Final report for SLF project H1333237

Quick and reliable detection of *Fusarium langsethiae* with "Loop-Mediated Isothermal Amplification" method

Zahra Omer[1], Jamshid Fatehi[2] and Ann-Charlotte Wallenhammar[1]
[1]HS Konsult AB /Hushållningssällskapet; [2]Lantmännen BioAgri

1. Background

Fusarium langsethiae is commonly found in Swedish cultivated oats and is known to produce type A mycotoxins, T-2 and HT-2 (Langseth & Rundberget., 1999; Thrane et al., 2004). The fungus is also involved in the *Fusarium* species complex causing the economically important Fusarium head blight (FHB) disease in cereals. It was first in 2004 that the incidence of *F. langsethiae* was directly connected to high T-2 and HT-2 content in oats kernels in a survey conducted in Norway (Langseth & Rundberget., 1999). In Sweden *F. langsethiae* was detected as one of the most common *Fusarium* species in harvested oats kernels between 2006-2008 (Fredlund et al., 2010) and between 2009-2011 (Fredlund & Lindblad., 2014) where significant correlation between the presence of *F. langsethiae* and T-2 and HT-2 levels was found. T-2 and HT-2 negatively affect both animals and humans health and are more toxic than the type B mycotoxin deoxynivalenol (DON) (Widestrand & Pettersson., 2000).

Unlike other *Fusarium* species involved in FHB, *F. langsethiae* infects plants without causing visible symptoms on the spikes (Imathiu, 2008). In one study when oats plants were artificially infected with *F. langsethiae*, typical FHB symptoms were developed (Divon., 2012) whereas under field conditions, visible symptoms were not developed on oats spikes although the fungus was detected by PCR. This make it impossible to visually detect *F. langsethiae* infection in oats. Investigations on the pathogenicity of *F. langsethiae*, has also demonstrated that infection at the seedling stage "seedling blight" is not part of the disease cycle (Imathiu et al., 2010). Furthermore, it was found that even apparently healthy cereal kernels could be contaminated with *F. langsethiae* (Parry., 1995), meaning that conventional filter paper methods can easily overlook the fungus. Consequently, the epidemiology of *F. langsethiae* i.e how the fungus spreads and how the plants are infected under the field conditions, is still unclear. Development of a fast, cheap and reliable method for detection of this pathogen in seed, soil or other plant material can be of great value for further epidemiological studies.

Loop-Mediated Isothermal Amplification (LAMP) is a novel nucleic acid amplification method which involves strand displacement autocycling DNA synthesis under constant temperature "isothermal". LAMP was reported as a simple and rapid diagnostic method for early detection of microorganisms and was first developed for rapid detection of bacteria, viruses and fungi in clinical samples (Notomi et al., 2000). The method is based on the amplification and specific detection of genomic DNA using four to six primers. Using a polymerase from "*Geobacillus stearothermophilus*" those primers hybridizes specifically to different target regions on a genomic DNA. Amplification of DNA can easily be performed on a heat block or water bath without thermocycling and the DNA product can easily be visualized by adding intercalating dyes as Calcein or a metal indicator such as Hydroxy Nathphol Blue and without performing gel electrophoresis (Goto et al., 2009). In addition, simple and visible detection could be achieved by observing the turbidity of the solution after LAMP reaction, which results from the accumulation of the byproduct magnesium pyrophosphate. Recently, LAMP was developed for specific detection of some fungal and bacterial plant pathogens (Niessen and Vogel., 2010; Moradi et al., 2013) as well as few plant parasitic nematodes (Niu et al., 2011) and plant viruses (Peng et al., 2012).

The aim of this project is to design species specific primers and to develop a quick and reliable LAMP PCR that can exclusively detect *F. langsethiae*.

2. Materials and Methods

2.1. Microorganisms

F. langsethiae and *F. sporotrichioides* strains originally isolated from cereal plants in Sweden, Norway and Austria were obtained from the National Food Agency (NFA), Sweden. Two strains of *F. graminearum* and *F. culmorum* were obtained from Dr. Thomas Börjesson (Agroväst). Several other *Fusarium* strains were obtained from Russia (Dr. Tatiana Gagkaeva), the United Kingdom (Dr. Simon Edwards) at Harper Adams University and (Dr. Paul Nicholson) at John Innes Institute. Several other reference strains of *Fusarium* species were purchased or obtained from CBS fungal collection (The Netherland), NIAS Genebank (Japan) and ARS Culture Collection (USA). Working cultures were maintained on PDA (Potato Dextrose Agar, Difco) and kept at 4º C. For long time preservation, the cultures were preserved as mycelia in 17% glycerol at -80º C.

2.2. Isolation and identification of *Fusarium* spp from oats kernels

Fusarium species were isolated from eight different seed batches of oats cultivar Belinda, Ingeborg, Scorpion and Kerstin from Frökontrollen Mellansverige AB and one seed batch of cultivar Belinda obtained from Lantmännen spannmål (Dr. Thomas Börjesson). Oats kernels were surface sterilized first in 1% sodium hypochlorite (commercial bleach) (v/v), washed three times with sterile distilled water and dried in a sterile bench. Approximately, 50 seeds from each seed lot were analyzed on PDA amended with chloramphenicol (100 mg/L) (five kernels/plate). The plates were incubated at 23° C for 3-7 days in the dark. The growing fungal mycelia were transferred into new PDA plates. Pure cultures were finally obtained by single spore isolation. Morphological characteristics of the isolates were investigated by growing them in different culture media (such as Potato sucrose Agar and SNA) and inspection under light microscope. The isolates were further characterized and identified with standard PCR using species specific primers developed for *F. langsethiae*, *F. sprotrichioides* and *F. poae* (Wilson et al., 2004; Parry and Nicholson., 1996). Furthermore a standard PCR was used for the identification of two subgroup IGS types in *F.langsethiae* isolates. These two subgroups are differentiated by the presence of a long deletion in IGS region in subgroup I (Konstantinova and Yli-Mattila., 2004).

2.3. Primers design

All available DNA nucleotide sequences of *F. langsethiae* in Genbank at NCBI (National Center for Biotechnology Information) were investigated and compared with DNA homologous regions of the closely related species of *F. sporotrichioides*, *F. sibiricum* and *F. poae*, in order to identify variable genomic regions, which could potentially be targeted as primer sites for LAMP PCR. Among those, the nucleotide sequences of the two trichothecene genes T*RI1* and *TRI16* and their intergenic region were selected for further analysis and designing of LAMP primers. DNA sequences of *F. langsethiae* (Genbank accession number HQ594538.1, HQ594539.1 and HQ594543.1) and *F. sporotrichiodes* (Genbank accession number HQ594537.1) were used. Multiple Sequence Alignment performed with ClustalW2 http://www.ebi.ac.uk/Tools/msa/clustalw2/. Several different primer sets were designed using either Primer Explorer V. 4 software (http://primerexplorer.jp/e/) (Eiken Chemicals Co., LTD, Tokyo, Japan) or LAMP Designer software (PREMIER Biosoft, USA). The BLAST®-algorithm function under (http://www.blast.ncbi.nlm.nih.gov/) was used for searching the GenBank for matches with other DNA sequences.

2.4. DNA extraction

Fungal cultures were grown in 50 ml liquid GYEP medium (0.3% glucose, 0.1% yeast extract, and 0.1% peptone) for up to five days. Cultures were centrifuged and the resulting mycelia were kept at -20ºC until farther use. DNA was extracted according to protocol described by Lee and Taylor (1990) or by using two commercial kits, Plant DNA extraction kit (Qiagene) and GeneJet Genomic DNA Purification kit (ThermoFisher Scientific). The DNA quality and quantity was evaluated in 1% agarose gel electrophoresis.

2.5. LAMP PCR

2.5.1. The LAMP master mix and reaction conditions

Several DNA polymerases with strand displacement activity were tested in this study. These included Bst2 from NEB and Lucigen, WarmStart Bst2 from NEB and Bsm DNA polymerase from ThermoFisher Sceintific. The colorimetric detection of LAMP reaction was initially evaluated by using two metal indicators of Calcein dye and Hyroxy Naphthol Blue (HNB), however HNB was used as the standard colorimetric dye. The LAMP reaction consisted of 1X polymerase buffer, 1,6 mM FIP, 1,6 mM BIP, 0,2 mM F3, 0,2 mM B3, 0,8 LoopF, 0,8 LoopB, 1,4 mM of each of dNTP (Thermo Fisher), 8 mM Mg+ supplied by $MgCl_2$ or $MgSO_4$, HNB (120 µM) or Calcein (50 µM), 0,2 M Betaine (Sigma), 2 µl DNA and H_2O to obtain a final reaction volume of 25 µl. To select the optimum reaction temperature, the reaction was run at different temperatures between 60 and 65º C for 1 hr in a thermo cycler (DNA Engine® Peltier Thermal Cycler).

2.5.2. Specificity of F. langsethiae LAMP PCR

Five different primer sets were preliminary evaluated in two isolates of *F. langsethiae* and two isolates of *F. sporotichioides*. Then only two sets of primers were selected for further screening of more isolates. Later on only one set of primers were thoroughly screened in all available *Fusarium* isolates in this study, which included a broad range of *F. langsethiae* and other closely related *Fusarium* spp or those commonly associated with cereal grains.

2.5.3. Sensitivity of F. langsethiae LAMP PCR

DNA from *F. langsethiae* ARS53439 was subjected to 10-folds serial dilutions from 12.5 ng/µl up to 1.25 fg/µl and 2 µl of the dilution preparations were added into each LAMP reaction. LAMP reaction was performed at 65 C for 1 h. The positive amplification was evaluated by the colour change of HNB and amplicons were further analyzed by electrophoresis in 2% agarose gel.

3. Results

3.1. Isolation and identification of *Fusarium* spp from oats kernels

Fungal isolation from the nine oats seed batches resulted in approx. 30 different *Fusarium* isolates, of which 6 were isolated from one seed batch of cv. Belinda (Lantmännen spannmål) and morphologically identified as *F. langsethiae.* These isolates had a flat fungal colony on agar with powdery appearance which is typical phenotypic growth for this *Fusarium* species (Fig. 1A). Production of globose to napiform microconidia was observed under the microscope, however macrocondia were totally absent, which is also considered as a typical morphological criterion for *F. langsethiae* (Fig. 1B) (Torp and Nirenberg. 2004). In addition, the IGS types of the 6 *F. langsethiae* isolates were also determined (Fig. 2). The rest of the tested seed batches were on the hand dominated by fast growing *Fusarium* spp e.g. *F. graminearum* and *F. poae* (results not shown).

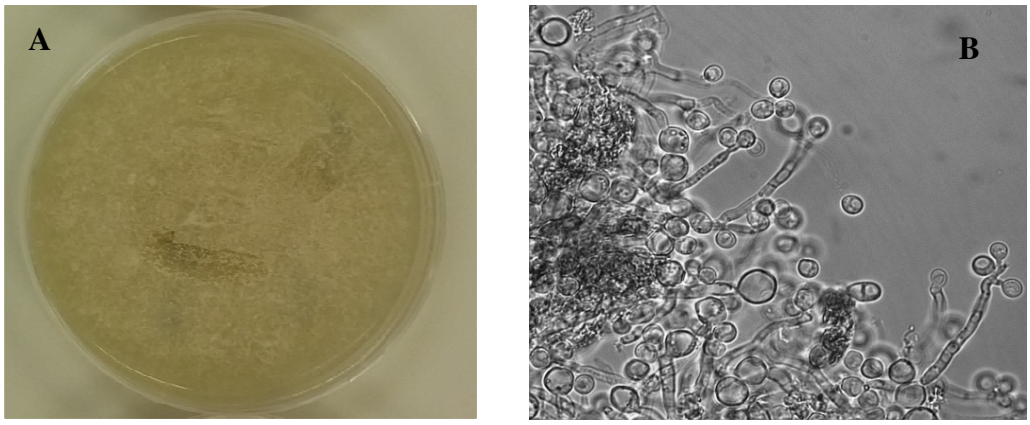


Figure.1. **A**: Fungal growth of *F. langsethiae* on PDA; **B**: Globose to napiform microconidia of *F. langsethiae*.

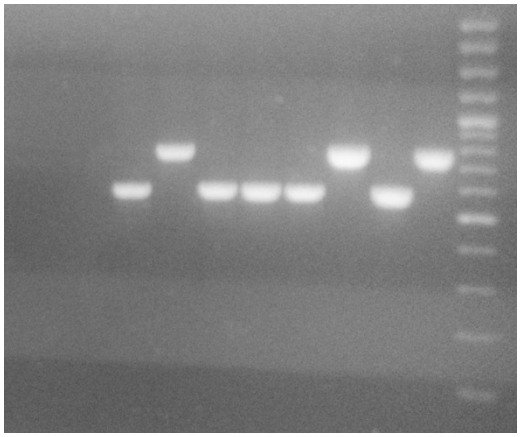


Figure.2. IGS-type (I or II) of some of *F. langsethiae* and one *F. sporotichioides* characterized by standard PCR using primer pair of CNL12/PulvIGSr. Left to right: JF2015/14 (II), JF2015/02 (I), JF2015/14 (II), JF2015/30 (II), F2015/32 (II), Fl04/02 (I), Fl041/11 (II), and VI 01304.

3.2. Primers design

Nucleotide sequences of the trichothecene C-8 hydroxylase cytochrome P450 monooxygenase gene (*TRI1*) and trichothecene C-8 acyl transferase (*TRI16*) gene and the their intergenic region of *F. langsethiae* were selected as a potential genomic region for designing LAMP primers. The two primer design programs used for this purpose suggested a number of LAMP primers including F3, B3, FIP and BIP. Two forward and reverse Loop primers were only constructed by LAMP Designer program. Two primer sets from Primer explorer program and 3 primer sets from Primer Design software designated as Fl1, Fl2 and Fl-LAMP were tested in this study. Sequence of primer set Fl1 shown as an example (Table 1).

Table 1. Primer set Fl1 designed based on the intergenic region between T*RI1* and *TRI16* genes

Primer set		Primers sequence (5′-3′)	Position	Software
F1	F3	ACTAAGGGCTGAGCTTTAATAC	3534-3555	LAMP Designer
	B3	AGCAGTACTTAGGTGTATTCG		
	FIP	ATTATAGTAAGCGTGCCCCAGGAGCCTC ATTGTCTGTCCT		
	BIP	TTAACTGGGACAATGGGCTTCCTCGGGCT AACTGAAAGTAGTA		
	LoopF	TCATGCGGTTCTTTATACGAGT		
	LoopB	GCATGTTTGGGGATTACTGC		

3.3. LAMP PCR

3.3.1. Specificity of LAMP

The two LAMP primer sets designed by the Primer explorer did not amplify *F. langsethiae* DNA with both Calcein and HNB (results not shown). All three primer sets designed by LAMP Designer software resulted in positive LAMP reactions. Two primer sets (Fl1 and Fl2) gave similar reactions in the first screenings, therefore, Fl1 in addition to primer set FL-LAMP were selected for further testing. Amplification with primer set Fl1 was successful both in Calcein and HNB LAMP (Fig. 3), however positive LAMP reaction was also noticed in two *F. sporotrichiodes* isolates. Primer set Fl-LAMP successfully amplified LAMP products in the first set of *F. langsethiae* isolates too. This primer set was further screened with DNA from all other isolates of *Fusarium* spp available in this study. LAMP reaction with all other *F. langsethiae* and also isolates belonging to *F. sibiricum* were exclusively positive and the amplicons were easily detectable by the colour change of HNB to sky blue colour. This primer set did not react with DNA from any other Fusarium species tested in this study (Table 2).

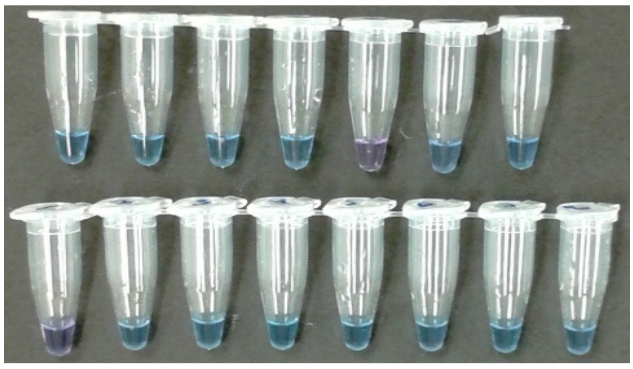


Figure.3. HNB LAMP reaction with primer set Fl1. Positive reaction with *F. langsethiae* (tubes with blue colour); negative reaction with water controls (purple colour).

3.3.2. Sensitivity of F. langsethiae LAMP with primer set Fl-LAMP

10 folds dilution series of DNA from *F. langsethiae* NRRL 53439 were tested with primer set FL-LAMP. The DNA used in reaction ranged approximately from 250 pg up to 2.5 fg (Dilutions included 250 pg, 25 pg, 2.5 pg, 250 fg, 25 fg, 2,5 fg) (Fig. 4. A). The LAMP products of these reactions were verified with agarose gel electrophoresis (Fig. 4. B). When DNA was freshly diluted and used in reaction the detection limit was at 250 fg, and below that no LAMP product was formed. The level of detection was reduced to 25 pg (100 times less sensitivity) after 2 times freezing and thawing of DNA solutions.

Table 2 Fungal isolates used in this study and their reaction in *F. langsethiae* LAMP PCR

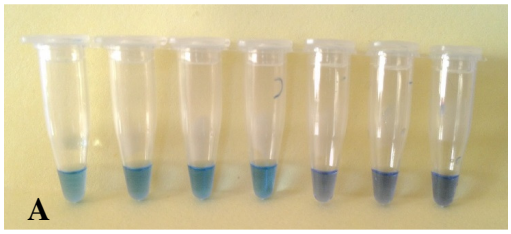
	Accession no.	**Name**	**Origin**	**LAMP-HNB**	Fl-IGS type	**Country**
1	NRRL 43641	*F. armeniacum*		-		USA
2	06:03	*F. culmorum*	T. Börjesson	-		Sweden
3	06:06	*F. graminearum*	T. Börjesson	-		Sweden
4	MAFF 240372	*F. kyushuense*	NIAS[1]	-		Japan
5	Fl04/02	*F. langsethiae*	S. Edwards	+	I	UK
6	Fl041/11	*F. langsethiae*	S. Edwards	+	II	UK
7	Fl04/01	*F. langsethiae*	S. Edwards	+	I	UK
8	Fl026/1	*F. langsethiae*	S. Edwards	+	II	UK
9	CC328	*F. langsethiae*	P. Nicholson	+	II	UK
10	CC321	*F. langsethiae*	P. Nicholson	+	II	UK
11	CC329	*F. langsethiae*	P. Nicholson	+	I	UK
12	CC324	*F. langsethiae*	P. Nicholson	+	II	UK
13	M560	*F. langsethiae*	NFA[2]	+	I	Sweden
14	M561	*F. langsethiae*	NFA	+	I	Sweden
15	60A/2010	*F. langsethiae*	NFA	+	II	Sweden
16	M562	*F. langsethiae*	NFA	+	II	Sweden
17	VI 01268	*F. langsethiae*	NFA	+	II	Sweden
18	VI 01287	*F. langsethiae*	NFA	+	II	Sweden
19	VI 01274	*F. langsethiae*	NFA	+	I	Sweden
20	VI 01288	*F. langsethiae*	NFA	+	II	Sweden
21	VI 01281	*F. langsethiae*	NFA	+	I	Sweden
22	VI 01272	*F. langsethiae*	NFA	+	I	Sweden
23	49A/2010	*F. langsethiae*	NFA	+	II	Sweden
24	54d/2010	*F. langsethiae*	NFA	+	I	Sweden
25	MFG11019	*F. langsethiae* I	T. Gagkaeva	+	I	Russia, Central, Orel
26	MFG11003	*F. langsethiae* II	T. Gagkaeva	+	I	Russia, South-European Kransnodar
27	CBS113234	*F. langsethiae*	CBS[3]	+	I	Norway (ex-type)
28	NRRL 53409	*F. langsethiae*	ARS[4]	+	I	Finland
29	NRRL 53410	*F. langsethiae*	ARS	+	I	Finland
30	NRRL 53439	*F. langsethiae*	ARS	+	I	Russia
31	NRRL 53411	*F. langsethiae*	ARS	+	II	Finland
32	NRRL 53417	*F. langsethiae*	ARS	+	I	Finland
33	NRRL 53419	*F. langsethiae*	ARS	+	I	Finland
34	JF-2015/02	*F. langsethiae*	This study	+	I	Sweden
35	JF-2015/1	*F. langsethiae*	This study	+	II	Sweden
36	JF-2015/14	*F. langsethiae*	This study	+	II	Sweden

[1]NIAS (NIAS Genebank, Japan); [2]NFA (National Food Agency, Sweden); [3]CBS (CBS fungal collection, The Netherland); [4]ARS (ARS culture collection, USA); Positive LAMP reactions indicated as (+); negative LAMP reactions indicated as (-).

Table 2 (Continuation) Fungal isolates used in this study and their reaction in *F. langsethiae* LAMP PCR

37	JF-2015/14	*F.langsethiae*	This study	+	II	Sweden
38	JF-2015/30	*F.langsethiae*	This study	+	II	Sweden
39	JF-2015/32	*F.langsethiae*	This study	+	II	Sweden
40	JF-2015/3	*F. poae*	This study	-		Sweden
41	JF-2015/4	*F. poae*	This study	-		Sweden
42	JF-2015/05	*F. poae*	This study	-		Sweden
43	JF-2015/11	*F. poae*	This study	-		Sweden
44	JF-2015/17	*F. poae*	This study	-		Sweden
45	JF-2015/18	*F. poae*	This study	-		Sweden
46	JF-2015/37	*F. poae*	This study	-		Sweden
47	JF-2015/35	*F. poae*	This study	-		Sweden
48	JF-2015/38	*F. poae*	This study	-		Sweden
49	NRRL 53421	*F. sibiricum*	ARS	+		Siberia
50	NRRL 53423	*F. sibiricum*	ARS	+		Siberia
51	NRRL 53427	*F. sibiricum*	ARS	+		Siberia
52	NRRL 53433	*F. sibiricum*	ARS	+		Siberia
53	MFG11012	*F. sibiricum*	T. Gagkaeva	+		Far East, Vladivostok
54	MFG11005	*F. sibiricum*	T. Gagkaeva	+		Siberia
55	VI 01304	*F. sporotrichioides*	NFA	-		Sweden
56	VI 01290	*F. sporotrichioides*	NFA	-		Sweden
57	VI 01292	*F. sporotrichioides*	NFA	-		Sweden
58	VI 0310	*F. sporotrichioides*	NFA	-		Sweden
59	VI 01314	*F. sporotrichioides*	NFA	-		Sweden
60	F627	*F. sporotrichioides*	P. Nicholson	-		France
61	NRRL 36461	*F. tricinctum*	ARS	-		Denmark
62	NRRL 54840	*F. tricinctum*	ARS	-		Switzerland

[1]NIAS (NIAS Genebank, Japan); [2]NFA (National Food Agency, Sweden); [3]CBS (CBS fungal collection, The Netherland); [4]ARS (ARS culture collection, USA); Positive LAMP reactions indicated as (+); negative LAMP reactions indicated as (-).



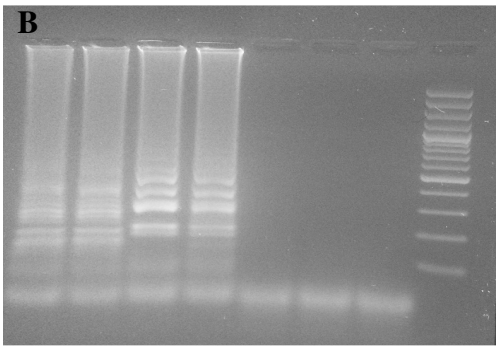


Figure.4. **A**. Loop-Mediated Isothermal Amplification of *F. langsethiae* NRRL 53439 with primer set of FL-LAMP. 10 fold serial dilutions of DNA was prepared in water, including 250 pg, 25 pg, 2.5 pg, 250 fg, 25 fg, 2,5 fg DNA per reaction (from left to right). The detection limit was at 250 fg. **B**. LAMP products were analyzed in 2% agarose gel.

4. Discussion

F. langsethiae is commonly found in Swedish cereals, especially oats. It is also reported as the main producer of T-2 and HT-2 in European cereal production (Edwards et al., 2009). Despite the frequency of *F. langsethiae* occurrence, its epidemiology is still unclear. Efforts have been made to identify factors that can influence disease development and T-2 and HT-2 mycotoxins accumulation in oats. Weather conditions (Hjelkrem et al., 2014) and agricultural practices (Hofgaard et al., 2014) were among those studied factors. Accumulation of DON was found to be generally correlated with weather conditions around flowering, however, few correlations were found between weather conditions and T-2 and HT-2. Cultural practices such as crop rotation, tillage practice and straw management were also investigated. More specifically, the amount of straw on the soil surface was used to predict the infection by *F. langsethiae* later during the growing season. Interestingly, although high contents of T-2 and HT-2 in oats kernels were found, only 1 % of *F. langsethiae* was detected in crop resides (Hofgaard et al., 2014). This is also puzzling, since it is generally known that removal of straw from the soil surface reduces H-2 and HT-2. Questions regarding, the source of initial inoculum and how oats plants become infected in the field, are remained to be answered. Tools such as LAMP PCR can help in understanding the epidemiology of this fungus. The method is quick, relatively cheap, highly specific and sensitive due to the use of 4-6 primers in the same reaction. The practical implementation of LAMP PCR in detection and quantification of plant fungal pathogens with minimal sample preparation was highlighted in some studies. For example, Moradi et al (2013) detected *Verticillium dahlia* in soil and Niessen and Vogel (2010) detected *F. graminearum* in cereal grains with simple treatments and without DNA extractions, which will save both time and resources.

In this study, a primer set FL-LAMP, was designed based on the intergenic region between the two trichothecene T*RI1* and *TRI16* genes. Despite the fact that this region varies in length within *F. langsethiae* (758-2299 bp) (Yli-Mattila et al., 2011), this primer set successfully amplified DNA from all tested *F. langsethiae* isolates. To insure its specificity, a collection of 35 *F. langsethiae* isolates obtained from different research groups, isolated from oats kernels and obtained from type culture collections were used. Specificity of the primer set was also tested against a broad range of 27 isolates representing different *Fusarium* species. The two closely related *Fusarium* spp to *F. langsethiae* are *F. sporotrichioides* and *F. poae*. The former is morphologically different, but has a similar mycotoxin profile as that of *F. langsethiae*, where as the latter has spore morphology similar to *F. langsethiae*, but is known to produce DON (Imathiu et al., 2013). FL-LAMP was highly specific to *F. langsethiae* hence none of the six tested *F. sporotrichioides* or the nine *F. poae* isolates detected in LAMP PCR. Furthermore, other T-2 and HT-2 producers such as *F. armeniacum* and *F. kyushuense* and DON producers such as *F. graminearum* and *F. culmorum* were not detected either in the LAMP reactions using primer set FL-LAMP.

Another *Fusarium* species, *F. sibiricum* was included in this study. DNA from the six tested isolates was also amplified by primer set FL-LAMP. *F. sibiricum* was described as a new species in 2011 and it was shown to have similar morphology as *F. langsethiae*, besides its ability to produce high levels of T-2 (Yli-Mattila et al., 2011). This species differ in few morphological characteristics from *F. langsethiae* e.g. the bent cylindrical monophialides which are present in *F. langsethiae* but not in *F. sibiricum* (Yli-Mattila et al., 2011). The two species differ however, in their geographical distribution, where *F. sibiricum* is restricted to Siberia and Far East Russia and *F. langsethiae* is restricted to Europe and West Siberia (Yli-Mattila et al., 2011). The IGS-typing performed in this study revealed that none of the European isolates belonged to this species. Since both *F. langsethiae* and *F. sibiricum* are

detected by primer set FL-LAMP and due to their prevalence in two different geographical regions, the primer set is useful for studying both species.

As other molecular diagnostic methods, LAMP PCR has also some drawbacks. The high sensitivity of LAMP PCR though regarded as an advantage, contamination with previous LAMP PCR products may occur in new reactions, resulting in false positives even in water control without DNA template (Sano and Itano., 2010). Contamination may also occur due to cross-contamination with DNA or fungal material (Storari et al., 2013). The positive LAMP reaction with *F. sporotrichiodes* using primer set Fl1 could be due to such cross-contamination.

Isolation of *F. langsethiae* from cereal grains was reported to be low. This is partially due to the slow growth rate of the fungus, which can be easily overgrown by other fast growing species such as *F. graminearum* (Torp and Nirenberg 2004).We succeeded in isolating few *F. langsethiae* isolates from one seed batch of cv. Belinda. This seed batch in particular was contaminated with T2 and HT-2 (Dr. Thomas Börjesson, personal communication). Our results are in agreement with previous studies (Imathiu et al. 2013; Lukanowski and Sadowski, 2008). Imathiu et al. (2013) isolated *F. langsethiae* from only 5-10% of seeds from oats and wheat seed batches with >500 µg/kg and >50 µg/kg T-2 and HT-2 respectively, but none from seed batches with lower T-2 and HT-2 content. We also realized that unlike other tested seed batches, which were highly dominated by fast growing *Fusarium* spp, this seed batch was dominated by slow growing isolates with flat colonies and powdery appearance on agar. In general, DON content is not correlated with T-2 and HT-2 (Hofgaard et al., 2014), which may also reflect the occurrence of the producing *Fusarium* species in cereal grains. Before the fungus was formally described as *F. langsethiae* in 2004 by Torp and Nirenberg, it was named as "powdery *F. poae*" due to its distinctive powdery appearance which is considered as an important criterion for the identification of the fungus (Imathiu et al. 2013). Tentative identification of the isolates as *F. langsethiae,* according to the above mentioned morphological characteristics was further confirmed by species specific primers and their IGS types were determined by PCR.

Conclusion:

We developed a LAMP PCR with specific primer set that can be used to detect and quantify the fungal DNA in soil and plant material and hence it can be implemented in further studies to understand the epidemiology of *F. langsethiae*.

Reference group meeting

The reference group consisted of Tomas Börjesson (Agroväst), Anders Jonsson (SLU, Skara) and Kenneth Alness (Lantmännen BioAgri). The group had a physical meeting on the 25th of august 2014 at Lantmännen BioAgri, Uppsala, where the achieved results and planned research activities were discussed.

Deviation from the project plan

The part concerning isolation of *Fusarium* spp from seedlings grown in the climate chamber was not performed since enough isolates were obtained by isolation from oats kernels. This in addition to several *Fusarium* spp obtained from different research groups and culture collections.

Plan for scientific deliverables

A manuscript is under preparation and will be submitted to International Journal of Food Microbiology.

References

Divon, H., Razzaghian, H., Udnes-Aamot, J., Sletner, H & Klemsdal, S. 2012. *Fusarium langsethiae* (Torp and Nirenberg), investigation of alternative infection routes in oats. European Journal of Plant Pathology. 132:147-161.

Edwards, S., Barrier-Guillot, B., Clasen, P. E., Hietaniemi, V& Pettersson, H. 2009. Emerging issues of HT-2 and T-2 toxins in European cereal production. World Mycotoxin J. 2**,** 173–179.
Fredlund, E., Gidlund, A., Pettersson, H., Olsen, M & Börjesson, T. 2010. Real-time PCR detection of Fusarium species in Swedish oat and correlation to T-2 and HT-2 toxin content. World Mycotoxin Journal. 3: 77-88.
Fredlund, E & Lindblad, M. 2014. Fusariumsvampar och dess toxiner i svenskodlad vete och havre - rapport från kartläggningstudie 2009-2011. Livsmedelsverkets rapportserie nr 2/2014. ISSN 1104-7089: 1-37.
Goto, M., Honda, E., Ogura, A., Nomoto, A and Hanaki, KI. 2009. Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxyl naphthol blue. Biotechniques. 46:167–172.
Hjelkrem, A-G., Torp, T., Bordal, G., Aamot, H.U., Nordskog, B., Strand, E and Hofgaard, I. 2014. Influence of weather conditions at different plant growth stages on mycotoxin content in oat. NJF Report. Volume 8, page 28.
Hofgaard, I., Seehusen, T., Aamot, H.U., Abrahamsen, U., Razzaghian, J., Le, V., Riley, H., strand., E., Gauslaa, E., Åssveen, M., Brurberg, M.B., Steen., H.S and Bordal, G. 2014. Influence of agricultural practices on prevalence of *Fusarium* and mycotoxins. NJF Report. Volume 8, page 27.
Imathiu, S. M . 2008. Fusarium langsethiae Infection and Mycotoxin Production in Oats. Newport, UK, Harper Adams University College. PhD Thesis.
Imathiu, S. M., Hare, M. C., Ray, R. V., Back, M. & Edwards, S. G. 2010. Evaluation of pathogenicity and aggressiveness of F. langsethiae on oat and wheat seedlings relative to known seedling blight pathogens. European Journal of Plant Pathology. 126:203-216.
Imathiu, S., Edwards, S., Ray, R and Back, M. 2013. *Fusarium langsethiae* – a HT-2 and T-2 Toxins Producer that Needs More Attention. J Phytopathol. 161:1–10.
Konstantinova, P and Yli-Mattila, T., 2004. IGS-RFLP analysis and development of molecular markers for identification of *Fusarium poae*, *Fusarium langsethiae*, *Fusarium sporotrichioides* and *Fusarium kyushuense*. International Journal of Food Microbiology. 95: 321–331.
Langseth, W and Rundberget, T. 1999. The occurrence of HT-2 toxin and other trichothecenes in Norwegian cereals. Mycopathologia. 147: 157–165.
Lee and Taylor. 1990. Isolation of DNA from fungal mycelia and single spores. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 282–287.
Lukanowski, A and Sadowski, C. 2008. *Fusarium langsethiae* on kernels of winter wheat in Poland – occurrence and mycotoxigenic abilities. Cereal Research Communications. 36 (Suppl. B):453–457.
Moradi, A., Almasi, M.A., Jafary, H & Mercado-Blanco, J. 2013. A novel and rapid loop-mediated isothermal amplification assay for the specific detection of *Verticillium dahliae*. J of Applied Microbiol. 1-13.
Niessen, L & Vogel, R. F. 2010. Detection of *Fusarium graminearum* DNA using a loop-mediated isothermal amplification (LAMP) assay. International Journal of Food Microbiology. 140: 183-191.
Niessen, L. 2013. Loop-Mediated Isothermal Ampli fi cation-Based Detection of *Fusarium graminearum*. Louise O'Connor and Barry Glynn (eds.), *Fungal Diagnostics: Methods and Protocols*, Methods in Molecular Biology, vol. 968, Springer Science+Business Media, New York.
Niu, J-H., Guo Q-X., Jian, H., Chen, C-L., Yang, D., Liu, Q & Guo, Y-D. 2011. Rapid detection of *Meloidogyne* spp by LAMP assay in soil and roots. Crop Protection. 30: 1063-1069.
Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T. 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Research 28: E63.
Parry, D.W and Nicholson, P. 1996. Development of a PCR assay to detect *Fusarium poae* in wheat. Plant Pathology. 45: 383–391.
Parry, D.W., Jenkinson, P. & McLeod. 1995. Fusarium ear blight (scab) in small grain cereals – a review. Plant Pathology. 44: 207-238.
Peng, J., Zhang, J., Xia, Z., Li, Y., Huang. J and Fan, Z. 2012. Rapid and sensitive detection of banana bunchy top virus by loop-mediated isothermal amplification. J Virol Methods. 185: 254–258.
Sano, A and Itano, E.2010. Applications of Loop-Mediated Isothermal Amplificaton Methods (LAMP) for Identification and Diagnosis of Mycotic Diseases: Paracoccidioidomycosis and Ochroconis gallopava infection. Gherbawy and Voigt (eds). Molecular Identification of Fungi. Springer-Verlag, Berlin Heidelberg.
Storari, M., von Rohr, R,, Pertot, I., Gessler, C and Broggini, G.A. 2013. Identification of ochratoxin A producing Aspergillus carbonarius and A. niger clade isolated from grapes using the loop-mediated isothermal amplification (LAMP) reaction. Journal of Applied Microbiology. 114: 1193-1200.
Torp, M and Nirenberg, H.I. 2004. Fusarium langsethiae sp. nov. on cereals in Europe. Int. J. Food Microbiol. 95: 247–256.
Thrane, U., Adler, A., Clasen, P., Galvano, F., Langseth, W., Lew, H., Logrieco, A., Nielen, K. F & Ritien, A. 2004. Diversity in metabolite production by Fusarium langsethiae, Fusarium poae and Fusarium sporotrichioides. Int J Food Microbiol. 95: 257–266.
Widestrand, J and Pettersson, H. 2000. Cytotoxicity screening of trichtecens using BrdU colorimetric bioassay. Abstract 6th European Fusarium seminar Third COST 835 workshop, Berlin, Germany 11-16 September 2000.
Wilson, A., Simpson, D., Chandler, E., Jennings, P., Nicholson, P. 2004. Development of PCR assays for the detection and differentiation of Fusarium sporotrichioides and *Fusarium langsethiae*. FEMS Microbiology Letters. 233: 69–76.
Yli-Mattila, T., Ward, T.J., O'Donnell, K., Proctor, R.H., Burkin, A., Kononenko, G.P., Gavrilova, O.P., Aoki, T., McCormick, S and Gagkaeva, T. 2011. *Fusarium sibiricum* sp. nov, a novel type A trichothecene-producing Fusarium from northern Asia closely related to *F. sporotrichioides* and *F. langsethiae*. International Journal of Food Microbiology. 147: 58–68.