Interaction between Mycotoxin Producing Fusarium Species in Different Oat Cultivars

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Interaktioner mellan mykotoxinproducerande Fusarium-arter i olika havresorter

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Abstract

Oat is the third most important cereal crop in Sweden and *Fusarium* head blight (FHB) disease makes the oat grain incompatible for animal and human consumption by producing secondary metabolites called mycotoxins.T-2, HT-2 and deoxynivalenol (DON) are the most common mycotoxins in oats produced by *Fusarium* spp. In this experiment, eight cultivars of oat plants were inoculated with *F. langsethiae* and/or *F. graminearum* and grown in a growth chamber. The *Fusarium* infection were quantified by using real time PCR and T-2 and DON concentrations were determined by using enzyme immunoassay. The result demonstrates that infection level varied for *F. langsethiae.* In some oat cultivars there was a higher infection level of *F. langsethiae* when this species were single inoculated and for some cultivars the infection of *F. langsethiae* was more severe when co-inoculated with *F. graminearum*. The same pattern is also true for *F. graminearum*. The amount of *F. graminearum* DNA and the DON concentration were strongly correlated ($r = 0.618$, $P=0.00$) as were the correlation between the amount of *F. langsethiae* DNA and T-2 toxin ($P=0.002$, $r = 0.517$).

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Introduction

Fusarium head blight (FHB) is a global fungal disease of small grain cereals. It affects not only wheat, barley, oats, rye and triticale, but also maize, canary seed and some forage grasses (Saskatchewan Agriculture and Food, 2007). The infection is favored by moist and warm conditions during flowering, and onwards during the growing period (Xu *et al*. 2007a). The first symptoms of FHB include a tan or brown discoloration at the base of a floret within the spikelet's of the head (Wolf & Lipps, 2003). Affected kernels become shriveled with a chalky (tombstone) appearance (Fanning, 2012) and loose the required weight for a successful harvest. *Fusarium* species is responsible for reducing seed germination and cause seedling blight. Seedling blight is responsible for seedling death and turns the plant stand become thinner. They drastically decrease the quality of the grain by producing mycotoxins and make the grain unsuitable for animal and human consumption (Xu & Nicholson, 2009).

Oat is the third most important cereal crop in Sweden. Approximately 20% of the land used for small grain cereals in Sweden is used for oat production (Jordbruksstatistisk årsbok 2012). Oat has been shown to be more contaminated by *Fusarium* toxins than any other cereals (Langseth & Rundberget, 1999; Scudamore *et al*. 2007). The mycotoxins T-2, HT-2 and deoxynivalenol (DON) are the most dominant mycotoxins in oats (Fredlund *et al*. 2010). For oat for human consumption the European Union has decided on legal limits for DON concentrations at 1,750 µg/kg (EC 2006), while limits for HT-2+ T-2 are under discussion, and 500 µg/kg has been suggested (Edwards *et al*. 2009).

Trichothecenes are a common group of mycotoxins produced by *Fusarium* species (Langseth & Rundberget, 1999). Trichothecene mycotoxins are divided into type- A and type-B. Type-A trichothecenes includes diacetoxyscirpenol (DAS), T-2 and HT-2 and type-B trichothecenes are DON and nivalenol (NIV) (Wilson *et al*. 2004). All trichothecenes have the same cyclic sesquiterpene structure and differs only in the form of functional group that is attached to the carbon backbone. Type-B trichothecenes have a carbonyl group, which is absent in type-A trichothecenes. The trichothecene producing *Fusarium culmorum, F. graminearum, F. langsethiae, F. poae* and *F. sporotrichioides* are all commonly found in grain (Fredlund *et al*. 2010).

The most common trichothecene in cereals is DON, produced by certain isolates of *F. graminearum, F. pseudograminearum* and *F. culmorum* (Nicolaisen *et al*. 2009). The highest concentration of DON is found during dry and warm summers rather than cold (Scudamore *et al*. 2007). DON inhibits DNA, RNA and protein synthesis in eukaryotes. It is also involved in the activation of several defense responses such as hydrogen peroxide production and programmed cell death, which may support it's colonization of a host plant (Desmond *et al.* 2008). DON is also involved in reducing levels of the serum protein albumin and responsible for transitory drop of packed blood cell volume, and

phosphorus and calcium concentration in the blood of pigs (Bergsjø *et al*. 1993). In human liver cells, DON has a diverse cytotoxic effect. Reduced cell viability, protein content and albumin secretion occurred depending on dose (Königs *et al*. 2008). Microbial transformation of DON to de-epoxy-DON caused a major loss of cytotoxicity in pig kidney cells (Kollarczik *et al*. 1994).

T-2 and HT-2 toxins are produced by *F. sporotrichioides* and *F. langsethiae* (Thrane *et al*. 2004). The T-2 toxin is cytotoxic and inhibits synthesis of DNA and RNA both in vivo and vitro, and inhibits protein synthesis in eukaryotes (Placinta *et al.* 1999). T-2 toxin can rapidly be metabolised to HT-2 toxin when ingested and therefore they have a common risk assessment (Eriksen & Alexander, 1998; Edward *et al.* 2009). Pigs are very sensitive towards the effects of T-2 toxin, and T-2 induces programmed cell death which affects the integrity of the cell membrane. T-2 toxin also stimulates both a toxin that destroy red blood cells, and bone marrow suppression which causes injury of the formation of blood cells in bone marrow (EFSA Panel, 2011). In human monocytes, T-2 toxins interfere in the differentiation process into macrophages and dendritic cells. This effect is responsible for the immunosuppressive properties of this alimentary toxin (Hymery *et al*. 2009).

For identifying *Fusarium* spp. in infected grain polymerase chain reaction, PCR is commonly used. For the quantification of individual *Fusarium* species real time PCR (qPCR) assays have been developed. The advantage of qPCR compared to common PCR is that it does not require post-PCR handling, which reduces the risk of cross-contamination and handling errors. It also has a faster throughput and is less labor intensive (Heid *et al*. 1996). A multiplex qPCR is developed to identify the major species within the complex (Waalwijk *et al.* 2003)

For identification and quantitative determination of mycotoxins in cereals the most exact method is different types of chromatography. They are able to, very accurately, separate different mycotoxins in a complex mixture. These methods are, however, time consuming and expensive. For shorter handling times commercial immunometric assays, such as enzyme-linked immunosorbent assays (ELISA) are frequently used for monitoring of mycotoxin content in raw materials (Pascale, 2009). The advantages are speed, high sensitivity, simple sample preparation, simultaneous analysis of multiple samples and limited use of organic solvents (only DON is dissolved in water) (Pascale, 2009).

Studies have found a correlation between fungal DNA from single species or groups of *Fusarium* species and their corresponding mycotoxin (Waalwijk *et al.* 2004; Fredlund *et al*. 2008). To calculate mycotoxin content in small grain cereals by quantification of fungal DNA could be a useful way of screening a large number of samples. Good correlation between the amount of toxin and DNA content helps to avoid the false negative results (Fredlund *et al*. 2010). This correlation is not always satisfactory to estimate the mycotoxin content. Fredlund *et al*. (2010) proved that some samples showed high level of mycotoxin although they have very low amount of DNA.

The aim of this project was to study the infection and mycotoxin production of *F. graminearum* and *F. langsethiae* in different oat cultivars. Also, the project aimed at monitoring the interactions between the two fungi and between fungi and oat. The approach to study many cultivars regarding susceptibility towards FHB using more than one *Fusarium* species is novel.

This study focus on these questions:

1. Is there a difference in susceptibility between oat cultivars towards *F. langsethiae* and *F. graminearum*?
2. How is the susceptibility affected by single species inoculation compared to paired inoculation of *F. graminearum* and *F. langsethiae*?
3. Is there an interaction between *F. graminearum* and *F. langsethiae* or do they compete with each other? How do mycotoxin production relate to this?
4. Is there a correlation between DNA content of *F. graminearum* and *F. langsethiae* and their mycotoxins DON and T-2?

Materials and methods

Plant material

Eight oat cultivars from Lantmännen SW Seed, Svalöv, were chosen for the experiment based on mycotoxin content from field trials in Bavaria in southern Germany during 2010. The cultivars were chosen according to their level of DON or T-2 using ELISA (Table 1).

Commercial garden peat soil (Hasselfors AB, Sweden) was used for the green house experiment, containing 85% peat and 15 % sand. Before sowing, oat seeds were surface sterilized by soaking in 0.5 % Sodium hypochlorite (NaClO) for 1 min and there after thoroughly washed with de-ionized water for 4 min. Four seeds were sown in a 13*13*20

Table 1 Oat cultivars used in a climate chamber experiment, and their mycotoxin content analysed with ELISA (in this study called low or high DON and T-2) from 2010 field trial in southern Germany. Data from Lantmännen SW Seed, Alf Ceplitis.

Variety	DON µg/kg	T-2 µg/kg	Selection
SW 100919	191		High DON
SW 100910	427		High DON
SW 100804	18		Low DON
SW 101006	18		Low DON
Circle	77	88	High T-2/HT-2
Cilla	19	9	low T-2/HT-2
SW Betania	62	14	low T-2/HT-2
SW Ingeborg	97	88	High T-2/HT-2

cm pot with presoaked soil, and there after covered with 3 cm soil layer. Additional water was spread on the top. Plants were grown in a growth chamber with a climate simulating the Swedish growing period (Figure 1, Table 2).

Figure 1 Oat plants were grown in a growth chamber with a climate mimicking the Swedish growing period. The plants were inoculated with different *Fusarium* spp. at flowering stage. This picture was taken 6 weeks after sowing.

Plants were watered when needed. After 3 weeks of emergence, addition of nutrients (Blomstra, Cederroth AB, Stockholm) was started. 102 mg N/l water was used when watering the plants. Nutrients were added until the experiment ended. Pots were rotated twice a week to make sure that the replicates were exposed at the same environmental condition and reduce variation between replicates.

Table 2. Applied temperature, light and humidity in a growth chamber. The experiment studying *Fusarium* spp. infection on eight oat cultivars.

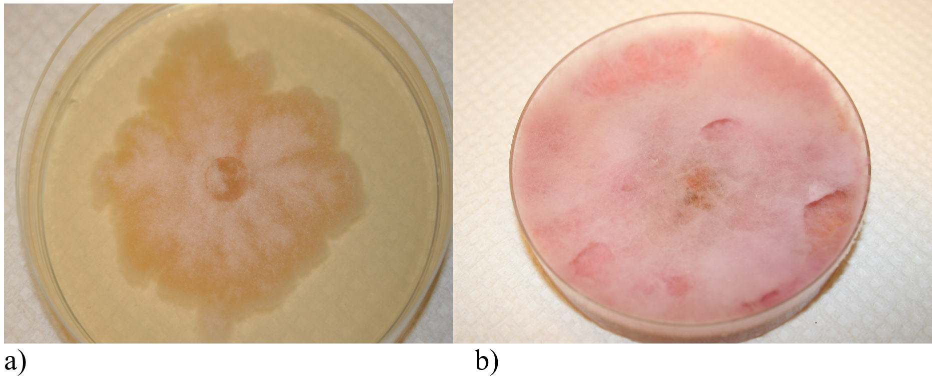
Time period (weeks)	Temperature	Light	Humidity
Two	10°C/8°C	17h/7h	70%RH/60%RH
Three	15°C/12°C	18h/6h	70%RH/60%RH
Two	18°C/15°C	18h/6h	70%RH/60%RH
Four	20°C/15°C	17h/6h	70%RH/60%RH

Inoculum production

Plants were inoculated using two species of *Fusarium*, *F. langsethiae* strain no. IBT01005 isolated from oat and *F. graminearum* strain no. PH1 isolated from wheat. Both strains provided by Erik Lysøe, Bioforsk, Norway and used in an ongoing research project.

Conidial suspension for inoculation was produced using carboxymethylcellulose (CMC) in 500 ml medium (recipe in appendix 1). *Fusarium langsethiae* and *F. graminearum* were grown on Potato dextrose agar (PDA) in 9 cm Petri dishes for 2 weeks (Figure 2) before samples were added to the CMC medium. Flasks were placed on a shaker for 2 weeks in room temperature and under normal day light conditions. The spore

concentrations were determined using a Bürker haemacytometer and then adjusted to working concentration using CMC. The spore concentration for *F. graminearum* and *F. langsethiae* were 5* 10^4 spores /ml and 2 * 10^6 spores /ml respectively. The working solutions were stored in -20^0C until use.

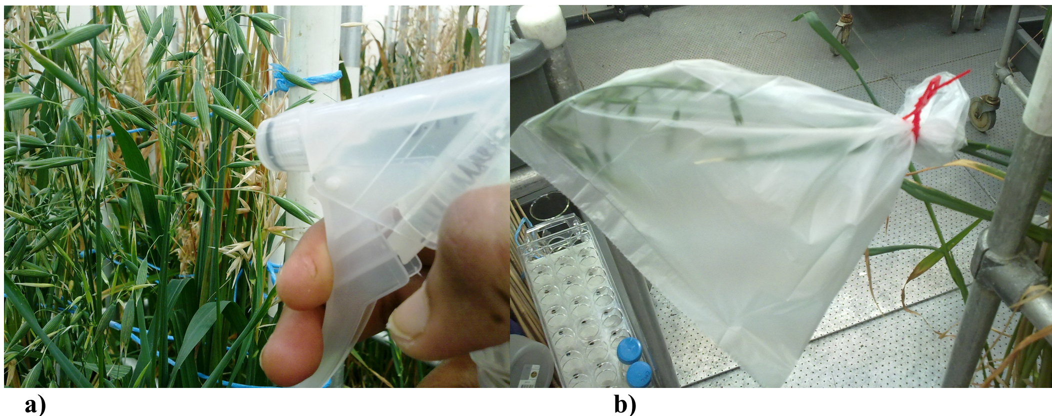


a) b)

Figure 2 a) *Fusarium langsethiae* and b) *Fusarium graminearum* grown on Potato dextrose agar (PDA).

Inoculation and sampling

At flowering, approximately DC 65-69 (Tottman, 1987) (approximately 2 months after sowing), the panicles were spray inoculated with *F. graminearum* and *F. langsethiae* (Figure 3). Approximately 1ml conidial suspension was used per panicle. Each panicle was covered with a 2-liter plastic bag for 4 days. Covering the panicle with a plastic bag ensure high humidity (about 100% RH) during the early infection, and enhance infection success. The different treatments were: inoculation with *F. graminearum* only*,* with *F. langsethiae* only*,* with a mixture of *F. graminearum*/*F. langsethiae*, and with CMC as a control. One to four panicles per replicate was inoculated. Panicles were harvested 14 days after inoculation, and immediately stored at - 80°C until DNA extraction. Each oat cultivar was subjected to the four treatments, and each treatment had 3 replicates, in total 96 pots.



a) b)

Figure 3 a) At flowering, (approximately 2 months after sowing), the oat panicles were spray inoculated with *Fusarium graminearum* and *Fusarium langsethiae.* b) Panicles were covered with plastic bag for 4 days to ensure high humidity during the early infection.

DNA extraction and real-time PCR

Plant and fungal DNA was extracted from 100 mg fresh tissue using DNeasy Plant Mini kit according to manufacturer's protocol (Qiagen, Hilden, Germany). The spikelets were removed from the panicles, and ground using liquid nitrogen. 530 µl of the buffer AP1 was used instead of 400 µl as recommended by the manufacturer. The extraction method was evaluated with respect to total DNA yield. DNA concentration and purity of each DNA template was measured using Nanodrop ND- 1000 Spectrophotometer (NanoDrop technologies, USA).

Quantification of plant DNA was investigated by using qPCR. *Fusarium langsethiae* primers were chosen according to Wilson *et al*. (2004) and qPCR protocol according to Fredlund *et al*. (2010). Primers used for *F. graminearum* was according to Reischer *et al*. (2004) and PCR protocol from Divon *et al*. (2012). qPCR was performed on a Real-Time Detection System (Biorad , CA, USA). qPCR reaction was performed in duplicate on all samples as technical replicate.

Amount of fungal DNA in inoculated plants were normalized comparative to plant DNA by dividing mean total quantity of two fungal replicates with mean total quantity of the responding two technical plant replicates (Divon *et al*. 2012).

Enzyme immunoassay for detection of DON and T-2 toxin

Quantification of DON and T-2 toxins in the samples was performed using the ELISA Ridascreen® DON and T-2 toxin kit (R-Biopharm AG, Germany) according to the manufacturer's protocol. Absorbance was measured at 450 nm with a spectrophotometer (Thermoscan, Australia). The data were evaluated by the software RIDA®SOFT Win (R-Biopharm AG, Germany). The three replicates of each treatment were pooled together due to scarcity of material.

Statistical analysis

Statistical analysis was performed using Minitab 16 (Minitab Inc., USA). To test for differences between the parameters treatment, and cultivar and their interaction, Generalized Linear Model (GLM) was used. Tukey's HSD was applied for pairwise comparisons. Pearson correlation analysis was used to test for correlation between the amount of fungal DNA and toxin concentration. To achieve normal distribution all data were log transformed. Square root transformation was done while doing the correlation between fungal DNA content and mycotoxin. A principal components analysis (PCA) was conducted for the analysis of correlation coefficient between the quantitative variables of fungal DNA amount and mycotoxin quantity. Categorical variables, all treatments were evaluated by one way analysis of variance with the coordinates of the quantitative variables. Student t-test was used to compare the mean value of each treatment with the general mean of all treatments. The analysis of PCA was performed by R (The R foundation, 2012) statistical software v 2.15.1, where 'FactoMinR' package was used as library function.

Results

The different treatments (control, single inoculations, and paired inoculation) had a significant effect on the infection of both *F. langsethiae* and *F. graminearum* on oat measured as the amount of fungal DNA in the panicle (P=0.00, $F_{3,62}$ = 8.40 and P=0.00, $F_{3,62}$ = 9.86 respectively). For *F. langsethiae* DNA, treatments C and G are significantly different from L and LG (Tukey's HSD). For *F. graminearum* DNA, treatments C and L are significantly different from G and LG (Tukey's HSD). For cultivars, only the infection of *F. langsethiae* measured as amount of fungal DNA was significantly different between cultivars (P<0.05, $F_{7,62}$ = 2.15). However, Tukey's HSD could not distinguish between the variables, probably because replicates were few and showed too much variation. There was no significant effect of the interaction between treatment and oat cultivar on the infection for either of the fungi. The individual infection of *F. langsethiae* was higher than when paired with *F. graminearum* for cvs 100919, 100910, Circle and Betania (Table 3). The individual infection of *F. graminearum* was less than when co-inoculated with *F. langsethiae* for all cvs but 100919 and 100910 (Table 3).

Analysis of DON concentration showed a significant effect of treatments (P=0.00, $F_{3,28}$ = 37.14). Also for T-2 concentration treatments had a significant effect (P=0.00, $F_{3,28}$ = 26.09). For DON concentration Tukey's HSD showed that the treatments with pair inoculation and single inoculation with *F. graminearum* had significantly higher concentrations of DON compared to the control and single inoculation with *F. langsethiae* (Table 4). Tukey's HSD showed for T-2 concentration that the control and the single inoculation with *F. graminearum* had significantly lower T-2 concentrations compared to the single inoculation with *F. langsethiae* and the paired inoculation of *F. langsethiae* and *F. graminearum* (Table 4).

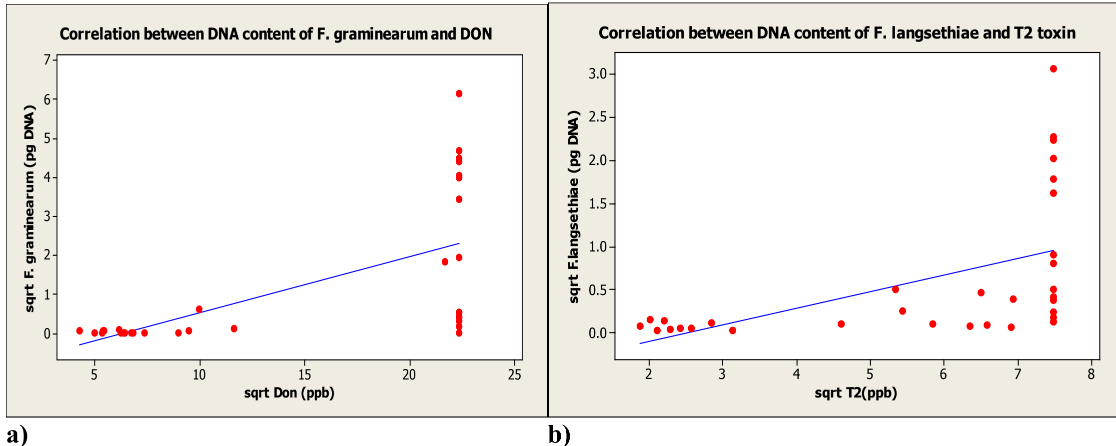
Pearson correlation analysis showed that the amount of *F. graminearum* DNA and the DON concentration in the eight oat cultivars studied were strongly correlated (P<0.01, r = 0.618) (Figure 4 a). Correlation was found between the amount of *F. langsethiae* DNA and concentration of T-2 toxin (P=0.002, r = 0.517) (Figure 4 b). No correlation was found between *F. langsethiae* DNA and DON concentration and or between amount of *F. graminearum* DNA and T-2 concentration. This can also be seen in figure 5. Three samples showed high levels of T-2 toxin (> 40 ppb) though the level of DNA was low (<0.007 pg). Two samples contained high DON (<500 ppb) whereas the DNA content was very low (<0.2 pg) (Table 3).

Table 3 The amount of DNA (picogram, pg) of *Fusarium langsethiae* and *Fusarium graminearum* in different oat cultivars and concentrations of *F. langsethiae* produced T-2 (ppb) and *F. graminearum* produced DON (ppb) mycotoxins. The values for DNA are means over three replicates, while in the toxin analysis the three replicates were pooled for each cultivar and treatment. Treatments: C - control, G - *F. graminearum*, L - *F. langsethiae* and LG - co-inoculation of *F. langsethiae* and *F. graminearum*. Normalized values were not shown here.

Cultivar	Treatments	T-2 (ppb)	*F. langsethiae* (pg)	*F. graminearum* (pg)	DON (ppb)
100919	C	4	0	0	≥500
100919	G	40	0	20	≥500
100919	L	≥56	4	0	40
100919	LG	≥56	5	3	≥500
100910	C	28	0	<0	90
100910	G	29	0	0	≥500
100910	L	≥56	0	0	81
100910	LG	≥56	2	0	≥500
100804	C	5	0	<0	29
100804	G	4	0	3	470
100804	L	≥56	0	0	47
100804	LG	≥56	0	19	≥500
101006	C	6	0	0	41
101006	G	3	0	0	≥500
101006	L	≥56	0	0	28
101006	LG	42	0	22	≥500
Circle	C	34	0	0	25
Circle	G	5	0	0	≥500
Circle	L	≥56	0	0	45
Circle	LG	≥56	0	16	≥500
Cilla	C	21	0	0	29
Cilla	G	8	0	16	≥500
Cilla	L	≥56	5	0	135
Cilla	LG	48	0	38	≥500
Betania	C	9	0	0	18
Betania	G	47	0	0	≥500
Betania	L	≥56	0	0	54
Betania	LG	≥56	0	11	≥500
Ingeborg	C	4	0	0	38
Ingeborg	G	43	0	0	100
Ingeborg	L	≥56	9	0	39
Ingeborg	LG	≥56	3	0	≥500

Table 4 Average of amount of fungal DNA over three replicates and eight oat cultivars. Average of mycotoxin concentration over three pooled replicates and eight oat cultivars.

Treatment	*Fusarium langsethiae* (pg)	T-2 (ppb)	*Fusarium graminearum* (pg)	DON (ppb)
C	0	14	0	96
FL	2	≥56	5	59
FG	0	22	0	446
FL+FG	1	53	13	≥500



a) **b)**

Figure 4 **a)** Correlation between the DNA content of *Fusarium graminearum* and DON in 8 cultivars of oat. The Pearson coefficient was 0.618 (P = 0.000) **b)** Correlation between the DNA content of *Fusarium langsethiae* and T-2 toxin in 8 cultivars of oat. The Pearson coefficient was 0.517(P = 0.002). DNA content of *Fusarium graminearum* and *Fusarium langsethiae* was analyzed by real time polymerase chain reaction, qPCR. DON and T-2 toxin was analyzed by ELISA Ridascreen®.

The PCA also show close positive correlation between *F. graminearum* DNA and mycotoxin DON concentration, but negative between *F. graminearum* DNA and mycotoxin T-2 concentration (Figure 5, Table 5). As seen in Table 5 the first dimension is also significantly influenced by the treatments LG, G, C and L (Figure 6). The significant correlation between *F. graminearum* DNA and DON toxin is mainly caused by the co-inoculation treatment (*F. graminearum* and *F. langsethiae*), since this treatment is clustered in the same quadrant (Figure 6) as the *F. graminearum* DNA and DON toxin arrows (Figure 5).

The correlation coefficient in the PCA between *F. langsethiae* DNA and the mycotoxin T-2 concentration was also closely related (Figure 5, Table 5). As seen in Table 5 the first dimension is also significantly influenced by the treatments LG, G, C and L (Figure 6). The treatment *Fusarium langsethiae* is the main reason behind this correlation since this treatment is clustered in the same quadrant (Figure 6) as the *F. langsethiae* DNA and T-2 toxin arrows (Figure 5).

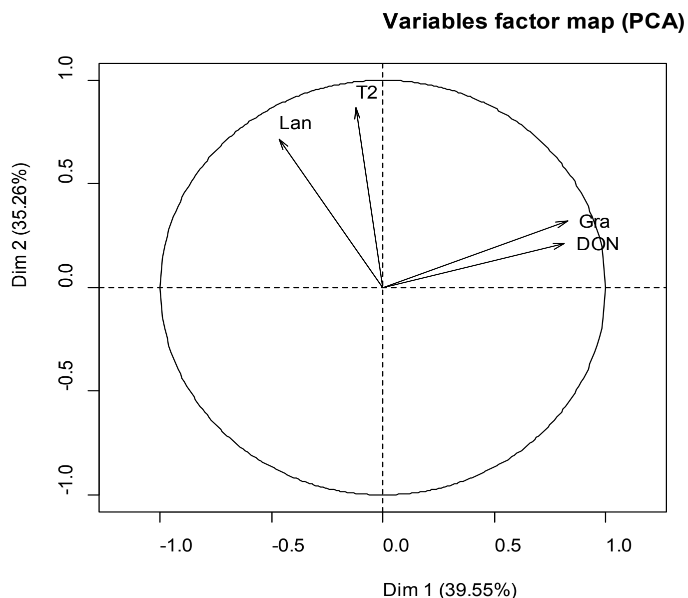


Figure 5 Correlation between *Fusarium* species and mycotoxin in infected oat plants. Abbreviations: Lan; *Fusarium langsethiae*, Gra; *Fusarium graminearum*, T-2; mycotoxin T-2, produced by *Fusarium langsethiae*, DON; mycotoxin DON, produced by *Fusarium graminearum*.

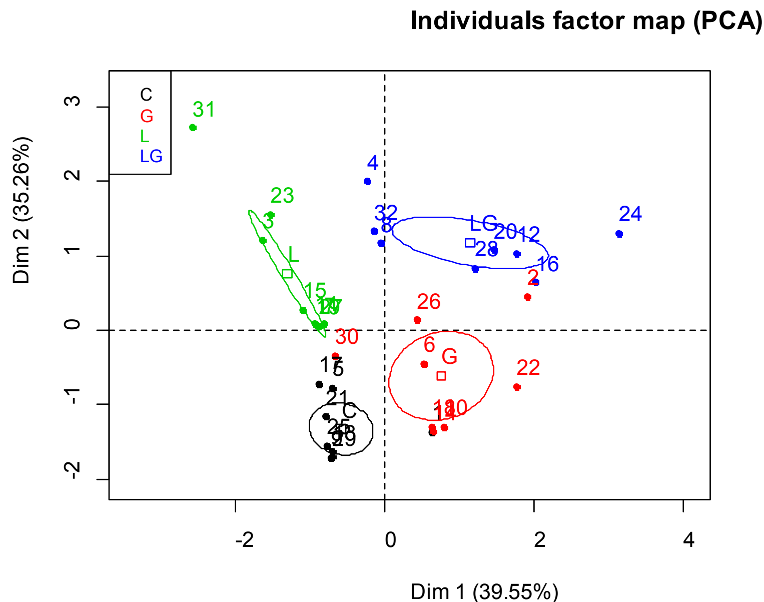


Figure 6 Projection of amount of *Fusarium langsethiae* DNA and *F. graminearum* DNA between treatments in a growth chamber experiment on eight different oat cultivars. Treatments: C- control, L- *Fusarium langsethiae*, G- *Fusarium graminearum*, LG- co-inoculation (*Fusarium langsethiae* and *Fusarium graminearum*). Serial numbers are samples ID.

Table 5 Correlation coefficient (R^2) and estimated point of quantitative and qualitative variables in the dimensions 1 and 2 of PCA

Dimension-1 (Eigenvalue λ 0.40)		
Quantitative variables	**Correlation (R^2)**	**p.value**
F. graminearum	0.832	<0.001
DON	0.817	<0.001
F. langsethiae	-0.457	<0.001
Treatments	0.625	<0.001
Categorical variables	**Estimated point**	**p.value**
LG	1.157	<0.001
G	0.747	0.006
C	-0.604	0.023
L	-1.300	0.001
Dimension-2 (Eigenvalue λ 0.35)		
Quantitative variables	**Correlation (R^2)**	**p.value**
T-2	0.868	<0.001
F. langsethiae	0.721	<0.001
Treatments	0.722	<0.001
Categorical variables	**Estimated point**	**p.value**
LG	1.163	<0.001
L	0.776	<0.001
G	-0.622	<0.001
C	-1.317	<0.001

Discussion

It is important to assess competitive interactions between FHB pathogens in terms of disease, fungal development and mycotoxin production in oat. In this study, some of the oat cultivars (100804, 101006, Circle, Cilla and Betania, (Table 3)) showed that mixed inoculation of *F. graminearum* with *F. langsethiae* creates generally a larger *F. graminearum* infection than single inoculation of *F. graminearum*. This is not so pronounced for *F. langsethiae*. Higher *F. graminearum* infection indicates that *F. graminearum* works as a better competitor than *F. langsethiae* or that *F. langsethiae* stimulates the growth of *F. graminearum*. Xu *et al*. (2007b) found that the growth rate of *F. graminearum* was unaffected, in maize grain, when *F. proliferatum* and *F. moniliforme* were present. In addition, the study showed that growth of the two latter fungi was reduced when *F. graminearum* was present. Xu *et al.* (2007b) also showed that mixed inoculation of *F. graminearum* with either *F. proliferatum* or *F. moniliforme* caused a larger total infection than when *F. graminearum* was alone.

In this experiment *F. graminearum* strain was used, isolated from wheat, known to be pathogenic to this species. *F. graminearum* is known to infect many different hosts and is commonly isolated from all cereal species. In 2011 Swedish oat was heavily contaminated and showed high DON levels. It is possible that *F. graminearum* may be

adapted to a specific host and this may have affected the experiment. Different strains are also known to have different aggressiveness. In cultivar screenings for *Fusarium* or mycotoxin production resistance it is therefore recommended to use a collection of strains. This and other studies show larger infections when *Fusarium* species are co-inoculated than when they are inoculated singly. A multi *Fusarium* species inoculum would therefore be preferred in cultivar screenings.

The cultivars in this study were based on either low or high DON and/or T-2 toxin content in harvested grain, based on toxin analyses from the field grown crops, season 2010 (Ceplitis, pers. com). In this experiment, using artificial inoculation, high DON content was registered for all cultivars. This is contradictory to results from the field material from 2010 showing that cvs 100804 and 101006 contained low DON concentrations. In this experiment, the plants were inoculated with high amounts of inoculum, which may be the reason for general high mycotoxin content. Also, in other studies it has been found that some FHB resistant cultivars may possess mechanisms to metabolize DON and convert it to deoxynivalenol-3-beta-glucopyranoside (D3G). DON and D3G concentration were tested by Liquid chromatography–mass spectrometry (LC-MS/MS) (Berthiller *et al*. 2007; Lemmens *et al*. 2005). Berthiller *et al*. (2007) found a close relation between D3G/DON ratio and most of the DON converted to D3G. D3G have been found in naturally infected wheat and maize, where it co-occur with DON. So, it is not known whether the degradation occurs or not either in the field trial or in the growth chamber. But one explanation to high content of DON in 100804 and 101006 in this growth chamber experiment could be that the process not can proceed as normal in the artificial experimental conditions. The cvs Cilla and Betania contained low T-2, according to the field trial 2010, but in this experiment high T-2 concentration is found in both cvs. Scudamore *et al*. (2007) found that HT-2 and T-2 were formed by the same fungal species but that HT-2 was always present at a higher concentration than T-2. Possible cross reaction with HT-2 in the ELISA analysis may be one reason for the high T-2 results. The HT-2 toxin was not analyzed in this experiment, and therefore the cross reaction ratio is unknown. According to the manufacturer of the ELISA Ridascreen® T-2 toxin kit (R-Biopharm AG, Germany) the cross reaction with HT-2 is 7%.

Working with wheat samples from the southern part of Sweden, Fredlund *et al*. (2008) found very few samples with DON content above the EC limit (for wheat used for food, the limit set by EU is 1250 ppb) . Several studies from UK have shown that, the average DON concentration is lower in oat samples than wheat and barley (Edwards, 2009). This is contradictory to the Swedish experience from the season 2011 where the DON levels in some oat samples were extremely high and 40-50 % of the oat production exceeded the EU limits for food products (for oat 1750 ppb). The experiences from UK may be due to a higher proportion of winter oat grown there and that flowers earlier in the spring than spring oat. On the other hand, higher levels of T-2 and HT-2 were detected in oat compared to barley and wheat samples in Norway (Langseth & Rundberget, 2000). In this study, mycotoxin analysis showed that 50% of the samples contained >450 ppb DON and 59% samples contained >40 ppb of T-2 (Table 3).

To investigate whether the *Fusarium* DNA levels could be correlated to the levels of DON and T-2 in the samples, Pearson correlation analysis, and PCA were performed on the data. The results showed a strong correlation between the DNA content of *F. graminearum* and DON (Figure 4a and Figure 5). It should be noted though that registration over 500 ppb could not be performed due to limitations in the method (out of range). It indicates that *F. graminearum* is an important source for DON production. Correlation was also found between DNA content of *F. langsethiae* and T-2 toxin concentration (Figure 4 b and 5), and *F. langsethiae* can be considered as an effective producer of T-2 toxin in the samples. In Figure 4, it can be seen that samples are aggregated to the right, i.e. they have high toxin concentrations (samples are out of range for the test kit). If they were diluted and reanalyzed more specific concentrations could be gained, and hence the correlation may give a different result.

Some samples contained high levels of either DON or T-2 toxins and low levels of *Fusarium* DNA (Table 3 and 4). The same result has been shown by Fredlund *et al*. (2010). For example, the control treatment of cv 100919 shows high DON content although no fungal DNA was detected in the PCR. As mycotoxin content in grain is a serious quality problem, this indicates the importance of measuring both fungal DNA and mycotoxin content studying the susceptibility of different cultivars.

This study applied a recent molecular method in order to quantify mycotoxin producing *Fusarium* species in oats. The same experiments can be repeated for other host plants for comparison of infection rate and fungal behavior in that host. When plants are grown in natural ecosystems, many different species of *Fusarium* can be involved in the infection process. For disease development up to 17 *Fusarium* species could be involved (Parry *et al*. 1995). It is difficult to identify which species or complex of species are more involved in disease development than other as different environmental condition also effects the interaction between species. This study focused on two-pathogen infections; different types of pathogens can give interesting synergistic interaction.

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Summary

The aim of this project was to study the infection and mycotoxin production of the plant pathogens *Fusarium graminearum* and *Fusarium langsethiae* on different oat cultivars. Mycotoxin is a secondary metabolite and it significantly decreases the quality of the grain and makes the grain unsuited for animal and human consumption. The plant disease, Fusarium head blight (FHB), has symptoms occurring on the head or spikelet. Oat is grown on a fifth of the arable area in Sweden. During the last decade problems with *Fusarium* infection in oats has increased, the mycotoxin production of these genus being the main problem. Damaged kernels become shriveled with a chalky appearance and loose the required weight for a successful harvest.

Oat cultivars for the experiment were selected based on mycotoxin content from field trials in Bavaria in southern Germany during 2010. Cultivars were chosen containing high or low concentration of DON and T-2 mycotoxin. Chosen cultivars were grown in a growth chamber with Swedish climate conditions and were inoculated with *F. langsethiae* and/or *F. graminearum* at flowering. For detection of fungal infection real-time polymerase chain reaction, was applied. These techniques amplify and simultaneously quantify the targeted DNA molecule. Enzyme-linked immunosorbent assays (ELISA) analysis was used for identification and quantitation of mycotoxins. ELISA is a test that uses antibodies and changes of color ensures the presence of substance.

The result showed that the individual infection of *F. langsethiae* was higher than when paired with *F. graminearum* for four cultivars. For *F. graminearum* the individual infection of was less than when co-inoculated with *F. langsethiae* for six cultivars. Higher *F. graminearum* infection indicates that *F. graminearum* works as a better competitor than *F. langsethiae* or that *F. langsethiae* stimulates the growth of *F. graminearum*.

Mycotoxin analysis showed that 50% of the samples contained >450 ppb DON mycotoxin and 59% samples contained >40 ppb of T-2 mycotoxin, i.e. they were out of range for the test method, indicating high concentrations of mycotoxins. These concentrations are contradictory compared to 2010 field data, and may be due to the artificial conditions compared to the field, i.e. larger amounts of fungi at infection compared to natural pathogen pressure.

It was investigated whether the fungal DNA levels could be correlated to the levels of mycotoxin in the samples. Strong correlation between *F. graminearum* and DON concentration and also strong correlation between *F. langsethiae* and T-2 concentration was found. This indicates that *F. graminearum* is the source for DON production and *F. langsethiae* the main producer of T-2 toxin in the samples, which has been shown in other studies.

Appendix 1

CMC medium was prepared by 30 g CMC , 4g yeast extract , 2g KH_2PO_4 , 2g NH_4NO_3, 3g $MgSO_4.7H_2O$ and de-ionized water was added to reach the total volume until 2 liter.