Agronomy Research **9** (Special Issue II), 521–526, 2011

Detection of Trichothecene-producing *Fusarium* Species in Cereals in Northern Europe and Asia

T. Yli-Mattila

Molecular Plant Biology, Department of Biochemistry and Food Chemistry, University of Turku, FI-20014 Turku, Finland.; e-mail: tymat@utu.fi

Abstract. Several toxigenic trichothecene-producing and nonproducing *Fusarium* species are involved in *Fusarium* head blight, which reduces both crop yield and quality in cereals. Climate change has altered crop production in many countries, and this in turn influences the pathogen populations. E.g. in northern areas a risk will be new toxigenic *Fusarium* species spreading to the north due to higher temperatures and the increased use of alternative hosts, such as maize, winter barley and winter oats. Traditional identifications and classifications of *Fusarium* species have been used for grouping isolates to species and grouping species according to shared morphological and cultural characteristics. During the last years researchers have started to use alternative ways for species identification and classification based on molecular data and phylogenetic analyses. The best way to identify and classify *Fusarium* isolates is the polyphasic approach by using all available characters.

Key words: deoxynivalenol, *Fusarium* head blight, *F. graminearum*, *F. langsethiae*, *F. sibiricum*, *F. sporotrichioides*, nivalenol, T-2 toxin, trichothecene

INTRODUCTION

Fusarium species are the most important phytopathogenic and toxigenic fungi in Nordic countries and globally. Several *Fusarium* species are involved in *Fusarium* head blight (FHB), which reduces both yield and quality in cereal crops. FHB was first described in England and the Russian Far East in the 1880's, but *Fusarium* outbreaks had already started much earlier, e.g. in the Far East. Since then FHB has increased worldwide (McMullen et al., 1997; Bottalico & Perrone, 2002; Goswami & Kistler, 2004; Desjardins, 2006; Yli-Mattila, 2010).

Fusarium toxins can be divided into those which already are under international regulation, i.e. deoxynivalenol (DON), zearalenone and fumonisins and to new emerging varieties, such as beauvericin, enniatins and culmorins. European regulations for nivalenol (NIV), T-2 toxin and HT-2 toxin, also produced by *Fusarium* species, are under evaluation. Chemically, trichothecenes can be divided into type A (e.g. T-2 and HT-2) and type B (e.g. NIV and DON) and their mono- and di-acetylated derivatives (Ueno, 1983).

The greatest tragedy due to the *Fusarium* toxins took place in former Soviet Union before and during World War II, when harvesting was delayed and overwintered mouldy grains were consumed. In the Orenburg region near the Ural River more than 10% of the population were affected by the disease called alimentary toxic aleukia and mortality was high. The ATA (Alimentary Toxic Aleukia) syndrome in Soviet Union

was probably due primarily to T-2 toxin (Sarkisov, 1954; Joffe, 1986; Yli-Mattila et al., 2011). In the 1960's high levels of T-2 toxin were also found in maize in USA (Mirocha & Patre, 1973).

In Nordic countries oats are more severely affected by mycotoxins than wheat and barley, which may result high DON and T-2/HT-2 levels (Yli-Mattila, 2010). In the 2000's, T-2 toxin levels have been rising in oats in northern Europe (Yli-Mattila et al., 2008, 2009a; Edwards et al.; 2009, Fredlund et al., 2010). In 2010 Norway had to import oat seed due to high *Fusarium* infection levels in Norwegian seed which inhibited germination. The increase of *Fusarium* toxin levels may be connected to climate change. The greatest oat producers in the world are in the Nordic countries. For example, in Finland about 8% of oats are used for human food (including baby food), thus the quality of the raw material for oat products is important.

Until now, only a few sources of resistance to FHB in oats have been demonstrated (Björnstad, et al., 2006; Tekauz et al., 2008; Gagkaeva et al., 2010). One of the problems in breeding resistant cultivars in oats and barley is the lack of clear visual symptoms, which could be scored to assess the level of FHB. That is why other parameters connected to mycotoxins, such as *Fusarium* DNA, should be evaluated. In the present review paper I will concentrate on trichothecene-producing *Fusarium* species.

Type A and B trichothecene-producing *fusarium* species

DON is the most important trichothecene in Europe and Asia. It is produced by type B trichothecene-producing species of the *F. graminearum* species complex and *F. culmorum*. In northern Europe the highest DON levels have been found in oats (Yli-Mattila, 2010). Nivalenol, which is also a type B trichothecene, is produced by *F. poae, F. cerealis* and NIV genotypes of *F. graminearum* species complex and *F. culmorum*.

T-2 toxin is the main type A trichothecene. The main *Fusarium* species producing T-2 toxin have been difficult to identify. The researchers have agreed that they belong to the *Sporotrichiella* section of the *Fusarium* species. In the Soviet Union the *Fusarium* species causing ATA was identified as *F. sporotrichioides* (Sarkisov, 1954), but for a long time the researchers occasionally thought that both *F. sporotrichioides* and *F. poae* isolates of *Sporotrichiella* section or even *F. tricinctum* might be effective in producing T-2 toxin (Joffe, 1986; Burkin et al., 2008) due to identification problems. Since then it has been found that T-2 producing *F. poae* isolates (Torp & Nirenberg, 2004; Yli-Mattila et al., 2011) differ from typical *F. poae* isolates, which do not produce high levels of T-2 toxin. Based on these investigations it seems that in Europe a new species *F. langsethiae* is the main T-2-producer, while in the Russian Far East another new species *F. sibiricum* is also involved in T-2 production together with *F. sporotrichioides*. There are two subgroups or populations of *F. langsethiae*, which are morphologically similar to *F. poae*, but phylogenetically are closer to *F. sporotrichioides* (Konstantinova & Yli-Mattila, 2004; Yli-Mattila et al., 2004; 2011). T-2 toxin production is affected by water activity and temperature and the toxin is rapidly metabolized to HT-2 toxin (Jestoi et al., 2004; 2008; Medina & Magan, 2011).

DNA extraction, detection and quantification

Different commercial kits are available for DNA extraction and should be optimised for the fungal DNA from plant material. The quality and quantity of DNA can be estimated by gel electrophoresis and spectrophotometer or by different

fluorescence methods. Fluorescence and gel electrophoresis are more specific than spectrophotometric analyses for quantification of DNA from grains. The amount of fungal DNA should be compared to the amount of total DNA obtained from plants (Yli-Mattila et al., 2011). Internal controls (Waalwijk, 2004; Kulik, 2011) should also be used in order to compensate for the effect of PCR inhibition.

TaqMan real-time PCR can be used for quantifying DNA of *Fusarium* species in plants and the correlation between *Fusarium graminearum* DNA and DON levels have been good (e.g. Waalwijk et al., 2004; Yli-Mattila et al., 2008). The advantage of TaqMan primers based on ribosomal DNA is that there are numerous ribosomal DNA copies in the genome, making the reaction more sensitive. Each *Fusarium* species has a species-specific mycotoxin profile, which means that in several cases there is a good correlation between *Fusarium* DNA and mycotoxins produced by them (Yli-Mattila et al., 2008).

Correlation between *fusarium* toxins and DNA levels

A highly significant correlation has been found between *F. graminearum* DNA and DON in Finnish oat, barley and spring wheat (Yli-Mattila et al., 2008, 2009a). *F. culmorum* seems to have a significant role in DON production only in barley. This is probably due to the fact that Finnish *F. graminearum* isolates are usually more effective in producing DON than *F. culmorum* isolates (Jestoi et al., 2008). The highly significant correlation between *F. graminearum*/*F. culmorum* DNA and DON is in agreement with previous results of Waalwijk et al. (2004) and Nicholson et al. (2003) in Europe and Sarlin et al., (2006) in North America. Highly significant correlations were also found between *F. langsethiae/F. sporotrichioides* DNA and HT-2/T-2 toxins and *F. avenaceum* DNA and enniatins/moniliformin (Yli-Mattila et al., 2008, 2009a). A slighter correlation has been found in Finland between NIV and *F. poae* DNA levels in barley and oats, while in Luxembourg *F. culmorum* seems to be the main NIV producer in winter wheat (Pasquali et al., 2010).

***Fusarium* DNA levels during growing season**

F. langsethiae is one of the pioneer *Fusarium* fungi (together with *F. poae*) in cereals during flowering (Wilson et al., 2004; Jestoi et al., 2008), especially in oats. It is a weak competitor, easily overgrown and replaced by other *Fusarium* species based on qPCR results. *F. poae* and *F. langsethiae* may be competitors in cereal grains, because it has been found that tillage with ploughing increases the amount of *F. poae*, while the amount of *F. langsethiae* decreases as compared to direct drilling (Yli-Mattila, 2010). After flowering, *F. graminearum, F. culmorum, F. sporotrichioides* and *F. avenaceum* cause higher infection than *F. langsethiae* (Yli-Mattila et al., 2009a).

F. *graminearum* species complex and multilocus genotyping

Multilocus genotyping and molecular phylogenetics are very useful in identification of *Fusarium* isolates (O'Donnell et al., 2000, 2008). *F. graminearum* (sexual state *Gibberella zeae*) is the most important *Fusarium* species in central Europe and in large areas in North America and Asia (O'Donnell et al., 2000; 2008; Gagkaeva and Yli-Mattila, 2004; Goswami & Kistler, 2004; Yli-Mattila et al, 2009a,b). In recent years *F. graminearum* has been spreading to the north in Europe in the Netherlands and England (e.g. Waalwijk et al., 2003; Nicholson et al., 2003), Norway (Elen et al., 2007, personal communication), Poland (Stepien et al., 2008) and north-western Russia

(Gagkaeva et al., 2009) and has been replacing the closely related *F. culmorum*, which is less effective in producing DON. *F. graminearum* has been divided into 13 species, of which one, still called *F. graminearum* (=lineage 7) is dominant in Europe and North America (O'Donnell et al., 2000; 2004; Gagkaeva & Yli-Mattila, 2004; Yli-Mattila et al., 2009b). In North America dramatic changes have been found in the toxin chemotypes of *F. graminearum*. The 3ADON genotype has been found to grow more quickly and to produce more trichothecenes and conidia than the 15ADON genotype. It explains why the 3ADON genotype is spreading in North America and why a major shift from 15ADON to the more competitive 3ADON-producing strains has taken place. In the Russian Far East the 3ADON genotype frequency increased between the years 1998–2006, and could reflect a shift in trichothecene chemotype composition similar to that observed within North America (Yli-Mattila & Gagkaeva, 2010).

Problems in cereal resistance to toxigenic *fusarium* species

The detection of *Fusarium* species in plants, which is necessary for breeding resistant cultivars, is currently based on morphological, cultural and antibody-based techniques. Only very few people are able to use morphological methods, which can only detect living mycelia and spores or are semi-quantitative, while cross reactions may be a problem in antibody-based techniques. Chromatographic analyses are the most exact methods for mycotoxin detection and quantification, but these methods are often too slow and expensive. The TaqMan real-time PCR is a quicker method, which will be used and developed in the present proposal for detecting and quantifying toxigenic *Fusarium* species in cereals during or even before harvesting. According to the latest investigations about one third of DON is converted to deoxynivalenol-3-glucoside (D3G) in wheat (especially in highly *Fusarium*-resistant lines) and TaqMan real-time PCR gives a better overview of the total content of DON and D3G than DON alone or visual scoring of *Fusarium* disease symptoms in wheat resistant assessments (Brunner et al., 2009).

CONCLUSIONS

In the future the current molecular methods will be improved, validated and automated, making it possible to use them in the routine identification, detection and quantification of *Fusarium* species, e.g. for resistance studies. Molecular analyses require expensive equipment, but they allow quicker and more reliable identification with fewer people than traditional morphological identification. Internal standards should also be used in the future to discriminate between uninfected samples and possible PCR inhibition (Waalwijk et al., 2004; Kulik 2011). The computer analysis of molecular data is expected to improve and the total amount of sequence data in the databases will increase. The whole genome of *F. graminearum*, for instance, has already been sequenced (Cuomo et al., 2007) and more *Fusarium* genomes will be sequenced soon. The sequenced genome of *Brachypodium* (the International Brachypodium Intitiative, 2010) will also help researchers in investigating the resistance in the subfamily Pooidae, including wheat, barley, rye and oat.

ACKNOWLEDGEMENTS: The work of T. Yli-Mattila was supported by the Nordic network project New Emerging Mycotoxins and Secondary Metabolites in Toxigenic Fungi of Northern Europe (project 090014), which was funded by the Nordic Research Board.

REFERENCES

Bjornstad, Å., Tarkegne, Y. & Sinnes, H. 2006. FHB resistance in oats derived from *Avena sterilis*. –9th European *Fusarium* seminar (book of abstracts): 87.

Bottalico, A. & Perrone, G. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-cereals in Europe. *Eur. J.Plant Pathol.* **108,** 611–624.

Brunner, K., Kovalsky, M. P., Paolino, G., Burstmayr, H., Lemmens, M., Berthiller, F., Schuhmacher, R., Krska, R. & Mach, R.,L. 2009. *Anal. Bional. Chem.* **395,** 1385–1394.

Burkin, A. A., Soboleva, N. A. & Kononenko, G. P. 2008. T-2 toxin production by *Fusarium poae* from cereal grain in Siberia and Far East regions. *Mycol. Phytopathol.* **42,** 354–358 (in Russian).

Cuomo, C. A., Güldener, U., Xu, J. R.Trail, F., Turgeon, G., Di Pietro, A., Walton, J. D., Ma, L-J., Baker, S. E., Rep, M., Adam, G., Antoniw, J., Baldwin, T., Calvo, S., Chang, Y.-L., DeCaprio, D., Gale, L. R., Gnerre, S., Goswami, R. S. Hammond-Kosack, K., Harris, L. J., Hilburn, K., Kennell, J. C., Kroken, S., Magnuson, J. K., Mannhaupt, G., Mauceli, E., Mewes, H.-W., Mitterbauer, R., Muehlbauer, G., Münsterkötter, M., Nelson, D., O'Donnell, K., Ouellet, T., Qi, W., Quesneville, H., Roncero, I. G., Seong, K.-Y., Tetko, I. V., Urban, M., Waalwijk, C., Ward, T. J., Yao, J., Birren, B. W. & Kistler, H. C 2007. The *Fusarium graminearum* genome reveals Cuomo, C.A. et al., 2007. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science **317,** 1400–1402.

Goswami, R. S. & Kistler, C., 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.*, **5,** 515–525.

Desjardins, A. E. 2006. *Fusarium* Mycotoxins Chemistry, Genetics and Biology. The American Phytopathological Society, 260 pp.

Edwards, S. G., B. Barrter-Guillot, B., Clasen, P.E. Hietaniemi, V. & Pettersson, H. 2009. Emerging issues of HT-2 and T-2 toxins in European cereal production. *World Mycotox. J.* **2,** 173–179.

Fredlund, E., Gidlund, A., Pettersson, H., Olsen, M., & Börjesson, T. 2010. Real-time PCR detection of *Fusarium* species in Swedish oats and correlation to T-2 and HT-2 toxin content. *World Mycotox. J.* **3,** 77–88.

Gagkaeva, T. & Yli-Mattila, T. 2004. Genetic diversity of populations of *Fusarium graminearum* in Europe and Asia. *Eur. J. Plant Pathol.,* **110,** 551–562.

Gagkaeva, T. Yu., Levitin, M. M., Sanin, S. S. & Nazarova, L. N. 2009. *Fusarium* head blight of small cereals and causal organisms associated with this disease on the territory of Russia during 2004–2006. *Agro XXI:* 4–6 (in Russian).

Gagkaeva, T., Gavrilova, O., Yli-Mattila, T. & Loskutov, I. Comparison of different approaches for estimation of resistance to Fusarium scab in oats. 11th European *Fusarium* seminar in Warsaw September 20–23, 2010 (abstract).

Joffe, A. Z, 1986. *Fusarium* species: Their biology and toxicology. J. Wiley & Sons, New York, USA., 588 pp.

Konstantinova, P. & Yli-Mattila, T. 2004. IGS-RFLP analysis and development of molecular markers for identification of *Fusarium poae, Fusarium langssethiae, Fusarium sporotrichioides* and *Fusarium kyushuense – Int. J. Food Microbiol.* **95,** 321–331.

Kulik, T. 2011. Development of TaqMan Assays for 3ADON, 15ADON and NIV *Fusarium* genotypes based on *Tri12* gene. *Cer. Res. Comm.* **39,** 200–214.

McMullen, M., Jones, R., & Gallenberg, D., 1997, "Scab of Wheat and Barley: A Re-emerging Disease of Devastating Impact". *Plant Dis.* **81,** 1340–1346.

Medina, A. .& Magan, N. 2011. Temperature and water activity effects on production of T-2 and HT-2 by *Fusarium langsethiae* strains from north European countries. *Food Microbiol.* **28,** 392–398.

Mirocha, C. J. & Pathre, S. 1973. Identification of the tixuc principle in a sample of poaefusarin. *Appl. Microbiol.* **26,** 719–724.

Nicholson, P., Chandler, E., Draeger, R. C., Gosman, N. E., Simpson, D. R., Thomsett M. & Wilson, A. H. 2003. Molecular tools to study epidemiology and toxicology of *Fusarium* head blight of cereals. *Eur. J. Plant Pathol.* **109,** 691–703.

O'Donnell, K., Kistler, H. C., Tacke, B. K. & Casper, H. H. (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proc. Natl.. Acad. Sci.* USA **97,** 7905–7910.

O'Donnell, K., Ward, T., Aberra, D., Kistler, H. C., Aoki, T., Orwig, N., Kimura, M., Bjørnstad,

Å. & Klemsdal, S. S., 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. *Fung. Gen. Biol.* **45,** 1514–1522.

Pasquali, M., Giraud, F., Brochot, C., Cocco, E., Hoffmann, L. & Bohn, T. 2010. Genetic *Fusarium* chemotyping as a useful tool for predicting contamination in winter wheat. *Int. J. Food Microbiol.* **137,** 246–253.

Sarkisov, A. Ch. 1954. Mycotoxicoses. Moscow, Russia. 216 pp. (in Russian).

Sarlin, T, Yli-Mattila T., Jestoi, M, Rizzo A., Paavanen-Huhtala, S. & Haikara, A. 2006. Real-time PCR for quantification of toxigenic *Fusarium* species in barley and malt. European *J. Plant Pathol.* **114,** 371–380.

Stępień, L., Popiel, D., Koczyk, G. & Chełkowski, J. 2008. Wheat-infecting *Fusarium* species in Poland — their chemotypes and frequencies revealed by PCR. *J. Appl. Genet.* **49**, 433–441.

Tekauz, A. B., Fetch, J. M., Rossnagel, B. G. & Savard, M. E. 2008. Progress in assessing the impact of *Fusarium* head blight on oat in western Canada and screening of *Avena* germplasm for resistance. *Cer. Res. Commun*. **36,** Suppl. B(8): 49–56.

Torp, M., & Nirenberg, H.I., 2004. Description of *Fusarium langsethiae* sp. nov. *Int. J. Food Microbiol.* **95,** 89–96.

Ueno, Y., 1983, Trichothecenes: Chemical, Biological and Toxicological Aspects. Elsevier, Amsterdam, The Netherlands, 313 pp.

Waalwijk C., Kastelein P., de Vries I., Kerenyi Z., van der Lee T., Hesselink T., Kohl J.& Kema G. 2003. Major changes in Fusarium spp. in the Netherlands. *Eur. J. Plant Pathol.* **109,** 743–754.

Waalwijk, C., van der Heide, R., de Vries, I., van der Lee, T., Schoen, C., Costrel-de Corainville, G., Häuser-Hahn, I., Kastelein, P., Köhl, J, Lonnet, P., Demarquet, T. & Kema, G. H. J. 2004. Quantitative Detection of *Fusarium* Species in Wheat Using TaqMan. *Eur. J. Plant Pathol*. **110,** 481–494.

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 Microbiol. Lett.* **233,** 69–76.

Yli-Mattila, T., Paavanen-Huhtala, S., Jestoi, M., Parikka, P., Hietaniemi, V., Gagkaeva, T., Sarlin, T., Haikara, A, Laaksonen, S. & Rizzo, A. 2008. Real-time PCR detection and quantification of *Fusarium poae, F. graminearum, F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. *Arch. Phytopathol. Plant Prot.* **41,** 243–260.

Yli-Mattila, T., Parikka, P, Lahtinen, T., Ramo, S., Kokkonen. M., Rizzo, A., Jestoi, M. & Hietaniemi, V. 2009a. Fusarium DNA levels in Finnish cereal grains. In: Gherbawy, Y., Mach, L. & Rai, M. (eds.), Current Advances in Molecular Mycology, Nova Science Publishers Inc., New York, USA.

Yli-Mattila, T., Gagkaeva, T., Ward, T. J., Aoki, T., Kistler, C. & O'Donnell, K. 2009b. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Far East. *Mycologia* **101,** 841–852.

Yli-Mattila, T. & Gagkaeva, T. 2010. Molecular chemotyping of *Fusarium graminearum, F. culmorum* and *F. cerealis* isolates from Finland and Russia. In Gherbawy, Y. & Voigt, K. (eds.) Molecular identification of fungi. Springer Verlag, Berlin, Heidelberg, New York: 159–177.

Yli-Mattila, T. 2010. Ecology and evolution of toxigenic *Fusarium* species in cereals in northern Europe and Asia. *J. Plant Pathol.* **92,** 7–18.

Yli-Mattila, T., Ward, T., O'Donnell, K., Proctor, R. H., Burkin, A., Kononenko, G., Gavrilova, O., Aoki, T., McCormick, S. P., & Gagkaeva, T. (2011). *F. sibiricum sp. nov;* a novel type A trichothecene-producing *Fusarium* from northern Asia closely related to *F. sporotrichioides* and *F. langsethiae*. *Int. J. Food Microbiol*. **147,** 58–68.