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Identifying inoculation methods for screening of resistance to *Fusarium langsethiae* in selected oat varieties

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Plant sciences/ Pathology

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ABSTRACT

F. langsethiae is reported as the main producer of HT-2+T-2 toxins in Norwegian oats (Hofgaard *et al.*, 2016). This investigation aimed to identify whether ranking of oat varieties according to the content of HT-2+T-2 toxins in grains from *F. langsethiae* inoculated plants differs from the ranking of cultivars according to deoxynivalenol (DON) in *F. graminearum* inoculated plants. Also, developing a method for screening of resistance to *F. langsethiae/ F. graminearum* in oats and ranking selected oats varieties according to the content of *F. langsethiae/ F. graminearum* DNA and mycotoxins produced by these fungus (HT-2 +T-2/ DON) in grains harvested from *F. langsethiae/ F. graminearum* inoculated plants.

In the course of three experiments, three oats varieties (Vinger, Odal, Belinda) were inoculated by injecting or spraying *F. langsethiae/ F. graminearum* spore suspension into the flag leaf sheath at late boot stage (GS45) or flag leaf sheath opening (GS47). Plants were also inoculated by spraying a *F. langsethiae* spore suspension at late boot stage (GS45), middle of heading (GS55) and full flowering (GS65).

Grain samples from plants that were spray inoculated with a *F. langsethiae* spore suspension at full flowering or middle of the heading, contained high levels of *F. langsethiae* DNA and HT-2+T-2, while only low levels of HT-2+T-2 were detected in oats that were inoculated at late boot stage or at flag leaf sheath opening. A significantly higher content of *F. langsethiae* DNA was detected in the grains harvested from *F. langsethiae* inoculated Belinda than from Odal, whereas Vinger contained the least content of *F. langsethiae* DNA. The grains harvested from *F. langsethiae* inoculated Belinda also contained more of HT-2+T-2 than Odal, whereas Vinger contained the least content of HT-2+T-2, however this difference was not significant between varieties.

Inoculation by spraying or injecting with *F. graminearum* spore suspension at flag leaf sheath opening or late boot stage were found to significantly increase the content of *F. graminearum* DNA and DON in comparison to non-inoculated plants in selected oats varieties. The grains harvested from Belinda contained higher amount of *F. graminearum* DNA and DON than Odal and Vinger, whereas Vinger contained the least content of *F. graminearum* DNA and DON, however the differences were not significant between varieties.

Inoculation by spraying with a *F. langsethiae* spore suspension at full flowering and middle of heading found to be the best method for inoculation of plants with *F. langsethiae*. Belinda was the most susceptible variety to both *F. langsethiae* and *F. graminearum* between the tested oat varieties, while Vinger showed better resistance to both of the *Fusarium* species than Odal and Belinda. Vinger also had less of HT-2+T-2 toxins than Odal and Belinda, while the content of DON was less in Vinger and Odal compared to Belinda, whereas Belinda contained highest levels of HT-2+T-2 and DON.

Key words: *Fusarium, langsethiae, graminearum,* HT-2+T-2, DON, Oats.

Introduction

Oats (*Avena sativa L.*) is a small grain cereal that is among the most consumed cereals. It is used both for human and animal diet and it is known for it's high nutritional values (Redaelli *et al.*, 2009). Oats is used as an important source of food especially in northern hemisphere and Nordic countries (Andon and Anderson, 2008). Oats contains ß-glucans, unsaturated fatty acids, antioxidants, dietary fiber, proteins, minerals and vitamins which are making the oats a nutritious cereal (Wood, 1990). Oats food products are recognized for their positive effects on health such as reducing total cholesterol of the blood and especially LDL (Low-density lipoprotein) cholesterol (Andon and Anderson, 2008).

Fusarium head blight (FHB) is a destructive disease of cereals with a worldwide expansion, which is caused by a number of *Fusarium* species as well as *Microdochium majus* (Glynn *et al.*, 2005). Generally in cereals, grains that are affected by *Fusarium* head blight, lose weight and quality, and their appearance become pale and dried-up (Xu *et al.*, 2007). FHB is responsible for the reduction in germination of the seeds and causes seedling blight. Seedling blight is the cause for death of the seedlings and thinner stems (Xu and Nicholson, 2009). *Fusarium* species also reduce the quality of the grains by producing mycotoxins and make them harmful for human and animal consumption (Xu and Nicholson, 2009).

Most FHB species are toxigenic and are found in wheat, oats and barley (in addition to other crops than cereals) widely in the world and also in central and Northern Europe (Langseth *et al.*, 1998). Parry *et al.* (1995) has widely investigated the cycle of the disease initiated by *Fusarium spp.*, in small seed cereals. The *Fusarium spp.* that remain in the soil as the saprophytic mycelium or thick walled spores (chlamydospores) on the plant residues, is the primary basis of infection of new plants. Infested seeds or plant residues may result in *Fusarium* seedling blight or foot rot, which can cause FHB by producing air born spores. The other routes to infect plants' head may be dispersal through splashing spores by rain drops, infested arthropod vectors and systemic growth of the pathogen (Parry *et al.*, 1995).

Between the FHB causing species, *F. langsethiae* is probably the one we least understand (Imathiu, 2008). FHB caused by *Fusarium langsethiae* can reduce the grain yield, but the most problematic concern is the production of mycotoxins that can have adverse effects on health

(Rocha *et al.*, 2005). Infection of *Fusarium* is favored by moist and warm condition at the flowering stage and later on during the growing period (Xu and Nicholson, 2009), but the severity of the disease may vary from region to region and year to year, because it is so dependent on climatic condition (Xu *et al.*, 2007). The optimum temperature for *F. langsethiae* infection is 25°C (10-35°C min-max) (Medina and Magan, 2010).

F. langsethiae was classified as a new species in 2004 (Torp and Nirenberg, 2004). Before, the *F. langsethiae* was known as powdery *F. poae,* because of the similarities in morphological profile (Torp and Langseth, 1999). The properties that differs *F. langsethiae* from *F. poae* are slower growth, less aerial spores and lack of odor (Yli-Mattila *et al.*, 2004). It also has powdery appearance when it is growing on artificial growth media and it does not produce sporodochial spores (Torp and Langseth, 1999; Torp and Nirenberg, 2004).

Despite the similarities in morphological profile to *F. poae*, the mycotoxin that is produced by *F. langsethiae* is comparable to those of *F. sporotrichioides* (Thrane *et al.*, 2004). *F. langsethiae* is known as the most important producer of the Trichothecene mycotoxins HT-2+T-2 in Norwegian cereals (Langseth and Rundberget, 1999). *F. langsethiae* also reported as the main producer of HT-2+T-2 toxins in Norwegian and Swedish cereals (Fredlund *et al.*, 2010; Hofgaard *et al.*, 2016). Additionally, a study by Edwards *et al.* (2012) claimed that in UK oats *F. langsethiae* is the main fungus liable for the production of HT-2+T-2 toxins.

Trichothecenes are common mycotoxin groups that are generated by *Fusarium* species (Langseth and Rundberget, 1999). Trichothecenes are two major types: Type A and Type B. Type-A trichothecenes consist of Diacetoxyscirpenol (DAS), HT-2+T-2, and Type-B trichothecenes include DON and nivalenol (NIV). Trichothecenes have an identical structure but with a difference in the form of the functional group. Type A trichothecenes have a carbonyl group less than type B (Wilson *et al.*, 2004). The mycotoxins HT-2+T-2 and Deoxynivalenol (DON) are the dominant mycotoxins in oats varieties in Norway and Sweden (Fredlund *et al.*, 2010; Hofgaard *et al.*, 2016).

DON is the most abundant trichothecene that can be found in cereals, and it is produced by *F. graminearum*, *F. culmorum* and *F. pseudograminearum* (Nicolaisen *et al.*, 2009). DON mycotoxins are harmful for livestock and humans because of inhibition in synthesis of DNA, RNA and albumin protein (Rocha *et al.*, 2005). According to Hofgaard *et al.* (2016) occurrence

of *F. graminearum* has increased significantly during the last years in Norwegian oats and wheats, which caused an increase in DON contamination of the grains.

HT-2+T-2mycotoxins are produced by *F. langsethiae* and *F. sporotrichioides, F. Poa, F. equiseti* (Bernhoft et al., 2013). The T-2 toxin is cytotoxic and inhibits synthesis of DNA and RNA in the eukaryote cells, and it also prevents the synthesis of protein (Placinta *et al.*, 1999; Rocha *et al.*, 2005). T-2 toxins can transform to HT-2 toxins in the ingestion system and consequently their risk assessment are similar (Eriksen and Alexander, 1998).

Oats are more exposed to contamination by *Fusarium* mycotoxins than any other cereals (Langseth and Rundberget, 1999; Scudamore *et al.*, 2007). Oats has appeared to be more influenced by HT-2+T-2 toxins than wheat and barley (Imathiu *et al.*, 2013). Oats is one of the most appropriate hosts for *F. langsethiae* (Edwards et al., 2009a). Oats and barley contain the highest amounts of HT-2+T-2 mycotoxins (Edwards *et al.*, 2009a). Like other small grain cereals, oats are more susceptible to *Fusarium* infection around anthesis (Tekle *et al.*, 2012). Edwards (2009b) measured the *Fusarium* mycotoxins in oats, barley and wheat during 2002 to 2005 period in the UK and reported that the levels of DON were much lower in barley and oats than in wheat, but the levels of HT-2+T-2toxins were much higher in oats in comparison with barley and wheat. However, some studies (Edwards, 2009b; Xu *et al.*, 2014) claimed that samples from organic fields of oats had approximately four times lower levels of HT-2+T-2 toxins compared to conventional fields. Related study in Norway confirmed these results (Bernhoft *et al.*, 2012). Elevated air humidity before or during flowering (Xu *et al.*, 2014) and before harvesting favors the accumulation of HT-2+T-2 in oats (Bernhoft *et al.*, 2012). European Union has set legal limits for the level of contamination with DON in the oats that are used for human consumption. Based on EuropeanCommission (2006), The DON concentration has to be less than 1750 µg/kg, while the suggested limits for HT-2+T-2toxins is 1000 µg/kg (EuropeanCommission, 2013).

Dependency on visual symptoms for quantifying the degree of FHB infection can be challenging (Parry *et al.*, 1995). Oats inoculated with *F. langsethiae* do not necessarily produce symptoms (Imathiu, 2008; Edwards *et al.*, 2012). Simulated panicle inoculation by *F. langsethiae* in the greenhouse or field experiments, produced lower levels of infection in comparison with untreated commercial fields (Imathiu, 2008).

PCR (polymerase chain reaction) is method that is widely used to identify *Fusarium spp.* infected grains. Real time PCR (qPCR) has been developed for the quantification of individual *Fusarium* species. There are some advantages in qPCR over common PCR. The first advantage is that it is faster and needs less processing time. The other advantage is that it does not need post-PCR handling which reduce the possibility for cross contamination and handling errors (Heid *et al.*, 1996; Williams, 2009).

The most exact method for quantification of mycotoxins in cereals is chromatography. This method is able to separate different mycotoxins in the mixture accurately but it is expensive and time consuming (Pascale, 2009; Aamot *et al.*, 2013). Several immunological methods are known for quantifying of mycotoxins in cereals. Enzyme linked immunosorbent assay (ELISA) is frequently used for monitoring of the mycotoxins in the samples (Pascale, 2009). The advantages of ELISA kits are high speed and sensitivity, simple sample preparation, simultaneous analysis of multiple samples and limited use of organic solvents (DON is dissolved in water and other mycotoxins are not) (Pascale, 2009; Aamot *et al.*, 2012). The disadvantage of this method is expensive ELISA kits.

Some fungi may develop as a saprophyte without initiating any disease symptom, perhaps because it is a weak pathogen, but can still produce mycotoxins in the cereals (Edwards *et al.*, 2012), while some others may generate severe symptoms of the disease without any mycotoxins e.g. *M. nivale* (Edwards *et al.*, 2012). Study by Waalwijk *et al.* (2004) have suggested a correlation between fungal DNA of a single or group of *Fusarium* species and their related mycotoxin, but this correlation is not always suitable to evaluate the amount of mycotoxins. Fredlund *et al.* (2010) identified grain samples with low levels of fungus DNA show high amounts of mycotoxins. Amounts of HT-2+T-2 toxins are decreasing considerably throughout the oats processing, particularly in de-hulling step, therefore the mycotoxins passing to the human food chain are highly downgraded compared to the primary amounts in unprocessed oats (Scudamore *et al.*, 2007).

The following hypothesis was to be tested in this study: “Ranking of oat varieties according to the content of HT-2+T-2 toxins in *F. langsethiae* inoculated plants differs from the ranking of cultivars according to deoxynivalenol (DON) in *F. graminearum* inoculated plants”. The secondary objectives of this study were therefore to:

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1. Develop a method for screening of resistance to *F. langsethiae* in oats.

2. Rank selected oats varieties according to the content of *F. langsethiae* DNA and mycotoxins produced by this fungus (HT-2 +T-2) in grains from *F. langsethiae* inoculated plants.

3. Rank selected oats varieties according to the content of *F. graminearum* DNA and mycotoxins produced by this fungus (DON) in grains from *F. graminearum* inoculated plants.

Material and methods

Experimental setup

Three experiments were done in this study during 2015-2016. The studies were done at NIBIO (Norwegian Institute for Bioeconomy Research) in Ås, Akershus, Norway (59°39′37″N 10°47′1″E). Properties and procedures of experiments can be found in Table 1.

Table 1: Detailed procedures and time points of the experiments

Exp.	Start date	Oats varieties	Fungal species	Inoculation Method	Harvest date	Incubation	Temperature
1	18 Feb 2015	Odal Belinda Vinger	*F. langsethiae* *F. graminearum*	Sprayed into the flag leaf sheath at GS47*	3 June 2015	3 days	18/12 °C Day/ Night
2	2 March 2015	Odal Belinda Vinger	*F. langsethiae* *F. graminearum*	Injected into the flag leaf sheath at GS45*	15 June 2015	3 days	18/12 °C Day/ Night
3	4, 16 and 25. September 2015	Odal Belinda Vinger	*F. langsethiae*	Sprayed at GS 45, 55, 65* or Injected into the flag leaf sheath at GS45	18. Jan, 25. Jan 2016 and 3 Feb 2016	6 days	18/12 °C Day/ Night (15/12 °C Day/ Night during bagging period)

*GS47: flag leaf sheath opening. GS45: late boot stage. GS55: middle of heading and GS65: full flowering

Plant material

All experiments were conducted using three varieties of oats: Odal, Belinda and Vinger. The seeds were selected to get a low percentage of *Fusarium*-infested seeds according to analysis performed at Kimen Såvarelaboratoriet AS. Ten seeds were sown in each pot (each replication). The pots standard 3.5 L and peat mixed soil (10% soil, Degernes Go' Jord) were used in these studies. The temperature was 18/12°C 16h day/8h night with relative humidity of 75% and 16 hours' light day time (105 nm/cm). White light was used during growth period (Philips Master HPI-T plus 400w/645). Plants were supported by bamboo sticks and ropes to support the stems, from six weeks after sowing. For the first and second experiments plants were irrigated when required and fertilized (Fertilizer water EC =1.8 S/m) five times during vegetative growth in both

experiments. For the third experiment plants were irrigated whenever they required. The plants were fertilized weekly from 3 weeks after sowing/ 8 weeks in total 0.5 L each time. The experiments were done with randomized block design plan with four replications (each block was mentioned as a replication).

Fungal isolates

F. langsethiae (isolate 201086) and *F. graminearum* (isolate 200628) were used in the first and second experiment. Two isolates of *F. langsethiae* (isolate 201086 and isolate 201058) were used in the third experiment. All isolates had been stored at -80°C.

Inoculum

The fungal mycelium was transferred from -80°C to mung bean agar and incubated for 10-14 days at 22°C with 12 Hours light (white+NUV by Osram L36-73 32W). Inoculum suspensions with 10^6 spores/ml for *F. langsethiae* and 10^5 spores/ml for *F. graminearum* were prepared by washing the fungal spores off the MBA surface by adding 10 ml water. The final concentration of inoculum suspensions was calculated using Hycor glasstic slides with grids. The prepared suspensions were transferred to new MBA plates and were incubated at 22°C with 12 hours light (white+NUV by Osram L36-73 32W) for 10-14 days. The inoculum suspension was diluted with distilled water to reach the correct concentration of 10^6 spores/ml for *F. langsethiae* and 10^5 spores/ml for *F. graminearum.* The prepared inoculum suspensions were stored at -20°C in 30 or 500ml containers. In the third experiment *F. langsethiae* inoculum was a mixture of two isolates 50 % of each. Viability and germination test for inoculum suspension was done by growing the inoculum suspension on three parallel water agar plates and counting the number of germinated spores after twenty hours.

Inoculation

In the first and second experiments, plants were inoculated with *F. langsethiae* or *F. graminearum*. Water was used as control (non-inoculated). In the first experiment oats varieties were inoculated by spraying the inoculum suspension into the boot at flag leaf sheath opening (Zadox growth stage 47) by using atomizers (Devilblis 151 by Sunshine co.). In the second experiment the plants were inoculated by injecting the spore suspensions into the flag leaf sheet by a syringe at late boot stage (Zadox growth stage 45).

Inoculation for the first experiment was done by using bike pumps for blowing the air pressure into the atomizers. In this experiment, most of the plants were inoculated at GS47 but there were also some plants which already passed that stage had been inoculated at GS49 (about 14%). Pots were covered with moisturized plastic bags for 72 hours.

Oats for the second experiment were inoculated a week later than the first experiment, at GS45 by using syringes for injection of the inoculum suspension into the boots. Pots were covered with moisturized plastic bags for 72 hours and checked for any diseases symptoms five and eight days after inoculation. The tests for germination and contamination of inoculum suspension were done after the inoculation process by using two PDA plates and two WA plates in both experiments. The length of panicles and the distance between flag lead node to top of the plants was measured at the end of flowering (GS70) for both experiments.

In the third experiment plants were inoculated with a *F. langsethiae* (mixture of two isolates) and water (non-inoculated plants). Oats from three different sowing dates were inoculated at the same time. Plants from the first sowing date were at full flowering (GS65), the second sowing date plants were at middle of heading (GS55) and the third sowing date plants were at late boot stage (GS45) at the time of inoculation. The spray inoculation method was used for the first and the second sowing date plants (four pots with inoculum suspension and four pots with water for each sowing date). Oats from the third sowing date were inoculated at GS45 by spray inoculation or by boot injection method using a syringe. The amount of inoculum suspension or water that used per panicle was 1 ml in spray method and 200 µl in injection method. One ml of tween 20 was added per liter of inoculum suspension and water before inoculation (0.01%). Pots were covered with moisturized plastic bags for six days. Five thermo loggers were installed inside the plastic bags randomly and five other were installed at random places in the glasshouse, to measure the temperature inside and outside the plastic bags during the cover period. In the third experiment the room temperature had been set to 15/12°C day/night during the bagging period. The tests for germination and contamination of inoculum suspension were done after the inoculation process by using two PDA plates and two WA plates. The length of panicles and the distance between flag lead node to top of the plants were measured at the end of flowering (GS70).

Disease symptom observation and measurements

Plants were observed for any disease symptoms five/six and eight days after inoculation. Plants were examined for any discoloration, necrosis or spots around the flag leaf and panicle. Symptoms were scored on the basis of one and zero (presence or absence of symptoms in the panicle). Panicle length and the length from flag leaf node to the top of the plant were measured at GS70.

Harvesting and milling

At maturity, grains were harvested and dried at 22°C for 48 hours. The grains were cleaned using a Minibat® sample harvester in the two first experiments and a grain cleaner machine in the third experiment. For all experiments the number of grains harvested from each pot was counted and weighted. Yield (gram per plant) and 1000 seeds weight per plant were calculated. Then grains were milled by Retsch® ZM200 milling machine using 1mm milling sieve size. Milled samples were kept in 150 mg portions at -80°C until DNA extraction and in 5 g portions at -20°C until analyses of mycotoxins.

Analysis of fungal DNA

In all experiments, total genomic DNA was extracted from 150 mg milled samples using Fast DNA spin kit for soil (MP Biomedicals®, France). The procedures were according to the protocol provided with the kit. Extracted DNA were diluted ten times with distilled H_2O and kept in -20 °C before qPCR. For the dilution, 10 µl DNA was added to 90 µl of distilled water. The following qPCR assays were used:

- *F. langsethiae (Hofgaard et al., 2016)*: Probe: 5`-6FAM-CAC ACC ***C***AT A***C***C TAC ***G***TG TAA-TAMRA-3`, Forward: 5`-GTT GGC GTC TCA CTT ATT C-3`, Reverse: 5`-TGA CAT TGT TCA GAT AGT CC-3`(Locked nucleic acid nucleotides, have been shown by bold italic letters).
- *F. graminearum* (Waalwijk *et al.*, 2004): Probe: AGA TAT GTC TCT TCA AGT CT, Forward: GGC GCT TCT CGT GAA CACA, Reverse: TGGCTA AAC AGC ACG AAT GC.
- *F. culmorum* (Waalwijk *et al.*, 2004): Probe: FAM-CACTTGGATATATTTCC-MGB, Forward: TCACCCAAGACGGGAATGA. Reverse: GAACGCTGCCCTCAAGCTT.

- The assay for oats DNA (Divon *et al.*, 2012): Probe: AM-CTCCTATTAAGCTCAGCCTT-MGB. Forward: GGTTGTTGCCACCAAGTCTCTT. Reverse: TGCCGCTGCCAACTTC

In these experiments, analyses of *F. langsethiae* and plant DNA were done by duplex reaction. The total volumes for qPCR were 25µl that comprised 4µl of genomic DNA (mean of 23 ng plant DNA), 300 nM of each *F. langsethiae* primer, 75nM of each plant primer, 100nM of each probe and 1xiQ™ Multiplex Powermix (BioRad, Hercules, CA, USA). *F. graminearum* and *F. culmorum* were analyzed by single reactions. qPCR was done in total volumes of 25 µl that comprised 4µl of genomic DNA, 300 nM of each *F. graminearum and F. culmorum* primer, 100nM of each probe and Sso Advanced™ Universal Probes Supermix (Biorad, Hercules, CA, USA). The amount of fungal or plant DNA was quantified using a standard curve algorithm with defined dilution of fungal or plant standard DNA in the range of 0.1 - 4 ng fungal DNA or 8 pg – 32 ng plant DNA. The amounts were given as pg of fungal DNA per µg of plant DNA. Standards that were prepared according to Divon *et al.* (2012), obtained from NIBIO. A C1000 touch term cycler combined with a CFX96TM Real-Time system (Biorad, USA) was used for the qPCR process and the probes for detection were labeled 6-FAM (for fungal DNA),(Applied Biosystems by Thermo Fisher Scientific, Waltham, CA, USA) and VIC (for plant DNA). The used parameters were 95^0C for 3 min + 45 cycles of 95^0C for 10 s and 60^0C for 30 s. The data were analyzed using BioRad CFX manager 3.1 (BioRad).

Analysis of Deoxynivalenol and HT-2+T-2 toxins

Quantification of DON and HT-2+T-2 toxins in the samples was performed using the ELISA Romerlabs® AgraQuant® DON (Assay 0.25/5.0) and Agraquant® HT-2+T-2 (Assay 25/500) toxin kits (Romer Labs®, Singapore), along with the manufacturer's protocol and absorbance was measured at 450 nm using a spectrophotometer (Spectra max190, Molecular devices). The data were analyzed using RomerLabs® software for windows. For statistical analyzes the amounts of DON and HT-2+T-2 toxins less than 250 and 150 µl/kg were corrected to 42 and 25 µl/kg, respectively.

Statistical analyzes

Various morphological and physiological traits of plants and also the amount of DNA and mycotoxins were subjected to statistical analyzes. Minitab 16 Statistical Software (2010)

[Computer software], State College, PA: Minitab, Inc. (www.minitab.com) was used for statistical analyzes in all experiments. General linear model (GLM) was used to test the differences between treatments, varieties, replications and interaction between varieties and treatments. The model for analyzes was variety, replication, treatments and the interaction between varieties and treatments. Replication was used as the random factor. Tukey pairwise comparison method was used for pairwise comparisons between the significant means at 95% confidence level. In experiment 3, for better understanding of the influence of the methods and also behavior of varieties, different inoculation time points were analyzed separately. In addition to regular analyzes for the amount of DNA and mycotoxins, due to the absence or very low levels of fungal DNA and mycotoxins, non-inoculated plants were deleted from statistical analyzes and the t-test was also performed between varieties in inoculated plants. Pearson correlation method was used to investigate the correlation between quantities of DNA and the amounts of mycotoxins in samples. Standard deviations between samples were calculated by excel software for windows. All the figures were designed and drawn in excel.

Results

Symptom development

Disease symptoms (necrosis, discoloration or spots around the oats panicle and flag leaf sheath) were recorded within eight days after inoculation. For each plant, symptoms were recorded as 1 or 0 for presence or absence of symptoms, and the percentage of plants with symptoms were then calculated per pot. No major differences in symptom development were detected between observations at 5 and 8 days after inoculation (table 2), hence only the symptoms recorded at 8 days after inoculation will be referred to hereafter.

Experiment 1

In experiment 1, a significant higher percentage of the *F. graminearum* inoculated plants (37%) and *F. langsethiae* inoculated plants (25%) developed symptoms compared to the non-inoculated plants (0%) ($P < 0.0001$, Figure 1A). Within the *F. graminearum* inoculated plants, the proportions of plants with symptoms were highest in Belinda (40%), followed by Odal (37%), and Vinger (34%), though this difference was not significant ($P = 0.3$). Within the *F. langsethiae* inoculated plants, the proportions of plants with symptoms were highest in Belinda (32%), followed by Odal (23%), and Vinger (22%), though this difference was not significant ($P = 0.3$, Table 2). There was no significant interaction between varieties and treatments ($P = 0.8$). No symptoms were observed on the non-inoculated plants.

Experiment 2

In experiment 2, a significant higher percentage of the *F. graminearum* inoculated plants (36%) developed symptoms compared to the *F. langsethiae* inoculated plants (1%) and non-inoculated plants (0%) ($P < 0.0001$, Figure 1B). Within the *F. graminearum* inoculated plants, the proportions of plants with symptoms were highest in Belinda (38.5%), followed by Odal (35%), and Vinger (35%), though this difference was not significant ($P = 0.7$). Within the *F. langsethiae* inoculated plants, symptoms were detected only in Belinda (3.5%), though the difference was not significant between varieties ($P = 0.7$, Table 2). There was no significant interaction between varieties and treatments ($P = 0.5$). No symptom was observed on the non-inoculated plants (Table 2).

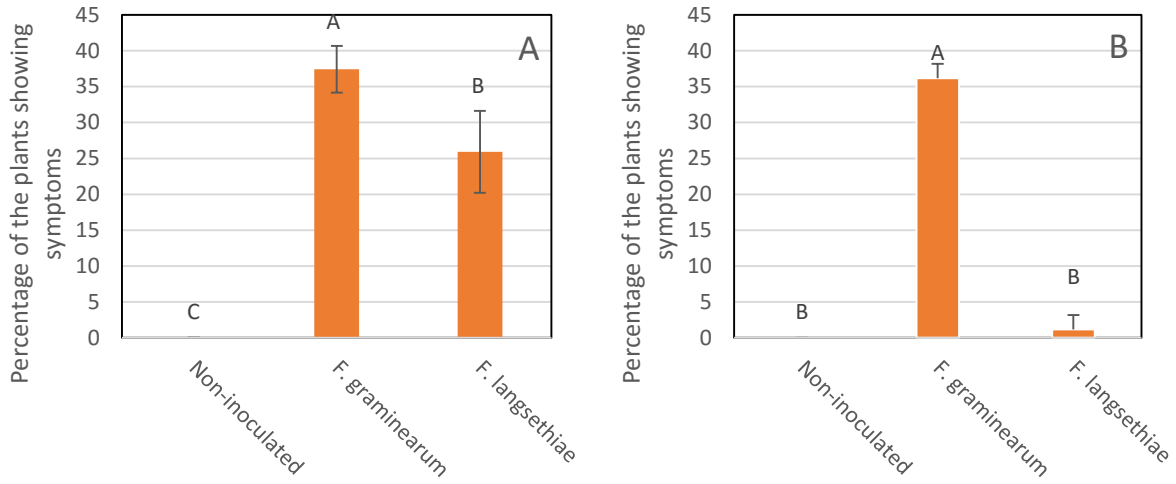

Figure 1: Percentage of plants with symptoms after inoculation with *Fusarium graminearum*, *Fusarium langsethiae* or water (non-inoculated control treatment) in three oats varieties (Vinger, Odal and Belinda) in Exp1 (A) and Exp.2 (B). The plants were inoculated by spraying a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1, and injecting a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at late boot stage (GS45) in Exp. 2. Means that are sharing the same letters in each experiment are not significantly different. Error bars are represented as standard deviations.

Experiment 3

In experiment 3, plants that were inoculated by injecting a spore suspension into the flag leaf sheath at GS45 (GS45I) showed no symptoms, neither for *F. langsethiae* inoculated, nor for non-inoculated plants, though the difference was not significant between treatments and varieties (Table 2).

Plants that were inoculated by spraying with *F. langsethiae* spore suspension at GS45 (GS45S) developed insignificant symptoms (4%), whereas no symptoms were observed on the non-inoculated plants (Table 2). The proportions of plants with symptoms were highest in Odal (5 %), followed by Belinda (3%), and Vinger (0%), though this difference was not significant (P = 0.6).

Plants that were inoculated by spraying with *F. langsethiae* spore suspension at GS55 (GS55S) developed symptoms only in Belinda (9%) and no symptoms were observed on the non-inoculated plants (Table 2), though the difference was not significant between treatments and varieties (P = 0.1 and P = 0.8, Table 2)

Between the plants that were inoculated with *F. langsethiae* spore suspension at GS65 (GS65S) a significant higher percentage of the *F. langsethiae* inoculated plants (32%) developed symptoms compared to the non-inoculated plants (0%) ($P < 0.0001$). Within the *F. langsethiae* inoculated plants, the proportions of plants with symptoms were highest in Belinda (44%), followed by Odal (32%), and Vinger (20%), though the difference between varieties was not significant ($P = 0.07$). There was no significant interaction between varieties and treatments ($P = 0.07$). No symptoms were observed on the non-inoculated plants (Table 2).

Table 2: Scoring of different traits

Exp.	Variety	Inoculation method and stage[a]	Pathogen[b]	Symptoms[c] after 5/6 days (%)	Symptoms after 8 days (%)	Yield per plant (g/plant) [d]	1000 Seeds weight (g)	Number of grains per plant
Exp. 1	Vinger	GS47SI	C	0 ^{ns}	00 ^{ns}	3.33^{AB}	40.2^{ns}	83.08^{ns}
		GS47SI	F. g	27 ^{ns}	34 ^{ns}	3.33^{AB}	43.4 ^{ns}	76.6 ^{ns}
		GS47SI	F. l	22 ^{ns}	22 ^{ns}	3.67^{AB}	46.9 ^{ns}	78.7 ^{ns}
	Odal	GS47SI	C	00 ^{ns}	00 ^{ns}	3.41^{AB}	44.98^{ns}	75.8 ^{ns}
		GS47SI	F. g	32 ^{ns}	37 ^{ns}	3.05^{B}	43.48^{ns}	71.2 ^{ns}
		GS47SI	F. l	23 ^{ns}	23 ^{ns}	3.39^{AB}	47.6^{ns}	70.5 ^{ns}
	Belinda	GS47SI	C	00 ^{ns}	00 ^{ns}	4.19^{A}	46.9 ^{ns}	88.7 ^{ns}
		GS47SI	F. g	34 ^{ns}	40 ^{ns}	4.5^{A}	46.5 ^{ns}	97.06 ^{ns}
		GS47SI	F. l	32 ^{ns}	32 ^{ns}	3.74^{A}	49.6 ^{ns}	76.1 ^{ns}
Exp. 2	Vinger	GS45I	C	00 ^{ns}	00 ^{ns}	3.80^{AB}	43.3 ^{ns}	90.2^{ns}
		GS45I	F. g	35 ^{ns}	35 ^{ns}	3.69^{AB}	45.6 ^{ns}	80.96 ^{ns}
		GS45I	F. l	00 ^{ns}	00 ^{ns}	3.8^{AB}	45.6 ^{ns}	83.96 ^{ns}
	Odal	GS45I	C	00 ^{ns}	00 ^{ns}	3.75^{AB}	42.8 ^{ns}	88.1 ^{ns}
		GS45I	F. g	38 ^{ns}	38 ^{ns}	3.25^{B}	40.3 ^{ns}	81.01 ^{ns}
		GS45I	F. l	00 ^{ns}	00 ^{ns}	3.62^{AB}	49.04 ^{ns}	74.35 ^{ns}
	Belinda	GS45I	C	00 ^{ns}	00 ^{ns}	4.19^{A}	45.3 ^{ns}	92.2 ^{ns}
		GS45I	F. g	31 ^{ns}	35 ^{ns}	4.24^{A}	44.4 ^{ns}	97.02 ^{ns}
		GS45I	F. l	3.5 ^{ns}	3.5 ^{ns}	3.99^{A}	51.6 ^{ns}	77.57 ^{ns}
Exp. 3	Vinger	GS45I	C	00^{ns}	00 ^{ns}	4.1^{A}	45.9^{AB}	89.6 ^{ns}
		GS45I	F. l	00 ^{ns}	2 ^{ns}	3.9^{A}	44.3^{AB}	87.31 ^{ns}
	Odal	GS45I	C	00 ^{ns}	00 ^{ns}	3.6^{AB}	41.2^{B}	90.38 ^{ns}
		GS45I	F. l	00 ^{ns}	00 ^{ns}	2.9^{B}	39.56^{B}	72.5 ^{ns}
	Belinda	GS45I	C	00 ^{ns}	00 ^{ns}	4.2^{A}	47.4^{A}	88.8 ^{ns}
		GS45I	F. l	00 ^{ns}	00 ^{ns}	4.08^{A}	46.76^{A}	87.5 ^{ns}
	Vinger	GS45S	C	00 ^{ns}	00 ^{ns}	4.02^{A}	45.2^{ns}	89.18 ^{ns}
		GS45S	F. l	00 ^{ns}	00 ^{ns}	3.7^{B}	40.00 ^{ns}	94.4 ^{ns}
	Odal	GS45S	C	00 ^{ns}	00 ^{ns}	3.5^{B}	42.4 ^{ns}	83.38 ^{ns}
		GS45S	F. l	00 ^{ns}	5 ^{ns}	3.4^{B}	41.6 ^{ns}	81.2 ^{ns}
	Belinda	GS45S	C	00 ^{ns}	00 ^{ns}	4.3^{A}	51.9 ^{ns}	85.13 ^{ns}
		GS45S	F. l	3 ^{ns}	3 ^{ns}	4.4^{A}	43.7 ^{ns}	1.3.2 ^{ns}
	Vinger	GS55S	C	00 ^{ns}	00 ^{ns}	3.98^{ns}	43.0^{AB}	93.19 ^{ns}
		GS55S	F. l	00 ^{ns}	00 ^{ns}	4.03 ^{ns}	46.06^{A}	87.6 ^{ns}
	Odal	GS55S	C	00 ^{ns}	00 ^{ns}	3.6 ^{ns}	41.3^{B}	88.35 ^{ns}
		GS55S	F. l	5 ^{ns}	9 ^{ns}	3.7 ^{ns}	41.70^{B}	89.6 ^{ns}
	Belinda	GS55S	C	00 ^{ns}	00 ^{ns}	4.09 ^{ns}	44.7^{AB}	91.46 ^{ns}
		GS55S	F. l	00 ^{ns}	00 ^{ns}	4.4 ^{ns}	48.03^{A}	93.01 ^{ns}
	Vinger	GS65S	C	00 ^{ns}	00 ^{ns}	3.8^{AB}	41.6^{AB}	92.05 ^{ns}
		GS65S	F. l	5 ^{ns}	20 ^{ns}	3.6^{AB}	41.1^{AB}	90.27 ^{ns}
	Odal	GS65S	C	00 ^{ns}	00 ^{ns}	3.6^{AB}	42.00^{AB}	85.75 ^{ns}
		GS65S	F. l	12 ^{ns}	32 ^{ns}	2.8^{B}	39.96^{B}	71.15 ^{ns}
	Belinda	GS65S	C	00 ^{ns}	00 ^{ns}	4.6^{A}	45.77^{A}	101.08^{ns}
		GS65S	F. l	13 ^{ns}	44 ^{ns}	4.2^{AB}	46.1^{A}	92.07 ^{ns}

Effect of *Fusarium* inoculation on symptom development and yield parameters in three oats varieties.

[a] GS47SI = Inoculated by spraying a spore suspension into the flag leaf sheath at GS47. GS45I= Inoculated by injecting a spore suspension into the flag leaf sheath at GS45. GS45S, GS55S and GS65S = Inoculated by spraying whole plants with a spore suspension at GS45, GS55 or GS65, respectively.

[b] *F. g: Fusarium graminearum, F. l: Fusarium langsethiae*, C: non-inoculated

[c] The average percentage of inoculated plants showing symptoms (necrosis, discoloration and the spots around the panicle and the flag leaf sheath).

[d] Means that are sharing the same letters in each experiment (Exp. 1 and 2) or inoculation method and time point (GS45I, GS45S, GS55S and GS65S in Exp. 3) are not significantly different (grouping by Tukey's pairwise comparison at 95% confidence level)

Yield parameters

Experiment 1

In experiment 1, the average yield per plant ranged from 3.60 g to 3.64 g between the different inoculation treatments, and treatment had no significant influence on the average yield per plant (P = 0.9, Figure 2A). Between the non-inoculated control plants, Belinda had higher yield per plant (4.19 g) than Vinger (3.33 g) and Odal (3.41 g). Within the *F. graminearum* inoculated plants, the yield per plant was highest in Belinda (4.5 g), followed by Vinger (3.33 g), and Odal (3.05 g). Within the *F. langsethiae* inoculated plants, the yield per plant was highest in Belinda (3.74 g), followed by Vinger (3.67 g), and Odal (3.39 g). There was significant difference between varieties within inoculation treatments (P = 0.01, Table 2). No significant interaction was detected between varieties and treatments (P = 0.4).

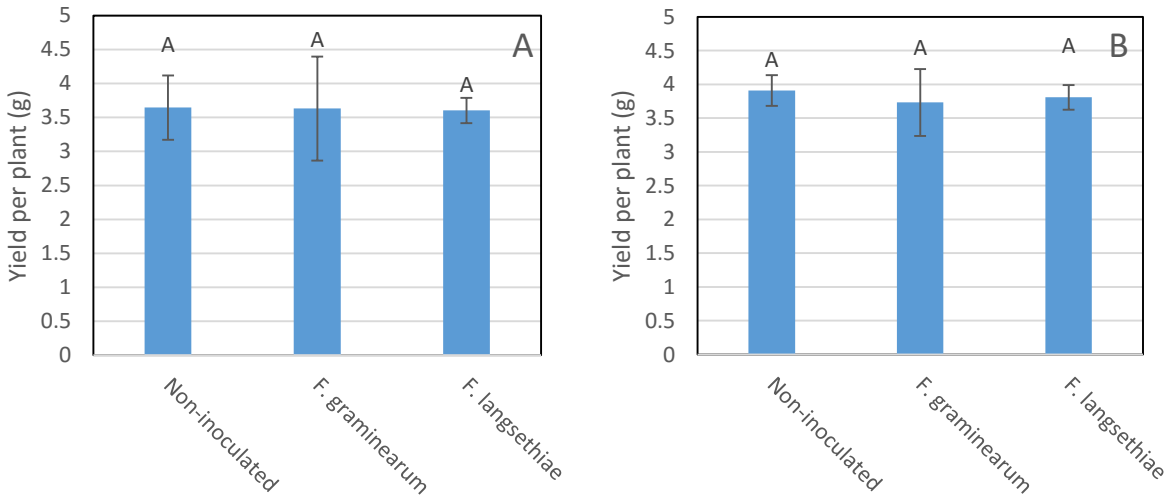

Figure 2: Average yield per plant (g) in three oats varieties (Vinger, Odal and Belinda) inoculated with *Fusarium graminearum*, *Fusarium langsethiae* or water (non-inoculated control treatment) in Exp1 (A) and Exp.2 (B). The plants were inoculated by spraying a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1, and injecting a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at late boot stage (GS45) in Exp. 2. Means that are sharing the same letters in each experiment are not significantly different. Error bars are represented as standard deviations.

The average 1000 seeds weight varied from 44.07 g to 48.07 g between the different inoculation treatments, however treatment had no significant influence on the average 1000 seeds weight (P = 0.07, Figure 3A). Between the non-inoculated control plants Belinda had the highest 1000 seeds weight (46.9 g), followed by Odal (44.98 g) and Vinger (40.2 g). Within the *F. graminearum* inoculated plants, the 1000 seeds weight was highest in Belinda (46.5 g), followed by Odal (43.48 g), and Vinger (43.4 g). Within the *F. langsethiae* inoculated plants, the 1000 seeds weight was highest in Belinda (49.6 g), followed by Odal (47.6 g), and Vinger (46.9 g). There was no significant difference between varieties within either inoculation treatment (P = 0.08, Table 2). Interaction between varieties and treatments was not significant (P = 0.8).

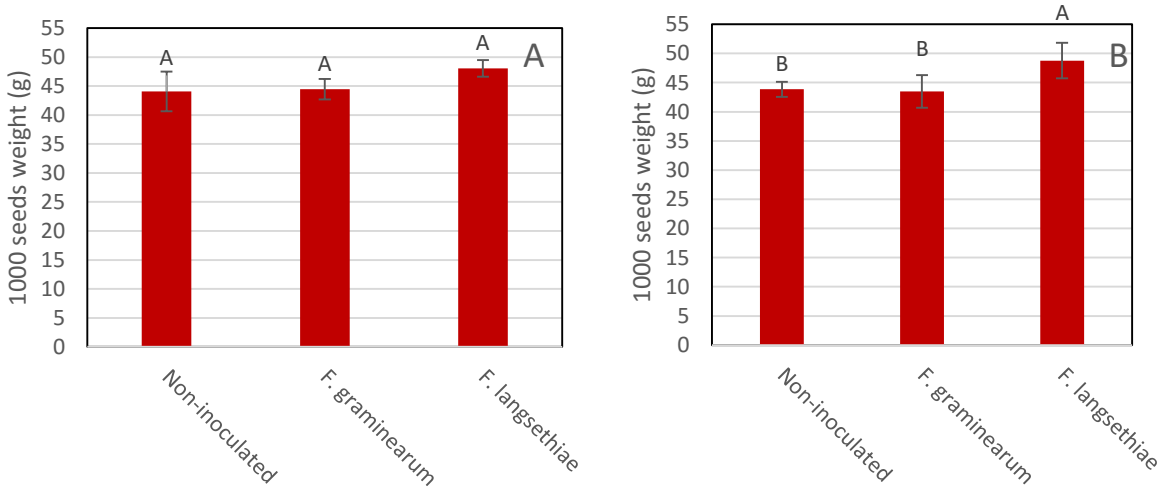

Figure 3: Average 1000 seeds weight (g) in three oats varieties (Vinger, Odal and Belinda) inoculated with *Fusarium graminearum*, *Fusarium langsethiae* or water (non-inoculated control treatment) in Exp1 (A) and Exp.2 (B). The plants were inoculated by spraying a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1, and injecting a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at late boot stage (GS45) in Exp. 2. Means that are sharing the same letters in each experiment are not significantly different.

The average number of grains per plant fluctuated from 75.17 to 82.56 grains per plant between the different inoculation treatments, however treatment had no significant influence on the average number of grains per plant (P = 0.3, Figure 4A). In the non-inoculated control plants, Belinda had more grains per plant (88.7) than Vinger (83.8) and Odal (75.8). Within the *F. graminearum* inoculated plants, the number of grains per plant was highest in Belinda (97.06), followed by Vinger (76.6), and Odal (71.2). Within the *F. langsethiae* inoculated plants, the number of grains per plant was highest in Vinger (78.7), followed by Belinda (76.1), and Odal (70.5). There was no significant difference between varieties within either inoculation treatment (P = 0.06, Table 2). No significant interaction was detected between varieties and treatments (P = 0.6).

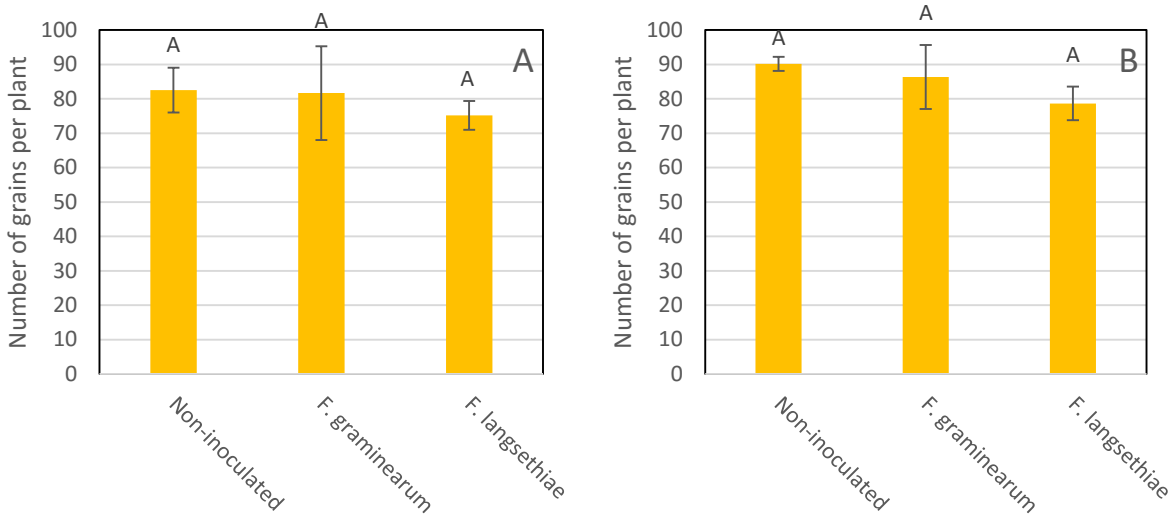

Figure 4: Average number of grains per plant in three oats varieties (Vinger, Odal and Belinda) inoculated with *Fusarium graminearum*, *Fusarium langsethiae* or water (non-inoculated control treatment) in Exp1 (A) and Exp.2 (B). The plants were inoculated by spraying a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1, and injecting a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at late boot stage (GS45) in Exp. 2. Means that are sharing the same letters in each experiment are not significantly different. Error bars are represented as standard deviations.

Experiment 2

In experiment 2, the average yield per plant ranged from 3.73 g to 3.90 g between the different inoculation treatments, however treatment had no significant influence on the average yield per plant (P = 0.2, Figure 2B). In the non-inoculated control plants, Belinda had higher yield per plant (4.19 g) than Vinger (3.80 g) and Odal (3.75 g). Within the *F. graminearum* inoculated plants, the yield per plant was highest in Belinda (4.24 g), followed by Vinger (3.69 g), and Odal (3.25 g).

Within the *F. langsethiae* inoculated plants, the yield per plant was highest in Belinda (3.99 g), followed by Vinger (3.8 g), and Odal (3.62 g). There was significant difference between varieties within inoculation treatments ($P < 0.0001$, Table 2). Interaction between varieties and treatments was not significant ($P = 0.1$).

A significant higher average 1000 seeds weight of the *F. langsethiae* inoculated plants (48.8 g) was recorded compared to *F. graminearum* inoculated plants (43.4 g) and the non-inoculated plants (43.8 g) ($P= 0.01$, Figure 3B). In the non-inoculated control Belinda had the highest 1000 seeds weight (45.3 g), followed by Vinger (43.3 g) and Odal (42.8 g). Within the *F. graminearum* inoculated plants, 1000 seeds weight was highest in Vinger (45.6 g), followed by Belinda (44.4 g), and Odal (40.3 g). Within the *F. langsethiae* inoculated plants, 1000 seeds weight was highest in Belinda (51.6 g), followed by Odal (49.4 g), and Vinger (45.6 g). There was no significant difference between varieties within either inoculation treatment ($P = 0.2$, Table 2). Interaction between varieties and treatments was not significant ($P = 0.3$).

The average number of grains per plant fluctuated from 78.63 to 90.19 grains per plant between the different inoculation treatments, however treatment had no significant influence on the average number of grains per plant ($P = 0.06$, Figure 4B). In the non-inoculated control plants, Belinda had more grains per plant (92.2) than Vinger (90.2) and Odal (88.1). Within the *F. graminearum* inoculated plants, the number of grains per plant was highest in Belinda (97.02), followed by Odal (81.01), and Vinger (80.96). Within the *F. langsethiae* inoculated plants, the number of grains per plant was highest in Vinger (83.96), followed by Belinda (77.57), and Odal (74.35). There was no significant difference between varieties within either inoculation treatment ($P = 0.2$, Table 2). No significant interaction was detected between varieties and treatments ($P = 0.3$).

Experiment 3

In experiment 3, when plants were inoculated at GS45 by injection (GS45I), the average yield per plant was 4 g for non-inoculated plants and 3.6 g for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average yield per plant ($P = 0.2$). Between the non-inoculated control plants, Belinda had higher yield per plant (4.2 g) than Vinger (4.1 g) and Odal (3.6 g). Within the *F. langsethiae* inoculated plants, the yield per plant was highest in Belinda (4.8 g), followed by Vinger (3.8 g) and Odal (2.9 g). There was significant difference between varieties

within inoculation treatments (P = 0.04, Table 2). Interaction between varieties and treatments was not significant (P = 0.6).

The average 1000 seeds weight for GS45I plants was 44.87 g for non-inoculated plants and 43.56 g for *F. langsethiae* inoculated plants, however treatment had no significant influence on 1000 seeds weight (P = 0.4). In the non-inoculated control plants, 1000 seeds weight was highest in Belinda (47.4 g), followed by Vinger (45.9 g) and Odal (41.2 g). Within the *F. langsethiae* inoculated plants, 1000 seeds weight was highest in Belinda (46.76 g), followed by Vinger (44.3 g) and Odal (39.56 g). There was significant difference between varieties within inoculation treatments (P = 0.01, Table 2). Interaction between varieties and treatments was not significant (P = 0.9).

For the GS45I treatment, the average number of grains per plant was 89.6 grains for non-inoculated plants and 82.4 grains for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average number of grains per plant (P = 0.3). In non-inoculated control plants, Odal had more grains per plant (90.38) than Vinger (89.6) and Belinda (88.8). Within the *F. langsethiae* inoculated plants, the number of grains per plant was highest in Belinda (87.5), followed by Vinger (87.31) and Odal (72.5). There was no significant difference between varieties within either inoculation treatment (P = 0.5, Table 2). Interaction between varieties and treatments was not significant (P = 0.4).

When plants were inoculated at GS45 by spraying (GS45S), the average yield per plant was 3.96 g for non-inoculated plants and 3.86 g for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average yield per plant (P = 0.6). In non-inoculated control plants, Belinda had higher yield per plant (4.3 g) than Vinger (4.02 g) and Odal (3.7 g) (Table 2). Within the *F. langsethiae* inoculated plants, the yield per plant was highest in Belinda (4.4 g), followed by Vinger (3.7 g), and Odal (3.4 g). There was significant difference between varieties within inoculation treatments (P = 0.01, Table 2). Interaction between varieties and treatments was not significant (P = 0.7).

For GS45S, a significant higher average 1000 seeds weight of non-inoculated plants (46.5 g) compared to *F. langsethiae* inoculated plants (41.76) was recorded (P = 0.04). In the non-inoculated control plants, 1000 seeds weight was highest in Belinda (51.9 g), followed by Vinger (45.2 g), and Odal (42.4 g). Within the *F. langsethiae* inoculated plants, 1000 seeds weight was

highest in Belinda (43.7 g), followed by Odal (41.6 g), and Vinger (40 g). There was no significant difference between varieties within either inoculation treatment (P = 0.1, Table 2). Interaction between varieties and treatments was not significant (P = 0.4).

For GS45S, the average number of grains per plant was 85.9 grains for non-inoculated plants and 92.9 grains for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average number of grains per plant (P = 0.3). In the non-inoculated control plants, Vinger had more grains per plant (89.1) than Belinda (85.1) and Odal (83.3). Within the *F. langsethiae* inoculated plants, the number of grains per plant was highest in Belinda (103.2), followed by Vinger (94.4), and Odal (81.2). There was no significant difference between varieties within either inoculation treatment (P = 0.3, Table 2). Interaction between varieties and treatments was not significant (P = 0.5).

For plants inoculated at GS55 by spraying (GS55S), the average yield per plant was 3.9 g for non-inoculated plants and 4.07 g for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average yield per plant (P = 0.3). In the non-inoculated control plants, Belinda had higher yield per plant (4.09 g) than Vinger (3.98 g) and Odal (3.6 g). Within the *F. langsethiae* inoculated plants, the yield per plant was highest in Belinda (4.4 g), followed by Vinger (4.03 g), and Odal (3.7 g). There was no significant difference between varieties within either inoculation treatment (P = 0.07, Table 2). Interaction between varieties and treatments was not significant (P = 0.7).

For GS55S, the average 1000 seeds weight was 43.02 g for non-inoculated plants and 45.27 g for *F. langsethiae* inoculated plants, however treatment had not significant influence on the average 1000 seeds weight (P = 0.1). In the non-inoculated control plants, 1000 seeds weight was highest in Belinda (44.7 g), followed by Vinger (43 g), and Odal (41.3 g). Within the *F. langsethiae* inoculated plants, 1000 seeds weight was highest in Belinda (48.03 g), followed by Vinger (46.06 g), and Odal (41.7 g). There was significant difference between varieties within inoculation treatments (P = 0.03, Table 2). Interaction between varieties and treatments was not significant (P = 0.6).

For GS55S, the average number of grains per plant was 91.01 grains for non-inoculated plants and 90.1 grains for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average number of grains per plant (P = 0.7). In non-inoculated control plants, Vinger had

more grains per plant (93.19) than Belinda (91.46) and Odal (88.35). Within the *F. langsethiae* inoculated plants, the number of grains per plant was highest in Belinda (93.01), followed by Odal (89.6), and Vinger (87.6). There was no significant difference between varieties within either inoculation treatment (P = 0.7, Table 2). Interaction between varieties and treatments was not significant (P = 0.6).

For plants inoculated at GS65 by spraying (GS65S), the average yield per plant was 4.01 g for non-inoculated plants and 3.59 g for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average yield per plant (P = 0.1). In non-inoculated control plants, Belinda had higher yield per plant (4.6 g) than Vinger (3.8 g) and Odal (3.6 g). Within the *F. langsethiae* inoculated plants, the yield per plant was highest in Belinda (4.2 g), followed by Vinger (3.6 g), and Odal (2.8 g). There was significant difference between varieties within inoculation treatments (P = 0.01, Table 2). Interaction between varieties and treatments was not significant (P = 0.7).

For GS65S, the average 1000 seeds weight was 43.16 g for non-inoculated plants and 42.38 g for *F. langsethiae* inoculated plants, however treatment had not significant influence on average 1000 seeds weight (P = 0.6). In non-inoculated control plants, 1000 seeds weight was highest in Belinda (44.7 g), followed by Vinger (43 g), and Odal (41.3 g). Within the *F. langsethiae* inoculated plants, 1000 seeds weight was highest in Belinda (46.1 g), followed by Vinger (41.6 g), and Odal (39.96 g). There was significant difference between varieties within inoculation treatments (P = 0.03, Table 2). Interaction between varieties and treatments was not significant (P = 0.8).

For GS65S, the average number of grains per plant was 92.9 grains for non-inoculated plants and 84.5 grains for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average number of grains per plant (P = 0.2). In non-inoculated control plants, Belinda had more grains per plant (101.8) than Vinger (92.05) and Odal (85.75). Within the *F. langsethiae* inoculated plants, the number of grains per plant was highest in Belinda (92.7), followed by Vinger (90.27), and Odal (71.15). There was no significant difference between varieties within either inoculation treatment (P = 0.1, Table 2). Interaction between varieties and treatments was not significant (P = 0.7).

Panicle length and the length between flag leaf node to the top of the plants

Panicle length (cm from the panicle base to the top) and the length between flag leaf node to the top of plants (cm) were measured at the end of flowering (beginning of fruit development, GS70) in all experiments.

Experiment 1

In experiment 1, the average panicle length varied from 16.30 cm to 16.41cm between the different inoculation treatments, however treatment had no significant influence on the average panicle length (P = 0.6, Figure 5A). In the non-inoculated control plants, Odal (15.40 cm) had shorter panicle length than Belinda (16.15 cm) and Vinger (17.36 cm). Within the *F. graminearum* inoculated plants, the panicle length was longest in Vinger (17.31 cm), followed by Belinda (16.92 cm) and Odal (14.84 cm). Within the *F. langsethiae* inoculated plants, the panicle length was longest in Vinger (17.8 cm), followed by Belinda (15.98 cm) and Odal (15.45 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 7A). No significant interaction was detected between varieties and treatments (P = 0.3).

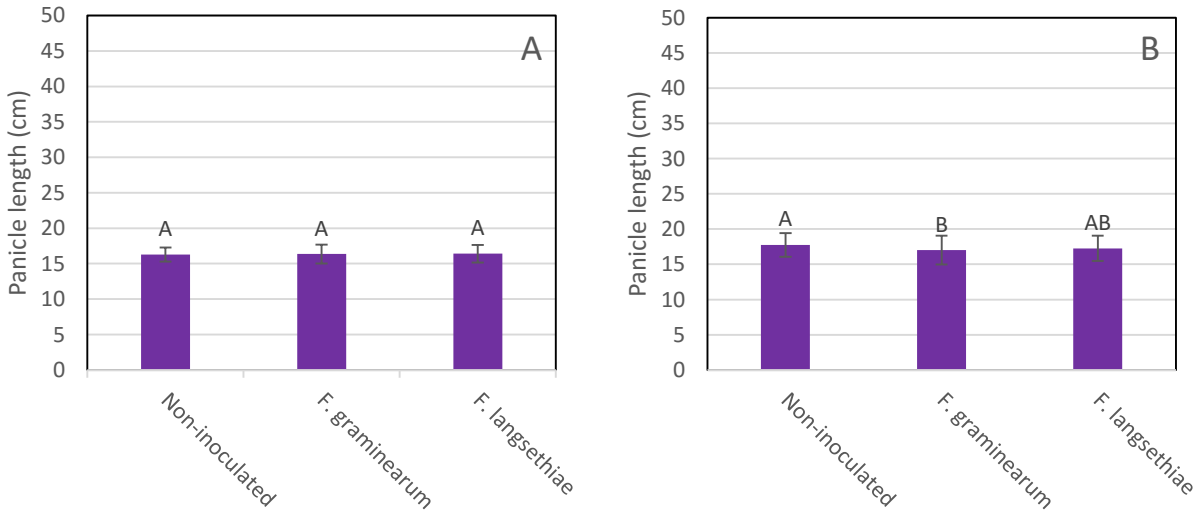

Figure 5: Average panicle length (cm) in three oats varieties (Vinger, Odal and Belinda) inoculated with *Fusarium graminearum*, *Fusarium langsethiae* or water (non-inoculated control treatment) measured at GS70 in in Exp1 (A) and Exp.2 (B). The plants were inoculated by spraying a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1, and injecting a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at late boot stage (GS45) in Exp. 2. Means that are sharing the same letters in each experiment are not significantly different. Error bars are represented as standard deviations.

A significant shorter average length between the flag leaf node to the top of the plant was recorded in the *F. graminearum* inoculated plants (32.15 cm) compared to the non-inoculated plants (34.55 cm) and *F. langsethiae* inoculated plants (33.28 cm) (P= 0.009, Figure 6A). In the non-inoculated control plants, Odal (35.2 cm) and Belinda (31.75 cm) had shorter length between flag leaf node to the top of the plant than Vinger (36.7 cm). Within the *F. graminearum* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (34.3 cm), followed by Odal (31.27 cm), and Belinda (30.88 cm). Within the *F. langsethiae* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (37.08 cm), followed by Odal (33.57 cm), and Belinda (29.2 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 7B). No significant interaction was detected between varieties and treatments (P = 0.1).

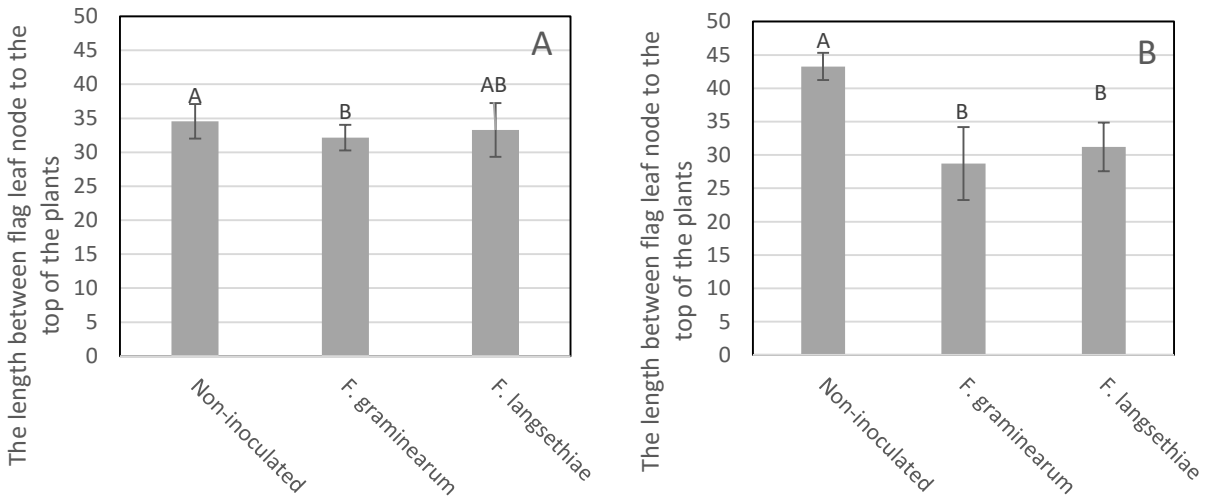

Figure 6: Average length between flag leaf node to the top of the plants (cm) in three oats varieties (Vinger, Odal and Belinda) inoculated with *Fusarium graminearum, Fusarium langsethiae* or water (non-inoculated control treatment) measured at GS70 in in Exp1 (A) and Exp.2 (B). The plants were inoculated by spraying a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1, and injecting a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at late boot stage (GS45) in Exp. 2. Means that are sharing the same letters in each experiment are not significantly. different. Error bars are represented as standard deviations.

Experiment 2

In experiment 2, a significant shorter average panicle length of the *F. graminearum* inoculated plants (17.02 cm) was recorded compared to the non-inoculated plants (17.77 cm) and *F. langsethiae* inoculated plants (17.26 cm) (P= 0.01, Figure 5B). In non-inoculated control plants,

the panicle length was longest in Vinger (19.42 cm) followed by Belinda (17.82 cm) and Odal (16.6 cm). Within the *F. graminearum* inoculated plants, the panicle length was longest in Vinger (18.98 cm), followed by Belinda (17.19 cm), and Odal (14.88 cm). Within the *F. langsethiae* inoculated plants, the panicle length was longest in Vinger (19.07 cm), followed by Belinda (17.22 cm), and Odal (15.49 cm). There was significant difference between varieties within inoculation treatments ($P < 0.0001$, Figure 8A). No significant interaction was detected between varieties and treatments ($P = 0.6$).

A significant shorter average length between flag leaf node to the top of the plant was recorded in both the *F. langsethiae* (31.19 cm) and the *F. graminearum* inoculated plants (28.72 cm) compared to the non-inoculated plants (43.27 cm) ($P < 0.0001$, Figure 6B). In non-inoculated control plants, the length between flag leaf node to the top of the plant was longest in Vinger (45.20 cm), followed by Odal (43.45 cm) and Belinda (41.15 cm). Within the *F. graminearum* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (34.35 cm), followed by Odal (28.40 cm), and Belinda (23.40 cm). Within the *F. langsethiae* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (34.92 cm), followed by Odal (31.02 cm), and Belinda (27.62 cm). There was significant difference between varieties within inoculation treatments ($P < 0.0001$, Figure 8B). No significant interaction was detected between varieties and treatments ($P = 0.2$).

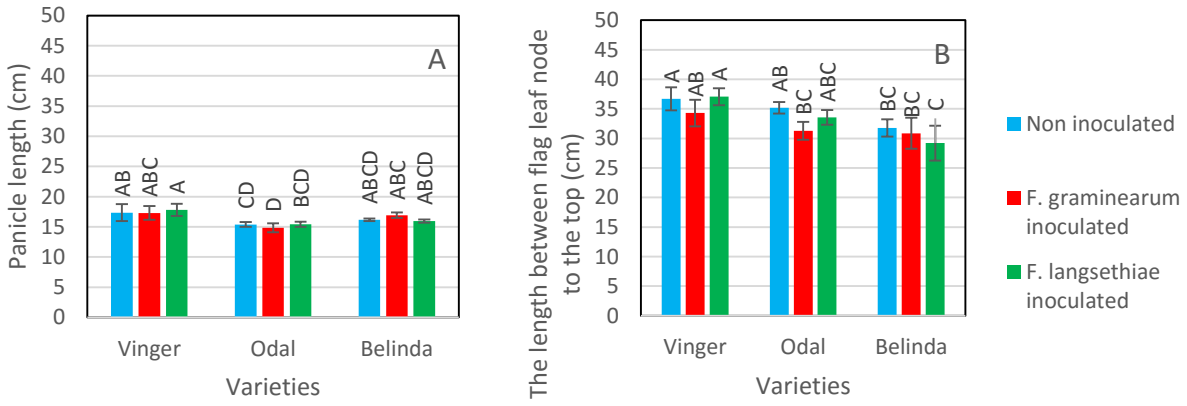

Figure 7: Average panicle length (A)(cm from the base of panicle to the top) and the length between flag leaf node to the top of the plants (cm)(B) in three oats varieties (Vinger, Odal and Belinda) inoculated with *Fusarium graminearum*, *Fusarium langsethiae* or water (non-inoculated control treatment) measured at GS70 in in Exp1. The plants were inoculated by spraying a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated)

into the flag leaf sheath at flag leaf sheath opening (GS47). Means that are sharing the same letters in each experiment are not significantly different. Error bars are represented as standard deviations.

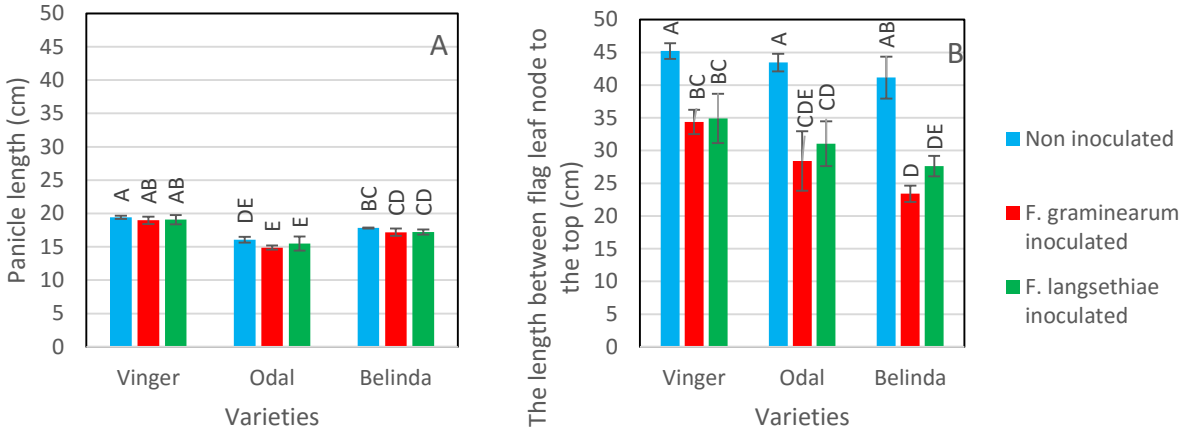

Figure 8: Average panicle length (A)(cm from the base of panicle to the top) and the length between flag leaf node to the top of the plants (cm)(B) in three oats varieties (Vinger, Odal and Belinda) inoculated with *Fusarium graminearum, Fusarium langsethiae* or water (non-inoculated control treatment) measured at GS70 in in Exp2. The plants were inoculated by injecting a spore suspension of *F. graminearum*, *F. langsethiae* or water (non-inoculated) into the flag leaf sheath at late boot stage (GS45). Means that are sharing the same letters in each experiment are not significantly different. Error bars are represented as standard deviations.

Experiment 3

In experiment 3, when plants were inoculated at GS45 by injection (GS45I), a significant shorter average panicle length of the *F. langsethiae* inoculated plants (16.15 cm) was recorded compared to the non-inoculated plants (17.52 cm) ($P < 0.0001$). In non-inoculated control plants, Belinda (18.32 cm) and Odal (15.79 cm) had shorter panicle length than Vinger (18.44 cm). Within the *F. langsethiae* inoculated plants, the panicle length was longest in Vinger (18.33 cm), followed by Belinda (15.54 cm), and Odal (14.59 cm). There was significant difference between varieties within inoculation treatments ($P < 0.0001$, Figure 9A). No significant interaction was detected between varieties and treatments ($P = 0.01$).

For GS45I, the average length between flag leaf node to the top of the plant in GS45I, a significant shorter length of the *F. langsethiae* inoculated plants (27.19 cm) was recorded compared to the non-inoculated plants (34.30 cm) ($P < 0.0001$). In non-inoculated control plants, Odal had longest length between flag leaf node to the top of the plant (35.68 cm) compared to Vinger (34.4 cm) and Belinda (32.81 cm). Within the *F. langsethiae* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (29.3 cm), followed by Odal (26.9 cm), and Belinda

(25.36 cm). There was no significant difference between varieties within either inoculation treatment (P = 0.3, Figure 9B). No significant interaction was detected between varieties and treatments (P = 0.6).

When plants were inoculated at GS45 by spraying (GS45S), the average panicle length was 17.26 cm for non-inoculated plants and 17.47 cm for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average panicle length (P = 0.4). In non-inoculated control plants, Belinda (17.73 cm) and Odal (15.67 cm) had shorter panicle length than Vinger (18.38 cm). Within the *F. langsethiae* inoculated plants, the panicle length was longest in Vinger (19.03 cm), followed by Belinda (17.39 cm), and Odal (15.99 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 9A). No significant interaction was detected between varieties and treatments (P = 0.3).

For GS45S, the average length between flag leaf node to the top of the plant was 34.97 cm for non-inoculated plants and 34.42 cm for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average length between flag leaf node to the top of the plant (P = 0.4). In non-inoculated control plants, Vinger (36.28 cm) and Belinda (31.56 cm) had shorter length between flag leaf node to the top of the plant than Odal (37.05 cm). Within the *F. langsethiae* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (36.94 cm), followed by Odal (34.81 cm), and Belinda (31.52 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 9B). No significant interaction was detected between varieties and treatments (P = 0.2).

When plants were inoculated at GS55 by spraying (GS55S), the average panicle length was 18.64 cm for non-inoculated plants and 18.81 cm for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average panicle length (P = 0.4). In non-inoculated control plants, Belinda (18.82 cm) and Odal (17.16 cm) had shorter panicle length than Vinger (19.95 cm). Within the *F. langsethiae* inoculated plants, the panicle length was longest in Vinger (20.29 cm), followed by Belinda (18.91 cm), and Odal (17.23 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 9A). No significant interaction was detected between varieties and treatments (P = 0.8).

For GS55S, the average length between flag leaf node to the top of the plant was 39.58 cm for non-inoculated plants and 40.28 cm for *F. langsethiae* inoculated plants, however treatment had

no significant influence on the average length between flag leaf node to the top of the plant (P = 0.5). In non-inoculated control plants, Vinger (41.94 cm) and Belinda (34.71 cm) had shorter length between flag leaf node to the top of the plant than Odal (42.16 cm). Within the *F. langsethiae* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (43.58 cm), followed by Odal (42.16 cm), and Belinda (35.11 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 9B). No significant interaction was detected between varieties and treatments (P = 0.8).

When plants were inoculated at GS65 by spraying (GS65S), the average panicle length was 17.17 cm for non-inoculated plants and 16.91 cm for *F. langsethiae* inoculated plants, however treatment had no significant influence on the average panicle length (P = 0.3). In non-inoculated control plants, Belinda (17.5 cm) and Odal (15.53 cm) had shorter panicle length than Vinger (18.5 cm). Within the *F. langsethiae* inoculated plants, the panicle length was longest in Vinger (18.14 cm), followed by Belinda (17.72 cm), and Odal (14.89 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 9A). No significant interaction was detected between varieties and treatments (P = 0.4).

For GS65S, the average length between flag leaf node to the top of the plant in GS65S, a significant shorter length of the *F. langsethiae* inoculated plants (39.5 cm) was recorded compared to the non-inoculated plants (40.71 cm) (P= 0.01). In the non-inoculated control plants, Odal (40.46 cm) and Belinda (39.24 cm) had shorter length between flag leaf node to the top of the plant than Vinger (42.45 cm). Within the *F. langsethiae* inoculated plants, the length between flag leaf node to the top of the plant was longest in Vinger (40.58 cm), followed by Odal (39.84 cm), and Belinda (38.07 cm). There was significant difference between varieties within inoculation treatments (P < 0.0001, Figure 9B). No significant interaction was detected between varieties and treatments (P = 0.4).

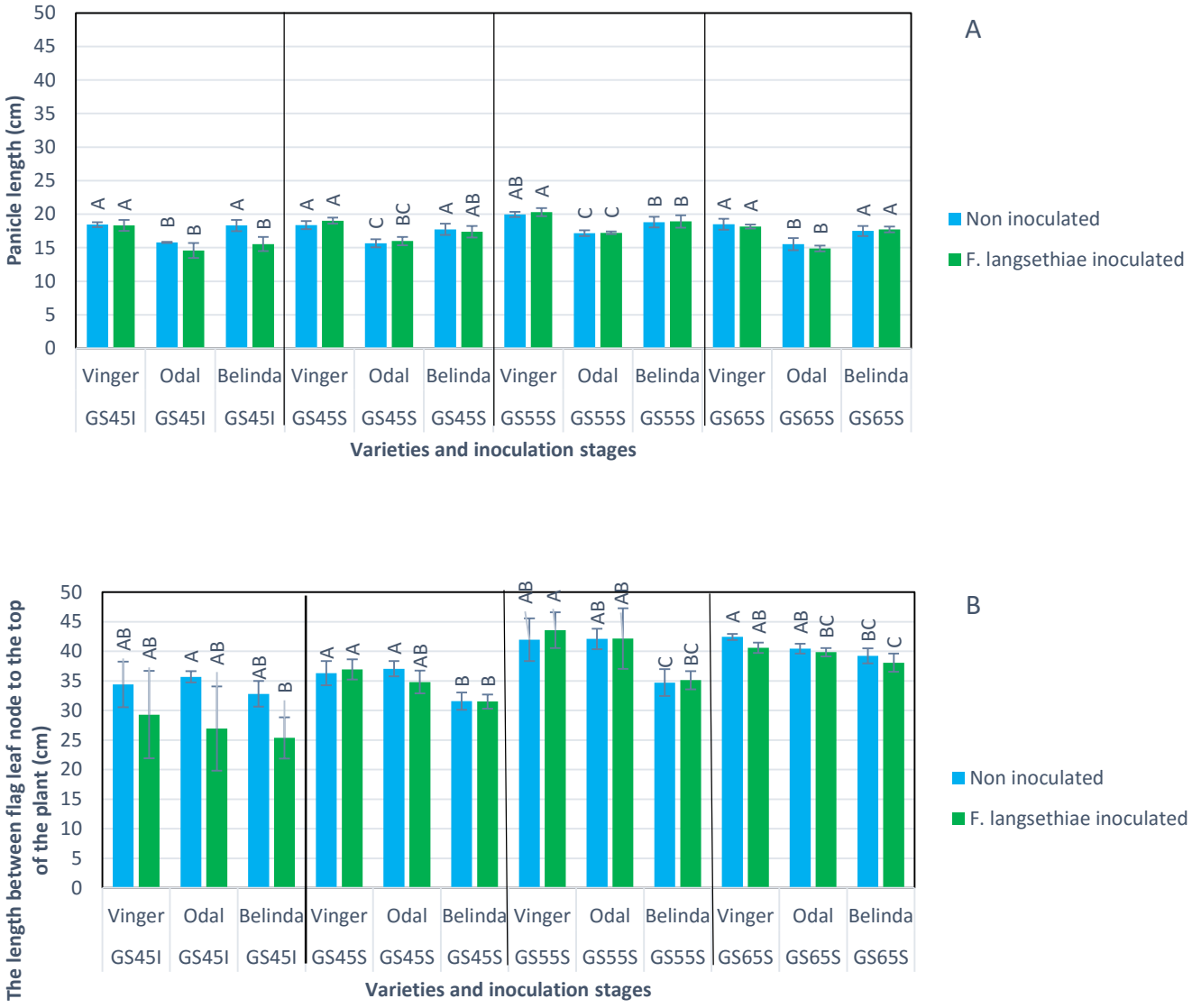

Figure 9: Average panicle length (A)(cm from the base of panicle to the top) and the length between flag leaf node to the top of the plants (cm)(B) in three oats varieties (Vinger, Odal and Belinda) inoculated with *F. langsethiae* measured at GS70 in Exp. 3. The plants were inoculated at different growth stages by different inoculation methods (GS45I: Inoculated by injecting a spore suspension into the flag leaf sheath at GS45, GS45S: Inoculated by spraying a spore suspension at GS45, GS55S: Inoculated by spraying a spore suspension at GS55, GS65S: Inoculated by spraying a spore suspension at GS65) with a spore suspension of *F. langsethiae* or water (non-inoculated). Means that are sharing the same letters in each inoculation method and time point (GS45I, GS45S, GS55S and GS65S) are not significantly different. Error bars are represented as standard deviations.

Fungal DNA and mycotoxins in the harvested grains

Experiment 1

In experiment 1, plants were inoculated by spraying a spore suspension of *F. graminearum* or *F. langsethiae* into the flag leaf sheath at flag leaf sheath opening (GS47). Only low levels of *F. langsethiae* DNA, and HT-2+T-2 toxins were detected in the *F. langsethiae* inoculated samples. Therefore, no statistical analyzes were performed to study the effect of *F. langsethiae* inoculation on development of fungal DNA, and HT-2+T-2 toxins in this experiment.

F. graminearum DNA was detected in all grain samples from plants inoculated with *F. graminearum* by atomizer at GS47. However, small amounts of *F. graminearum* DNA were also detected in the non-inoculated samples. A significantly higher content of *F. graminearum* DNA (96.3%) was detected in oats grains from *F. graminearum* inoculated (485.5 pg/µg plant DNA) versus non-inoculated plants (17.58 pg/µg plant DNA, $P < 0.0001$). Within the *F. graminearum* inoculated plants, Belinda had highest content of *F. graminearum* DNA (953.5 pg/µg plant DNA), followed by Odal (318 pg/µg plant DNA), and Vinger (185 pg/µg plant DNA). In the non-inoculated control plants, Vinger had highest content of *F. graminearum* DNA (42.5 pg/µg plant DNA), followed by Belinda (7.5 pg/µg plant DNA), and Odal (2.5 pg/µg plant DNA). though no significant difference in quantities of DNA was detected between the varieties ($P = 0.1$), nor between the different replications ($P = 0.3$). Since a significant interaction was detected between varieties and *F. graminearum* inoculation treatment ($P = 0.04$), *F. graminearum* inoculated plants were tested separately for the quantity of fungal DNA.

In the separate test, although Belinda had more than double the amount of *F. graminearum* DNA (953.5 pg/µg plant DNA), compared to Odal (318 pg/µg plant DNA) and Vinger (185 pg/µg plant DNA) (Figure 10A), the oats variety had no significant influence on the amount of *F. graminearum* DNA ($P = 0.1$).

A significant higher content of *deoxynivalenol* (DON) was detected (57.1%) in oats grains from *F. graminearum* inoculated (574.08 µg/kg) versus non-inoculated plants (246.21 µg/kg) ($P < 0.0001$). Within the *F. graminearum* inoculated plants, Odal had highest content of DON (864.5 µg/kg), followed by Belinda (433.98 µg/kg), and Vinger (423.68 µg/kg). In the non-inoculated control plants, Vinger had highest content of DON (350.1 µg/kg), followed by Odal (278.95 µg/kg), and Belinda (109.54 µg/kg). There was significant difference between varieties within

inoculation treatments (P = 0.04). Since a significant interaction was detected between varieties and *F. graminearum* inoculation treatments (P = 0.04), *F. graminearum* inoculated plants were tested separately for the amount of DON.

In the separate test, although Odal had higher content of DON (864.6 µg/kg), compared to Belinda (434 µg/kg) and Vinger (423.7 µg/kg), the oats variety had no significant influence on the amount of *F. graminearum* DNA (P = 0.1, Figure 10B).

Since relatively high amounts of DON were detected in non-inoculated samples, these were tested for *Fusarium culmorum* contamination (another producer of DON). The results confirmed the presence of *F. culmorum* in non-inoculated samples (174.08 pg/µg). The variety Vinger had highest content of *F. culmorum* DNA (362.5 pg/µg plant DNA), followed by Odal (97.5 pg/µg plant DNA), and Belinda (62.25 pg/µg plant DNA), though no significant difference in quantities of *F. culmorum* DNA was detected between the varieties (P = 0.4).

The Pearson correlation between the content of *F. graminearum* DNA and DON was significant in experiment 1 (r = 0.42, P = 0.01) (Figure 11).

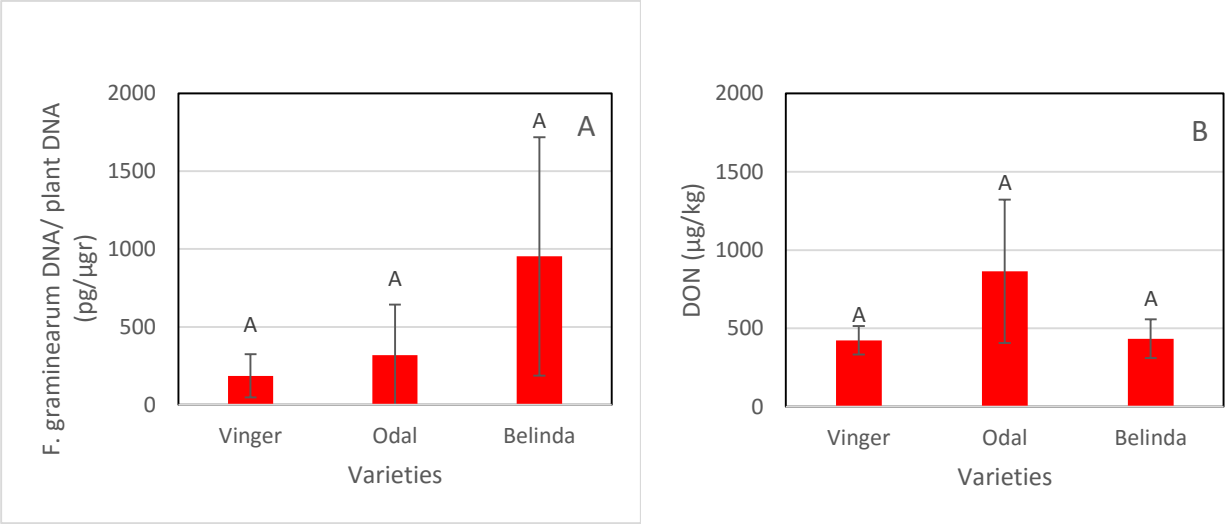

Figure 10: The quantity of *F. graminearum* DNA (pg/µg plant DNA) (A) and the amounts of deoxynivalenol (DON) (µg/kg) (B) in harvested grains of three oats varieties (Vinger, Odal and Belinda)(results from separate test). The plants were inoculated by spraying a spore suspension of *F. graminearum* into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1. Water was used as a non-inoculated control treatment. Means that are sharing the same letters in each experiment are not significantly different. Standard deviations are shown as error bars.

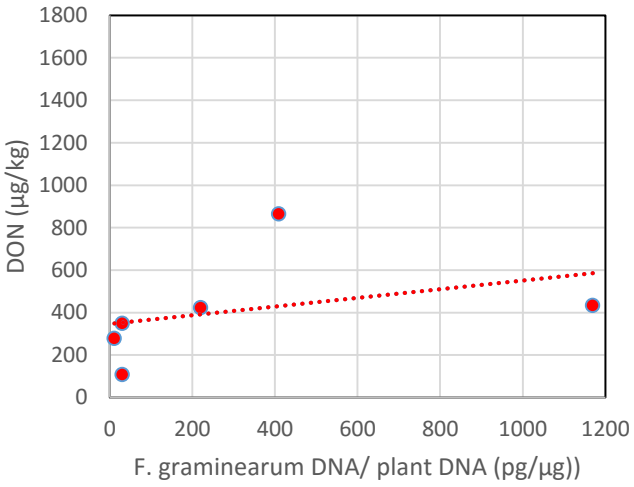

Figure 11: Correlation between the quantity of *F. graminearum* DNA (pg/µg plant DNA) and the amounts of deoxynivalenol (DON) (µg/kg) in harvested grains of three *F. graminearum* inoculated oats varieties (Vinger, Odal and Belinda). The plants were inoculated by spraying a spore suspension of *F. graminearum* into the flag leaf sheath at flag leaf sheath opening (GS47) in Exp. 1.

Experiment 2

In experiment 2, plants were inoculated by injecting a spore suspension of *F. graminearum* or *F. langsethiae* into the flag leaf sheath at late boot stage (GS45). Only low levels of *F. langsethiae* DNA, and HT-2+T-2 toxins were detected in the *F. langsethiae* inoculated samples. Therefore, no statistical analyzes were performed to study the effect of *F. langsethiae* inoculation on development of fungal DNA, and HT-2+T-2 toxins in this experiment.

F. graminearum DNA was detected in all grain samples from plants inoculated with *F. graminearum* by syringe at GS45. However, small amounts of *F. graminearum* DNA were also detected in the non-inoculated samples. A significantly higher content of *F. graminearum* DNA (97.49%) was detected in oats grains from *F. graminearum* inoculated (638.66 pg/µg plant DNA) versus non-inoculated plants (16 pg/µg plant DNA) ($P < 0.0001$). Within the *F. graminearum* inoculated plants, Belinda had highest content of *F. graminearum* DNA (920 pg/µg plant DNA), followed by Odal (578.5 pg/µg plant DNA), and Vinger (417.5 pg/µg plant DNA). In the non-inoculated control plants, Belinda had highest content of *F. graminearum* DNA (43.5 pg/µg plant DNA), followed by Odal (4.5 pg/µg plant DNA), and Odal (0 pg/µg plant DNA), though no significant difference in quantities of DNA was detected between the varieties ($P = 0.1$), nor interaction between varieties and inoculation treatments ($P = 0.2$). To have a more precise

overview of the effect of *F. graminearum* inoculation on the quantity of *F. graminearum* DNA, results from the *F. graminearum* inoculated oats grains were tested separately.

In the separate test, although Belinda contained higher amount of *F. graminearum* DNA (920 pg/µg plant DNA), than Odal (578.5 pg/µg plant DNA) and Vinger (417.5 pg/µg plant DNA), oats variety had no significant influence on the amount of *F. graminearum* DNA ($P = 0.247$, Figure 12A).

A significant higher (83.49%)content of DON was detected in oats grains from *F. graminearum* inoculated (1082.65 µg/kg) versus non-inoculated plants (178.68 µg/kg, $P < 0.0001$). Within the *F. graminearum* inoculated plants, Belinda had highest content of DON (1423.18 µg/kg), followed by Odal (960.15 µg/kg), and Vinger (864.62 µg/kg). In the non-inoculated control plants, Vinger had highest content of DON (211.16 µg/kg), followed by Odal (193.90 µg/kg), and Belinda (130.98 µg/kg), and significant difference was detected in amounts of DON between the varieties ($P = 0.04$). To have a more precise overview of the effect of *F. graminearum* inoculation on the quantity of DON, results from the *F. graminearum* inoculated oats grains were tested separately.

In the separate test, although Belinda had more content of DON (1423.18 µg/kg), compared to Odal (960.15 µg/kg) and Vinger (864.62 µg/kg), the oats variety had no significant influence on the amount of *F. graminearum* DNA ($P = 0.1$, Figure 12B).

DON was also detected in the samples from non-inoculated oats grains. Therefore, the non-inoculated samples were tested for *F. culmorum* contamination (another producer of DON). The results confirmed the presence of *F. culmorum* in non-inoculated samples (79.75 pg/µg). In the non-inoculated control plants, Belinda had highest content of *F. culmorum* DNA (132.5 pg/µg plant DNA), followed by Odal (69.25 pg/µg plant DNA), and Vinger (37.5 pg/µg plant DNA), though no significant difference in quantities of *F. culmorum* DNA was detected between the varieties ($P = 0.3$).

The Pearson correlation between the quantities of *F. graminearum* and DON was significant in experiment 2 ($r = 0.751$, $P < 0.0001$) (Figure 13).

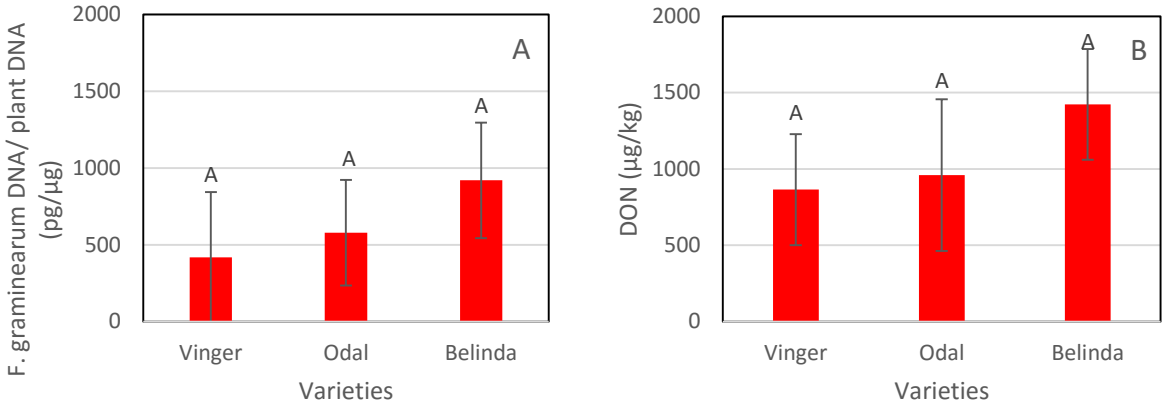

Figure 12*:* The quantity of *F. graminearum* DNA (pg/µg plant DNA) (A) and the amounts of deoxynivalenol (DON) (µg/kg) (B) in harvested grains of three *F. graminearum* inoculated oats varieties (Vinger, Odal and Belinda) (results from separate test). The plants were inoculated by injecting a spore suspension of *F. graminearum* into the flag leaf sheath at late boot stage (GS45) in Exp. 2. Water was used as a non-inoculated control treatment. Means that are sharing the same letters in each experiment are not significantly different. Standard deviations are shown as error bars.

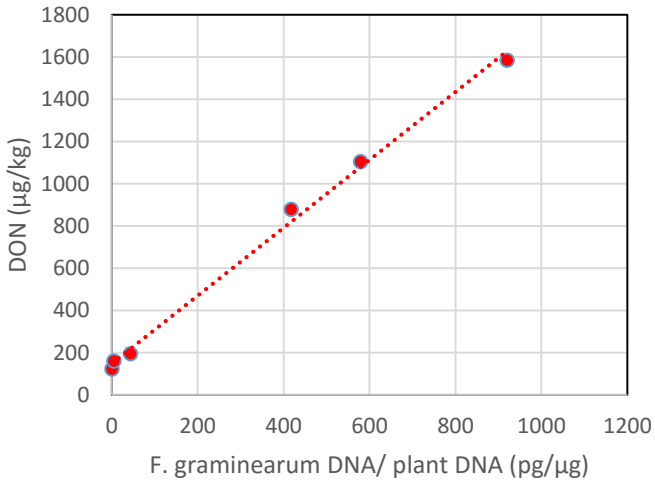

Figure 13: Correlation between the quantity of *F. graminearum* DNA (pg/µg plant DNA) and the amounts of *deoxynivalenol* (DON) (µg/kg) in harvested grains of three *F. graminearum* inoculated oats varieties (Vinger, Odal and Belinda). The plants were inoculated by injecting a spore suspension of *F. graminearum* into the flag leaf sheath at late boot stage (GS45) in Exp. 2.

Experiment 3

In experiment 3, plants were inoculated by injecting a spore suspension of *F. langsethiae* into the flag leaf sheath at late boot stage (GS45) or spraying at late boot stage (GS45), middle of heading (GS55) and full flowering (GS65).

There were no *F. langsethiae* DNA, and HT-2+T-2 toxins detected in the grains from non-inoculated samples. Therefore, no statistical analyzes were performed to study these samples in this experiment.

A significantly higher content of *F. langsethiae* DNA (29.45%) was detected in oats grains from *F. langsethiae* spray inoculated plants at GS65 (585.74 pg/µg plant DNA) than spray inoculated plants at GS55 (413.2 pg/µg plant DNA), while only low levels of *F. langsethiae* DNA was detected in injection inoculated plants at GS45 (30.7 pg/µg plant DNA) and spray inoculated at GS45 (3.39 pg/µg plant DNA) ($P < 0.0001$, Figure 14A). Within the *F. langsethiae* inoculated plants, Belinda had highest content of *F. langsethiae* DNA (401.88 pg/µg plant DNA), followed by Odal (296.78 pg/µg plant DNA), and Vinger (76.15 pg/µg plant DNA), and significant difference was detected in quantities of *F. langsethiae* DNA between the varieties ($P < 0.0001$). Since significant interaction was detected between varieties and inoculation time point and method ($P < 0.0001$), the *F. langsethiae* inoculated plants from each inoculation method and time point were tested separately.

For plants that were inoculated with *F. langsethiae* at GS45 by injection (GS45I), only low levels of *F. langsethiae* DNA was detected in the samples. Within the *F. langsethiae* inoculated plants Odal had higher content of *F. langsethiae* DNA (54.5 pg/µg plant DNA) than Belinda (32.6 pg/µg plant DNA) and Vinger (4.98 pg/µg plant DNA), but the difference between varieties was not significant ($P = 0.2$, Figure 14A).

Between all different inoculation time points and methods for the *F. langsethiae* inoculated plants, the lowest levels of *F. langsethiae* DNA was detected in samples from the GS45S plants. Within the *F. langsethiae* inoculated plants Odal had higher content of *F. langsethiae* DNA (4.49 pg/µg plant DNA) than Belinda (3.03 pg/µg plant DNA) and Vinger (2.66 pg/µg plant DNA), but the difference between varieties was not significant ($P = 0.8$, Figure 14A).

For samples from GS55S plants, inoculated with *F. langsethiae*, Belinda had the highest content of *F. langsethiae* DNA (671.8 pg/µg plant DNA) followed by Odal (459.4 pg/µg plant DNA) and Vinger (108.5 pg/µg plant DNA), and difference between varieties was significant ($P = 0.02$, Figure 14A).

For samples from GS65S plants, inoculated with *F. langsethiae*, Belinda had the highest content of *F. langsethiae* DNA (900.1 pg/µg plant DNA) followed by Odal (668.7 pg/µg plant DNA) and Vinger (188.5 pg/µg plant DNA), and difference between varieties was significant (P = 0.01, Figure 14A).

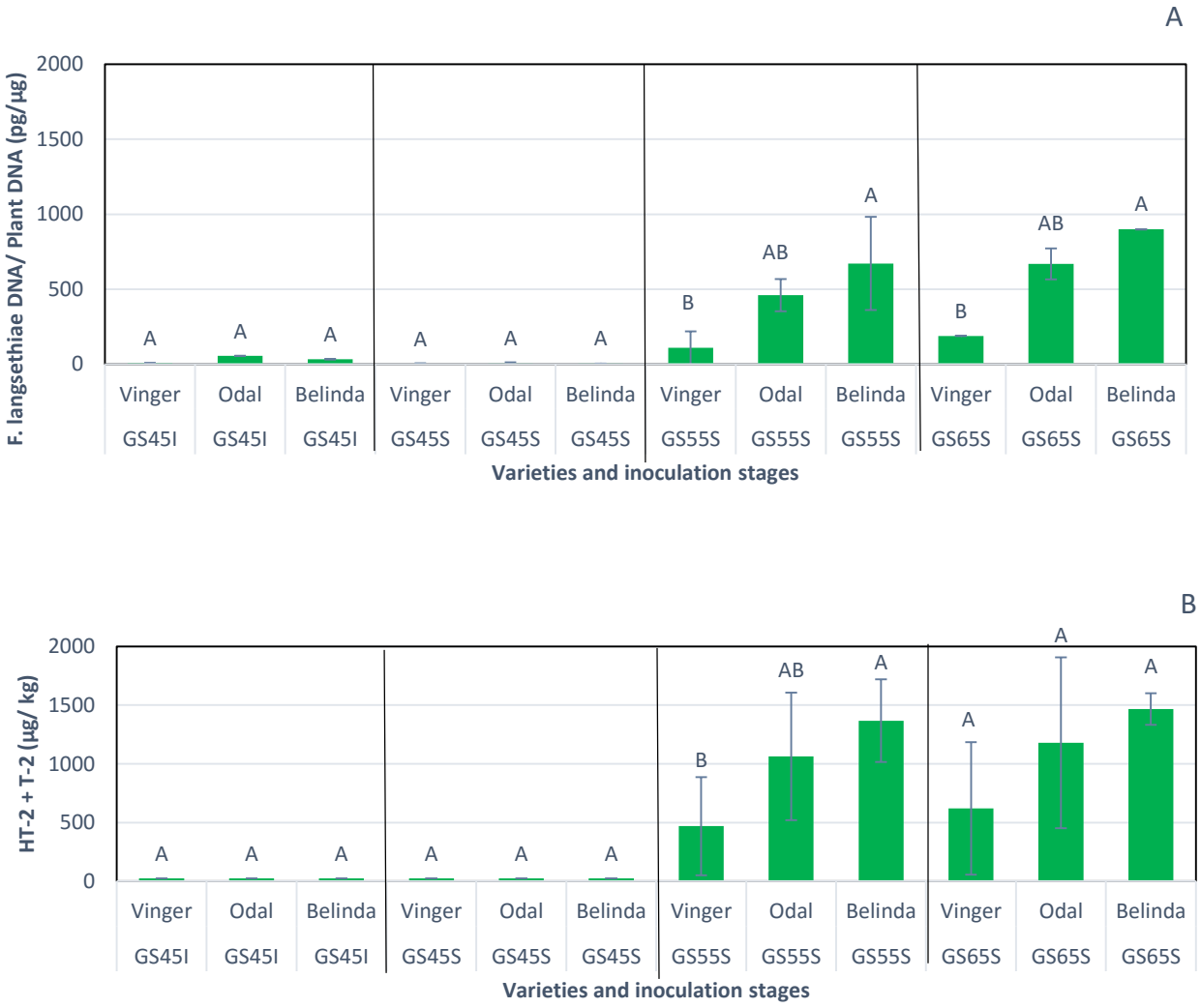

Figure 14: The quantity of *F. langsethiae* DNA (pg/µg plant DNA) (A) and the amounts of HT-2+T-2 (µg/kg) (B) in harvested grains of three *F. langsethiae* inoculated oats varieties (Vinger, Odal and Belinda). The plants were inoculated at different growth stages by different inoculation methods (GS45I: Inoculated by injecting a spore suspension into the flag leaf sheath at GS45, GS45S: Inoculated by spraying a spore suspension at GS45, GS55S: Inoculated by spraying a spore suspension at GS55, GS65S: Inoculated by spraying a spore suspension at GS65) with a *F. langsethiae* spore suspension in Exp. 3. Means that are sharing the same letters in each inoculation method and time point (GS45I, GS45S, GS55S and GS65S) are not significantly different. Standard deviations are shown as error bars.

A significant higher content of HT-2+T-2 was detected in oats grains from *F. langsethiae* inoculated at GS65 (1088.81 µg/kg), than in *F. langsethiae* inoculated samples at GS55 (966.48 µg/kg), GS45I (88.56 µg/kg) and GS45S (18.75 µg/kg), and the difference between inoculation time points and methods was significant ($P < 0.0001$, Figure 14B). Within the *F. langsethiae* inoculated plants, Belinda had the highest content of HT-2+T-2 (726.53 µg/kg), followed by Odal (577.1 µg/kg), and Vinger (318.3 µg/kg), though no significant difference in content of HT-2+T-2 was detected between the varieties ($P = 0.1$), nor in interaction between varieties and inoculation treatments ($P = 0.3$). Pearson correlation was significant between the content of *F. langsethiae* DNA and the amounts of HT-2+T-2 ($r= 0857$, $P < 0.0001$, Figure 15). To have a more precise overview on the effect of *F. langsethiae* inoculation on the quantity of HT-2+T-2, results from the *F. langsethiae* inoculated oats grains were tested separately.

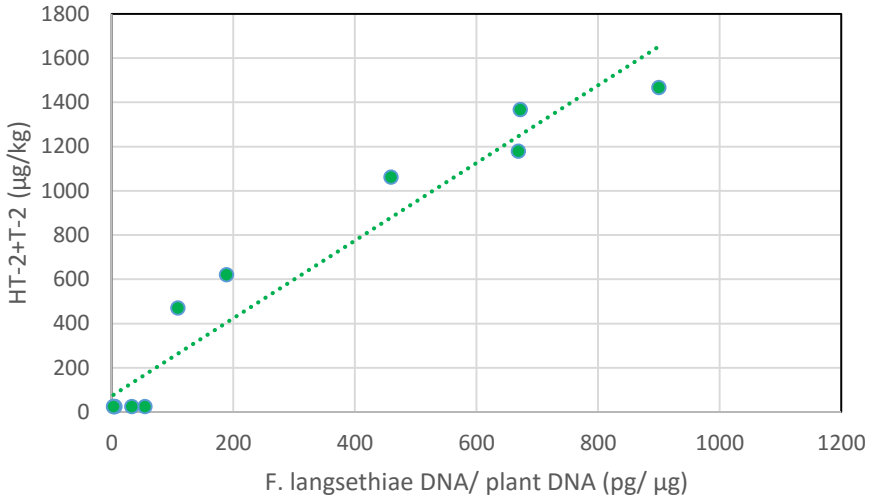

Figure 15: Correlation between the quantity of *F. langsethiae* DNA (pg/µg plant DNA) and the amounts of HT-2+T-2 (µg/kg) in harvested grains of three *F. langsethiae* inoculated oats varieties (Vinger, Odal and Belinda). The plants were inoculated at different growth stages by different inoculation methods (GS45I: Inoculated by injecting a spore suspension into the flag leaf sheath at GS45, GS45S: Inoculated by spraying a spore suspension at GS45, GS55S: Inoculated by spraying a spore suspension at GS55, GS65S: Inoculated by spraying a spore suspension at GS65) with a *F. langsethiae* spore suspension in Exp. 3.

For plants that were inoculated with *F. langsethiae* at GS45 by injection (GS45I), only low levels of *HT-2+T-2* were detected in the samples. All three varieties had equal content of HT-2+T-2 (25 µg/kg), though the difference was not significant between varieties ($P =1.00$, Figure 14B).

Also or plants that were inoculated with *F. langsethiae* at GS45 by spraying (GS45S), only low levels of *HT-2+T-2* was detected in the samples. All three varieties had equal content of HT-2+T-2 (25 µg/kg), though the difference was not significant between varieties (P = 1.00, Figure 14B).

Among the *F. langsethiae* inoculated GS55S plants, Belinda had the highest content of HT-2+T-2 (1367.4 µg/kg) followed by Odal (1062.3 µg/kg) and Vinger (469.8 µg/kg), and the difference between varieties was significant (P = 0.04, Figure 14B).

Among the *F. langsethiae* inoculated GS65S plants Belinda had the highest content of HT-2+T-2 (1466.9 µg/kg) followed by Odal (1179.3 µg/kg) and Vinger (620.2 µg/kg), but difference between varieties was not significant (P = 0.09, Figure 14B).

Discussion

In this study, different inoculation methods were tested in order to identify a method for *Fusarium langsethiae* inoculation of oats. Oats plants were inoculated by injecting fungal spore suspensions into the flag leaf sheath at late boot stage (GS45), by spraying fungal spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47), and by spray-inoculation of plants at late boot stage (GS45), middle of the heading (GS55) or full flowering (GS65). Resistance to *F. langsethiae* was identified and compared with resistance to *Fusarium graminearum* in three oats varieties.

Identifying a suitable method for *F. langsethiae* inoculation

F. langsethiae

High amounts of *F. langsethiae* DNA and HT-2+T-2 were detected in oats grains harvested from plants that were spray inoculated with *F. langsethiae* at middle of heading (GS55) or full flowering (GS65). The greatest quantities of *F. langsethiae* (900.1 pg/µg plant DNA) and HT-2+T-2 (1088.81 µg/kg) were detected in grains harvested from oats that were spray inoculated at full flowering. Only low quantities of *F. langsethiae* DNA and HT-2+T-2 were detected in oats grains from the plants inoculated by injecting or spraying with a spore suspension of *F. langsethiae* into the flag leaf sheath at late boot stage (GS45) or flag leaf sheath opening (GS47). In our study, the amounts of HT-2+T-2 toxins were correlated with the quantities of *F. langsethiae* in the samples. Similar results, with a high correlation of HT-2+T-2 toxins with *F. langsethiae* infection in oats were reported by (Imathiu, 2008). That study found that bagging with plastic bags increased the *F. langsethiae* infection and resulted in higher production of HT-2+T-2. Imathiu (2008) also reported that injection with *F. langsethiae* spore suspension was resulting in higher quantities of *F. langsethiae* DNA and HT-2+T-2 in harvested oats grains than spray inoculation, which is similar to our results from late boot stage inoculation (GS45I and GS45S). Opoku (2012) hardly detected any *F. langsethiae* DNA in oats when the plants inoculated before head emergence, while excessive amount was detected in head samples that inoculated at at GS59 and continued accumulating up to GS92. The study by Opoku (2012) also documented the high correlation between *F. langsethiae* DNA and HT-2+T-2 mycotoxins. *F. langsethiae* was showed to be in charge for excessive concentrations of HT-2+T-2 toxins in the field and glasshouse experiments, although injection inoculation with *F. langsethiae* in

glasshouse experiments was unsuccessful in attaining the elevated amounts of HT-2+T-2 toxins generally found in UK commercial oats fields (Imathiu, 2008). Divon *et al.* (2012) observed the highest levels of *F. langsethiae* DNA in mature grains from oat plants spray inoculated at anthesis or early dough compared to boot injection at the late boot stage, even though the latter treatment gave significant levels of infection in this particular study. It seems as if neither inoculation with a single nor a mixed *F. langsethiae* isolate spore suspension could establish an effective infection when plants were inoculated at late boot stage and flag leaf sheath opening, with either of the two methods of inoculation that were tested. However, samples from plants that were spray inoculated with a *F. langsethiae* spore suspension at full flowering or middle of the heading, contained high levels of *F. langsethiae* DNA and HT-2+T-2.

The highest percentage of plants developing symptoms was observed in the plants inoculated by spraying with a *F. langsethiae* spore suspension at full flowering (GS65) (32%) and at flag leaf sheath opening (GS47) (25%). The symptoms that developed around the panicle and flag leaf sheath in the plants spray inoculated into the flag leaf sheath at flag leaf opening might be due to damages that atomizer made on the panicles during the inoculation. On plants inoculated with *F. langsethiae* spore suspension at late boot stage and middle of the heading, using either spray or injection, only a low percentage of symptoms developed. *F. langsethiae* is making insignificant or no disease symptoms on cereals, particularly in oats because it is not a strong pathogen (Opoku *et al.*, 2011). Another study also confirmed absence of visible symptoms in affected panicles of oats with *F. langsethiae* in glasshouse and commercial field surveys (Imathiu, 2008). There is an essential need for innovative ways of examining *F. langsethiae* infection other than visible symptoms (Imathiu, 2008). Similar to our results, Opoku (2012) and Imathiu *et al.* (2013) also detected excessive quantities of *F. langsethiae* DNA and HT-2+T-2 mycotoxins in symptomless panicles. The lack of symptoms in plants, while *F. langsethiae* DNA and mycotoxins were detected in the grains documents that it is crucial to find alternative ways of diagnosing *F. langsethiae* infection other than dependency on disease symptoms.

Yield and yield parameters were not extremely affected by *F. langsethiae* inoculation. Yield per plant, 1000 seeds weight and the number of grains per panicles were not significantly different between the non-inoculated and spray or injection inoculated plants with *F. langsethiae* spore suspension at late boot stage, flag leaf sheath opening, middle of heading or full flowering. A

study by Imathiu *et al.* (2013) indicated that *F. langsethiae* infection had no straight influence on the crop yield but it might lead to refusal or downgrade of the oats grains in the industry as a consequence of excessive levels of HT-2+T-2 mycotoxins. It seems that *F. langsethiae* infection does not lead to reduction in yield or yield parameters in oats even though it produces serious amounts of HT-2+T-2 toxins in the harvested grains.

Length of panicles was not altered significantly in the plants that were inoculated by spraying or injecting with *F. langsethiae* spore suspensions at late boot stage (GS45), flag leaf sheath opening (GS47), middle of heading (GS55) or full flowering (GS65). The length between flag leaf node to the top of the plants was not changed greatly in the plants inoculated by spraying a *F. langsethiae* spore suspension at late boot stage, middle of the heading or full flowering. On the other hand, the length between flag leaf node to the top of the plants were significantly decreased in the plants inoculated by injecting with *F. langsethiae* spore suspension into the flag leaf sheath at late boot stage, this however, might be the effect of the method. Opoku (2012) observed reduced panicle length when the plants were boot inoculated with *F. langsethiae* spore suspension at late boot stage and at flag leaf sheath opening, but this was not observed in our study. Despite the high content of *F. langsethiae* DNA and HT-2+T-2, no changes were recorded in the panicle length and the length between flag leaf node to the top of the plants between the plants that were inoculated by spraying with *F. langsethiae* spore suspension at full flowering and middle of heading.

F. graminearum

Higher quantities of *F. graminearum* DNA (638.66 pg/µg plant DNA) and DON (1082.65 µg/kg) were detected in oats grains harvested from the plants inoculated by injecting a *F. graminearum* spore suspension into the flag leaf sheath at late boot stage (GS45) by syringe (Exp. 2) than the plants inoculated by spraying a *F. graminearum* spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47) by atomizer (Exp. 1) (485.5 pg/µg plant DNA and 574.08 µg/kg). Due to recent changes in the incidence of FHB pathogens and the increasing importance of *F. graminearum* and DON in Norwegian oats (Hofgaard, 2010; Hofgaard *et al.*, 2016) and cereals worldwide (Martinelli *et al.*, 2014), identification of the time point that *F. graminearum* invade the oats and also more resistant species seems essential. Tekle *et al.* (2012) found that oats are most susceptible to *F. graminearum* infection during anthesis and inoculation

after anthesis could decrease the susceptibility and reduce DON accumulation. In agreement with results of our study a trial by Xue *et al.* (2015), has shown that inoculation of oats before flowering (GS51-59) did not result in extreme amounts of DON in the harvested grains. According to Xue *et al.* (2015), both field and glasshouse studies have confirmed that oats are most susceptible to the infection of *F. graminearum* and DON contamination once it is inoculated around anthesis, while the susceptibility is decreasing at later phases of growth. The higher detected quantities of *F. graminearum* DNA and DON in oats grains from injection inoculated plants in comparison with the plants inoculated by spraying a *F. graminearum* spore suspension into the flag leaf sheath at flag leaf sheath opening, might be because of the fact that the fungus can use the natural moisture inside the flag leaf sheath before emerging from the panicle and make a successful infection before appearance. However, in our study, the amounts of DON were not as high in any of the varieties and inoculation time points as to reach the legal limits (1750 µg/kg) of European food safety commission (EuropeanCommission, 2006).

The percentage of plants developing symptoms was almost equal in the plants inoculated by injecting a *F. graminearum* spore suspension into the flag leaf sheath at late boot stage (GS45) (37%) and sprayed into the flag leaf sheath at flag leaf sheath opening (GS47) (36%). *F. graminearum* is identified as the most pathogenic *Fusarium* species making visible symptoms in wheat and to some extent in oats (Imathiu, 2008). Conversely Xue *et al.* (2015) reported that there were not any obvious symptoms of FHB in the oats varieties that were inoculated before emergence of the ear (GS51-55), but the symptoms started to appear and increase when the oats were inoculated from GS61 to 50% anthesis (GS65-69). However, regarding the higher quantities of *F. graminearum* DNA and DON in oats grains from the plants inoculated by injecting a *F. graminearum* spore suspension at late boot stage (GS45) than the plants inoculated by spraying into the flag leaf sheath at flag leaf sheath opening (GS47), it is possible to conclude that some of the symptoms in the sprayed plants might be due to the damages that were made by the atomizer during inoculation.

Yield and yield parameters were not affected by *F. graminearum* infection. Yield per plant was slightly higher in plants inoculated by injecting a *F. graminearum* spore suspension at late boot stage (3.73 g) than the plants inoculated by spraying a *F. graminearum* spore suspension at flag leaf sheath opening (3.63 g). However, inoculating by *F. graminearum* spore suspension or

inoculation methods did not affect the 1000 seeds weight and number of grains per plant. According to Martinelli *et al.* (2014) only five out of twenty oats varieties showed reduced grain weight under the influence of FHB. Anyhow, despite the *F. graminearum* infection and production of DON in the oats grains harvested from *F. graminearum* inoculated plants in our study, inoculation with *F. graminearum* sore suspension did not lead to reduction in yield or yield parameters.

Longer panicle length was recorded in the plants inoculated by injecting a *F. graminearum* spore suspension into the flag leaf sheath at late boot stage (GS45) (17.02 cm) in comparison to the plants inoculated by spraying a *F. graminearum* spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47) (17.02 cm) by atomizer. Longer length between flag leaf node to the top of the plants was recorded in the plants inoculated by spraying a *F. graminearum* spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47) (32.15 cm) by atomizer in comparison to the plants inoculated by injecting a *F. graminearum* spore suspension into the flag leaf sheath at late boot stage (GS45) (28.72 cm). Shorter length between flag leaf node to the top of the plants was recorded for the plants inoculated by spraying a *F. graminearum* spore suspension at flag leaf sheath opening in comparison to the *F. langsethiae* and non-inoculated plants while the panicle length in the same plants was not altered significantly in comparison to *F. langsethiae* and non- inoculated plants. Opoku (2012) detected reduced panicle length in the boot inoculated oats with *F. langsethiae* at late boot stage, but to our knowledge no study has investigated the panicle length and the length between flag leaf node to the top of the plants in the oats inoculated with *F. graminearum* before the present study. Both panicle length and the length between flag leaf node to the top of the plants were decreased in the plants inoculated by injecting a *F. graminearum* spore suspension in comparison with *F. langsethiae* inoculated plants and non-inoculated plants.

Response to *F. langsethiae* in oats varieties

F. langsethiae

Higher quantities of *F. langsethiae* DNA and HT-2+T-2 were detected in oat grains of Belinda than Odal and Vinger, in the plants inoculated by spraying a *F. langsethiae* spore suspension at full flowering (900.1 pg/µg plant DNA and 1466.9 µg/kg) or middle of the heading (671.8 pg/µg plant DNA and 1367.4 µg/kg). On the contrary, in variety trials of non-inoculated Oats,

Hofgaard et al have detected high levels of HT-2+T-2 toxins in Odal compared to Vinger and Belinda (pers. comm.)". In our study, the variety Odal contained higher quantities of *F. langsethiae* DNA in the plants that were inoculated by spraying or injecting a *F. langsethiae* spore suspension at late boot stage than Belinda and Vinger, however the samples only contained low quantities of *F. langsethiae* DNA and HT-2+T-2. The oats variety Vinger seemed to be more resistant to *F. langsethiae* DNA and contained less amount of HT-2+T-2 in comparison with Odal and Belinda. It seems that the oats varieties used in this study were well-resistant to the *F. langsethiae* DNA and HT-2+T-2 accumulation when inoculated before or around leaf sheath opening (GS45 and GS47) but the resistance declined with plants aging.

The highest percentage of the plants showing symptoms were registered for Belinda in the plants inoculated by spraying with *F. langsethiae* spore suspension at full flowering (GS65) or the plants inoculated by spraying with *F. langsethiae* spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47). Only low levels of *F. langsethiae* DNA and HT-2+T-2 were detected in the plants inoculated by spraying with *F. langsethiae* into the flag leaf sheath at flag leaf sheath opening (GS47), therefore, it seems that the symptoms that were observed was caused by the damages that the atomizer made on the plants during the inoculation. The incidence of FHB is not as evident in oats as it is in barley and wheat (Browne and Cooke, 2005). In this study Belinda had a few more symptoms than Odal and Vinger that was in line with the content of *F. langsethiae* DNA and HT-2+T-2 toxins in the harvested grains.

Yield per plant was slightly higher in Belinda than Vinger and Odal both in non-inoculated and *F. langsethiae* inoculated plants. Inoculation with *F. langsethiae* spore suspension did not significantly reduce the yield per plant in the three oat varieties used in this study. Higher yield parameters of the variety Belinda compared to Vinger and Odal seems to be the properties of varieties and remained unchanged after inoculation with *F. langsethiae* spore suspension at late boot stage, flag leaf sheath opening, middle of the heading or full flowering. Furthermore, Belinda had marginally higher 1000 seeds weight and number of grains per plant in non-inoculated plants than *F. langsethiae* inoculated plants. The 1000 seeds weight and the number of grains per plant did not differ significantly among the selected oat varieties inoculated with a *F. langsethiae* spore suspension at late boot stage, flag leaf sheath opening, middle of the heading or full flowering compared with non-inoculated plants. A field experiment has showed

higher yield in the variety Odal than Belinda and Vinger (Assveen M., 2015). That finding is not in accordance with the results from the present study, and an explanation may be that Belinda has a better performance in the controlled environment of the glasshouse, as in our study. Given that the variety Belinda had higher yield parameters and also higher content of *F. langsethiae* DNA and HT-2+T-2 toxins compared to Odal and Vinger, it seems that Belinda is a variety with higher yield and higher susceptibility to *F. langsethiae* DNA and HT-2+T-2 compared to Odal and Vinger.

Vinger had longer panicles than Belinda and Odal in both non- inoculated and *F. langsethiae* inoculated plants. Inoculation with a *F. langsethiae* spore suspension at late boot stage, flag leaf sheath opening, middle of the heading or full flowering did not make any significant difference in the panicle length between these varieties. Vinger had longer length between flag leaf node to the top in comparison to Odal and Belinda. Neither non-inoculated control nor plants inoculated with *F. langsethiae* spore suspension at late boot stage, flag leaf sheath opening, middle of the heading or full flowering by different methods made any significant change in differences between the length between flag leaf node to the top of the plants of these varieties. Compared to other cereals, as wheat and barley, oats have longer pedicles and broadly distributed spikelet in the panicle (Bjornstad and Skinnes, 2008) that may be the reason for less FHB expansion to the adjacent spikelet and rachis (Kosova, 2009; Tekle *et al.*, 2012). In our study, the variety Vinger had longer panicles and less content of *F. langsethiae* DNA and HT-2+T-2 toxins compared to Belinda and Odal, that might be because of the broader panicles and less expansion of FHB in Vinger compared to Odal and Belinda.

F. graminearum

The variety Belinda had higher quantities of *F. graminearum* DNA than Odal and Vinger in the oats grains harvested both from plants that were inoculated by injecting or spraying with *F. graminearum* spore suspension at late boot stage (GS45) and at flag leaf sheath opening (GS47). Higher amounts of DON were detected in harvested oats grains from Belinda than Odal and Vinger in the plants inoculated by injecting a *F. graminearum* spore suspension into the flag leaf sheath at late boot stage. Higher amounts of DON were detected in harvested oats grains from Odal than from Belinda and Vinger in the plants inoculated by spraying a spore suspension into the flag leaf sheath at flag leaf sheath opening, despite the fact that Belinda grains contained

higher quantities of *F. graminearum* DNA. Examining the harvested oat grains with *Fusarium culmorum* DNA assay confirmed the unwanted contamination with *F. culmorum* (another producer of DON) in the harvested oat grains. Therefore, this might be the reason for higher amounts of DON in harvested oat grains from Odal even though the quantities of *F. graminearum* DNA were higher in Belinda. Anyways, more investigations are needed to identify the ranking of oat varieties according to the content of DON in harvested grains. According to a three-years experiment by Lillemo M. *et al.* (2013), in spray inoculated plants at flowering, Odal had less content of DON than Vinger, whereas Belinda contained significantly higher amount of DON than Odal and Vinger. In our study, like that study Belinda contained higher content of *F. graminearum* DNA and DON compared to Vinger and Odal, but conversely Vinger had less *F. graminearum* DNA and DON compared to Odal. In our study, Vinger seemed to be more resistant to *F. graminearum* DNA and had less content of DON compared to Odal and Belinda.

The percentage of the plants showing symptoms was almost equal between varieties. Insignificantly higher percentage of symptoms developed in Belinda in comparison to Odal and Vinger, when the plants were inoculated by spraying a *F. graminearum* spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47). Insignificantly higher percentage of symptoms developed in Odal in comparison to Belinda and Vinger, when the plants were inoculated by injecting a *F. graminearum* spore suspension into the flag leaf sheath at late boot stage (GS45). Regarding that higher content of *F. graminearum* DNA was detected in Belinda than Odal and Vinger, therefore, dependency on visual symptoms for quantifying the degree of FHB infection can be challenging and other new ways must be considered.

Belinda had higher yield parameters than Vinger and Odal in non-inoculated plants, and also both when plants were inoculated by spraying a *F. graminearum* spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47) or injecting into the flag leaf sheath by syringe at late boot stage (GS45). A field trial by Assveen M. (2015) indicated higher yield in Odal than Belinda and Vinger. This finding is not in line with results of this study, and might be due to a better performance of Belinda in the controlled environment. Given that the variety Belinda had higher yield parameters and also higher content of *F. graminearum* DNA and DON compared to Odal and Vinger, it seems that Belinda is a variety with higher yield and higher susceptibility to *F. graminearum* DNA and DON compared to Odal and Vinger.

Significantly longer panicles were recorded for Vinger in comparison to Belinda and Odal in non-inoculated plants and in the plants inoculated with a *F. graminearum* spore suspension. Moreover, Vinger had significantly longer length between flag leaf node to the top of the plant than Odal and Belinda both in non-inoculated plants and in the plants inoculated with a *F. graminearum* spore suspension. The results are in line with those for *F. langsethiae* inoculated plants. Compared to other cereals, as wheat and barley, oats have longer pedicles and broadly distributed spikelet in the panicle (Bjornstad and Skinnes, 2008) that may be the reason for less FHB expansion to the adjacent spikelet and rachis (Kosova, 2009; Tekle *et al.*, 2012). In our study, the variety Vinger had longer panicles and less content of *F. graminearum* DNA and DON compared to Belinda and Odal, that might be because of the broader panicles and less expansion of FHB in Vinger compared to Odal and Belinda.

Conclusion

The results of these experiments indicate that the oats that were inoculated by spraying with a *F. langsethiae* spore suspension at full flowering (GS65) and middle of heading (GS55) contained higher content of *F. langsethiae* DNA and HT-2+T-2 than the plants that were inoculated by injecting, spraying or spraying into the flag leaf sheath at late boot stage (GS45) or flag leaf sheath opening (GS47). The variety Vinger contained low amounts of *F. langsethiae* DNA and also contained less of HT-2+T-2 mycotoxins in the harvested grains compared to Odal and Belinda and therefore was regarded as the most resistant variety, whereas Belinda contained highest levels of *F. langsethiae* DNA and HT-2+T-2 mycotoxins. Plants contained higher levels of *F. graminearum* DNA and DON when they were inoculated by injecting a *F. graminearum* spore suspension at late boot stage (GS45) compared to the plants inoculated by spraying a *F. graminearum* spore suspension into the flag leaf sheath at flag leaf sheath opening (GS47). The varieties Vinger and Odal contained less *F. graminearum* DNA compared to Belinda and also contained less of DON in the harvested grains and therefore were regarded as more resistant varieties than Belinda. High levels of *F. graminearum* DNA and DON were detected in the harvested grains from the oats inoculated at late boot stage (GS45) and flag leaf sheath opening (GS47), while only low levels of *F. langsethiae* and H-2+T-2 were detected in the harvested grains from the oats inoculated at these two stages. However, this phenomenon was expected considering that the mycotoxins are producing by different *Fusarium* species and *F. langsethiae*

is known as a weaker pathogen and less competitiveness than *F. graminearum*. Vinger contained less of HT-2+T-2 than Odal and Belinda, while the content of DON was less in Vinger and Odal compared to Belinda, whereas Belinda contained highest levels of HT-2+T-2 and DON.

The results of these experiments also indicate that despite the existence of *F. langsethiae* and *F. graminearum* DNA and mycotoxins in the oats that were inoculated with *F. graminearum* and *F. langsethiae* by different methods at different time points, there were no considerable effects on yield, yield parameters and developmental properties of the plants in tested oat varieties. The symptoms produced after inoculation with *F. graminearum* are sparse, and even less or none after inoculation with *F. langsethiae*. There is an essential need for innovative ways of detecting *F. langsethiae* and *F. graminearum* infection other than examining visible symptoms. Given that these fungi produce mycotoxins (DON and HT-2+T-2) harmful for human and animal health but lead to limited amounts of symptoms, enhanced effort is needed for identification of resistant oat varieties.

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Appendix:

I. P-value Table: Effect of *Fusarium* inoculation on symptom development and yield parameters in three oats varieties *floridana* and infection % per sampling date.

II. Effect of *Fusarium langsethiae* inoculation on symptom development and yield parameters in three oats varieties inoculated by *Fusarium langsethiae* and non-inoculated in experiment 3.

III. Effect of *Fusarium graminearum* (left) and *Fusarium langsethiae* (right) inoculation on symptom development

IV. Inoculation by spraying with *F. langsethiae* spore suspension at full flowering (left) and injecting into the flag leaf sheath at late boot stage (right) in experiment 3.

Exp.	Inoculation time point and method[a]	Variables	Symptoms after 5 days[b]	Symptoms after 8 days	Panicle length	The length between flag leaf node to the top	Yield per plant	1000 Seeds weight	Number of grains per plant	DNA	Mycotoxin
Exp. 1	GS47 SI	Variety	0.625	0.352	0.000	0.000	0.015	0.089	0.055	0.125	0.117
		Treatment	0.000	0.000	0.948	0.023	0.990	0.070	0.392	_	_
		Replication	0.400	0.119	0.664	0.660	0.788	0.470	0.834	0.398	0.495
		Variety X Treatment	0.959	0.809	0.288	0.163	0.486	0.817	0.530	_	_
Exp. 2	GS45 I	Variety	0.811	0.724	0.000	0.000	0.000	0.254	0.255	0.247	0.103
		Treatment	0.000	0.000	0.009	0.000	0.285	0.015	0.053	_	_
		Replication	0.878	0.801	0.633	0.819	0.122	0.660	0.444	0.461	0.126
		Variety X Treatment	0.345	0.526	0.685	0.236	0.166	0.387	0.382	_	_
Exp. 3	GS45 I	Variety	0.391	0.391	0.000	0.397	0.048	0.008	0.555	0.439	1.00
		Treatment	0.333	0.333	0.000	0.001	0.182	0.409	0.240	_	_
		Replication	0.420	0.420	0.188	0.068	0.656	0.392	0.328	0.450	0.859
		Variety X Treatment	0.391	0.391	0.010	0.683	0.624	0.958	0.454	_	_
	GS45 S	Variety	0.391	0.600	0.000	0.000	0.012	0.090	0.363	0.877	1.00
		Treatment	0.333	0.202	0.490	0.460	0.683	0.044	0.328	_	_
		Replication	0.420	0.585	0.763	0.877	0.143	0.432	0.636	0.543	0.859
		Variety X Treatment	0.391	0.600	0.395	0.257	0.726	0.406	0.502	_	_
	GS55 S	Variety	0.391	0.084	0.000	0.000	0.071	0.039	0.750	5.37	0.046
		Treatment	0.333	0.108	0.450	0.594	0.372	0.134	0.797	_	_
		Replication	0.420	0.420	0.069	0.429	0.598	0.250	0.248	1.83	0.242
		Variety X Treatment	0.391	0.084	0.855	0.871	0.798	0.654	0.640	_	_
	GS65 S	Variety	0.053	0.072	0.000	0.000	0.014	0.037	0.133	4.50.	0.048
		Treatment	0.000	0.000	0.994	0.011	0.644	0.631	0.250	_	_
		Replication	0.395	0.169	0.376	0.432	0.161	0.826	0.724	2.09	0.203
		Variety X Treatment	0.053	0.072	0.468	0.490	0.701	0.827	0.762	_	_

Appendix I. P-value Table: Effect of *Fusarium* inoculation on symptom development and yield parameters in three oats varieties

[a] GS47SI = Inoculated by spraying a spore suspension into the flag leaf sheath at GS47. GS45I= Inoculated by injecting a spore suspension into the flag leaf sheath at GS45. GS45S, GS55S and GS65S = Inoculated by spraying whole plants with a spore suspension at GS45, GS55 or GS65, respectively.

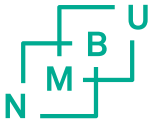
[b] The average percentage of inoculated plants showing symptoms (necrosis, discoloration and the spots around the panicle and the flag leaf sheath).

Exp.	Variety	Pathogen	Symptom after 5/6 days	Symptoms after 8 days	Yield per plant	1000 Seeds weight	Number of grains per plant
Exp. 3	Vinger	C	0 (0)	0 (0)	3.99 (0.11)	43.98 (1.97)	91.01 (1.9)
		F. l	1 (2)	5 (10)	3.83 (0.15)	42.87 (2.8)	89.9 (3.2)
	Odal	C	0 (0)	0 (0)	3.61 (0.05)	41.75 (0.5)	86.9 (3.0)
		F. l	4 (5)	12(15)	3.21 (0.43)	40.71 (1.1)	78.6 (8.6)
	Belinda	C	0 (0)	0 (0)	4.3 (0.21)	47.4 (3.18)	91.64 (6.81)
		F. l	4 (6)	11 (21)	4.3 (0.18)	46.1 (1.8)	93.9 (6.6)

Appendix II. Effect of *Fusarium langsethiae* inoculation on symptom development and yield parameters in three oats varieties inoculated by *Fusarium langsethiae* and non-inoculated plants in experiment 3.

Appendix III. Effect of *Fusarium graminearum* (left) and *Fusarium langsethiae* (right) inoculation on symptom development

Appendix IV. Inoculation by spraying with *F. langsethiae* spore suspension at full flowering (left) and injecting into the flag leaf sheath at late boot stage (right) in experiment 3.



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