea, sunflower and canola (Fernandez et al., 2008). Similarly, Pereyra and Dill-Macky (2004; 2008) detected low levels of *F. poae* DNA in straw residues of wheat, barley, sunflower and gramineous weeds. These results confirm the presence of *F. poae* in straw residues of a wide range of cultural plants, however because of the low levels of detection the probability of straw residues being the main source of *F. poae* inoculum is low. Despite the unlikelihood of straw residues being the main source of *F. poae* inoculum, the role of other plant residues as inoculum sources merits further research.

High levels of *F. langsethiae* DNA were observed in the oat plant material late season both years. With high levels of *F. langsethiae* DNA accumulating in the plant material late season in 2017 and 2018, infected plant material deposited on the soil surface could act as an inoculum source, responsible for causing field infection the subsequent year. Hofgaard et al. (2016b) investigated the role of straw residues as a potential source of *F. langsethiae* inoculum at two different locations in Norway. Their results did not

provide strong indications of stem residues being the main source of *F. langsethiae* inoculum because of the low levels of DNA detected.

Furthermore, Imathiu et al. (2013b) examined the presence of *F. langsethiae* DNA in dead oat leaves sampled at different growth stages. They failed to detect DNA of *F. langsethiae* in dead leaves collected at stem elongation, however high levels of *F. langsethiae* DNA were present in the dead leaves at oat flowering. Despite little evidence of straw residues being the main source of *F. langsethiae* inoculum, the results of Imathiu et al. (2013b) indicates that dead leaves, and possibly other plant residues, might indeed serve as a source of *F. langsethiae* inoculum. Further investigation on the role of plant residues including roots, leaves and spilled grains, as inoculum sources is important to gain insight into the epidemiology of *F. langsethiae*.

Mode of *F. poae* and *F. langsethiae* dispersal

An increase in *F. poae* and *F. langsethiae* DNA in the air samples occurred late season from oat ripening and onwards in 2017. This increase occurred parallel with the observed increase in DNA of *F. poae* and *F. langsethiae* in the oat plants, especially in the upper plant parts, around ripening. The increase in *Fusarium* DNA in the air samples at senescence could be related to sporulation of *F. poae* and *F. langsethiae* in the upper plant parts. Similarly, Opoku et al. (2013) recorded an increase in *F. langsethiae* DNA in cereal heads at senescence, suggesting that this increase in *F. langsethiae* DNA were linked to increased sporulation.

F. poae and *F. langsethiae* were without doubt the most prevalent *Fusarium* species in the air samples with an abrupt increase in *Fusarium* DNA at ripening in 2017. Conidia mainly rely on splash dispersal for inoculum dissemination in the field (Jenkinson and Parry, 1994b). For instance, conidia of *F. avenaceum* (Jenkinson and Parry, 1994b), *F. culmorum* (Jenkinson and Parry, 1994b; Hörberg 2002) and *F. poae* (Hörberg 2002) is found to move distances up to 60 cm vertically and 100 cm horizontally (70 for *F. poae*). To our knowledge, no studies on dispersal of *F. langsethiae* has yet been carried out. The weeks from ripening until harvest there were frequent events of precipitation, we can therefore speculate whether the increase in *F. poae* and *F. langsethiae* in the air samples was a result of conidia being dispersed by rain splash.

The high levels of *F. langsethiae* DNA recorded in the air samples in our study is rather unusual compared to other studies on *Fusarium* in air samples. For instance, a study looking at *F. langsethiae* in air samples from two different oat fields in Norway only

recorded low levels of *F. langsethiae* DNA in the samples (Hofgaard et al., 2016b). In this study, Hofgaard et al. (2016b) collected data over a long time period during the growth season, using similar active samplers as the ones we used, and recorded low levels *F. langsethiae* DNA in the samples at late developmental stage (4 weeks past heading). In a recent study from Switzerland, they failed to detect *F. langsethiae* spores in air samples collected in an oat field during flowering (Schöneberg et al., 2018). To our knowledge this is the first study of *F. langsethiae* DNA in air samples collected late season, and the remarkably high level of *F. langsethiae* DNA observed in the air samples highlights the necessity of more research *F. langsethiae* and in air samples, especially late season.

The high levels of *F. poae* DNA recorded in the air samples in our study is rather unusual compared to other studies on *F. poae* in air samples. Schöneberg et al., (2018b) passively collected air samples within an oat field and only observed a few colonies of *F. poae* deposited on the petri dishes during oat flowering. At flowering the level of *F. poae* DNA in our air samples was negligible which coincides with the results of Schöneberg et al., (2018b). Research on *F. poae* in air samples is limited, and the high level of *F. poae* DNA observed in our air samples late season emphasise the need of further investigation on *F. poae* in air samples, especially late season.

There are weaknesses related to our method of collecting air samples and the analysis of estimated number of spores in the air samples. The spore-traps collected air samples 1 m above ground which means that spores have to reach this approximate height to be captured. At the late growth stages, when the plant reaches it maximum height, around 90 cm for Belinda, the spore-traps are likely to capture the *Fusarium* in circulation. The spore-traps are designed to capture fungal material in circulation at cereal height, however if the inoculum source is close to- or on the soil surface, spores have to travel over 1m vertically to be captured. From stem elongation to ripening the number of spores in the air samples were low and one can question if the spore-traps were able to capture all *Fusarium* in circulation within the developing plant canopy, closer to the ground.

The spore-trap do not select for spores when collecting air samples and the observed DNA could be originating form *Fusarium*- mycelium or spores. In our investigation, the number of *Fusarium* spores in the air samples were calculated based on a known number of spores in a standard spore solution.

Furthermore, the calculations are not adjusted for the interspecies differences in spore morphology between *F. poae, F. langsethiae, F. avenaceum* and *F. graminearum*. While *F. poae* and *F. langsethiae* only are known to produce conidia, *F. avenaceum* and *F. graminearum* also produce ascospores in addition to conidia and this might influence total DNA in the samples. Ascospores usually consist of four cells (Booth 1971) whereas macroconidia of *F. graminearum* and *F. avenaceum* usually contain five to six and five cells respectively (Leslie and Summerell, 2008 p.132-180). Also the size of the macroconidia larger than ascospores (Booth, 1971). A possible difference in total DNA in ascospores and conidia will be unaccounted for in a qPCR analysis. In addition to differences between *Fusarium* ascospores and conidia, there are great inter- and intraspecies variation in conidia morphology between *Fusarium* species (Leslie and Summerell, 2008). For instance, *F. graminearum* is only known to produce macroconidia, whereas *F. avenaceum* and *F. poae* isolates can produce both macro- and microconidia (Leslie and Summerell, 2008) and *F. langsethiae* only produce microconidia (Torp and Nirenberg, 2004). These differences will also influence the spore count since macroconidia contain several septa whereas microconidia usually have none or one spetations, which might influence total DNA in each conidia. These differences in spore morphology between the four species will lead to uncertainties associated with comparison of number of spores in the air samples. Furthermore, the air sample analysis cannot differentiate between type of DNA arising from *Fusarium* spores or mycelium. Therefore, the results presenting an almost identical number of *F. poae* and *F. langsethiae* spores in the air samples (week 35) might not be an accurate representation as some *F. poae* isolates also produce macroconidia probably containing more DNA than the microconidia produced by *F. langsethiae*.

In 2018, the oat field was heavily infested with aphids. Aphids were first detected in week 23, at leaf development/tillering, and the extent of field infestation increased in severity until ripening (week 29). Insects are identified as possible transmitters of *Fusarium* inoculum and can therefore play an important role in *Fusarium* inoculum dispersal. In 2018, the oat plants were abnormally short and frail and one can speculate if heavy aphid infestation might have contributed to poor plant growth and increased *Fusarium* infection in the oat field. Aphid infestation might influence *Fusarium* head blight infection in cereals. Drakulic et al. (2015) reported an increased in disease progression of *F. graminearum* and increased mycotoxin accumulation (DON) in wheat correlated with

aphid infestation. The same was observed with increased *F. langsethiae* infection in wheat plants and increased contamination of T-2/HT-2 of harvested grains upon grain aphid (*Sitobion avenae*) infestation and feeding. In a detached leaf assay experiment, inoculating wounded oat and wheat leaves with a *F. langsethiae* conidial suspension caused substantial amounts of necrosis around the wound, with heavier necrosis on the oat leaves (Imathiu et al., 2009). On unwounded leaves, no symptoms of *F. langsethiae* infection were observed upon inoculation on wheat leaves, whereas necrotic lesions around the inoculation area were seen on oat leaves. Based on the observation of Imathiu et al. (2009) of considerable symptoms of *F. langsethiae* infection occurring on wounded leaves of oat and wheat, one can speculate if wounding caused by aphid feeding can facilitate and accelerate infection of *F. langsethiae* in the field.

In addition to aphids, other insects able to transmit *Fusarium* inoculum include the housefly (*Musca domestica,* Phoku et al., 2014), clover leaf weevil (*Hypera punctate)* and two-striped grasshopper (*Melanoplus bivittatus*) which are found to carry spores of *F. poae, F. avenaceum, F. graminearum,* and *F. culmorum* (Gordon, 1959, cited in Parry et al., 1995). Furthermore, Cooper (1940) and Cherewick and Robinson (1958) found evidence of mites (*Siteroptes graminum*) carrying spores of *F. poae* (cited in Parry et al., 1995). It is of interest and importance to further investigate the role of insects, and aphids, as possible transmitters of *F. poae* and *F. langsethiae* inoculum between- and within fields.

Oat growth stage at the time point of *F. poae* and *F. langsethiae* infection

In our field trial, the DNA of *F. langsethiae* was first detected around booting of the main shoots (GS 40-49) in 2017 and in 2018. DNA of *F. langsethiae* DNA were detected in the oat plant material from booting both years, however the levels were low. These findings suggest that infection of *F. langsethiae* in the oat main shoots might occur earlier than previously observed for cereal plants. For instance, Opoku et al. (2013) and Imathiu et al. (2013b) detected DNA of *F. langsethiae* in cereals in the period from heading (GS 50-59) to early flowering (GS 60-65) in cereals. Cereals are considered most susceptible to *Fusarium* head blight infection at anthesis and a short period thereafter (Parry et al., 1995; Xu, 2003; Osborne and Stein, 2007). Hence, in the light of our and others observations, initial infection of *F. langsethiae* occurs prior to anthesis, which indicates that its biology differs from that of other *Fusarium* species which infect cereal heads at- or a short period after flowering.

DNA of *F. poae* were first detected in the oat plant material at stem elongation (GS 30-39) in 2017 and around heading (GS 50-59) in 2018. Research of the time point of initial infection of *F. poae* in cereals is limited, however Sturz and Johnston (1983) detected *F. poae* in ears of wheat and barley at heading, hence suggesting *F. poae* to be a primary colonizer of cereal ears. Our results coincide with the finding of Sturz and Johnston (1983) suggesting that initial infection of *F. poae* occurs around heading. However, more research on the point of initial infection of *F. poae* in the field is required to confirm our results.

There were great differences in level of *F. poae* and *F. langsethiae* DNA between normal and abnormal plants with remarkably higher levels of *F. poae* and *F. langsethiae* DNA late season in 2017 and 2018. These differences were observed for all Fusarium species, but was significant only for *F. poae* and *F. langsethiae* in 2017. Abnormal plants possessed characteristics like early tillering, reduced- or stagnated growth and grain abortion. Divon et al. (2012) documented a correlation between the presence of *F. langsethiae* in node tissue of oat plants and stunting, hence pointing out the possibility of stunting being caused by *F. langsethiae*. Stagnated growth was one of the characteristics of the collected abnormal plants, this might explain the higher levels of *F. langsethiae* DNA found in abnormal plants compared to normal plants if stunting is a consequence of *F. langsethiae* infection.

Our results indicate that *F. poae* and *F. langsethiae* might have a preference for the upper plant parts, such as the oat panicle, followed by the flag leaf. In 2017, remarkably high levels of *F. poae* and *F. langsethiae* DNA were accumulated in the abnormal panicles and flag leaves whereas the DNA levels in the stems were low. The same trend was observed in 2018, but with lower DNA concentrations. Both years, there were little *F. poae* and *F. langsethiae* DNA in the stems of both normal and abnormal plants. This coincides with other studies documenting a preference of *F. poae* (Sturz and Johnston, 1983; Kiecana et al., 2012) and *F. langsethiae* (Divon et al., 2012; Opoku et al., 2013; Imathiu et al., 2013b) for cereal heads. Similar to our findings, Imathiu et al. (2013b) documented high levels of *F. langsethiae* DNA in the oat panicle whereas no DNA were detected in the lower plant parts. Imathiu et al. (2013b) could not find any strong evidence of *F. langsethiae* systematically ascending from the lower- to the upper plant parts. The putative preference of *F. poae* and *F. langsethiae* for the upper plant parts might suggest

that the infection of *F. poae* and *F. langsethiae* started from the upper plant parts. However, more research on the epidemiology *F. poae* and *F. langsethiae* is required to understand their mode of dispersal.

The concentration of *Fusarium* DNA in the main shoots of normal plants were low compared to in the abnormal main shoots both years. Furthermore, the level of *Fusarium* DNA in the oat grains harvested in 2017 was moderate whereas the DNA level was high in the oat grains harvested in 2018. As the majority of oat plants within the field were normal oat plants, there is reason to believe that the level of *Fusarium* DNA in the harvested oat grains are somewhat “diluted” compared to a grain sample hypothetically originating from highly *Fusarium* infected abnormal plants. Nevertheless, there were high level of *F. poae* DNA in the oat grains harvested in 2018, despite most grains arising from normal oat plants, this might indicate that the presence of *F. poae* in the oat field in 2018 was high.

Both years, the main shoots of the abnormal oat plants developed slower than the normal main shoots. Likewise, tillers of oat plants presumably develop slower than the main shoots, apart from damaged or dead main shoots. This arises the question if the normal tillers, which developed slower, like abnormal plants, were more contaminated with *F. poae* and *F. langsethiae* than the normal main shoot itself. As the development of oat- main shoots and tillers differs, infection of *F. poae* and *F. langsethiae* between main shoots and tillers might also differ. Hence, one can speculate whether the high level of *F. poae* and *F. langsethiae* DNA in the harvested oat grains in 2018 were influenced by infection tillers. Further investigation on level of *F. poae* and *F. langsethiae* DNA in the tillers of both normal and abnormal plants would be of great interest to investigate their role in the epidemiology of *F. poae* and *F. langsethiae*.

Fusarium species and mycotoxins in the harvested oat grains

In 2016, the harvested oat grains where highly contaminated with the mycotoxins DON, T-2 and HT-2. The high concentration of DON in the harvested oat grains could to some extent be expected as the neighbouring field to ours was inoculated with *F. graminearum,* the main DON producing *Fusarium* species in Norway (Bernhoft et al. 2010). The high levels of T-2 and HT-2 can possibly be attributed to the presence of *F. langsethiae* in the oat field (Imathiu et al., 2013a). The T-2 and HT-2 toxins could also have been produced by *F. sporotrichioides* (Bottalico and Perrone, 2002), however since *F. langsethiae* is

identified as the main T-2/HT-2 producer in Norwegian cereals (Bernhoft et al. 2010) it is most likely responsible for the accumulated T-2/HT-2 in the harvested oat grains. The high levels of T-2/HT-2 in the oat grains harvested in 2016 can therefore indicate that *F. langsethiae* was present in the oat field in 2016.

The oat grains harvested in 2017 contained high levels of NIV and AUF, and low levels of DON and T-2/HT-2. Several *Fusarium* species are known NIV producers, for instance *F. poae, F. graminearum* and *F. culmorum* (Bottalico and Perrone, 2002). The DNA levels of *F. poae* in the harvested oat grains was low, the level of *F. graminearum* DNA was negligible and the levels of *F. culmorum* DNA was below the limit of detection, we can assume that *F. poae* was responsible for the high levels of NIV in the harvested oat grains. Furthermore, the high levels of AUF accumulated in the oat grains can also be associated with *F. poae* (Thrane et al. 2004) which reinforces the likelihood of *F. poae* being the NIV producing *Fusarium* species in the oat grains harvested in 2017.

In 2018, there were generally low mycotoxins levels in the harvested oat grains, however NIV was the most accumulated mycotoxin followed by DON and AUF. There were high levels of *F. poae* DNA in the harvested oat grains in 2018, which coincides with high NIV contamination, likely produced by *F. poae*.

The highest concentration of NIV were observed in the oat grains harvested in 2017 despite low levels of *F. poae* DNA in the harvested oat grains. In 2018 however, there were high levels of *F. poae* DNA in the harvested oat grains and lower levels of NIV. Mycotoxin production and *Fusarium* biomass do not strictly correlate, meaning that high *Fusarium* biomass do not necessarily mean high mycotoxin contamination (Xu and Nicholson, 2009; Hofgaard et al. 2016a).

In 2018, infection of *F. poae*, *F. langsethiae* and *F. avenaceum* increased late season at oat plant senescence. In 2017, the field was harvested at grain ripening, whereas in 2018 harvest were postponed to investigate how delayed harvest influenced *Fusarium* infection of the oat plants. The oat grains would normally have been harvested in week 31, however the field were harvested in week 34, three weeks later. There was an increase in *F. poae* and *F. avenaceum* DNA in the oat plant material starting in week 31. The level of *F. langsethiae* and *F. graminearum* started to increase from week 33 and onwards. This observed increase in *Fusarium* DNA in the oat plant material late season in 2018 could be related to plant senesce, presumably weakening the host resistance, hence facilitating *Fusarium* infection

Choice of method for isolation of *F. poae* and *F. langsethiae* from oat grains

The two experiments were carried out aiming to investigate if one of the methods were more suited for isolation of *F. poae* and *F. langsethiae*. Incubation on *Fusarium* selective medium showed great variation in mycelial appearance which made it possible to differentiate between *Fusarium* colonies. Despite different morphological appearance the majority of the *Fusarium* colonies were identified as *F. poae*. The mycelial colour of several of the *F. poae* isolates showed great variation, hence all the different morphological groups. *Fusarium poae* is a known producer of the mycotoxin AUF which also act as a pigment (Thrane et al. 2004). In 2017, there were high levels of AUF in the harvested oat grains, one can therefore speculate if the observed variation in mycelial colour between the *F. poae* isolates can be attributed to AUF production. Furthermore, *F. poae* incubated in artificial medium might produce a characteristic sweet, peachy-like odor which can serve an additional sensory tool for indicating presence of *F. poae* (Leslie and Summerell, 2008). Because of more variation between fungal colonies incubated on *Fusarium* selective medium compared to on filter paper, our results found incubation on *Fusarium* selective medium better suited for isolation of *F. poae*.

No isolates of *F. langsethiae* were identified in the incubation experiment, neither on *Fusarium* selective medium or on filter paper. The qPCR analysis revealed that *F. langsethiae* was indeed present in the harvested oat grains by quantifying the level of *F. langsethiae* DNA in the oat grains. The difficulty of *F. langsethiae* isolation from oat grains incubated on *Fusarium* selective medium or on filter paper might be related to its modest growth rate, making it easily overgrown by other faster growing *Fusarium* species (Torp and Nirenberg, 2004). Our observation coincides with Schönenberg et al. (2018a) who also found isolation of *F. langsethiae* isolates from oat grains incubated on PDA plates difficult. Based on Schönenberg et al. (2018a) and our observations, the best method for analysis of *F. langsethiae* presence in harvested oat grains is by qPCR analysis.

Predictions about *F. poae* and *F. langsethiae* in the future

The weather conditions during the 2017 and 2018 growth season turned out very different with the 2018 season being one of the driest and warmest season for centuries (Skaland et al., 2019). Despite large weather differences, *F. poae* and *F. langsethiae* dominated in our oat field both years. The weather conditions during the 2017 season had events of

precipitations evenly distributed throughout the season with normal to high June-August temperatures. From June to August (stem elongation to ripening) the weakly mean temperatures ranged around 15°C and there were few and short events of precipitation. In 2018, the temperatures from May to August were 3°C higher than the normal with the precipitation during the same period being more than halved compared to normal years. Within the end of the century, the early average temperatures in Norway are projected increase with approximately 4.5°C and there is expected a yearly increase in precipitation, however not during the growth season (Hanssen-Bauer et al., 2015). With increasing temperatures, evaporation is expected to increase and even though an increase in yearly precipitation is expected, periods of drought is expected to increase in both severity and duration. Summer drought is projected to increase with up to 1-2 months in some parts of Norway by 2100 in the agricultural areas of Norway (Hanssen-Bauer et al., 2015). Based on this climate projection report, one can expect the future climate in Norway to resemble the conditions seen in the 2018 with warm and dry summers.

The trend of *F. poae* and *F. langsethiae* being prevalent in our oat field over the two years is in accordance with the current trend showing increased prevalence of *F. poae* and *F. langsethiae* in European (Xu et al., 2005; Stenglein 2009) and Nordic countries (Kosiak et al., 2003; Nilelsen et al., 2011; Karlsson et al., 2017) over the past decades. *Fusarium poae* is regarded as a *Fusarium* species mostly occurring in the warmer climatic zones with dry and warm weather (Xu et al. 2008). Also *F. langsethiae* is associated with warm weather conditions (Parikka et al. 2008). With an expected increase in average temperature in Norway as a consequence of climate change, *F. poae* and *F. langsethiae* and their associated mycotoxins may become more common in Norwegian cereal fields (Bernhoft et al., 2012; Xu et al., 2013).

Conclusion

In accordance with other studies, we found initial infection of oat plants by *F. poae* and *F. langsethiae* to occur prior to flowering, supporting the hypothesis of their biology differing from other *Fusarium* head blight pathogens, which mainly infect cereals at- or after flowering. To our knowledge, this is the first time abnormal plants from has been analysed for the presence of *F. poae* and *F. langsethiae*. Abnormal plants were significantly more infected with *F. poae* and *F. langsethiae* than normal plants.

Our results do not confirm *P. annua* as being the main source of *F. poae* or *F. langsethiae* inoculum. The inoculum source of *F. poae* and *F. langsethiae* is still a mystery and further research the role of weeds, plant residues and spilled grains as possible inoculum sources is required to understand their biology.

Results from this research on *F. poae* and *F. langsethiae* infection in an oat field carried out over a two years' period highlights the importance of in-depth research on the epidemiology of these *Fusarium* species. It would be of major interest to further investigate the role of abnormal plants in the epidemiology of *F. poae* and *F. langsethiae*.

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Appendix I

Tables of the chemicals, equipment, kits, mediums and primers and probes used in this investigation is presented in Table 1 to 5.

Supplementary table 1. List of chemicals used and their suppliers.

Chemical	Supplier
Agarose	Sigma-Aldrich, St. Louis, MO, USA
Chlorine	Orkla, Norway
Ethanol 96%	VWR Chemicals, Radnor, PA, Germany
Ethidium bromide	Merck KGaA, Darmstadt, Germany
Liquid Nitrogen	AGA, Norway
SsoAdvanced^{TM} Universal Probes Supermix	Bio-Rad Laboratories, Hercules, CA, USA

Supplementary table 2. List of the equipment and their suppliers.

Equipment (Model)	Supplier
FreeZone® 4.5 Liter Freeze Dry System	Labconco, Kansas city, MO, USA
Ultra Centrifugal Mill ZM 200	Retsch, Haan, Germany
FastPrep instrument-24™ Classic Grinder	MP Biomedicals, Solon, OH, USA
Automatic Multi-Vial Cyclone Samplers	Burkard Manufacturing, Rickmansworth, UK
T-100™ Thermal Cycler	Bio-Rad Laboratories, Hercules, CA, USA
Qubit® 2.0 fluorometer	Invitrogen, Carlsbad, CA, USA
NanoDrop™ 2000 spectrometer	Thermo Fisher Scientific, Waltham, MA, USA
Gel Doc™ EQ	Bio-Rad Laboratories, Hercules, CA, USA
Eppendorf® Centrifuge 5417R	Eppendorf Biotools, Hamburg, Germany
Leitz Dialux 20 microscope	Leica, Wetzar, Germany

Supplementary table 3. List of all the pre-prepared kits and their suppliers

Kit	Supplier
FastDNA® SPIN Kit for Soil	MP Biomedicals, Santa Ana, CA, USA
DNeasy® Plant Mini Kit	QIAGEN, Hilden, Germany
Qubit® dsDNA HS Assay Kit	Invitrogen, Carlsbad, CA, USA

Supplementary table 4. All artificial medium used in the investigation and the recipe of each medium.

Medium	**Chemical**	**Amount**
PDA (Potato Dextrose Agar)	Distilled water	1000 ml
	Potato starch	4.0 g
	Dextrose	20.0 g
	Agar	15.0 g
MBA (Mung Bean Agar)	Distilled water	1000 ml
	Green mung beans	40.0 g
	Agar	15.0 g
Water agar 1.5%	Distilled water	1000 ml
	Agar	15.0 g

Because I prepared the *Fusarium* selective medium (CZPD) and selective nutrient agar (SNA) their recipe and procedure is described in more detail.

Supplementary table 5. Recipe for preparation of 3 L Fusarium selective medium

CZPD (Czapek Propicanozol Dichloran agar)	
Ingredient	Amount
Distilled water	3000 ml
Czapek Dox Broth	105.0 g
Dichloran solution (DS)	3.0 ml
Chloramphenicol solution (CA)	3.0 ml
Trace metal solution (TM)	3.0 ml
Agar	24.0 g
Chlortetracycline solution (CT)	5.0 ml
Tilt top solution	0.5 ml

2L of distilled water were measured and poured into a large flask and placed on a magnetic stir. Then, Czapek-Dox were weighted and added to/into the flask under constant stirring. Furthermore, 3ml of dichloran solution, chloramphenicol solution and trace metal solution were measured and added to the solution. Bacto-Agar were then weighted and added to the solution, stirred and finally autoclaved. When the agar solution reached a temperature >50°C,

chlortetracycline solution and Tilt top were added under sterile conditions, stirred and poured into Petri dishes.

Supplementary table 6. Recipe for preparation of 3L Selective nutrient agar.

SNA (Selective Nutrient Agar)	
Ingredient	**Amount**
Distilled water	3000 ml
KH_2PO_4	3.0 g
KNO_3	3.0 g
$MgSO_4 \cdot 7H_2O$	1.5 g
KCl	1.5 g
Glucose	0.6 g
Sucrose	0.6 g
Agar	46 g

2L of distilled water were measured and poured into a large flask and placed on a magnetic stir. Then, the ingredients were weighted and added to the flask in chronologic order following the recipe. The SNA solution was then autoclaved, chilled down to >50°C and partitioned onto Petri dishes.

Appendix II

Groups of morphological characteristics of *Fusarium* observed on CZPD Agar

1. Fluffy mycelium of medium size, white colour and looks like cotton, quite compact mycelial growth.
   1.1. With dark brown/reddish colour under, no pattern
   1.2. Weak yellow colour, no pattern
2. “Cotton ball”, densely packed, white colour (shade of peach in some), round shape, high (stands out), “soft and clean look”.
   2.1. Yellow in the middle, red ring around, uneven shape (not rings)
3. Little to medium mycelial growth, mostly white colour with shades of pink/peachy, fluffy texture, not densely packed mycelium
   3.1. Red- to dark red colouring of the agar, no regular shape
   3.2. Orange colouring of the agar, no regular shape
   3.3. Yellowish colouring of the agar, no regular shape
4. Small size, compact and pink mycelium, low height
   4.1. Only yellowing of the agar, no distinct shape, clean appearance
5. White mycelium with peachy colour in the middle, fluffy texture, the mycelium is more compact in the peach-coloured zone (middle), fast growth (medium to large size)
   5.1. Ruby red, orange and yellowish ring underneath
6. Dark pinkish mycelium, yellowish in the middle (looks dirty), rapid growth (much mycelium)
   6.1. Yellow and/or reddish, looks patchy, no regular shape of the colouring
7. Medium sized mycelium, pink colour, compact mycelium
   7.1. Yellow and red rings
   7.2. Dark red colour
8. Peach colour, medium size, fluffy aerial mycelium which seems to have collapsed, looks powdery
   8.1. Yellow/red underneath, no pattern/ring
   8.2. Brownish/dark red ring
   8.3. Orange and ruby red concentric rings.
9. Much mycelial growth, pink/white colour and fluffy
   9.1. Orange ring, “clear/pure colour”
10. Fluffy mycelium, pink/peach colour, denser in the middle, medium to large size

10.1. Orange or peachy rings underneath, clean appearance

10.2. Yellow in the middle, red ring around, uneven shape (not rings)

11. Fungal growth but not fusarium

X. No fungal growth, bacterial growth also included in this group

Groups of morphological characteristics of *Fusarium* observed on Freezer Blotter Plates

I. Much mycelial growth, fluffy with white or pink color

II. Very little mycelial growth, white and observed under the loop

III. Pink powdery or flour-like mycelium

IV. Not *Fusarium* infection or no fungal infection

Appendix III

Supplementary table 7. Species specific primers and probes used in the qPCR reaction. Letters written in bold represents locked nucleic acid nucleotides.

Species	Primers/Probes	Sequence (5'-3')	Publications
Oat DNA	Cox 554- f	GGT TGT TGC CAC CAA GTC TCT T	Divon et al., 2012
	Cox 554- r	TGC CGC TGC CAA CTT C	
	Cox 554- p	FAM-CTC CTA TTA AGC TCA GCC TT-MGB	
F. avenaceum	TMAV- f	AGA TCG GAC AAT GGT GCA TTA TAA	Halstensen et al., 2006
	TMAV- r	GGC CCT ACT ATT TAC TCT TGC TTT TG	
	TMAV- p	Cy5-CTC CTG AGA GGT CCC AGA GAT GAA CAT AAC TTC-BHQ3	
F. graminearum	gramMGB- f	GGC GCT TCT CGT GAA CAC A	Waalwijk et al., 2004
	gramMGB- r	TGG CTA AAC AGC ACG AAT GC	
	gramMGB- p	6-FAM-AGA TAT GTC TCT TCA AGT CT-MGB	
F. culmorum	culmMGB- f	TCA CCC AAG ACG GGA ATG A	Waalwijk et al., 2004
	culmMGB- r	GAA CGC TGC CCT CAA GCT T	
	culmMGB- p	CAC TTG GAT ATA TTT CC	
F. langsethiae	F. lan- f	GTT GGC GTC TCA CTT ATT ATT C	Hofgaard et al., 2016 a
	F. lan- r	TGA CAT TGT TCA GAT AGT AGT CC	
	F.lan probe	FAM- CAC ACC **C**AT A**C**C TAC **G**TG TAA - TAMRA	
F. poae	poae1- f	AAA TCG GCG TAT AGG GTT GAG ATA	Waalwijk et al., 2004
	poae1- r	GCT CAC ACA GAG TAA CCG AAA CCT	
	poae probe	CAA AAT CAC CCA ACC GAC CCT TTC	
F. sporotrichioides	F.spor f	GGT TGG CGT CTC ACT TAT AC	Nazari et al., 2019
	F.spor rev	AAT TTC TGA TTC GCT AAA GTG G	
	spor probe	FAM- CCA CAC CC**A** TAG **T**TA C**G**T GTA A- TAM	
Broad primers	ITS4	TCCTCCGCTTATTGATATGC	White et al., 1990
	ITS5	GGAAGTAAAAGTCGTAACAAGG	

Appendix IV

Standard Mastermix recipe used for qPCR analysis.

The specific primers and probes varied according to the different *Fusarium* species (Appendix III).

Supplementary table 8. Recipe of the standard mastermix used in the qPCR reactions. The primers, probes and DNA were specific for each Fusarium species.

Ingredient	**Amount**
SsoAdvanced^{TM} Universal - Probes Supermix	12.5 µl
Primer- forward	1 µl
Primer- reverse	1 µl
Probe	1 µl
H_2O (Milli-Q)	5.5 µl
Extracted DNA	4 µl
Sum	**25 µl**

Appendix V

Supplementary table 9. Mycotoxin content in the harvested oat grains expressed as µg per kg oat flour. LOD= Level of Detected

Mycotoxin	Concentration (µg/kg) 2017	Concentration (µg/kg) 2018
Deoxynivalenol	101	75.6
DON-3-glucoside	12	13.7
15-Acetyldeoxynivalenol	<LOD	NA
3-Acetyldeoxynivalenol	<LOD	<LOD
Nivalenol	713	462.2
T-2 Toxin	23	<LOD
T-2 glucoside	28744	<LOD
HT-2 Toxin	51	3.4
HT-2 glucoside	4.5	<LOD
Zearalenone	<LOD	NA
Zearalenone sulfate	253720	NA
Beauvericin	85.5	50.2
Enniatin A	0.08	NA
Enniatin A1	1.36	0.9
Enniatin B	3.49	1
Enniatin B1	3.61	1.6
Enniatin B2	0.18	NA
Moniliformin	<LOD	4.8
Aurofusarin	696	65.7
Culmorin	43	<LOD
Apicidin	136	NA
Bicaverin	<LOD	NA
Chrysogin	7	8.6
Antibiotic Y	83	NA
Equisetin	<LOD	NA
Siccanol	153600	484640
Tenuazonic acid	32.99	NA
Alternariol	0.82	NA
Alternariolmethylether	<LOD	NA
Infectopyron	495	185.4
Altersetin	<LOD	NA
Calphostin	<LOD	NA
Pyrenocin A	13.98	NA
Linamarin	251	NA
Lotaustralin	126	166.1
Citreorosein	3.13	NA
Emodin	1.88	NA
ELISA (NIBIO)	270	NA

Appendix VI

Growth stage of sampled plant material

2017

Supplementary table 10. Week, date and growth stage of the plant material form each point of sampling.

Week	Date	Growth stage
33	16/08	Normal GS89
32	10/08	Normal GS 85-87 Abnormal GS 77-83
31	03/08	Missing data
30	25/07	Missing data
29	21/07	Normal GS65-69 Abnormal 67-69
28	12/07	Normal GS71 Abnormal GS63-67
27	04/07	Normal GS 59 Abnormal GS 47-51
26	27/06	Normal GS50-53 Abnormal 41-45
25	21/06	Normal GS37-39 Abnormal ≈31
24	13/06	Normal GS16-17 Abnormal GS22-24
23	06/06	Normal GS12-13 Abnormal ≈GS20-22
22	24/05	Normal GS11-12 No abnormal plants collected

2018

Supplementary table 11. Week, date and growth stage of the plant material form each point of sampling.

Week	Date	Growth stage	Comments
34	21/08	Normal 93 Abnormal 93	
33	14/08	Normal 93 Abnormal 92	All plants in the field are overripe
32	07/08	Normal GS 93 Abnormal GS92	
31	31/07	Normal GS89-92 Abnormal GS77 to GS87	Some stems are still visibly green, but grains are overly ripe
30	24/07	Normal GS87 Abnormal GS77	
29	18/07	Normal GS85 Abnormal GS55	Aphid infestation severity decreased from previous weeks.
28	10/07	Normal GS73 Abnormal GS 55-59	
27	04/07	Normal GS69 Abnormal GS43 to GS53-57	
26	24/06	Normal GS59-61 Abnormal GS49-57	Still heavily infested with aphids. Uneven growth between the ends of the field (more growth at the south end).
25	19/06	Normal GS45-59 Abnormal GS55 (25)	Uneven development in the field, much variation in growth stage. High infestation of aphids
23	05/06	Normal GS14-15 Abnormal GS21-22	Much Aphids in the field. Plants stressed, had purple leaf tips
22	29/05	Normal GS14 Abnormal ≈GS21-22	The abnormal plants had started tillering

21	22/05	Normal GS11-12 No abnormal plants collected	

Supplementary table 12. Date of sampling of the plant material and growth stage of the normal and abnormal plants from both growth seasons. Growth stage is registered from 0-99 according to Lancashire et al. 1991. Colouring is determined based on development of Normal plants. PY= Past Yellowing, N/A= No data (plants not collected or analysed), M.D= Missing Data.

2017				**2018**			
Date (Week)		**Growth Stage (GS)**		**Date (Week)**		**Growth Stage (GS)**	
Week	*Date*	*Normal*	*Abnormal*	*Week*	*Date*	*Normal*	*Abnormal*
18	2/5	Sowing		19	7/5	Sowing	
25	21/6	37-39	31	25	19/6	45-59	55 (25)
26	27/6	50-53	41-45	26	24/6	59-69	49-57
27	04/7	59	47-51	27	04/7	69	42-57
28	12/7	71	63-67	28	N/A	N/A	N/A
29	21/7	65-69	63-67	29	18/7	85	55
30	25/7	M.D	M.D	30	N/A	N/A	N/A
31	03/8	M.D	M.D	31	31/7	92	77-87
32	10/8	85-87	77-83	32	07/8	1 week PY	92
33	16/8	89	77-83	33	14/8	2 weeks PY	1 week PY
34	N/A	N/A	N/A	34	21/8	3 weeks PY	2 weeks PY
35	31/8	Harvest		34	22/8	Harvest	

Germination 0-9
Leaf development 10-19
Tillering 20-29
Stem elongation 30-39
Booting 40-49
Heading 50-59
Flowering 60-69
Fruit development 70-79
Ripening 80-89
Senescence 90-99

Appendix VII

Calculation of the number of *Fusarium* spores in the standard spore solution based on average spore count from KOVA slide.

Supplementary table 13. Average number of Fusarium spores found from spore count on the KOVA glassic slide.

	F. poae	***F. langsethiae***	***F. avenaceum***	***F. graminearum***
Number of spores	7.1	14.1	83.9	63.1

<u>Calculate spore concentration/ml:</u>

$$Spores\ pr\ ml = \frac{Number\ of\ spores}{0.01111\ \mu l\ *} \times 1000$$

**Small grid volume of KOVA® glasstic slide 10 with grids*

Example

F. gram: 63,1 spores

$$F.gram\ \left(\frac{spores}{ml}\right) = \frac{63.1}{0.01111\ \mu l} \times 1000 = 57.10^5\ spores/ml$$

Supplementary table 14. Calculated spore concentration of the four Fusarium species.

	F. poae	***F. langsethiae***	***F. avenaceum***	***F. graminearum***
Spores/ml	6.4x10^5	13x10^5	75.5x10^5	57x10^5

<u>Make eluates with concentration of 40x10^5, 200x10^5 and 400x10^5 spores/ml</u>

Formula used to calculate the amount of standard spore solution required to obtain desired spore concentration: $C_1V_1 = C_2V_2$

Example

F. gram: $C_1V_1 = 57x10^5$ spores/ml

$$V_2 = \frac{40x10^5}{57x10^5} = 0.7\ ml$$

$$V_2 = \frac{200x10^5}{57x10^5} = 3.5\ ml$$

Supplementary table 15. Amount of standard spore solution required to obtain desired spore concentration of 40, 200 or 400.10^5^.

Spores/ml	***F. poae***	***F. langsethiae***	***F. avenaceum***	***F. graminearum***
40.10^5	-	-	0.53	0.7
200.10^5	31.25	15.75	2.65	3.5
400.10^5	-	30.79	-	-
Volume of 1st + 2nd eluate (ml)	31.25	46.54	3.18	4.2

Find spore concentration in eluate (100 or 200 µl):

Formula: $Nr.spores = \frac{spores/ml}{ml\ suspension}$

Example

F. gram: $57x10^5$ spores/ml.

Totally 200 µl eluate (1st + 2nd eluate mixed)

$$Number\ of\ spores = 57x10^5 spores/ml \times 4.2\ ml = 2.39x10^7\ spores$$

$$Concentration = \frac{2{,}36x10^7\ sporess}{200\ \mu l} = 1.18x10^5\ spores/\mu l$$

Example

F. poae: $6.4x10^5$ spores/ml

Totally 100 µl eluate

$$Number\ of\ spores = 6.4x10^5 spores/ml \times 31.25\ ml = 2x10^7\ spores$$

$$C = \frac{2x10^7\ spores}{100\ \mu l} = 2x10^5\ spores/\mu l$$

Supplementary table 16. Number of Fusarium spores per µl solution.

	F. poae	***F. langsethiae***	***F. avenaceum***	***F. graminearum***
Spores/µl	$2x10^5$	$2.95x10^5$	$1.2x10^5$	$1.18x10^5$

Number of spores in standard 1 ($4\mu l$)

$$\frac{spores}{\mu l} \times 4\ \mu l\ eluate = Nr.spores$$

Example

F. gram: 1.18x10^5 spores/µl

$$1.18x10^5 \frac{spores}{\mu l} \times 4\ \mu l = 4.72x10^5\ spores$$

Supplementary tabell 17. Number of spores in standard 1.

	F. poae	***F. langsethiae***	***F. avenaceum***	***F. graminearum***
Number of spores in STD 1 (Spores in 4 ul)	7.99x10^5	1.18x10^6	4.8x10^5	4.77x10^5

DNA concentration in eluate measured with Qubit HS assay:

	F. poae	***F. langsethiae***	***F. avenaceum***	***F. graminearum***
Concentration ng/µl	4.37	0.916	2.91	6.19
Concentration pg/µl	4370	916	2910	6190

F. poae and *F. avenaceum* were diluted x4 and x2 respectively. *F. langsethiae* and *F. graminearum* were undiluted.

	***F. poae* x4**	***F. langsethiae***	***F. avenaceum* x2**	***F. graminearum***
Concentration ng/µl	$\frac{4.37}{4} = 1.0925$	0.916	$\frac{2.91}{2} = 1.455$	6.19
Concentration pg/µl	1092.5	916	1455	6190
pg/µl*4µl in qPCR	4370	3664	5820	24760

Number of spores into standard 1 in real-time qPCR reaction (make serial dilution curve)

$$\frac{Nr. of\ Spores}{pg\ DNA/\mu l} = \frac{Spores}{pg\ DNA}$$

Example

F. gram: Number of spores =4,77 x10^5

pg DNA/ µl = 24760

$$\frac{4,77x10^5}{24760} = 19,27\ spores/pg$$

	F. poae	***F. langsethiae***	***F. avenaceum***	***F. graminearum***
Spores/pg	182,8	322,41	82,52	19,27

To obtain number of spores collected in the spore trap, spores/pg is multiplied with pg DNA found in the sample (data from qPCR).

$$\frac{Spores}{pg} \times pg\ DNA\ in\ sample = number\ of\ spores\ in\ sample$$

Appendix VIII

Methodical experiment on oat test plants

A methodical experiment on test plants was conducted prior to dissection and grinding of the frozen plant material collected in 2017. Oat plants grown in the greenhouse was used as test plants. The plants were collected and put in the freezer at -20°C. The plants were dissected and divided into different groups of plant parts. The dissection took place in a cold working lab, 4°C, for the plant material to defrost slowly. Some plants were again put back into the freezer after dissection, while the remaining plant material were freeze dried. This test experiment aimed to investigate if freezing, thawing and re-freezing of plant material would affect the DNA quality of the material. The results from this experiment showed no significant difference in DNA quality between plants directly freeze dried and plants put back into the freezer.

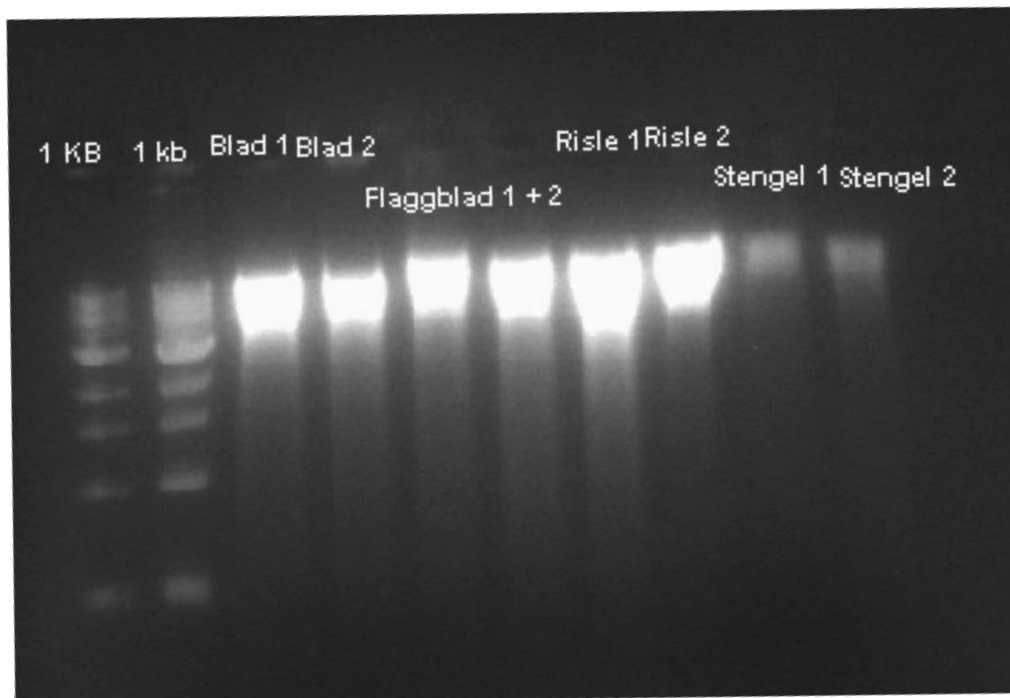


Figure 1. Picture of the PCR analysis showed against a 1 KB ladder visualized by the Gel Doc™ EQ (Bio-Rad Laboratories, Hercules, CA, USA). The numbers 1 and 2 represents the pre-treatment of the plant material. The first group (1) was taken from the freezer and directly freeze dried, whereas the second group (2) were freezed- thawed and freezed before freeze drying.
Blad= leaf, Flaggblad= flag leaf, Risle= panicle, Stengel = stem.

Appendix IX

Pictures from the oat field

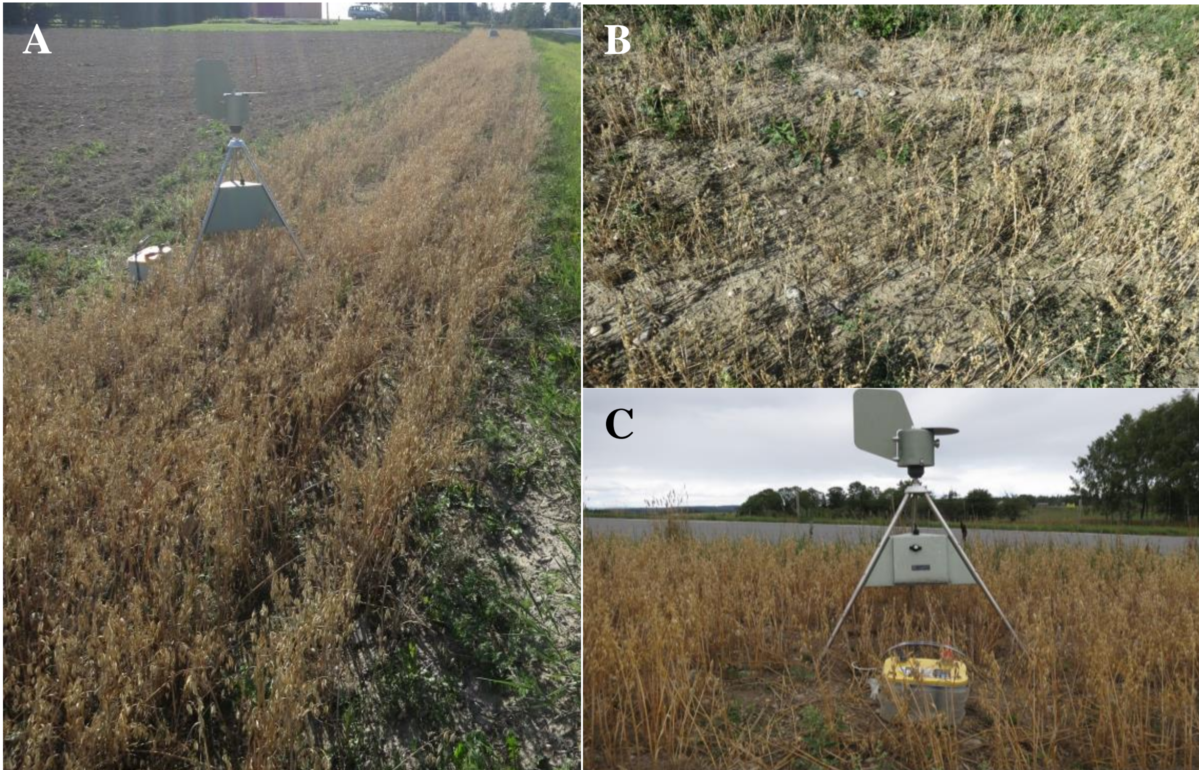


Figure 2. Pictures from the oat field (strip) taken mid-August. A) The field seen from the south end where the plant density was highest and the plants were taller (more irrigation). B) Picture from the middle part of the field taken from above as a representation of the scarce growth and small plants. The spore samplers (B-C) which were placed at the north and south end of the field were much higher than the oat plants.

Visual representation of normal and abnormal plants

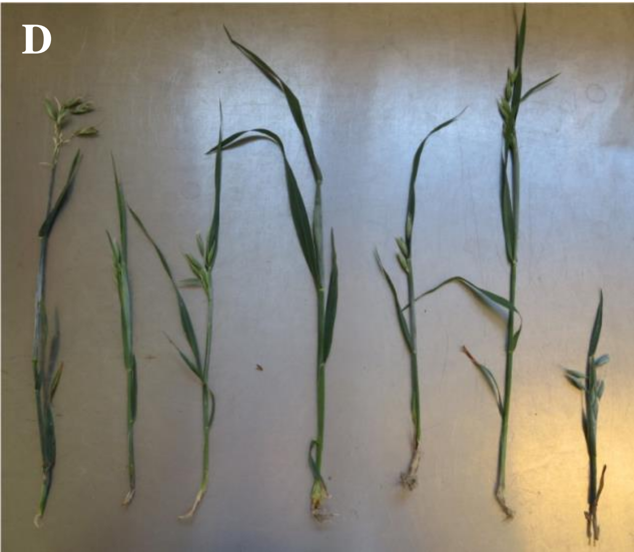
Pictures illustrating the difference between normal and abnormal plants collected the same day (A-B) and illustration of normal and abnormal plants at the same growth stage (GS) (C-D).

Normal Plants | **Abnormal Plants**

Collected 04/07-2018, at GS 69

Collected 04/07-2018, at GS 43-57

Collected 24/06-2018, at GS 59-61



Collected 10/07-2018, at GS 55-59

Appendix X

Spore trap data 2017

DNA concentration in the 1st and 2nd DNA eluates of air samples measured with the Qubit® dsDNA HS Assay kit.

Table 1 DNA concentration in 1st and 2nd eluate of the DNA extraction from the spore trap collected during the growth season 2017. The DNA concentration was measured with the Qubit® dsDNA HS assay kit.

Week	*Sample number*	*Concentration (ng/ul)*	
		1st eluate	2nd eluate
23	1001	1,33	0,72
24	1002	8,59	1,19
25	1003	11,3	2,82
26	1004	10,7	2,41
27	1005	4,16	1,14
28	1006	4,91	0,8
29	1007	2,1	0,23
30	1008	28,5	4,13
31	1009	10,9	3,2
32	1010	18,3	4,22
33	1011	8,87	2,73
34	1012	11,3	2,74
35	1013	15	3,79

Number of insects and DNA concentration (1st eluate) in the air samples.

Week	Number of Insects	DNA Concentration (ng/µl)	Notes
23	3	1.33	2 flies, 1 beetle
24	7	8.59	7 flies
25	7	11.3	7 flies
26	6	10.7	5 flies, 1 spider
27	3	4.16	3 small flies
28	5	4.91	4 flies, 1 spider
29	1	2.1	1 fly
30	8	28.5	8 flies (small and large)
31	2	10.9	2 flies
32	20	18.3	20 small flies
33	4	8.87	4 large flies
34	7	11.3	7 flies
35	8	15	8 different flies

Appendix XI

The raw sequence of the *Fusarium* isolate sendt to Eurofins for ITS identification.

TTAAGTTCAGCGGGTATTCCTACCTGATCCGAGGTCAACATTCAGAAGTTGGGGT
TTTACGGCATGGCCGCGCCGCGTTCCAGTTGCGAGGTGTTAGCTACTACGCAATG
GAGGCTGCAGCGAGACCGCCAATGTATTTCGGGGGCGGCACCGCCCAGAAGGGC
AGAGCCGATCCCCAACACCAAACCCGGGGGCTTGAGGGTTGAAATGACGCTCGA
ACAGGCATGCCCGCCGGAATACCAGCGGGCGCAATGTGCGTTCAAAGATTCGAT
GATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTGCTGCGTTCTTCATC
GATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTATTTGTTTGTTTTAC
TCAGAAGTTACAATAAGAAACATTAGAGTTTGGGTCCTCTGGCGGGCCGTCCCGT
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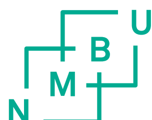

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