Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft
Berlin-Dahlem

6th European Fusarium Seminar
&
Third COST 835 Workshop of Agriculturally Important Toxigenic Fungi

(Berlin, Germany, 11 – 16 September 2000)

Book of Abstracts

Edited by

Helgard I. Nirenberg

Biologische Bundesanstalt für Land- und Forstwirtschaft
Institute for Plant Virology, Microbiology and Biological Safety, Berlin-Dahlem

Heft 377

Berlin 2000

Herausgegeben von der
Biologischen Bundesanstalt für Land- und Forstwirtschaft
Berlin und Braunschweig

Parey Buchverlag Berlin
Kurfürstendamm 57, D-10707 Berlin

ISSN 0067-5849 ISBN 3-8263-3352-7

Editor

Dr. Helgard I. Nirenberg
Biologische Bundesanstalt für Land- und Forstwirtschaft
Institut für Pflanzenvirologie, Mikrobiologie und Biologische Sicherheit
Königin-Luise-Str. 19
D-14195 Berlin
Germany
Tel. +49 30 8304 2210 – Fax. +49 30 8304 2203
e-mail: H.Nirenberg@bba.de

Cover design of the seminar edition

Anne Worbs

Authors are responsable for the contents of their manuscripts !

Die Deutsche Bibliothek - CIP-Einheitsaufnahme

European Fusarium Seminar <6, 2000, Berlin>:
Book of abstracts / 6th European Fusarium Seminar & Third COST 835
Workshop of Agriculturally Important Toxigenic Fungi : (Berlin,
Germany, 11th - 16th September 2000) / hrsg. von der Biologischen
Bundesanstalt für Land- und Forstwirtschaft. Ed. by Helgard I.
Nirenberg. - Berlin : Parey, 2000
(Mitteilungen aus der Biologischen Bundesanstalt für Land- und
Forstwirtschaft Berlin-Dahlem ; H. 377)
ISBN 3-8263-3352-7

Kommissionsverlag Parey Buchverlag Berlin, Kurfürstendamm 57, 10707 Berlin

Printed in Germany by Arno Brynda, Berlin.

Table of Contents

LECTURES

Taxonomy and Genetics 14

POSTERS

Taxonomy and Genetics 52

Breeding 93

Plant Protection 101

LECTURES

Taxonomy and Genetics

Schnarrenberger, C.

Institute of Biology, Free Univ. of Berlin, Königin-Luise-Str. 12-16a, 14195 Berlin, Germany,
e-mail: schnarre@zedat.fu-berlin.de

Evolution of pathways: Gene transfers from prokaryotes to eukaryotes

Evolutionary trees of all genes of a metabolic pathway can give clues about the origin of eukaryotic genes from prokaryotes. One would expect the following sources in prokaryotes: nucleo-cytoplasmatic genes should have evolved from archaebacteria since rRNA genes imply this closest organismic relationship between prokaryotes and eukaryotes; plastidic genes should have evolved from cyanobacteria and mitochondrial genes from α-proteobacteria because the respective rRNAs imply them as progenitor organisms of plastids and mitochondria, respectively. When testing these hypotheses we found an evolution of Calvin cycle genes in the plastids of plants from cyanobacteria for 6 genes, but at least 2 genes must have originated from α-proteobacteria. When analyzing the genes of glycolysis/gluconeogenesis in the cytosol they evolved from either cyanobacteria or α-proteobacteria. The genes of the TCA cycle in the mitochondria originated mostly from α-proteobacteria, occassionally from γ-proteobacteria. The genes of the glyoxylate cycle in peroxisomes or in the cytosol evolved either from γ-proteobacteria or through gene duplications of mitochondrial genes. In sum most genes for pathways were transferred from cyanobacteria and proteobacteria to eukaryots either directly or were, after gene duplications in early eukaryotic evolution, randomly distributed to either plants, animals and/or fungi. Only the gene of the cytosolic transketolase in animals evolved for sure from archaebacteria. There are many cases of cell-compartment specific isoenzymes for enzymes of the above mentioned pathways. The gene duplications for such isoenzymes have occurred at all times of evolution, i.e. in prokaryotic evolution, early eukaryotic evolution or within the early evolution of animals, plant and fungi, respectively. Particularly fungi, as mainly represented by yeast and fission yeast, were extremely successful in generating their own compartment-specific isoenzymes. In another example of plant aldolase, class I and class II isoenzymes exist which even do not show any sequence homology. Both classes form plastid and cytosol specific isoenzymes, class I aldolases in higher plants and class II aldolases in the primitive alga *Cyanophora paradoxa*.- Yet, we know only a limited number of gene sequences in the major taxa. However, the analyses indicate that pathway evolution and the evolution of compartment-specific isoenzymes is far more complex than was expected from the uniform and functional appearance of pathways. It would be attractive to start over with comparative enzymology and to follow the changes in regulatory enzyme properties along the genetic evolution.

Chelkowski, J.

Institute of Plant Genetics , Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznan, Poland

Fusarium species - Genome and molecular identification: A review

The genome of *Fusarium* species contains whole DNA consisting of 8 to 12 chromosomes in the haploid nucleus. The genome size is approximately 20-50 M base pairs (Mbp). Size of chromosomes ranges from

0.4 to 12 Mbp. The size of the haploid genome of wheat – an important host plant of *Fusarium* spp. is about 16.000 Mbp and is composed of genomes A, B and D each one with 7 chromosomes.

It was possible to resolve chromosomes of six different mating populations (biological species) of the *Liseola* section as well as species *F. avenaceum, F. camptoceras, F. chlamydosporum, F. fusarioides, F. oxysporum, F. pallidoroseum, F. poae, F. sporotrichioides and F. tricinctum*.

Basic genome organization: preserved regions (sequences), arrangements, deletions and translocations were examined using RFLP probes. Several DNA assays were developed for *Fusarium* species identification both in cultures and in infected plant tissues. Specific SCAR primer pairs sequence is known for sensitive detection of six species of *Discolor* section, and: *F. avenaceum, F. tricinctum, F. poae, F. moniliforme* and *F. subglutinans*, as well as formerly belonging to *Fusarium* - *Microdochium nivale* var. *nivale* and var. *majus*. SCAR method to identify other pathogens contributing to foot rot and root rot of cereals are available.

Most *Fusarium* species can be actually distinguished using RAPD-PCR assay. Decamer primers have been used to examine genetic structure of some *Fusarium* species.

Usage of PCR assay to identify toxigenic *Fusarium* species in cultures and in cereal tissues will be presented.

Geiser, D.

Fusarium Research Centre, The Pennsylvania State University, USA

Molecular database: Tools for the *Fusarium* biologist

Fusarium systematics has always been difficult. The difficulty of identifying species using morphology has led to over-simplified taxonomic systems that obfuscate the variation that exists in this genus. Molecular data interpreted using a phylogenetic species concept are showing that even the most species-rich morphological taxonomic treatments vastly underestimate species diversity. However, in *Fusarium* we now have available, perhaps the largest systematic dataset available, for any organism, and this provides an excellent framework for strain identification, identification of new species, and exploration of speciation and other evolutionary processes. I will first discuss what tools are available. I will give a few examples of how these tools work and a few specific *Fusarium* applications. Included will be investigations of *F. redolens* and its newly identified sister species, *F. hostae*, and speciation processes in *F. proliferatum* and *F. fujikuroi*. I will then talk about additional tools that are now being developed.

Nirenberg, H. I.

Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, D-14195 Berlin, Germany

When taxonomic results of classical and modern techniques do not correlate

Most of the times classical and modern techniques like DNA sequencing and secondary metabolites profile analyses lead to the same taxonomic results. Even more, sometimes these data from these modern techniques point out that one morphological entity is truly a good species – even if the differences of the morphological characters from its sister species are minor like in the case of *F. circinatum* and *F. pseudocircinatum; F. nygamai* and *F. pseudonygamai* etc.

With four examples I would like to show that different base pairs at the loci in two strains do not necessarily mean that they belong to different species (*F. fujikuroi, F. pseudoanthophilum*), that the same base pairs do not necessarily mean that the strains belong to the same species (*F. nisikadoi* and *F. misanthi*) or most similar sequences mean that they belong to the same genus (*Neocosmospora* and *Haematonectria*). I will try to find some answers to these discrepancies.

To avoid errors I recommend a multi-disciplinary (polyphasic) approach to describe new genera and species at present until we have sequenced the whole genomes of the fungi.

Baayen, R. P.[1)]; O'Donnell, K.[2)]; Waalwijk, C.[3)]; Bonants, P. J. M.[3)]; Cigelnik, E.[2)]; Kroon, L. P. N. M.[3)]; Roebroeck, E. J. A.[4)]

[1)] Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, The Netherlands
[2)] National Center for Agricultural Utilization Research, U.S. Department of Agriculture – Agricultural Research Service, 1815 North University Street, Peoria (IL), U.S.A.
[3)] Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[4)] Bulb Research Center, P.O. Box 85, 2160 AB Lisse, The Netherlands

Practical implications of changing views on the population structure and biology in *Fusarium oxysporum* and related species

In the past decade the view has become established that the population biology of *Fusarium oxysporum* is largely dictated by the asexual nature of the fungus, and the selection of evolutionary successful clonal lineages known as vegetative compatibility groups or VCGs. In some of the over 150 formae speciales a close correspondence was observed between VCGs and virulence groups (races). In others this was not the case, a phenomenon that has been attributed to lack of differentials (i.e., hidden races exist in the current ones) or to monogenic resistance in host cultivars that is easily overcome by adaptation of the fungus. While the monophyly of VCGs is strongly supported by RFLP, RAPD and AFLP fingerprinting, the monophyly of formae speciales is being questioned. Practical diagnosis of races and formae speciales through rapid molecular methods such as PCR with specific primers depends on the monophyly of the target groups. O'Donnell et al. (1998) were the first to demonstrate that formae speciales may be polyphyletic. Their study involved two formae speciales, f. sp. *cubense* and f. sp. *lycopersici*. This study was recently extended by constructing nuclear (EF-1α) and mitochondrial (mtSSU rDNA) gene genealogies and AFLP-based phylogenies for eight formae speciales associated with rot and wilt diseases: *F. oxysporum* f. spp. *asparagi*, *dianthi*, *gladioli*, *spinaciae*, *lilii*, *tulipae*, *opuntiarum*, and *lini* (Baayen et al., 2000). Reference strains from each of the three main clades identified previously within the *F. oxysporum* complex were included. Strains within vegetative compatibility groups (VCGs) shared identical sequences, supporting the monophyly of the two single-VCG formae speciales, *lilii* and *tulipae*. Identical genotypes were also found for the three VCGs in *F. oxysporum* f. sp. *spinaciae*. In contrast, multiple evolutionary origins were apparent for *F. oxysporum* f.spp. *asparagi*, *dianthi*, *gladioli*, *lini* and possibly *opuntiarum*, although different VCGs within each of these formae speciales often clustered close together or shared DNA haplotypes. Parsimony analyses of AFLP fingerprint data supported the gene genealogy based cladogram. The predictive value of the forma specialis naming system within the *F. oxysporum* complex is therefore questioned. Prospects for development of molecular diagnostic methods are restricted to monophyletic groups such as f. spp. *lilii*, *tulipae* and *spinaciae*, or to monophyletic lineages within polyphyletic formae speciales such as VCGs. In saprophytic soil-inhabiting populations of the fungus, many more VCGs are present than in populations occurring as a pathogen on agricultural host species. Both in such populations and in agricultural ones strong evidence has been presented that the sexual cycle may still be active (Taylor et al., 1999). Altogether, the concept of *F. oxysporum* as an asex-

ual fungus consisting of clonal lineages may have to be replaced by a concept of a species complex with active sexuality comprising myriads of VCGs and ony a few clonal lineages selected by agriculture.

Literature

Baayen, R. P., O'Donnell, K., Waalwijk, C., Bonants, P. J. M., Cigelnik, E., Kroon, L. P. N. M., and Roebroeck, E. J. A. 2000. Gene genealogies and AFLP analyses within the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. Phytopathology **90**, in press.

O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. USA **95**: 2044-2049.

Taylor, J. W., Jacobson, D. J., and Fisher, M. C. 1999. The evolution of asexual fungi: reproduction, speciation and classification. Annu. Rev. Phytopathol. **37**: 197-246.

Gunn, L. V.[1]; Summerell, B. A.[1]; Burgess, L. W.[2]

[1] Royal Botanic Gardens, Mrs Macquaries Rd, Sydney, NSW, 2000, Australia

[2] Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney, NSW, 2006, Australia.

Variability in *Fusarium oxysporum* f. sp. *canariensis* in Australia

Fusarium wilt of *Phoenix canariensis* (Canary Island Date Palm) is caused by *Fusarium oxysporum* f. sp. *canariensis* (Foc) and occurs in the USA, France, Canary Islands, Japan and Australia. In Australia the fungus has killed hundreds of palms since 1982 when it was first reported. Palms affected by the disease show a characteristic one-sided death of the pinnae, with fronds sequentially dying from the oldest upwards until finally the whole plant dies. The fungus is spread in soil, by transplanting palms, on pruning implements and possibly by seed, birds and insects.

Over 100 isolates of *F. oxysporum* were collected from fronds of diseased palms at sites around Sydney and different parts (non-frond) of individual palms within a site. Three techniques were used to assess diversity of these isolates; vegetative compatibility groupings (VCGs), pathogenicity testing using a PCR-based technique (1) and PCR fingerprinting using ERIC primers. *F. oxysporum* isolates from palms in other countries and from non-palm collections in Australia were included.

VCG testing showed there are two major clonal groups in Sydney (A and B) and three minor ones (C, D and E) two of which (C and D) contained some members which reacted weakly in both groups. The largest group (B) contained all frond isolates from one intensively sampled site, a small percentage of non-frond isolates from that site, isolates from other sites around Sydney and the first samples collected in Australia in 1982. Initial testing of six overseas isolates including one from the Canary Islands suspected of representing the ancestral form also showed weak reactions with members of this group. However, subsequent attempts to repeat this were unsuccessful and indicate that some, but not all loci for compatibility are common to the two groups. All other isolates, including the majority of non-frond isolates and all non-palm isolates showed no cross-reactivity (single-member group).

PCR-pathogenicity testing using primers specific to a unique sequence associated with a library of Foc isolates (1) produced a positive response for 80% of Australian isolates including all members of VCG types A, B (frond and non-frond), C and D. Negative results were found for non-palm isolates, as expected. However, some palm isolates also tested negative. Some were from non-frond isolates which did not fall into any VCG group and were therefore likely to be saprophytes. Three others were from fronds which were the members of the distinct VCG type E, illustrating sequence differences for these Australian isolates. A small percentage of unexpected positive results also occurred in some of the single-member VCG palm isolates - the true status of the pathogenicity of these isolates therefore remains ambiguous.

PCR fingerprinting results confirmed these basic groupings and further illustrated the genetic variation in single-member VCG isolates. VCG types A, B and E had unique banding patterns, C and D shared the

same. Overseas isolates also shared the same pattern which was different to any Australian isolate. Single VCG types (non-frond isolates) which were PCR positive had a small but different range of ERIC patterns, single VCG types which were PCR negative had the greatest range of patterns with (not surprisingly) non-palm isolates being totally different to any from palms.

Literature

[1] Plyler ,T .R., Simone, G.W., Fernandez, D and Kistler, H.C. 1999 Rapid Detection of the *Fusarium oxysporum* lineage containing the Canary Island Date Palm wilt pathogen. Phytopathology 89: 407-413.

Kerényi, Z.[1]; Moretti, A.[2]; Logrieco, A.[2]; Hornok, L.[1]

[1] Agricultural Biotechnology Center, 2100 Gödöllő, Hungary
[2] Istituto Tossine e Micotossine da Parassiti Vegetali, CNR, 70125 Bari, Italy

Mating type diversity within *Gibberella fujikuroi* MP 'D' (*Fusarium proliferatum*) assessed by PCR amplification of MAT-specific sequences

Gibberella fujikuroi is a species complex comprising at least eight different, reproductively isolated mating populations (MPs) which are equivalent to biological species. Members of mating population D (anamorph: *Fusarium proliferatum*) have a widespread occurrence and have been isolated from various host plants, including asparagus, maize, sorghum and wheat. In order to assess the mating type distribution in different sub-populations of *G. fujikuori* MP `D` a PCR-based method was used to amplify unique fragments of the two mating type idiomorphs (*MAT-1* and *MAT-2*) from DNA samples of a representative collection of isolates from various sources and geographic origins.

Two pairs of PCR primers (mat1-f and mat1-r, as well as GfHMG1 and GfHMG2) were designed based on MAT-1 alpha-box motifs (EMBL accession No. AB 015641) and HMG box sequences (accession No. AJ 131527) of *G. fujikuroi*, respectively. The diagnostic value of these primers was tried on seven pairs of mating type tester strains (A–G) of *G. fujikuroi*. All testers yielded only a single band when reacted with the four primers. The size of the amplification product clearly indicated the mating type: the MAT-1 specific primers generated a 320 bp band, whereas the MAT-2 specific ones amplified a 210 bp fragment. A single PCR-reaction is thus sufficient to determine the mating type of any isolate within the *G. fujikuroi* species complex.

Fifty-eight strains of *G. fujikuroi* MP `D` isolated from asparagus, date palm, fig, maize, reed (*Arundo donax* L.) and tomato were subjected to mating type assessment using the PCR-based method. All strains but one produced a single, characteristic PCR-fragment and were identified as MATD-1 or MATD-2 isolates according to the size of the fragment. (In a subset of the samples traditional crossings were made to confirm the PCR-based results.) Both mating types have been found to occur among the isolates collected from asparagus, maize and reed while all strains from fig, date palm and tomato proved to be MATD-2 isolates.

Torp, M.[1]; Langseth, W.[1]; Yli-Mattila, T.[2]; Klemsdal, S. S.[3]; Mach, R. L.[4]; Nirenberg, H. I.[5]

[1] National Veterinary Institute, Dept. of food- and feedhygiene, P.O.Box 8156 Dep., N-033 Oslo, Norway
[2] University of Turku, Dept. of Biology, FIN-20014 Turku, Finland
[3] Norwegian Crop Research Institute, Plant Protection Center, N-1432 Ås, Norway
[4] IBTM Technical University of Vienna, Getreidemarkt 9/172/5, A-1060 Wien, Austria
[5] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Strasse 19, D-14195 Berlin, Germany

Section *Sporotrichiella* – identification of a new *Fusarium* species, or a polyphasic approach to its taxonomy

A *Fusarium* species with a micro morphology similar to *F. poae* and a metabolite profile resembling that of *F. sporotrichioides* has been identified as the main producer of T-2 toxin and the deacetylated form HT-2 toxin in Norwegian cereals [1, 2]. Isolates collected from other countries indicate that this species routinely has been identified as *F. poae* based on morphological criteria. Through collaboration within COST action 835 "Agriculturally Important Toxigenic Fungi", 23 strains of this *Fusarium* (*"*powdery *F. poae"*), 49 strains of *F. poae,* 35 strains of *F. sporotrichioides,* and 2 strains of *F. kyushuense* has been collected from different countries for a polyphasic approach to the taxonomy of these species. This is a preliminary report of some results from the study.

The strains were incubated at 25 °C for seven days on Potato Sucrose Agar (PSA) and Synthetischer Nährstoffarmer Agar and the morphology was described. Production of trichothecenes and other metabolites was analyzed by gas chromatography-mass spectrometry (GC-MS) from cultures grown on PSA and Yeast Extract Sucrose Agar [1]. The internal transcribed spacer (ITS) regions I and II of ribosomal DNA (rDNA), intergenic spacer (IGS) rDNA and β-tubulin of the same strains were sequenced. The strains were also studied by random amplified polymorphic (RAPD) DNA analysis.

Comparison of the results so far indicates that the strains classified according to morphology and growth characteristics as "powdery *F. poae*", are much closer genetically to *F. sporotrichioides* than to *F. poae* and with *F. kyushuense* further apart. These results are also in harmony with the results of the metabolite profiling. According to the preliminary sequencing results, all the "powdery *F. poae*" strains are closer to *F. sporotrichioides* than to *F. poae,* but a part of the "powdery *F. poae*" strains form a well-supported group, which can be separated from the *F. sporotrichioides* group. This is also supported by the results of the RAPD analyses. The impact of these results on the taxonomy of Section *Sporotrichiella* is discussed.

Literature

[1] Torp, M., Langseth, W. 2000. Production of T-2 toxin by a *Fusarium* resembling *Fusarium poae.* Mycopathologia, in press.
[2] Langseth, W., Rundberget, T., Torp, M. 2000. Production of T-2 toxin by a *Fusarium* resembling *F. poae*, and the occurrencer in Norwegian cereals. X Internat. IUPAC symp. on mycotoxins and phycotoxins, Sao Paulo, Brazil 2000, 167.

Yli-Mattila, T.[1]; Paavanen-Huhtala, S. M.[1;]; Bulat, S. A.[2]; Alekhina, I. A.[2]; Nirenberg, H. I.[3]

[1] Lab. of Plant Phys. and Mol. Biol., Dept. of Biology, 20014 Univ. of Turku, Finland, e-mail: tymat@utu.fi
[2] Lab. of Eukaryote Genetics, Dept of Mol. and Radiation Biophysics, Petersburg Nuclear Physics Institute, Gatchina 188350, Russia
[3] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Berlin, Germany

Molecular and phylogenetic analysis of the *Fusarium avenaceum/F. arthrosporioides/F. tricinctum* species complex

According to previous RAPD-PCR [1] and UP-PCR [2] results, Finnish *F. avenaceum* strains can be divided into two main groups (I and II), which can be identified by strain-specific primers [3]. The two most pathogenic *F. avenaceum* strains (a17 and a38) belong to the main group II [2]. More strains of main groups I and II were found by using strain-specific primers. By the specific primers it was also possible to detect and identify strains a17 and a38 directly from infected potato tubers [3]. The strains a43 and a49 differed clearly from these two main types [1] and later a43 was morphologically identified as *F. tricinctum* (t43), which according to the present classification belongs to Sporotrichiella section, while *F. avenaceum* and *F. arthrosporioides* belong to Arthrosporiella/Roseum section.

In the present work more strains of *Fusarium avenaceum/F. arthrosporioides/F. tricinctum* species complex from different origins have been analyzed by sequencing ITS, IGS and beta-tubulin regions and by UP-PCR hybridization analysis. According to the preliminary results the strains from all three species are closely related to each other. *F. avenaceum* and *F. arthrosporioides* strains do not form their own monophyletic groups, but are grouped together. The two main groups of *F. avenaceum* are separated from each other in the tree based on beta-tubulin sequences and less clearly also in the trees based on IGS and ITS sequences. Most of the *F. tricinctum* strains form in IGS and beta-tubulin trees a well-supported cluster, which is nested within the large cluster of *F. avenaceum* and *F. arthrosporioides* strains consisting of several subclusters. Strain a49 may be an intermediate between *F. avenaceum/F. arthrosporioides* and *F. tricinctum* clusters, since it was not clearly clustered with any other strain in beta-tubulin and IGS trees. There are also a couple of other strains, which are less tightly connected to the main clusters and might thus be intermediates between *F. avenaceum/F. arthrosporioides* and *F. tricinctum.* These results are partially supported by the results of UP-PCR hybridization analysis, according to which most of the *F. avenaceum*, *F. arthrosporioides* and *F. tricinctum* strains belong to the same "genospecies" [4]. The close connection between the strains of these three species is also supported by ITS sequence data. Thus according to our molecular results a revision may be necessary and it might be necessary to place *F. avenaceum*, *F. arthrosporioides* and may be also most of the *F. tricinctum* strains to the same species.

Literature

[1] Yli-Mattila, T., Paavanen, S., Hannukkala, A., Parikka, P., Tahvonen, R., Karjalainen, R. 1996. Isozyme and RAPD-PCR analyses of *Fusarium avenaceum* strains in Finland. Plant Pathol. 45, 126-134.
[2] Yli-Mattila, T., Mironenko, N.V., Alekhina, I.A., Hannukkala, A., Bulat, S.A. 1997. Universally primed polymerase chain reaction analysis of *Fusarium avenaceum* isolated from wheat and barley in Finland. Agr. Food Sci. Finland 6, 25-36.
[3] Paavanen-Huhtala, S., Hannukkala, A., Yli-Mattila ,T. 2000. RAPD-PCR based assays for the development of strain-specific primers for pathogenic *Fusarium avenaceum* isolates. (In preparation).
[4] Bulat, S.A., Lubeck, M., Alekhina, I.A., Jensen, D.F. and Lubeck, P.S. 1998. UP-PCR analysis and ITS1 ribotyping of *Trichoderma* and *Gliocladium* fungi. – Mycol. Res. 102, 933-943.

Thrane, U.[1)]; Seifert, K. A.[2)]; Bissett, J.[2)]; Blackwell, B. A.[2)]; Louis-Seize, G.[2)]; Nikiforuk, J. H.[2)]; Savard, M. E.[2)]; McDonald, J.[3)]; Wong, E.[3)]

[1)] Mycology Group, Department of Biotechnology, Technical University of Denmark, Søltofts Plads, Building 221, DK 2800 Kgs. Lyngby, Denmark
[2)] Eastern Cereal and Oilseed Research Centre, K.W. Neatby Building, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6
[3)] Centre for Plant Quarantine Pests, Canadian Food Inspection Agency, Ottawa, Ontario, Canada K2H 8P9

A multidisciplinary taxonomic study of *Fusarium avenaceum*, *F. tricinctum* and *F. stilboides*

A common set of 50 strains of *Fusarium avenaceum*, *F. tricinctum* and *F. stilboides* were examined by colony characters, image analysis of sporodochial conidia and conidia from the aerial mycelium, substrate utilisation profiles (BIOLOG™), rep-PCR (BOX, ERIC, REP primers), DNA sequencing (beta-tubulin, alpha-elongation factor) and metabolite profiling (HPLC, NMR). Most strains identified as *F. avenaceum* and *F. arthrosporioides* clustered together in the molecular analyses, but three small groups of strains from Australia were potentially different. A population isolated from Turkish wheat formed a distinct cluster. *F. tricinctum* and *F. stilboides* formed distinct clusters except for a single strain of each species that appeared to be more distantly related. Two subspecies of *F. avenaceum*, *F. avenaceum* subsp. *aywerte* and *F. avenaceum* subsp. *nurragi* were distantly related to *F. avenaceum*. Analyses of combined data sets and correlations between molecular, morphological and physiological data sets will be used to refine species concepts for these taxa.

Mishra, P. K.; Fox, R. T. V.; Culham, A.

School of Plant Sciences, University of Reading, Whiteknights, Reading, RG6 6AS, U.K.

Taxonomic relationships of *Fusarium culmorum*: a comparison of nrDNA ITS and IGS sequences

The genus *Fusarium* contains many species of economic importance as pathogens of crop plants. *Fusarium culmorum* is of particular importance because of its significance not only in plant pathology but also in mycotoxicology. In this study the use of nuclear DNA sequences has been investigated to establish markers to identify genetic variation that can be used to investigate transfer and migration of *Fusarium culmorum*. Isolates from around the world have been extracted and sequenced for both ITS and IGS regions of the nuclear ribosomal repeat unit. The ITS regions were 570 bases long and the IGS regions varies from approximately 2.2 to 2.5 kilobases in length. Variation in ITS was very low (2 base substitutions within *F. culmorum*) but higher in IGS (116 substitutions and 12 indels) both in absolute and proportional terms. Molecular markers to *F. culmorum* have been identified from ITS sequences. It is planned that strain specific markers will be developed from the IGS sequences. ITS and IGS regions correlate in the groups identified but differ in their resolving power.

Aoki, T.[1]; O'Donnell, K.[2]

[1] National Institute of Agrobiological Resources, Department of Genetic Resources I, Ministry of Agriculture, Forestry and Fisheries, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
[2] Microbial Properties Research, National Center for Agricultural Utilization Research, United States Department of Agriculture, Agricultural Research Service, Peoria, Illinois 61604, USA

Re-characterization of Group 1 population of *Fusarium graminearum* and its *Gibberella* teleomorph

Two distinct populations, Group 1 and Group 2, have been recognized within *Fusarium graminearum*. Group 2 population is famous as a major pathogen, causing wheat head scab throughout the temperate regions of the world. In contrast, Group 1 population has been reported as the causal pathogen of crown rot (or dryland foot rot) of wheat in Australia, Africa and the Northwest United States. Up till now, these two populations have been differentiated based on colony growth rates, colony morphology on PDA slants, and presence or absence of homothalic perithecial production, in addition to the different disease symptoms.

In the present study, strains of Group 1 and Group 2 populations were re-examined morphologically and molecular-phylogenetically. Strains of the two populations differed in mycelial growth rates on some, but not all, culture media. No significant difference could be found in conidial size or in colony morphology on PDA. Group 1 population could, however, be distinguished from Group 2 population based on widest position of conidia. Conidia of Group 1 strains are widest mostly at their mid-region and conidia of Group 2 strains are widest mostly at their upper position. Maximum parsimony analysis of DNA sequences from the β-tubulin gene introns and exons indicate that Group 1 strains represent a phylogenetically distinct species that is a sister group to a *F. graminearum* Group 2-*F. lunulosporum*-*F. culmorum*-*F. cerealis* clade. Based on the above differences, a new species, *F. pseudograminearum* sp. nov., was described for the *F. graminearum* Group 1 population [1].

Mating experiments were performed among 18 strains of *F. pseudograminearum*. Heterothallic production of perithecia was observed in eight out of all 153 possible combinations. Mature asci and viable ascospores were recovered in seven of the combinations. Perithecia in the fertile pairings were subglobose to ovoid, dark, 120-370 μm in diam. and formed directly on the surface of rice stems placed on the culture media. Asci were unitunicate and 8-spored when mature. Mature ascospores were primarily hyaline, fusoid, straight or curved, with rounded ends and (1-)3-septate. Microdimensions of the teleomorph obtained for *F. pseudograminearum* were different from those of the *G. zeae* teleomorph of *F. graminearum*. A new species of *Gibberella*, *G. coronicola* was described for the teleomorph of *F. pseudograminearum* [2]. Group 1 and Group 2 populations recognized previously within *F. graminearum* differ from each other in their anamorphic and teleomorphic morphology, ecological habitats, pathogenicity, mode of sexual reproduction and phylogenetic relationships.

A PCR primer pair that specifically amplified DNA from all isolates of *F. pseudograminearum* tested was developed based on sequence data from the translation elongation factor (EF-1α) gene.

Literature

[1] Aoki, T., O'Donnell, K. 1999. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum* and PCR primers for its identification. Mycologia 91, 597-609.

[2] Aoki, T., O'Donnell, K. 1999. Morphological characterization of *Gibberella coronicola* sp. nov., obtained through mating experiments of *Fusarium pseudograminearum*. Mycoscience 40, 443-453.

Schlacht, U. F.; Möller, E. M.; Geiger, H. H.

University of Hohenheim, 350 Institute of Plant Breeding, Seed Science, and Population Genetics, 70593 Stuttgart, Germany

Identification of *Fusarium anamorphs* of the *Gibberella fujikuroi* species complex and differentiation of *F. verticillioides* and *F. subglutinans* by species-specific primer pairs

The *Gibberella fujikuroi* species complex can be subdivided into eight distinct mating populations (MP A - H) comprising several *Fusarium* anamorphs most of which are known to produce toxins representing a serious risk to human and animal health [1, 2, 3]. Among the different *Fusarium* anamorphs, *F. verticillioides, F. subglutinans*, and *F. proliferatum* are most frequently found on maize causing destructive diseases known as *Fusarium* seedling blight, stalk, kernel, and ear rot. Since much expertise and time is required for the identification of the individual anamorphs of the *G. fujikuroi* species complex if only morphological characters are used, we applied random amplified polymorphic DNA (RAPD) markers to characterize monosporic isolates of the *Fusarium* section *Liseola* [4]. Our worldwide collection consists of 160 isolates from ten different geographic origins, among them field isolates sampled at three different locations, and mating population tester strains as references [1]. As previously reported, RAPD markers proved well suited to characterize the genetic diversity of several *Fusarium* anamorphs (secton *Liseola*) [5] and to serve as basis for the development of specific primer pairs [6]. This abstract presents additional specific RAPD markers lending themselves to a reliable identification of individual *Fusarium* anamorphs. Their specificity was tested by Southern hybridizations. Based upon the sequences of the amplified DNA fragments, specific primer pairs for several *Fusarium* anamorphs were developed and tested by applying them to 73 - 96 *Fusarium* isolates (section *Liseola*), 20 isolates of other *Fusarium* species, and five non-*Fusarium* isolates infecting maize (Schlacht *et al*., 1997 unpublished data). The first primer pair, 17.6F-17.6R amplifies a fragment of about 225 bp, allowing the identification of seven anamorphs belonging to mating populations A-G of the *G. fujikuroi* species complex. The second primer pair, 3.10F-3.10R, amplifies a fragment of nearly 800 bp which is specific for *F. verticillioides* (MP A). Two further primer pairs, 7.2F-7.2R and 16.4F-16.4R, amplify fragments of *F. subglutinans* isolates (MP E) of approximately 550 bp and 750 bp, respectively. The smaller fragment was shown to be specific for all *F. subglutinans* isolates (MP E) tested, whereas the larger amplicon was found with *F. subglutinans* isolates belonging to a MP E subcluster according to an UPGMA dendrogram based on RAPD data. The specific PCR assays were successfully applied to naturally *Fusarium*-infected maize kernels from Austria. Only *F. subglutinans* was detected in this sample. The specific primer pairs proved to be reliable and time-efficient diagnostic tools for an early detection of anamorphs belonging to MP A - G of the *G. fujikuroi* species complex and, more specifically, for the anamorphs *F. subglutinans* and *F. verticillioides.* The PCR assay is directly applicable to host tissue without any need for previous fungus isolation.

Literature

[1] Leslie, J.F. 1995. Available populations and variable traits. Can. J. Bot. 73, S282-S291.

[2] Britz, H., Coutinho, T.A., Wingfield, M.J., Marasas, W.F.O., Gordon T.R., Leslie J.F. 1999. *F. subglutinans* f. sp. *pini* represents a distinct mating population in the *G. fujikuroi* species complex. Appl. Environ. Microbiol. 65 (3): 1198-1201

[3] Marasas, W.F.O., Nelson, P.E., Toussoun, T.A. 1984. Toxigenic *Fusarium* species: Identity and Mycotoxicology. University Park, PA: Pennsylvania State University Press.

[4] Schlacht, U.F., Möller, E.M., Geiger, H.H. 1996. RAPD analysis of *G. fujikuroi* isolates sampled from German and Italian maize fields. Genes, Genomes, and Chromosomes. Abstracts of the Meeting of the Genetic Society, Sept. 23-27, Jena, Germany. Vol. 4.

[5] Schlacht, U.F., Möller, E.M., Geiger, H.H. 1997. Genetic diversity of *G. fujikuroi* isolates from different geographic origins. Proceedings of the 5th European *Fusarium* Seminar on *Fusarium* Mycotoxins, Taxonomy and Pathogenicity, Aug. 29 - Sept. 5, Szeged, Hungary.

[6] Möller, E.M., Schlacht, U.F., Geiger, H.H. 1997. Molecular diagnosis of toxigenic *Fusarium* species by PCR. Proceedings of the 5th European *Fusarium* Seminar on *Fusarium* Mycotoxins, Taxonomy and Pathogenicity, Aug. 29 - Sept. 5, Szeged, Hungary.

Schnerr, H.; Niessen, L.; Vogel, R. F.

Lehrstuhl für Technische Mikrobiologie, Technische Universität München, Weihenstephaner Steig 16
85350 Freising-Weihenstephan, Germany

Quantification of *Fusarium* species producing trichothecene by LightCycler™-PCR, - using SYBR®Green I for continuous fluorescence monitoring

The LightCycler™ technology combines rapid *in vitro* amplification of DNA with real time detection and quantification of the amount of target molecules present in a sample. The system enables a 35 cycle PCR to be completed in 20 min and is therefore well suited for routine anlysis of bigger samples numbers. Based on *tri5* specific PCR primers previously described (Tox5-1/Tox5-2) [1], a quantitative group specific assay for trichothecene producing *Fusarium* spp. was established. In the assay, SYBR®Green I was used as fluorescent dye enabling online detection of PCR products. Characterization of the amplicons was achieved by online analysis of the product´s melting point at 85 °C. Unspecific products like primer dimers could readily be distinguished from the product by their lower melting point. Coposition of the amplification buffer was optimized and various hot start methods were tested in order to achieve highest sensitivity of the assay. Uracil DNA glycosylase was added to prevent amplification of unspecific products due to DNA carry-over. The spectrum of species detected was in accordance to the results found in conventional PCR using the Tox5 primer pair [1]. Reproducibility of the assay developed was determined to be 98 % in the range between 0.05 ng and 6 ng of purified *Fusarium graminearum* DNA when 6 parallel experiments were run. The assay developed was used to analyze 67 wheat samples with known DON concentrations, 26 of which had also been characterized for *Fusarium* contamination. Correlation between DNA content analyzed with the new assay and DON concentrations was calculated to be R=0.83. The coefficient for DNA content and mycological data was R=0.67. With both parameters, correlations were higher in low to medium contaminated samples campared to high contaminated material. This is the first report on the use of the Light Cycler™ system in combination with SYBR®Green I for the quantification and identification of fungal DNA in pure cultures and sample material.

Literature

[1] Niessen, L. Vogel, R.F. 1998. Group specific PCR-detection of potential trichothecene-producing *Fusarium* species in pure cultures and cereal samples. System. Appl. Microbiol. 21, 618-631.

Plant Pathology

Levitin, M. M.

All-Russian Institute of Plant Protection, St. Petersburg – Pushkin, Russia

Recent developments in research on *Fusarium* head blight of cereals in Russia

Fusarium head blight (FHB) of cereals is very widely spread in Russia. The loss of wheat crop reached 25-50%. In 25-80% of wheat samples the concentration of DON exceeded the permissible level. Research of FHB in Russia includes the following problems: 1) study of species composition of *Fusarium* fungi and their toxigenicity, 2) study of biology of the most dangerous species, 3) development of cultural control techniques, chemical and biological methods of disease control, 4) breeding of resistant cultivars.

The study of *Fusarium* species carried out on cereals in Russia has allowed to identify 17 species. Great variability of *Fusarium* species was discovered on the territory of Russia and predominance of some species in different climatic conditions was observed. For example, *F. graminearum* was dominant in the warm and humid conditions of North Caucasus and the Far East; *F. culmorum* and *F. tricinctum* spread more in the ecological conditions of the Central and North-West regions. However there are species characterized by a wide adaptability. For example, *F. avenaceum* and *F. poae* were found in all cereal-producing areas of Russia. Climatic conditions of a certain growing season greatly influence the diversity of *Fusarium* species. In warm humid years *F. graminearum* was dominant in South Russia, but in dry years *F. sambucinum* and *F. moniliforme* prevailed. *F. graminearum* is a more severe pathogen. The biology of pathogens and epidemiology of diseases were studied. New host plants for the ascogenous stage and the correlation between resistance of maize to stalk rot and number of perithecia, formed in autumn, were established [1].

The research was carried out for studying the spectrum of mycotoxins in different *Fusarium* species. The ability of fungal isolates, selected in the same region, to produce mycotoxins can vary widely. For example, *F. culmorum* isolates, selected from wheat seeds in the Moscow district (Central Russia) produced DON in amounts of 16,9 - 1850,0 mg/kg, 3AcDON at levels of 1,8-21,9 mg/kg and moniliformin at levels of 0,7 - 3,7 mg/kg [2]. The analysis of *F. graminearum* strains isolated from the infected grain in North Caucasus showed that they contained DON at levels ranging from 1.3 - 4830.0 mg/kg [3]. The problem of cereal grain contamination by mycotoxins is very important and actual for Russia. 23% samples of wheat, barley and rye were contaminated by DON. Among them 9% of samples contained DON in concentrations exceeding the permissible level. In 0,4% of samples of bread and groat products concentrations of mycotoxins exceeded hygienic standards [4].

Different measures for the control of FHB were studied. Cultural control techniques decreased severity of disease by 36% and among the fungicides more effective against this disease tubeconazole (folicur), prochloraz (sportak), frutriafol (impact) chemicals [5]. Breeders developed the cultivars of winter wheat tolerant to FHB: Dacha, Delta, Echo. The cv. Frontana, Ringo Star, Chine 7, Bizel has been used as a sources of FHB resistance in breeding program [6].

Literature

[1] Ivaschenko V.G., Shipilova N.P, Nazarovskaya L.A. 1994.The estimation of infectional potential of *Gibberella zeae* (Schw.) Petch – the main pathogen causing FHB of cereals. Mycol. and Phytopathol. 28, 45-49 (in Russian).

[2] Kononenko G.P., Malinovskaya L.S., Pirjazeva E.A., Soboleva N.A. 1998. Species diversity and toxigenesity of wheat seedling fusariosis agent in Moscow region. Mycol. a. Phytopath. 32, 37-41 (in Russian).

[3] L'vova L.S., Kizlinko O.L., Shulgina A.P., Omelchenko M.D., Zakharova L.P., Piminova V.V., Gagkaeva T.Yu. 1997. Peculiarities of deoxynivalenol and zearalenon synthesis in wheat grain infected by fusariosis of spike. Mycol. and Phytopathol. 1997, 31, 52- 58 (in Russian).

[4] Tuteljan V. 1995. Medical-biological requirements to quality and safety of grain products and bread-loaf goods. J. bread baking in Russia, 2, 17-19 (in Russian).
[5] Zazimko M.I. 1995. Ecological system of control of diseases and pests of cereals in North Caucasus. Doct. thesis. VIZR, S. Petersburg, 416 pp. (in Russian).
[6] Ablova I. 1998. The resistance of winter wheat to FHB and the possibility of its increase by breeding and immunological methods. Ph.D. Krasnodar, 24 pp. (in Russian).

Lienemann, K.; Oerke, E. C.; Dehne, H.-W.

Institut für Pflanzenkrankheiten, Universität Bonn, Nußallee 9, 53115 Bonn, Germany

Effect of differences among wheat genotypes on the occurrence and damage caused by *Fusarium* head blight

The occurrence and disease frequency of *Fusarium* spp. on winter wheat largely depends on weather conditions but can also be influenced substantially by cultivar selection. In 1999 the correlations between morphological cultivar characteristics and ear infestation by *Fusarium* spp. and *Microdochium nivale* of 15 wheat genotypes has been investigated under field conditions. Additionally, all cultivars were artificially inoculated with a mixed conidia suspension (5 x 10^5 conidia/ml) of *Fusarium graminearum*, *Fusarium avenaceum*, *Fusarium poae* and *Fusarium cerealis* (one isolate of each fungus). Disease assessment was carried out by incubating kernels on selective media to determine *Fusarium* infection.

The influence of the stem length on ear infestation with *Fusarium* spp. was determinated by the use of growth regulators (a: untreated; b: Cycocel® applied in EC32 and Juwel Top® applied in EC 37 at 1l/ha). The use of growth regulators lead to a cultivar-specific reduced stem length ranging between 0 and 8.5 cm with an average of 4.2 cm. This treatment resulted in a higher *Fusarium* infestation as compared to the untreated control by most of the cultivars (a: 8.7 % *Fusarium* spp.; b: 13.2 % *Fusarium* spp.). Only 4 out of 15 cultivars showed a reduced *Fusarium* infestation. Genotypes with the shortest stem length showed the highest degree of ear infestation (< 70 cm: 29 %, 70-80 cm: 8% and > 80 cm 5 % *Fusarium* spp.). *Microdochium nivale* only occurred occassionally throughout the entire field (< 1 %).

There was a close correlation between the distance of flag leaf and spike (r = 0.8). This was also depending on flag leaf arrangement – the more erectophil varieties had a higher disease index compared with more planophil genotypes This was most pronounced with short-stem cultivars. Additionally, erectophil varieties may also lead to changes in microclimate close to the ear and by this may have an indirect effect on infection intensity.

The density of the spike is represented by the D-factor (D = spikelets per spike x 100/spindle length [mm]). There was no correlation among the wheat cultivars between spike density and *Fusarium* infection of ears. The *Fusarium* species naturally occurring in the field were identified microscopically. There was no influence of cultural measures as well as cultivars on the occurrence of different *Fusarium* species.

The most pronounced effect of *Fusarium* head blight was observed on thousand-kernel weight (TKW). Artificial inoculation at EC 65 lead to infestation rates with an average of 73.2 % *Fusarium* spp.. The examined genotypes showed differences in the level of resistance to *Fusarium* infection. At 11 cultivars TKW was significantly reduced from 50.8 g to 47.3 g compared to the non-inoculated control. TKW of 4 genotypes (cv. `Renan`, `Asketis`, 2 breeder-lines) was not affected by inoculation.

Giese, H.; Olsson, S.; Stimmann, B. M.; Grell, M.

Section of Genetics and Microbiology, Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Strategies for the study of stress induced responses in *Fusarium culmorum*

Stress response processes in filamentous fungi are poorly understood and deserve attention in relation to their pathogenic properties and their production of toxic secondary metabolites. *Fusarium culmorum* is believed to be the major cause for *Fusarium* head blight of barley in Denmark and is the most frequent *Fusarium* species in Danish soils. *Fusarium* species are also used as production organisms by the biotechnological industry and it is important to gain insight into the processes that lead to mycotoxin production.

The aim of our research is to characterize genes and processes in *Fusarium culmorum* that are induced when the fungus is subjected to different stress conditions.

Defined culture systems for *F. culmorum* and methods of stress application that can be reproduced at the physiological level will be established. Stress applications such as nutrient and oxygen starvation, heat and chemical compounds will be tested. The entire mycelium will be stressed simultaneously to get a definite starting point to facilitate molecular analyses of fungal stress reactions as a series of events triggered by a specific external factor. The fungal stress responses will be characterised by fluorescent and confocal laser scanning microscopy to monitor cytological changes that can be used to verify reproducible stress treatments.

2-D electrophoreses of proteins extracted from fungal cultures subjected to different types of stress will be carried out. The gels will be scanned and a databank of protein patterns derived from the different stress treatments will be generated. A long term perspective is to analyse individual protein spots by mass spectrometry to identify the proteins that are up- or down-regulated.

Differential display technology will be applied to RNA from fungal cultures subjected to different types of stress. Confirmed differentially expressed sequences will be cloned and characterised by sequencing. Gene expression studies will be performed and it will be attempted to classify genes according to their response to particular types of stress. Eventually transformation experiments including reporter genes will be carried out to monitor stress induction.

Goliński, P. K.[1)]; Kiecana, I.[2)]; Kaczmarek, Z.[3)]; Kaptur, P.[1)]; Kostecki, M.[1)]; Golińska, B.[4)]; Wisniewska, H.[2)]; Chelkowski, J.[2)]

[1)] Dept. of Chemistry , A. Cieszkowski Agricultural University, Wojska Polskiego 75, 60-625, Poznan, Poland
[2)] Department of Phytopathology, Agricultural University, Leszczynskiego 7, 20-069 Lublin, Poland
[3)] Institute of Plant Genetics Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland
[4)] Dept. of Biochemistry and Biotechnology, A. Cieszkowski Agr. Univ., Wolynska 35, 60-637 Poznan, Poland

Fusarium head blight of common Polish winter wheat cultivars – comparison of the effects of *Fusarium avenaceum* and *F. culmorum*

Fusarium head blight (scab) of 14 winter wheat cultivars was investigated in two different regions of Poland with different climate, at localities with different scab incidence. Single isolates of *F. avenaceum* producing moniliformin (MON) and *F. culmorum* producing both deoxynivalenol (DON) and nivalenol (NIV) were independently used in inoculation of winter wheat heads. Percentage of scabby kernels as well as reduction of the following yield traits: 1000 kernels weight (TKW), weight (WKH) and number (NKH) of kernels per head and *Fusarium* toxins (MON, DON, NIV) accumulation in kernels after inocu-

lation were analyzed statistically. The results indicate differences between both pathogens in their scab effects. In a three-factor analysis of variance the following general hypotheses were tested: 1° no difference between *F. avenaceum* and *F. culmorum* in their effects on yield traits reduction; 2° no difference between climatic conditions (years); 3° no interaction between climatic conditions and aggressiveness of *F. avenaceum* and *F. culmorum* in yield reduction; 4° no difference between tested cultivars in their susceptibility to the disease caused by *F. avenaceum* and *F. culmorum*; 5° no interaction between pathogens (*F. avenaceum* and *F. culmorum*) and cultivars in their susceptibility to scab; 6° no interaction between climatic conditions, and cultivars and 7° no interaction between cultivars x pathogens x years with regard to reduction of yield components.

All hypotheses were rejected at the 0.01 level of significance. All results of analysis of variance give a general information concerning the differences between tested cultivars. Differences of TKW and WKH on rejection of the hypothesis about equality of *F. avenaceum* and *F. culmorum* in producing scab had the highest influence. Aggressiveness of *F. avenaceum* was higher than of *F. culmorum* during 1996 and 1997 when differences in yield reduction were the most significant. Comparison of the cultivars indicates the lowest susceptibility of Begra cultivar to scab induced by both tested *Fusarium* pathogens and this genotype as well as several others were more susceptible to *F. avenaceum* than to *F. culmorum*, while Elena was the only cultivar with an opposite tendency.

Nicholson, P. J. P.[1]; Carter, H. N.[1]; Rezanoor, A. E.[1]; Desjardins, A. E.[2]; Plattner, R. D.[2]

[1] John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK
[2] Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, Illinois 61604, USA

Genetic diversity, toxin production and pathogenicity of *Fusarium graminearum* subgroups from Europe, America and Nepal

Isolates of *Fusarium graminearum* obtained from maize, rice and wheat in Nepal were analysed by random amplified polymorphic DNA (RAPD), IGS-RFLP and PCR polymorphisms. Isolates could be divided into two groups (A and B), one of which (B) contained the majority of isolates from maize. Isolates of *F. graminearum* from Europe and USA were then compared to isolates of the two Nepalese subgroups by RAPD analysis, polymorphic PCR, production of deoxynivalenol (DON), nivalenol (NIV) and pathogenicity to wheat and maize seedlings. All of the European and USA isolates formed a single subgroup (C) that was distinct from the two groups identified in Nepal. All of the USA and European isolates (C), except for one, produced DON, whereas isolates from Nepal produced predominantly NIV or DON. All of the toxigenic isolates in sub-group B produced nivalenol. It was concluded that chemotypes of *F. graminearum* are not distributed evenly around geographic range or across subgroups and may have some affect on disease severity and host preference. The relationship between the sub-group, origin of host and toxin production will be discussed.

Inch, S.[1)]; Fernando, D.[2)]; Gilbert, J.[1)]; Tekauz, A.[1)]

[1)] Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB, Canada
[2)] University of Manitoba, Winnipeg MB, Canada

Relationship between environmental variables and spore release by *Gibberella zeae* and *Fusarium graminearum*

Rotorod spore samplers were used to assay for ascospores of *Gibberella zeae* and macroconidia of *Fusarium graminearum*. Ten rotorod samplers were placed 10 m apart in a line transect running northeast to southwest across a wheat field. An area 30 x 30 m was artificially inoculated in the second week of July 1999 with corn kernels infested with *F. graminearum*. Four spore traps extended 40 m, downwind and upwind, from the inoculated site. The spore traps collected spores from 1800h to 0200h, to coincide with known timing of ascospore release. The rods were collected daily during July and August 1999 and examined for ascospores and macroconidia. Temperature, rainfall, relative humidity, wind speed and direction were recorded every half hour. Ascospore discharge occurred between 1 to 4 days after 25 mm or more of rain; release was inhibited during rainfall periods. Hourly spore concentrations ranged from 0-214 ascospores/m3. Similar trends were observed with macroconidia release, although fewer conidia were trapped. Hourly spore concentrations ranged from 0-42 conidia/m^3.

Hysek, J.[1)]; Vanova, M.[2)]; Sychrova, E.[1)]; Koutecka-Brozova, J.[1)]; Radová, Z.[3)]; Hajšlová, J.[3)]

[1)] Research Institute of Crop Production, Drnovská 507, 161 06 Praha 6, Czech Republic
[2)] Agricultural Research Institute, Havlíčkova 2787, 767 41 Kroměříž, Czech Republic
[3)] Institute of Chemical Technology, Technická 3, 166 28 Praha 6, Czech Republic

Fusarioses of barley – the spectrum of species and the levels of mycotoxins (trichothecenes)

We studied the spectrum of the genus *Fusarium* on barley and the levels of trichothecenes in cultures and the grain of barley. The fungi were isolated from 9 varieties (360 samples) of barley in the Agricultural Research Institute at Kroměříž. [1]

The spectrum of species in the years 1997 – 1998 were: *Fusarium culmorum* (over 70% of the isolates), *F. poae* (over 20% of the isolates), *F. avenaceum* (over 2%). Much less frequent were: *F. stilboides* var. *stilboides, F. aquaeductuum* var. *aquaeductuum, F. merismoides* var. *merismoides* and *F. gigas*.

For determination of six trichothecene mycotoxines (nivalenol, deoxynivalenol, diacetoxyscirpenol, T-2 toxin, HT-2 toxin, FUS-X toxin) in fungal mycelium, macroconidia and spring barley high resolution capillary gas chromatography with electron capture detection was used. Only toxin T-2 and nivalenol were detected in fungal mycelium [2]. Low level of nivalenol was found in spring barley, variety Rubín (lower than legislated limit – 2mg/kg of cereals).

Literature

[1] Hýsek, J., Váňová, M., Hajšlová, J., Radová, Z., Koutecká, J., Tvarůžek, L. 1999. Fusarioses of Barley with Emphasis on the Content of Trichothecenes. Plant Protection Science 3, 35, 96 – 102.
[2] Seidel, V., Lang, B., Fraissler, S., Lang, C., Schiller, K., Filek, G., Lindner, W. 1993. Analysis of trace levels of trichothecene mycotoxins in Austrian cereals by gas chromatography with ECD. Chromatographia, 37, 119 – 128.

Kang, Z.; Buchenauer, H.

Institute of Phytomedicine , University Hohenheim, D-70593, Stuttgart, Germany

Ultrastructural and immunocytochemical studies on resistance mechanism of wheat to *Fusarium* head blight

Epidemics of *Fusarium* head blight (FHB) of wheat, generally caused by *Fusarium graminearum* and *F. culmorum*, not only result in a significant yield reduction and low quality of grains, but also contaminate grains with trichothecene mycotoxins. Because of its economic importance, much research has been carried out on the disease concerning different aspects worldwide. However, the knowledge about the resistance mechanism of wheat to FHB is still limited. Recently, we elucidated the infection process and pathway of spreading of *F. culmorum* in wheat spikes using light and electron microscopy. Our immunogold labelling studies suggested a close relationship between the accumulation of *Fusarium* toxins in the infected wheat spike and the pathogenic changes in the host cells, symptom appearance and the colonisation of the pathogen in the host tissues. In addition, our cytochemical analyses confirmed that *F. culmorum* also produced cell wall degrading enzymes at early stages of infection. To reveal the resistance mechanism of wheat to FHB, the present studies were conducted to compare the pathogen development and host responses, the toxin distribution in host cells and lignin content in host cell walls in the wheat spikes of resistant and susceptible cultivars by means of light and electron microscopy, as well as immunogold labelling technique.

Observations showed that the infection process and the initial spreading of *Fusarium culmorun* in wheat spikes were the same between resistant and susceptible cultivars, but the pathogen extended obviously more slowly in resistant cultivars as compared in susceptible one, indicating that the fungal spreading was restricted in resistant cultivars. The plant structural defense reactions such as formation of thick layered appositions and large papillae occurred in the infected host tissues of resistant cultivars, but not in the susceptible ones.

Immunogold labelling of lignin demonstrated that no correlation was found between the existing lignin content in the uninoculated healthy and the resistance of wheat spikes to FHB, but labelling densities of lignin in host cell walls of the infected wheat spikes differed distinctly between resistant and susceptible cultivars. In the susceptible wheat cultivar, the lignin content in the cell walls of the infected tissues showed only a slight increase as compared with that in the uninoculated tissues. In contrast, lignin accumulation in the host cell walls of the infected wheat spikes of the resistant cultivars was much larger than that in the uninoculated corresponding healthy tissues. These findings indicated that lignin accumulation in the infected wheat spikes may play an important role in resistance to the spreading of the pathogen in the host tissues.

Immunogold labelling of the *Fusarium* toxin DON in the infected lemma showed that the labelling patterns in the host tissue were the same between resistant and susceptible cultivars. The toxin was localized in host cell walls, cytoplasm, chloroplast, plasmalemma and vacuole as well as on endoplasmic reticulum and ribosome. However, there were distinct differences in the toxin concentration between the tissues of the resistant and the susceptible cultivars. At the early stage of infection, the labelling densities in resistant cultivars were significantly lower than that in susceptible ones.

The present studies indicate that the FHB resistant cultivars are able to develop active defense reactions during infection and spreading of pathogen in the host tissues. The lower accumulation of *Fusarium* toxin DON in the tissues of the infected wheat spikes of resistant cultivars may allow earlier and higher defense responses to the pathogen.

Bagga, P. S.; Kumar, V.

Punjab Agricultural University, Regional Research Station, Gurdaspur – 143521, Punjab, India

Bakanae and foot-rot (*Fusarium moniliforme* Sheld.) disease of basmati rice in India - Genetic resistance, cultural and fungicidal control

Bakanae disease of rice, also called foot-rot (*Fusarium moniliforme* Sheld) in India, is an important disease throughout much of the world's rice growing areas [1]. During the past decade, this disease has become an important constraint to basmati rice production, in the rice-wheat cropping system, in the north-western parts of India, especially in the Punjab state. None of the four recommended cultivars of basmati rice grown in the area viz., basmati 370, basmati 385, basmati 386, and Pusa basmati-1 was completely free (immune) from foot-rot. The cv. Basmati 385 was found to be highly susceptible and the remaining cvs. showed a high level of field tolerance to the disease. However, these cvs. become highly susceptible after artificial inoculation and thus lack adequate genetic resistance. Among the 85 genotypes of basmati rice, evaluated after artificial inoculation (by seedling – inoculation for 16 hrs.) in the field during 1997 and 1998, 11 (15.3%) genotypes were identified as highly resistant (0% infection) to foot-rot. These outstanding genotypes are potential source of resistance and can be utilized for improving resistance to foot-rot in the commerical cvs. of basmati rice. All four recommended cvs. of basmati rice became highly susceptible (78.3-91.9% infection) after artificial inoculation in the field. The effect of transplanting dates on foot-rot was investigated under natural field infection from 1996-1999 and it was found that the disease is much higher at the early transplanting dates (June 19–July 8) as compared to later dates of transplanting (July 10-22 and July 27-Aug 6). This appears to be primarily due to the higher temperature prevailing during the early dates of transplanting, which favours the pathogen. Experiments performed on fungicidal control demonstrated that the seed treatment with BENOMYL at 0.1%, TILT at 0.05% and EMISAN at 0.2% significantly improved seedling emergence and reduced the foot-rot disease. Seed treatment with TILT was found to be phytotoxic in the early stages of plant growth. Seedling treatment with BENLATE, BAVISTIN and TOPSIN-M at 0.1% and TILT at 0.05% for 8 hrs. significantly reduced foot-rot incidence to 4.09%, 4.42%, 5.64% & 2% respectively as compared to 25.98% in the control treatment, after artificial inoculation in the field. Seedling treatment with EMISAN was found to be phytotoxic. Results suggest that the foot-rot disease can be managed by late transplanting and by seed and seedling treatment with systemic fungicides.

Literature

[1] Ou, S.H. 1985, Rice diseases, CMI, Kew, Surrey, U.K. 380 pp.

Moretti, A.[1)]; Ferracane, R.[2)]; Ritieni, A.[2)]; Frisullo, S.[3)]; Lops, A.[3)]; Logrieco, A.[2)]

[1)] Istituto Tossine e Micotossine da Parassiti Vegetali, CNR, Viale Einaudi, 51, 70125, Bari, Italy
[2)] Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II" 80055, Portici, Italy
[3)] Dipartimento di Produzioni e Preparazioni alimentari, Facoltà di Agraria, Università di Foggia, 71100, Foggia, Italy

Fusarium species from fig in Apulia: Biological and toxicological characterization

Fig endosepsis, also called pink rot or soft rot, is a serious fungal disease of figs described in 1920 in California where the casual agents were identified mainly as *F. verticillioides* (Sacc.) Nirenb. (syn. *F. moniliforme* Sheldon), *F. solani* (Mart.) Appel & Wollenw. emend Snyd & Hans., and *F. dimerum* Penzig, and also as *F. proliferatum* (Matsushima) Nirenb. and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas [1]. Moreover, Nirenberg described two other *Fusarium* species colonizing fig fruits: *F. lactis* Pirrotta & Riboni and *F. ramigenum* O'Donnell et Nirenberg [2]. In a recent survey in fig orchards in the region of Apulia, Southern-Italy, where the fig plant is a common and natural component of local flora, and fig cultivation is economically very important, we isolated one hundred and

twenty *Fusarium* strains from rotted fig fruits. Eighty-seven samples were collected from nine localities all around Apulia and from different fig cultivars; each sample was composed of 15 fruits.

By using morphological criteria, we identified among the isolates mainly three species, *F. ramigenum*, *F. solani* and *F. subglutinans*, and at a lower frequency, *F. proliferatum*. Investigations on the fertility of the isolated strains belonging to *Liseola* [3], performed by sexual crosses on carrot agar medium [4], led to classification of the strains to the mating populations, E and D, but also a high level of infertility was observed.

The strains were then studied for their potential toxin production on maize kernel media. Single spore fungal cultures were grown on 100 g autoclaved maize kernels, for 4 weeks in the dark at 25 °C. Beauvericin, fumonisin B_1 and B_2, and fusaproliferin were analyzed by HPLC, and fusaric acid by GC.

The results showed that fusaric acid was produced by all species at very low amounts, but one strain of *F. subglutinans* produced a high level of the toxin. Only *Liseola* section species produced beauvericin, fumonisins, and fusaproliferin (26, 16, and 3 strains respectively).

Finally, due to the occurrence of some toxigenic strains among the populations isolated from the rotted fig fruits, toxin analysis were also perfomed on the fruits, showing a fumonisin contamination at a low level of some samples.

In conclusion this investigation shows:

a) high level of *Fusarium* contamination of fig fruits in Apulia;

b) the occurrence of several toxigenic strains among the *Fusarium* species isolated;

c) the fertility of several strains which suggests a potential for a high genetic recombination in fig orchards that could widened the genetic pool of the pathogenic and toxigenic population of the different species;

d) the risk of the consumption of dried figs because of the possible toxin occurrence in infected fruits.

Literature

[1] Michailides, T. J., Morgan D. P., and Subbarao K. V. 1996. Fig endosepsis: an old disease still a dilemma for California growers. Plant Dis. 80, 828-841.
[2] Nirenberg H.I., O'Donnell K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* complex. Mycologia, 90, 434-458.
[3] Leslie J.F. 1995. *Gibberella fujikuroi*: available populations and variable traits. Can. J. Bot. 73 (Suppl. 1): S282-S291.
[4] Klittich C.J.R. and Leslie J.F., 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics, 118, 417-423.

Curir, P.[1]; Danieli, B.[2] ; Dolci, M.[3]

[1] Istituto Sperimentale per la Floricoltura, Corso Inglesi 508, 18038 Sanremo, Italy
[2] Dipartimento di Chimica Organica e Industriale, Univ. di Milano, Via Venezian 21, 20133 Milano, Italy
[3] DI.VA.P.R.A., Univ. di Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy

Diversity in phenol composition reflects susceptibility differences to *Fusarium oxysporum* f. sp. *dianthi* in two carnation cultivars closely related genetically

Two carnation (*Dianthus caryophyllus*) cultivars, half-sib progeny, show a different resistance degree towards *Fusarium oxysporum* f. sp. *dianthi* (Fod): the first one, cv. Roland, is totally resistant and the second one, cv. Gloriana, is susceptible to this fungal disease. Due to their genetic affinity, it should be expected that the two cultivars share many biochemical characters and, among them, the phenol pattern. Phenols have been reported to play an important role in plant defensive processes (Biswas et al., 1981) and particularly in the Fod-carnation pathological interactions (Niemann et al., 1987). A research has been therefore undertaken to find out if possible differences in phenol composition between the two

above mentioned genetically related cultivars can explain a different resistance degree against Fod. Internodal explants of the two cultivars were aseptically cultured on a medium composed of Murashige and Skoog macro and microelements, iron chelates and vitamins (Murashige and Skoog, 1962), plus 50 mg/l ascorbic acid, 30 g/l sucrose, 5 µmol/l 2,4-dichlorophenoxyacetic acid, 1 µmol/l 6-benzylaminopurine, 8 g/l Difco Bacto agar, pH 6.8. After one month culture at 22 °C and 12 hrs photoperiod, each explant-developed callus was inoculated with a 100 µ/l drop of a water suspension of Fod race 2 conidia. Five days later, when the fungal mycelium was visually detectable along the callus surface, the explants were collected and analysed for their phenol composition: the calli which were not inoculated, kept as a control, were likewise analysed. Callus phenols were extracted, purified through column chromatography, identified by NMR and MS analyses and quantified according to an already described procedure (Curir et al., 1996). The results showed that the fungal infection induces an accumulation of constitutive phenols in both cultivars, and the biosynthesis of the new compound, 2,6-dimethoxybenzoic acid, in the cv. Gloriana. The fungitoxic activity towards Fod, assayed through in vitro trials, was low for vanillic and protocatechuic acid and quercetin 3-O-arabinoglucoside, and high (at the dosage of 20 µmol/l) for datiscetin and 2,6-dimethoxybenzoic acid. Datiscetin was detectable only in the tissues of cv. Roland, while 2,6-dimethoxybenzoic acid is synthesized de novo only by the Fod-susceptible cultivar as a response to the fungal infection and is not a constitutive compound. The latter acid is present in traces in the tissues, and for this reason it is likely that its in situ fungitoxic effect is irrelevant. On the contrary, the Fod-resistant cultivar contains high amounts of datiscetin, which could therefore behave as a phytoalexin counteracting the pathogen development. The slight differences in phenol composition, due to the genetic affinity of the two cultivars, seem enough to determine a different resistance degree towards Fod. Moreover, the results indicate, that the intrinsic fungitoxic properties of constitutive phenols can represent a fundamental aspect in the defensive processes of carnation towards Fod.

Tekauz, A.; McCallum, B.

Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB, Canada

Bipolaris sorokiniana may be antagonistic to *Fusarium graminearum* in spikes of barley and wheat

Fusarium head blight (FHB) is widespread and damaging to barley and wheat in the eastern Canadian prairies. Based on isolation frequency from seed, *Fusarium graminearum* Schwabe is the primary causal species [1]. In addition to *Fusarium*, *Bipolaris sorokiniana* (Sacc.) Shoemaker (spot blotch, common root rot) often infests a high proportion of kernels of barley and wheat grown in Manitoba. To determine whether *B. sorokiniana* may reduce levels of FHB or suppress the isolation of *F. graminearum* from infested seed, barley and wheat spikes were inoculated with the two pathogens singly and in combination, to compare FHB severity, and levels of seed microflora and DON.

Three spring barley cvs., Manley (2-row malt, moderately susceptible (MS) to FHB), AC Oxbow (2-row malt, MR) and Stander (6-row malt, S), and Roblin bread wheat (VS) were seeded in large plastic pots (10 plants per pot), grown to heading, and subsequently inoculated with *F. graminearum* (5×10^4 macrocondia per ml) and/or *B. sorokiniana* (5×10^3 conidia per ml). The six treatments were: 1) control - inoculated with sterile distilled water; 2) inoculation with *F. graminearum*; 3) inoculation with *B. sorokiniana*; 4) inoculation with *F. graminearum* followed 72 h later by *B. sorokiniana*; 5) inoculation with *B. sorokiniana* followed 72 h later by *F. graminearum*; and 6) joint single inoculation with *F. graminearum* and *B. sorokiniana*. Following each inoculation, plants were kept at 100% RH for 24 h. Plants were rated for FHB severity at 14-21 days by counting the total, and number of diseased spikelets in inoculated heads.

Disease symptoms appeared on all inoculated spikes. Symptoms of FHB were typical of the disease, i.e., a bleached or a light to dark-brown discoloration of most of the spikelet surface, in wheat and barley, respectively. In barley, affected spikelets appeared distinctly thinner and smaller than healthy ones. Symptoms following *B. sorokiniana* inoculation were manifested as a dark brown discolouration of the distal portion of some spikelets, with little obvious reduction in spikelet size. Severity of FHB, as determined by visual symptoms, was considerably reduced when *B. sorokiniana* preceeded inoculation with *F. graminearum* (Tab.1). There was an inconsistent effect on FHB severity when the pathogens were inoculated jointly, and no effect when *B. sorokiniana* followed inoculation with *F. graminearum*. Results of levels of seed microflora and DON are pending.

Table: Severity of *Fusarium* head blight in barley and wheat inoculated with *F. graminearum* and/or *B. sorokiniana*.

Cultivar	**Treatment**			
	F.g.	F.g. + B.s.	F.g. / B.s.	B.s. + F.g.
Barley				
Manley (2-row)	95.8[a]	75.4	51.5	1.4
AC Metcalfe (2-row)	70.9	68.1	65.5	1.4
Stander (6-row)	70.8	68.6	82.1	15.1
Wheat				
Roblin (hard red)	40.5	43.6	32.0	7.2

[a] percent spikelets visibly affected

Literature

[1] Tekauz, A., McCallum, B., Gilbert, J. 2000. Review: *Fusarium* head blight of barley in western Canada. Can. J. Plant Pathol. 22, 9-16.

Breeding

Mesterhazy, A.

Cereal Research non-profit Co., Szeged, Hungary

Latest results of breeding wheat cultivars resistant to FHB in Hungary

Following the large FHB epidemic of wheat in East Hungary (1970) a research program to develop more resistant cultivars was started. However, basic information was lacking and a scientific foundation for breeding had to be established. Now it was possible, like in other parts of the globe to breed highly resistant cultivars for commercial purposes. In the last decades the toxin problem enhanced the research, as food safety became a very important goal of food and feedstuff production.

Resistance is highly complicated; many components of resistance were described. The resistance to spreading is the most important. Concentrating on this fact, highly resistant wheats could be bred. Results showed that high resistance means low or no toxin contamination, however, in some genotypes lower or higher toxin amounts were found according to the actual infection severity.

Relations between FHB and seedlings, leaves, as well as stalk-crown rot are generally lacking, but plants exist in which some of these resistance parameters occur. This means that breeding for one of these traits does not allow an automatic improvement for other traits.

It is evident that resistance is not specific to *Fusarium* species. At least 7-8 *Fusarium* species belong to this group.

Based on winter wheat material, medium to good resistance was achieved for instance in 'Ringo Star' and 'Bence'. However, because of low baking quality or susceptibility to other diseases they were only of scientific significance. Besides very susceptible genotypes in many breeding programs genotypes with high resistance were detected in many countries and that was the first step to improve resistance.

More important results were achieved with the use of Asiatic spring wheat as resistance sources. Among Sumey-3 progenies several highly resistant winter lines were found with excellent resistance to FHB. Nobeoka Bozu, a Japanese cultivar was also successfully used. They were agronomically much better than the so far used sources for resistance and therefore were used to develop cultivars. Here not only FHB resistance was considered, but also leaf rust, stem rust, powdery mildew and others as well as yield ability and baking quality.

Now we have several lines with high resistance to FHB comparable to other sources of resistance and other important traits. They have no or only very low toxin contamination. We hope that some of them will become commercial cultivars.

Many hundreds of new lines will also be tested for FHB resistance which do not originate from crosses for FHB resistance. We have identified 10 cultivars that have higher resistance to FHB than the leading cultivars at present. Therefore a slow improvement is possible now and these new cultivars help decrease the risk of devastating epidemics. In several years the high resistant cultivars can replace those which are grown nowadays.

Of course, many remaining problems have to be solved. We have not enough information about the inheritance of FHB, even less for seedling and crown rot resistance and resistance genes determining them. Molecular breeding will be an important research field.

Heinrich, N.; Miedaner, T.; Oettler, G.

University of Hohenheim, State Plant Breeding Institute, D-70593 Stuttgart, Germany

Studies on the inheritance of *Fusarium* head blight in winter triticale

Fusarium head blight (FHB) infection is of growing importance world-wide in triticale (*Triticosecale* Wittm.). Studies on the inheritance of the disease were conducted in rye [1] and wheat [2], but are lacking for triticale. The aim of the present diallel cross analysis was, therefore, to estimate general and specific combining ability (GCA, SCA) effects for resistance and to correlate hybrid with parental performance.

Ten homozygous winter triticale genotypes, covering a range of resistance levels, were selected, and all possible 45 F_1 combinations, excluding reciprocals, were produced. Parents and F_1s were grown in micro-plots of 10 single plants with two replicates in three climatically diverse locations in southern Germany in 1999. During anthesis genotypes were inoculated twice with an aggressive isolate of *F. culmorum* at a concentration of $5x10^5$ spores ml^{-1}. Disease assessment was performed at four to five day intervals from the appearance of the first symptoms until maturity commenced. For each genotype and location, the average head blight rating (1 = no symptoms visible, 9 = 100% of spikelets and spikes per plot bleached) was calculated as the arithmetic mean from four to five single ratings and used for statistical analyses.

The mean head blight rating of the 45 F_1s across locations was 3.5, ranging from 2.9 to 4.4. The respective parental values were 3.8 and a range from 2.9 to 5.3. The combining ability analysis for the F_1 showed GCA to be the major source of variation (significant at $P = 0.01$), whereas SCA was small and of minor importance only ($P = 0.10$). GCA x location interaction was also highly significant. Heritability of head blight amounted to 0.8. Resistance between hybrids and their parents was highly correlated ($r = 0.8$, $P = 0.01$).

The preponderance of the GCA variance indicates that the resistance to FHB in triticale is primarily based on additive gene effects, the same as described previously for rye and wheat [1, 2]. Therefore, the performance of progenies can largely be predicted from their parental values. Some crosses, however, showed a higher resistance rating than would have been expected from their parental performance due to SCA effects. The high GCA x location interaction requires multi-environment tests to assess reliably triticale genotypes for FHB resistance.

Literature

[1] Miedaner, T., Geiger, H.H., 1996. Estimates of combining ability for resistance of winter rye to *Fusarium culmorum* head blight. Euphytica 89, 339-344.

[2] Snijders, C.H.A. 1990. Diallel analysis of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica 50, 1-9.

Buerstmayr, H.; Lemmens, M.; Doldi, L.; Stierschneider, M.; Steiner, B.; Ruckenbauer, P.

Institute for Agrobiotechnology, Department of Biotechnology in Plant Production, Konrad Lorenz Str. 20, A-3430 Tulln, Austria. www.ifa-tulln.ac.at e-mail: buerst@ifa-tulln.ac.at

Molecular mapping of *Fusarium* head blight (FHB) resistance in wheat

In our research group several topics on FHB are addressed e.g. optimization of resistance assessment, germplasm screening and improvement [1, 2] resistance mechanisms and role of fungal toxins and enzymes in pathogenesis, *in-vitro* selection for resistance [3] and research on the inheritance of resistance by classical [4, 5], cytogenetical [6] and molecular tools. Only few results have been published till now on molecular markers for FHB resistance in wheat [7, 8].

Our mapping populations comprise F1-derived doubled haploids (DH) from two crosses between two resistant cultivars ('Frontana' and 'CM82036') and one susceptible spring wheat cultivar ('Remus'). More than 200 DH-lines per population were evaluated for the expression of *Fusarium* head blight resistance traits in field trials in summer 1999. Inoculation and evaluation methods used were similar to [5]. The molecular genetic work concentrates on the 'Remus/CM-82036' population. RFLP, AFLP and SSR markers were used.

Both DH-populations showed significant quantitative variation in FHB severity as measured by visual symptoms. So far we analyzed 157 markers with ANOVA and mapmaker. Two linkage groups showed highly significant ($p < 0.0001$) association with visual FHB symptoms. They appear to mark two QTL for FHB resistance with additive effects, explaining 46% of the phenotypic variation for disease symptoms.

Literature

[1] Buerstmayr, H., M. Lemmens, H. Grausgruber & P. Ruckenbauer, 1996. Scab resistance of international wheat germplasm. Cereal Research Comm. 24, 195-202.
[2] Buerstmayr H., M. Lemmens, H. Grausgruber, & P. Ruckenbauer, 1996. Head blight (*Fusarium* spp.) resistance of wheat cultivars registered in Austria. Die Bodenkultur 47, 183-190.
[3] Lemmens, M., A. Reisinger, H. Buerstmayr & P. Ruckenbauer, 1994. Breeding for head blight (*Fusarium* spp.) resistance in wheat: development of a mycotoxin-based selection method of seedlings. Acta Horticulturae 355, 223-232.
[4] Buerstmayr, H., M. Lemmens, S. Berlakovich & P. Ruckenbauer, 1999a. Combining ability of resistance to head blight caused by *Fusarium culmorum* (W.G. Smith) in the F1 of a seven parent diallel of winter wheat (*Triticum aestivum* L.). Euphytica 110, 199-206.
[5] Buerstmayr, H., B. Steiner, M. Lemmens & P. Ruckenbauer, 2000. Resistance to *Fusarium* head blight in two winter wheat crosses: heritability and trait associations. Crop Sci. (in press).
[6] Buerstmayr, H., M. Lemmens, G. Fedak & P. Ruckenbauer, 1999. Back-cross reciprocal monosomic analysis of *Fusarium* head blight resistance in wheat (*Triticum aestivum* L.). Theor. Appl. Genetics 98, 76-85.
[7] Bai, G., F.L. Kolb, G. Shaner & L.L. Domier, 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89, 343-348.
[8] Waldron, B.L., B. Moreno-Sevilla, J.A. Anderson, R.W. Stack & R.C. Frohberg. 1999. RFLP mapping of QTL for *Fusarium* head blight resistance in wheat. Crop Sci. 39, 805-811.

Plant Protection

Munkvold, G. P.[1]; Hellmich, R. L.[2]; Biggerstaff, C. M.[1]

[1] Department of Plant Pathology, Iowa State University, 351 Bessey Hall, Ames, IA 50011, USA
[2] USDA-ARS Corn Insect and Crop Genetics Research Unit and Dept. of Entomology, Iowa State University, Genetics Laboratory, Ames, IA 50011, USA

Interactions among toxigenic *Fusarium* species, insect pests, and *Aspergillus flavus* in transgenic maize

Insect pests of maize play a major role in the development of *Fusarium* and *Aspergillus* ear rots and subsequent accumulation of mycotoxins in maize grain. Several insect species, including the European corn borer (*Ostrinia nubilalis*) and the corn earworm (*Helicoverpa zea*) have been shown to influence infection of maize by *Fusarium* spp. and *Aspergillus flavus*. In previous studies [1,2] we have observed consistently that control of the European corn borer with the use of transgenic Bt maize hybrids results in reductions in *Fusarium* ear rot severity and fumonisin concentrations in the grain. These hybrids express crystalline insecticidal proteins derived from the bacterium *Bacillus thuringiensis*. Bt hybrids currently available in the U.S. are of five different types: 176, BT11, CBH351, DBT418, and MON810. Only 176, BT11, and MON810 have been approved for sale in Europe. Bt proteins in these hybrids are effective against the European corn borer and partially effective against the corn earworm. The most effective control of insect damage to the maize kernels occurs with BT11, CBH351, and MON810. Because of their effectiveness against insects, these types of Bt maize also demonstrate the most effective control of *Fusarium* infection and fumonisin accumulation. In 1999, we studied the interactions among *Fusarium* spp., *A. flavus*, the European corn borer, and the corn earworm on Bt maize hybrids and near-isogenic conventional hybrids in the field. There were three insect infestation treatments: 1) natural, 2) manual European corn borer infestation, and 3) manual corn earworm infestation. Plants in one-half the experiment were inoculated with *A. flavus* by injecting a spore suspension into the silk channel of each ear, without damaging the kernels or cob. Only natural *Fusarium* inoculum was present. We recorded the severity of insect feeding and visible ear rot; internal infection of the kernels by *Fusarium* spp. and *A. flavus*; and samples of grain were analyzed for aflatoxins, fumonisins, deoxynivalenol (by Romer Labs, Union, MO), and fusaproliferin (by W.G. Hyde, Iowa State University Veterinary Diagnostic Laboratory). In 1999, the natural population of European corn borers was lower than usual but there was an unusually high natural infestation with corn earworms, and this resulted in more damage to the Bt maize ears than we have observed in previous years. *Fusarium* ear rot severity was generally low, and Aspergillus ear rot symptoms occurred only in the inoculated treatment. In general, the differences between Bt and non-Bt hybrids were less pronounced than in previous years. Insect infestation did not significantly affect the severity of Aspergillus ear rot. There were no significant differences in Aspergillus ear rot between Bt hybrids and non-Bt hybrids except that the MON810 hybrid had significantly lower severity than did its conventional counterpart. The BT11, CBH351, and MON810 hybrids had lower severity of *Fusarium* ear rot than their conventional counterparts in the insect-infested treatments. Infection by *Fusarium* species consisted primarily of *F. verticillioides*, with some *F. graminearum*, *F. proliferatum* and *F. subglutinans*. Fumonisin concentrations were lower in several of the Bt hybrids than in their conventional counterparts, but the difference was significant only for the BT11 hybrid. There were no significant differences in aflatoxin concentrations between Bt and non-Bt hybrids. The most unusual result was that *A. flavus* inoculation caused a significant increase in *Fusarium* ear rot severity and fumonisin concentrations. A possible explanation is that the infection by *A. flavus* predisposed the kernels to subsequent infection by *F. verticillioides*. A similar phenomenon was reported by Reid et al. [3], who reported increased infection by *F. moniliforme (F. verticillioides)* following inoculation with *F. graminearum*. In

our experiment, deoxynivalenol and fusaproliferin concentrations were low and there were no significant differences within the pairs of hybrids.

Literature
[1] Munkvold, G.P., Hellimich, R.L., and Showers, W.B. 1997. Reduced *Fusarium* ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology 87: 209-217.
[2] Munkvold, G.P., Hellmich, R.L., and Rice. L.G. 1999. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and non-transgenic hybrids. Plant Dis. 83: 130-138.
[3] Reid, L.M., Nicol, R.W., Ouellet, T., Savard, M., Miller, J.D., Young, J.C., Stewart, D.W., and Schaafsma, A.W. 1999. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: disease progress, fungal biomass, and mycotoxin accumulation. Phytopathology 89: 1028-1037.

Diamond, H.; Cooke, B. M.

Department of Environmental Resource Management, University College Dublin, Belfield, Dublin 4, Ireland,
Fax: +353–1–7067010, e-mail: Mike.Cooke@ucd.ie

Preliminary studies on biological control of the *Fusarium* ear blight complex of wheat

Pre-inoculation of wheat ears at anthesis in the glasshouse with two non-host pathogens and three cell-free germination fluids of *Fusarium* ear blight pathogens resulted in significant reductions in diseases severity caused by *Fusarium culmorum, Fusarium avenaceum*, *Fusarium poae* and *Microdochium nivale*. Ears pre-inoculated with *Phoma betae* and challenged with *F. culmorum* showed only 30% of the symptoms expressed by the control treatment after 25 days. A 40% increase in incubation period compared to the control occurred when wheat ears pre-inoculated with *Pythium ultimum* were challenged with *M. nivale*, and a 50% increase in latent period occurred when ears pre-inoculated with *M. nivale* germination fluid were challenged with conidia of the same organism. Although treatments did not significantly affect thousand grain weights, pre-inoculation of wheat ears with either *P. ultimum* or *P. betae* often resulted in a significant increase in number of grains/ear compared to equivalent germination fluid treatments, and a decrease in the level of *Fusarium* infection in harvested grain. Possible mechanisms for the observed effects are discussed.

Raman, N.; Gnanaguru, M.

Centre for Advanced Studies in Botany, University of Madras, Guindy campus, Chennai - 600 025, India
(Email: raman55n@hotmail.com), Fax:91-44-2352870)

Biocontrol of *Fusarium* wilt of tomato by the VA-mycorrhizal fungus, *Glomus fasciculatum*

Roots support the growth of a variety of microorganisms that in concert have a profound effect on the growth and survival of the plant. Vesicular arbuscular mycorrhizal (VAM) fungi are important in plant protection and the association is beneficial to crop plants through increased absorption of nutrients and water as well as protection against pathogens. Fungal wilt diseases continue to be important and frequently devaste plants in many areas of the world. *Fusarium* wilt of tomato (*Lycopersicon esculentum* Mill.) caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. is a world wide important disease. Some lines of evidence have shown that inoculation of host plants with VAM fungi could reduce incidence and severity of root diseases in a large number of crops. However, exact mechanisms by which VAM fungi contribute to restrict pathogen colonization in root tissues are not fully understood. As a prelude to further investigations of the effect of mycorrhizal infection on the induced resistance of plants

to pathogen attack, the present study was undertaken to gain better insight into the influence exerted by *Glomus fasciculatum* in stimulating defense mechanisms in tomato plants infected by *F. oxysporum* f. sp. *lycopersici*. Tomato plants were inoculated with *G. fasciculatum* alone (MI), *F. oxysporum* f. sp. *lycopersici* alone (PI), *G. fasciculatum* and *F. oxysporum* f. sp. *lycopersici* (DI), *F. oxysporum* f. sp. *lycopersici* prior to *G. fasciculatum* (PrI) and *F. oxysporum* f. sp. *lycopersici* after *G. fasciculatum* (PoI). A field trial was also conducted. Wilt severity was highly reduced in DI plants and was nearly absent in PoI plants, while PI and PrI plants were completely wilted. High content of chlorophyll followed by enhanced rate of photosynthesis was found in PoI plants followed by MI and DI plants. Higher amount of sugar, reducing sugar, P, cytokinin, protein and amino acids was found in PoI plants. High level of phenol, O-dihydric phenol and tomatine was observed in PoI plants. Generally, enzyme activities except acid and alkaline phosphatases increased initially and decreased in inoculated plants. The suppression of disease in DI and PoI plants may be due to the effect of *G. fasciculatum* because of increased level of P, reducing sugar, protein, cytokinins, phenols, O-dihydric phenols, tomatine and enhanced activity of defence enzymes such as catalase, peroxidase, phenol oxidase and phenylalanine-ammonia lyase along with the lignification and phenol deposition in mycorrhizal roots. Under field condition, *G. fasciculatum* not only increased the growth and yield of tomato, but also suppressed the wilt disease. So *G. fasciculatum* can be used as a biological control agent to control wilt disease caused by *F. oxysporum* f. sp. *lycopersici*.

Glynn, N. C.[1]; Edwards, S. G. [1]; Hare, M. C. [1]; Burke, R. [2]; Parry, D. W. [3]

[1] Harper Adams University College, Newport, Shropshire, TF10 8NB, UK
[2] Novartis Crop Protection UK Ltd., Whittlesford, Cambridge, CB2 4QT, UK
[3] HRI East Malling, West Malling, Kent, ME19 6BJ, UK

Quantification of *Fusarium* spp. and *M. nivale* in fungicide seed treatment efficacy trials

In the UK, *Fusarium* seedling blight of wheat is caused predominately by *Microdochium nivale* and *Fusarium culmorum* [1]. The control of seedling blight is primarily achieved through the use of seed treatments, however, as the two pathogens cause similar symptoms, the relative performance of any particular treatment against either pathogen in a mixed infection is difficult to assess. The development of quantitative PCR techniques has allowed the efficacy of fungicide seed treatments to be determined against specific pathogens. In the present investigation, competitive PCR assays were developed to allow the quantification of *Fusarium* spp. and *M. nivale* in infected plant material from fungicide seed treatment efficacy trials.

Oligonucleotide primers specific to *Fusarium* spp. and *M. nivale* were obtained from Novartis Agribusiness Biotechnology Research Inc. An internal standard was constructed for each primer pair for use in quantitative PCR according to the method of Förster [2]. These quantitative PCR assays were used in fungicide seed treatment efficacy trials.

Winter wheat seed cv. Equinox infected with *M. nivale* and *Fusarium* spp. was produced according to the method of Edwards *et. al.*, 1998 [3]. Seed was treated with either Beret Gold or Sibutol, untreated seed was used as a control. Seed was drilled according to a randomised block design with four replicate treatments. Thirty seedlings were removed from each plot at the third leaf stage, DNA was extracted and the amount of *Fusarium* spp. and *M. nivale* quantified using competitive PCR.

The results indicate that both seed treatments reduced significantly ($p<0.05$) the fungal biomass of each pathogen. Beret Gold appeared more active than Sibutol and the results suggest greater activity of both treatments towards *Fusarium* spp. than *M. nivale*. Further work will focus on assessing the performance

of seed treatments towards the two sub-species of *M. nivale* (var. *majus* and var. *nivale*) the persistence of *M. nivale* control and the performance of seed treatments under specific environmental conditions.

Literature

[1] Paveley, N. D., Rennie, W. J., Reeves, J. C., Wray, M. W., Slawson, D. D., Clark, W. S., Cockerell, V., Mitchell, A. G. 1996. Cereal seed health and seed treatment strategies. HGCA Project Report No. 34. Home-Grown Cereals Authority: London.

[2] Förster, E. 1994. An improved general method to generate internal standards for competitive PCR. Biotechniques, 16, 18-20.

[3] Edwards, S. G., Hetherington, R., Glynn, N. C., Hare, M. C., West, S. J. E., Parry, D. W. 1998. Evaluation of fungicide seed treatments against *Fusarium* diseases of wheat using PCR diagnostic tests. Brighton Crop Protection Conference. Pests and Diseases, 1017-1022.

Sanchis, V.[1)]; Magan, N.[2)]; Marín, S.[1)]; Ramos, A. J.[1)]

[1)] Food Technology Department, Lleida University, 25198 Lleida, Spain

[2)] Applied Mycology Group, Biotechnology Centre, Cranfield University, Bedford MK43 0AL, UK

Ecology and control of growth and fumonisin production by *Fusarium* spp. in maize

The ecology of fumonisin producing species on maize is predominantly determined by the prevailing environmental conditions pre- and post-harvest and by interactions with other contaminant fungi. We have over the last 6 years been conducting extensive ecophysiological studies to identify the environmental requirements for *F. verticilloides* and *F. proliferatum* for germination, growth and fumonisin production. The impact of interaction with other spoilage fungi on niche colonisation, and on mycotoxin production have been identified. This has enabled the detailed environmental profiles over which growth and mycotoxin production may occur on maize to be identified. More recently, the potential for effective control these species in maize using food grade preservatives has been investigated. The sensitivity and tolerance of different isolates if *F. verticilloides* and *F. proliferatum* to propionic acid and its salts under different water availability and temperature regimes using irradiated and natural maize grain have been studied. Growth of isolates of *F. proliferatum* were restricted at 0.98 a_w when the highest concentration of propionate (0.07%) was used, but not those of *F. verticilloides*. In the presence of lower concentrations (0.03%) growth was sometimes stimulated. However, there was little effect of treatments on concentrations of fumonisins produced. Subsequently, natural maize grain alone, or inoculated with additional *Fusarium* spores was treated with a commercial mixture of propionates (0.05 and 0.1%) and stored under different environmental conditions. Preservative treatment decreased the total fungal populations colonising maize, especially of *Penicillium* spp. However, again, fumonisin production was unaffected by treatment, regardless of environmental conditions. These studies suggest that alternative more effective post-harvest treatments are required to prevent fumonisins entering the human and animal food chain.

Toxicology

Marasas, W.F.O.

Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council, PO Box 19070, Tygerberg 7505, South Africa

Fusarium mycotoxins in the Third Millennium

The five most important mycotoxins are aflatoxin, ochratoxin, deoxynivalenol, zearalenone and fumonisin. The three most important toxigenic *Fusarium* species are:

- *F. sporotrichioides*. Causes alimentary toxic aleukia in humans and haemorrhagic syndromes in animals. Produces T-2 toxin and diacetoxyscirpenol.
- *F. graminearum*. Causes oestrogenic, feed refusal and emetic syndromes in animals. Produces deoxynivalenol and zearalenone.
- *F. verticillioides*. Causes leukoencephalomalacia in horses, pulmonary oedema syndrome in pigs, liver cancer in rats and is associated with oesophageal cancer in humans. Produces fumonisins.

The fumonisins were first isolated and chemically characterised in South Africa in 1988. Since that time fumonisin B_1 has been shown to cause field outbreaks of mycotoxicoses in animals, to be carcinogenic in animals and possibly carcinogenic to humans, and to occur naturally in maize (*Zea mays*) all over the world. These findings during the first 12 years after the discovery of these carcinogenic metabolites of *F. verticillioides* have created world-wide interest in the fumonisins. This interest in *Fusarium* mycotoxins in the Third Millennium will continue and grow.

Logrieco, A.

Istituto Tossine e Micotossine da parassiti vegetali, CNR, 70125 Bari, Italy

Advances on natural occurrence and production of beauvericin by *Fusarium* species

The extent of human, animal and plant exposure to beauvericin (BEA) is not yet well-established, although some recent reports indicate that the BEA occurrence is quite common particularly on cereals. Beauvericin is a toxic metabolite first reported to be produced by some entomopathogenic fungi such as *Beauveria bassiana* and *Paecilomyces fumosoroseum*. In 1991, Gupta et al. detected BEA in culture of entomopathogenic isolates of *F. subglutinans* from *Scirpophaga excerptalis* (Lepidoptera: Pyralide) and of *F. semitectum* from *Nilaparvata lugens* (Homoptera: Delphacidae) [1]. As *F. subglutinans* is a well known maize ear rot agent, extended investigation on BEA production by *F. subglutinans* strains isolated from maize in different geographical areas showed that BEA production is a common trait of *F. subglutinans* isolates from various countries with moderate climate, including Austria, Canada, Italy, Poland, Perù, South Africa. Subsequently, BEA was demostrated also to be produced by *F. proliferatum*, a species closely related to *F..subglutinans*, from Italian maize and then confirmed also by various strains isolated from different Italian sources, including maize, wheat and asparagus. On the basis of the teleomorphic state of *Fusarium* species belonging to *Liseola* Section (*Gibberella fujikuroi*), BEA was produced in large amount by isolates belonging to mating populations B,C, D, and E whereas isolates of A and F mating populations produced little, if any. Finally BEA proved to be a common metabolite of other

species in the genus [2]. In particular BEA was produced by *F. acuminatum* var. *acuminatum*, *F. acuminatum* var. *armeniacum, F. anthophilum*, *F. avenaceum*, *F. beomiforme, F. dlaminii*, *F. equiseti*, *F. longipes*, *F. nygamai*, *F. poae*, *F. sambucinum* and *F. oxysporum*. Studies conducted on various *formae speciales* of *F. oxysporum* (*F. oxy*.) have shown that BEA is commonly produced by *F. oxy*. f. sp. *asparagi*, *F. oxy*. f. sp. *gladioli*, *F. oxy*. f. sp. *opuntiarum*, *F. oxy*. f. sp. *melonis* and *F. oxy*. from lily, suggesting that BEA might play a role in the plant diseases induced by these fungi. In this respect BEA showed a certain toxicity towards protoplasts, with particular activity towards melon protoplasts [3].

Beauvericin was detected for the first time as a natural contaminant in amounts of 5-60 µg/g, together with moniliformin, in Polish maize ear rot mostly infected by *F. subglutinans*. Subsequently, selected maize ears, mostly colonized by *F. proliferatum* and collected from fields in Italy, were found to be contaminated by BEA (up to 40 µg/g) together with fumonisin B1 and moniliformin [4]. Various laboratories have been giving more attention to BEA occurrence in infected maize kernels and consequently the number of reports on its occurrence in major maize-producing areas has been increasing. Since BEA is produced by many species in the genus *Fusarium* it was supposed that it may be a contaminant of plants (in particular cereals) other than maize. In this respect BEA was commonly detected as natural contaminant of wheat kernels collected in Finland (up 3.5 µg/g) and most strains of *F. avenaceum* and *F. poae* isolated from these samples produced BEA and other esadepsipeptides in high amounts (up to 3,703 µg/g) [5]. Finally a study on the effects of cereal substrates and temperature on production of BEA by *F. subglutinans* showed that wheat and rice were the best substrates for BEA biosynthesis [6], suggesting investigations on the possible accumulation of this toxin in naturally infected rice kernels. In this respect strains of *F. fujikuroi* from Italy and Taiwan, isolated from rice were found to produce high levels of BEA [7]. These data indicate that BEA has a worldwide distribution and that it is a common contaminat of cereals, particularly maize and wheat.

Literature

[1] Gupta S., Krasnoff, S. B., Underwood, N.L., Renwick, J. A. A. Roberts, D. W. 1991. Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans*. Mycopathol. 123, 171-184.

[2] Logrieco, A., Moretti, A., Castella G., Kosteki , M., Golinski P., Ritieni, A., Chelkowski J.1998. Beauvericin production by *Fusarium* species. Appl. Environ. Microbiol. 64: 3084-3088.

[3] Sakaguchi, M., Moretti, A., Endo, M., Mastuda, Y., Nonomura, T., Toyoda, H. Ouchi, S. 1999 An approach to the use of plant simple detection of mycotoxins. October 99 third seminar on mycotoxins: Nara, Kinki University.

[4] Logrieco, A., Moretti, A., Ritieni, A., Bottalico, A., Corda, P. 1995. Occurrence and toxigenicity of *Fusarium prolifertaum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Dis. 79: 727-731.

[5] Logrieco, A., Rizzo A., Ferracane L..,Ritieni A., personal communication.

[6] Kosteki M., Wisniewska H., Perrone G., Ritieni A., Golinski P. Chelkowski J., A. Logrieco 1999. The effect of cereal and temperature on production of beauvericin, moniliformin and fusaproliferin by *Fusarium subglutinans* ITEM-1434. Food Addit. Contam. 16: 361-365.

[7] Moretti A., Logrieco A., Bottalico A., Ritieni A., Fogliano V., Randazzo G. 1996. Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*) Sydowia 48(1): 44-56.

Lemmens, M.[1]; Kouri, K.[2]; Lemmens-Gruber, R.[2]

[1] Institute of Agrobiotechnology, University of Agricultural Sciences, Konrad Lorenz Strasse 20, A-3430 Tulln, Austria
[2] Institute of Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Patch clamp studies on the electrophysiological properties of the beauvericin channel incorporated in artificial biological membranes

Beauvericin (BEA) is a mycotoxin produced by *Fusarium subglutinans* on maize in Austria. BEA is a hexacyclodepsipeptide with cytotoxic, antibiotic, insecticide and ionophoric properties. Ionophoric activities of a molecule can be explained by either carrier- or channel-building properties. With this study we wanted to provide direct evidence for the channel-forming properties of BEA, and an electrophysiological analysis of the behaviour of the BEA channel at the single-channel level is presented.

To reach our goal a reconstitution method to incorporate solubilized proteins in artificial membranes was developed in order to be able to study the characteristics of the channel with the patch clamp technique. This reconstitution procedure was successfully tested with well described channel-forming substances such as gramicidin and melittin.

BEA was incorporated in freeze-thaw liposomes (FTL). The membranes were composed of phosphatidyl-choline, -ethanolamine, -serine and cholesterol (5/5/2/2 w/w). Patch pipettes were sealed on the outer membrane of the FTL. Patches were excised by short exposure of the tip of the pipette to the air. Both bathing and pipette solution contained a standard solution of (in mM) 100 KCl, 1 $CaCl_2$, 5 Hepes brought at pH 7.5 with Tris. BEA (10 µM) was added in the pipette or in the bathing solution.

The following conclusions can be drawn from the results:

- Channel forming activity with clear transitions to different current levels was observed.
- Incorporation in the membranes occurred spontaneously. BEA is a very potent channel-forming molecule inducing pores even when present in trace amounts in the bathing solution.
- The conductance of the main open state of the BEA channel clamped between –100 and +100 mV was about 60 pS.
- At more extreme voltages the single channel current tended to saturate, *i.e.* the observed current was smaller than predicted by Ohm's law.
- Substates of the partially closed type were detected. The channel remained mostly in the main open state and switched to different lower current levels.
- The probability per unit time of a transition from the main open level to smaller current levels was voltage dependent and increased at large positive voltages (> +100 mV with reference to the pipette).
- At negative potential differences (<-50 mV with reference to the pipette) the channel activity was characterised by a pronounced flickery behaviour with high frequency oscillations and without any clear constant current level.

Preliminary experiments on the selectivity of the channel indicated that the pore is selective for cations (K^+ and Na^+) and not permeable for anions (Cl^-). Currently we are searching blockers for this pore. The non-selective toxic activity (towards microbes, insects and mammalian tissues) of BEA could, at least in part, be explained by its ability to induce pores in biological membranes, resulting in a disturbance of the normal gradients of physiological important monovalent cations across membranes. The closely related enniatins as well as a possibly phytotoxic activity of BEA and enniatins will also be investigated.

Srobarová, A.[2]; Logrieco, A.[1]; Ritieni, A.[3]; Ferracane, A.[3]; Morreti, A.[1]; Nadubinská, M.[2]

[1] Istituto Tossine e Micotossine da Parasiti Vegetali, C.N.R., Bari 70125, Italy
[2] Institute of Experimental Phytopatology and Entomology S.A.S., 900 28 Ivanka pri Dunaji, Slovakia
[3] Dipartmento di Scienza degli Alimenti, Universita degli Studi di Napoli "Federico", Portici 80055, Italy

Contamination of the maize ear by toxigenic *Fusarium* spp. in Slovakia

Three years observations carried out on the occurrence of *Fusarium* maize ear rot in Slovakia revealed a high incidence of the disease in maize fields of several localities (Šrobárová and Nadubinská 1999). Besides five other *Fusarium* spp. those of section Liseola were the prevalent fungi on maize. Eleven selected samples from different localities with well developed mouldy maize ears in 1996, 1998 were examined for causal species, associated mycotoxins and viability of infected kernels in connection to wheather conditions. Fungi were localized in different parts of caryopsis.

In both years the maize ears were contaminated by *F. moniliforme* from 4 % to 100 %. In addition to this fungus *F. proliferatum* and *F. subglutinans* were also present. *F. proliferatum* from 8 to 94 %. *F. subglutinans* has been very seldom identified in 1996, but more frequently in 1998. All samples were found to be contamined by FB_1 (up to 26,9 µg/g) and FB_2 (up to 6,30). BEA has been found just in one sample. Eight samples was contaminated by FP. Differences between level of toxins and species according to the years of sampling has been examined.

The production of FB_1, FB_2, BEA, FP was investigated for 28 strains (in 1996). FB_1 was produced by 20 strains of A and 2 of D population (up 1350 µg/g). FB_2 was not produced, BEA by one of A population (374 µg/g**)** and by sterile ones (2). FP was produced by A, D and sterile samples from 10 up to 1335 µg/g. All strains from 1998 belong to mating population A^+ or A^- and one strain (not fertile) is producing BEA (31 µg/g) and FP (22 µg/g). This toxin was not produced by MP A, but BEA has been in low concentration (from traces up to 3 µg/g). These high amounts of FB_1 (up to 5646 µg/g) and FB_2 (from traces up to 2017 µg) have been produced by strains of MP A in 1998, i.e. FB_2 was not produced in 1996. The intensity of infestation according to the viability of seedlings could be attributed to the temperature and precipitation in 1998 season.

Rossi, V.; Pattori, E.; Languasco, L.; Giosuè, S.

Istituto di Entomologia e Patologia vegetale, Università Cattolica S. Cuore, Via E. Parmense 84, I-29100 Piacenza, Italy

Dispersal of *Fusarium* species causing head blight of wheat under field conditions

Severe epidemics of *Fusarium* head blight (FHB) on wheat occur when environmental conditions after heading are moist and warm. Some works showed that high rainfall, air temperature, and length of the wet periods increase disease incidence. However, they did not explain the effect of weather on inoculum dispersal, because they considered the entire infection process. After the environment-controlled experiments of Stepanov (1935) and of Jenkinson and Parry (1994), splashing or wind-driven rain is widely regarded as the principal dispersal mechanism for *Fusarium* macroconidia, but it is not supported by studies carried out under natural crop conditions. Therefore, the dispersal of *Fusarium* conidia in wheat fields was studied by means of spore samplers, and related to the concomitant environmental conditions.

In a first experiment (1994 to 1997), a volumetric spore sampler was used to collect air above wheat crop, which was naturally infected by FHB, for a 3-week period around flowering. In a second experiment (1997 to 1999), microscope slides with an adhesive tape were placed within the wheat canopy and at the head height, in wheat plots that have been artificially inoculated by wheat straw previously infected with *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F.* (*Microdochium*) *nivale*. Slides were exposed daily starting from heading.

Results showed that, irrespective of the *Fusarium* species or the year, the dispersal of the conidia was firstly related to rainfall: the number of conidia sampled within or above the wheat canopy increased as the number of rainy days increased. Distribution of the rainy days over the sampling season was also important: i) prolonged intervals of dry days between two consecutive rainfalls reduced the total conidia sampled from the air more than some brief dry periods; ii) rain events of 2 or 3 days, increased the number of spores sampled, compared to many isolated rainfalls.

In each rain event the number of conidia progressively increased with the beginning of rainfall, especially after 3 or more hours after rain begun, but the highest numbers of conidia were sampled after rainfall had ceased. Dynamic of spore sampling after rain had ceased was related to wetness and relative humidity: spores continued to be sampled in the presence of wetness, with high relative humidity, and their density strongly decreased at the end of wet periods or when relative humidity dropped.

Based on the results obtained in the present work, the role of rainfall in the dispersal of *Fusarium* macroconidia within a wheat crop till the head height can be drawn as follows. At the beginning of a rain event, rain drops firstly disperse spores in to a film of water, then their impact produces many splash drops that carry spores directly on to the upper leaves. Later, rain drops resplash these spores previously deposited on leaves, for further upward movements till the head height. Atmospheric turbulence can assist the upward movement of droplets during their jumps. When the rain period cease, wind carried droplets can further disperse spores within the canopy, for some times.

High numbers of airborne conidia during no rainy days was associated with the presence of sporodochia on the scabbed heads: wet conditions caused by rainfalls favoured spore production on the scabbed head parts, and these conidia became airborne because of air currents also two or three days after rainfall had ceased.

Regression models were elaborated to estimate the density of conidia in to the air on the basis of the meteorological conditions, in either rainy or dry days. These models accounted for a great part of data variability and estimated patterns of airborne conidia accurately.

Amra, H. A.[1]; Aboul-Enein, A. M.[2]; Ragab, A. A.[2]; Mohamed, I. M.

[1] Mycotoxins Central Lab., National Research Center,Egypt
[2] Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt

Fumonisins and *Fusarium moniliforme* in Egyptian corn (*Zea mays)*

One hundred and twenty corn samples (white and yellow) were collected from markets of different governorates namely; Cairo, Giza, El-Sharkia, El-Monufyia, El-Beheara and El-Gharbia during 1995-1996 (summer and winter). The samples were examined for Fumonisn B_1 (FB_1) and *Fusarium moniliforme* infection.

The results indicated that in winter season the highest positive percentage for FB_1 in white corn was (40 %) of the total corn sample. The minimum and maximum concentrations were 100 and 3250 µg/ kg sample (average 1189.5 µg/ kg corn).In summer season the percentage of positive white corn samples for FB_1 was 10%. The minimum and maximum concentrations were 500 and 4100 µg/ kg sample (average 1726.7 µg/ kg).

The highest percentage of positive yellow corn samples for FB_1 (33.4%) was observed in winter season. The range concentration was 60-3800 µg/kg sample, with an average of 1328 µg/kg of corn sample, while in summer season, the minimum and maximum concentrations were 1450 and 7760 µg/kg sample, (average 2278 µg/kg sample). The percentage of positive yellow corn samples for FB_1 was (16 %).

From our data it is clear that the FB_1 is found in Egyptian white corn and yellow corn samples tested and the occurrence of FB_1 in the samples depend on the area of collection as well as the season (winter and summer).

The percentage of *F. moniliforme* to total fungal count on PDA ranged from 7.5 to 49.4 %. The total *Fusarium* spp. count varied from 34 to 244 colony/100 seeds in samples collected from Cairo (white corn) and El-Kalubia (white corn) on PCNB respectively. The relative percentages of *F . moniliforme* to *Fusarium* spp. ranged from 16.6 to 90 % for El-Beheara and El-Sharkia governorates respectively. Twenty isolates from 50 of *F. moniliforme* isolates (40 %) were found positive to FB_1 production. FB_1 production was ranged between 0.2 to 280.7 with an average of 82.4 mg/kg on corn sample. The most *F. moniliforme* isolates from yellow corn samples tested for FB_1 production showed higher percent (60-100 %) than the isolates from white corn samples (0-60 %).

Nadubinská, M.[1)]; Ritieni, A.[2)]; Srobarová, A.[1)]

[1)] Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, Nadrazna 52, SK-90028, Ivanka pri Dunaji, Slovakia
[2)] Dipartmento di Scienza degli Alimenti, Universita degli Studi de Napoli "Federico", Partici 80055, Italy

The influence of fusariotoxins on chlorophyll content of maize

Fusarium species are common maize pathogens. Many of them are known to produce significant levels of different toxins. The role of particular toxins in pathogenesis is not always completely understood. The aim of this work was to study the phytotoxic effect of fusariotoxins on maize plants. Two maize inbreds with different susceptibility to *Fusarium* infection were used: susceptible Pavla and resistant Lucia. Two weeks old intact plants were grown on solutions containing fumonisin B_1 (FB_1), moniliformin (MF), fusaproliferin (FP), zearalenone (ZEN), zearalenol (ZEA), deoxynivalenol (DON) at 30 $\mu g.ml^{-1}$ concentration. Controls were exposed to toxin diluent. After 72 hours chlorophyll was extracted in ethanol and its content per one gram of dry mass evaluated. The greatest decrease in chlorophyll levels was caused by MF and FP. Plants treated with ZEA and FB_1 contained significantly higher amounts of chlorophyll. DON has slightly decreased chlorophyll content in Lucia and ZEN in Pavla. Additionally toxins were added to chlorophyll extract to make final concentrations 20 $\mu g.ml^{-1}$ and their influence was observed and compared to *in vivo* experiments.

Langseth, W.[1)]; Ghebremeskel, M.[1)]; Kosiak, B.[1)]; Kolsaker, P.[2)]; Miller, J. D.[3)]

[1)] National Veterinary Institute, Dep. Feed and Food hygiene, P. Box 8156 Dep, N-0033 Oslo, Norway
[2)] Dep. Chemistry, University of Oslo, P. Box 1033 Blindern, N-0315 Oslo, Norway
[3)] Ottawa-Carleton Institute of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6, Canada

Production of hydroxy-culmorins and other metabolites by *Fusarium culmorum* and *F. graminearum* strains, and the occurrence of hydroxy-culmorins in artificially inoculated and naturally contaminated grain

Twenty-three *Fusarium culmorum* and 21 *F. graminearum* isolates were studied for their ability to produce mycotoxins and other secondary metabolites. The strains were cultivated on rice, and the extracts analysed by gas chromatography mass spectrometry (GC-MS) after purification on Mycosep™#225 columns and derivatization with pentafluoropropionic (PFP) reagent. Fourty-five samples of naturally contaminated grain, barley, wheat and oats, and three samples of mixed feed from 1988-1995, and 16 samples of grain artificially inoculated with *Fusarium culmorum* were also analysed for deoxynivalenol (DON), 3-acetyl-DON, culmorin, and hydroxy-culmorins. The amount of each of culmorin, 5-, 12-, 14- and 15- hydroxy-culmorin and one unknown hydroxy-culmorin were determined relative to the amount of DON plus 3-acetyl DON for each sample.

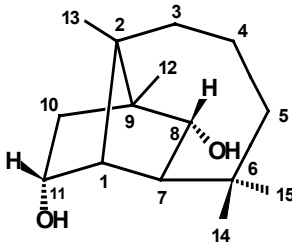


Figure: Culmorin

This study shows that there is a strong correlation between the amount of DON produced and the amount of culmorin and hydroxy-culmorins present. 15- hydroxy-culmorin, followed by 5-hydroxy-culmorin were the main metabolites produced by *F. culmorum* in addition to DON and 3-acetyl-DON, while 5-, 12- and an unidentified hydroxy-culmorin, suggested to be 14-hydroxy-culmorin, were the main ones of *F. graminearum*. The hydroxy-culmorin profile was found to be significant different for the two *Fusarium* species. Minor amounts of about ten other hydroxy-culmorins, and four hydroxy-culmorones were also detected in most cultures. The precursors in the biosynthetic sequence to 3-acetyl-DON, 7,8-dihydroxycalonectrin and 15-deacetyl-7,8-dihydroxycalonectrin, were detected in most cultures. Traces of sambucinol seemed to be present in some of the isolates, but were not detected in any significant amounts.

The ratio between the total amount of culmorin plus hydroxy-culmorins and the DON compounds ranged from 0.03 to 2.2 in the cultures and from 0.14 to 1.07 in the grain samples. The ratio of each of the culmorin compounds were in the same range in the grain inoculated by *F. culmorum* as found in for the *F. culmorum* strains cultivated on rice, while the profile in the naturally contaminated grain was more similar to that of *F. graminearum*, indicating that *F. graminearum* may be an important source for DON in grain also in relatively cold areas.

Muriuki, G.; Mirocha, C. J.

University of Minnesota, St. Paul, MN, USA

Time of deoxynivalenol and nivalenol biosynthesis during pathogenesis of *Fusarium* species in wheat

Fusarium graminearum Schw. and *F. culmorum* (W.G. Smith) Sacc. occur worldwide on wheat and barley. They are the principal pathogens causing *Fusarium* head blight [1]. Toxins they produce can accumulate in infected grains and are health hazards. Quality loss because of toxin contamination of harvest in wheat is probably the worst consequence of *Fusarium* head blight. The role of DON in pathogenicity and virulence of *Fusarium* species has been studied although it has not been clearly defined; moreover limited studies have not demonstrated the role of NIV in pathogenesis.

To study the role of DON and NIV in pathogenecity, toxin detection time and the relative aggressiveness of four nivalenol (NIV) producers (*F. graminearum*, ACR 2705-1, Japan; *F. culmorum*, France; *F. culmorum*, 30 & 56, Germany) were compared with aggressiveness of a deoxynivalenol (DON) producer (*F. graminearum* Butte 86 Ada-11, U.S.A.) on a resistant (Pioneer 2375) and a susceptible (Norm) wheat cultivars. Kinds and amounts of toxins produced on wheat by these *Fusarium* isolates were also studied. Disease severity symptoms caused by pure DON and NIV toxins was studied in these wheat genotypes and in Sumai # 3. DON and de-epoxyDON production was studied in the three wheat genotypes.

Fusarium isolates were first established on potato dextrose agar medium (PDA) and cultured on mung bean agar for sporulation [2]. Conidia were harvested 14 days post inoculation and the concentration adjusted to 10^6 macroconidia ml^{-1}. Experimental plants were grown both in the greenhouse and in the field. At anthesis a central spikelet to be inoculated was marked by cutting the horns and inoculating with 10ul of inoculum using a Hamilton mirosyringe. Disease severity in inoculated spikelets was assessed visually on a scale of 0-5. Toxins were analyzed using a single kernel method as described [3]. Single spikelets of wheat the inoculated and the two adjacent (apical and basal) were detached from the spike, weighed and placed individually in culture tubes for analysis. All samples were analyzed on a Shimadzu QP- 500 GC/MS using selected ion monitoring (SIM)

Toxins were detected within 48 h while visible symptoms occurred at 72 h post inoculation. Disease symptoms caused by pathogens were necrosis and bleaching whereas those caused by pure toxins was bleaching. The DON- producing isolate (Butte 86) was more aggressive than the NIV producers; infection spread was noted 10 days post inoculation. Pure DON injected into spikelets declined over time indicating metabolic degradation. Both DON and NIV caused visible disease symptoms (bleaching) on Pioneer 2375 and Norm, but no symptoms on Sumai 3.

Literature

[1] Eeuwijk, F.A., Mesterhazy A., Kling Ch. I., Ruckenbauer P., Saur, L., Burstmayr H. Lemmens M., Keizer, L. C.P., Maurin, N. and Snijders C.H.A. 1995. Assessing non- specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theor Appl. Genet 90: 221-228.

[2] Evans, C. K., Xie, W., Dill-Macky, R., and Mirocha, C.J. 2000. Biosynthesis of deoxynivalenol in spikelets of barley inoculated with macroconidia of *Fusarium* graminearum. Plant Dis. 84: In publication.

[3] Mirocha, C.J., Kolaczkowski, E., Xie, W., Hui, Y., and Jelen, H. 1998. Analysis of deoxynivalenol and its derivatives (Batch and Single Kernel) using Gas Chromatography/ Mass Spectrometry. J. Agric.Food Chem. 46: 1414-1418.

Klerks, M.; Schoen, C.; Waalwijk, C.

Wageningen University and Research Centre, Plant Research International, Business Unit Biointeractions and Plant Health, P.O. Box 16, 6700 AA Wageningen, The Netherlands

The detection of trichodiene synthase mRNA in *Fusarium* on seeds using AmpliDet RNA

NASBA is an isothermal nucleic acid amplification method, where single stranded RNA is amplified [1]. Since all steps of the amplification are carried out at the same temperature (41^0C) this technology is specifically suited for large-scale screenings. The NASBA based-system was standardized and adapted for detection of trichothecene toxin producing *Fusarium* species in indexing programs for *in vitro* material after the effectiveness of the system has been evaluated. Different sets of primers for the amplification of trichothecene producing *Fusarium* species have been designed on basis of published sequences of trichodiene synthase, *tri5*, genes. With a detection limit of less than 100 molecules of *in vitro tri5* RNA, the NASBA system could clearly detect *in vitro tri5* RNA from *F. culmorum*, *F. graminearum*, *F. poae, F. sambucinum* and *F. venenatum*. Moreover, detection of *tri5* mRNA in cultures of *F. culmorum* and *F. sporotrichioides* was also successful.

To improve the applicability of the system for large-scale screenings, NASBA was combined with molecular beacons [2], enabling simultaneous real-time amplification and detection in a single tube, called AmpliDet RNA [3]. Using AmpliDet RNA the sensitivity remained 100 copies of *in vitro tri5* RNA. Detection of *tri5* mRNA expression in cultures of *Fusarium* was already possible after 8 hours after inoculation with 4e4 spores per milliliter.

Finally, production samples of wheat contaminated with different levels of DON were analysed by AmpliDet RNA and a good correlation with signal intensity was observed.

Literature

[1] Kievits, T., van Gemen, B., van Strijp, D., Schukkink, R., Dircks, M., Malek, L., Sooknanan, R. and Lens, P. (1991) NASBA^{TM} isothermal enzymatic in vitro nucleic acid amplification optimized for HIV-1 diagnosis. J. Virol. Meth. 35: 273-286.

[2] Tyagi, S., and Kramer, F.R. (1996) Molecular beacons: Probes that fluoresce upon hybridization. Nature Biotechnol. 14: 303-308.

[3] Leone, G., van Schijndel, H., van Gemen, B., Kramer, F.R., Schoen, C.D. (1998) A molecular beacon probe combined with amplification by NASBA enables homogeneous, real-time detection of RNA. Nucl. Acids Res. 26: 2150-2155.

Garaleviciene, D.; Pettersson, H.

Department of Animal Nutrition and Management, Swedish University of Agriculture Sciences, P.O. Box 7024, SE 750 07 Uppsala, Sweden

Effect of adsorbents (MYCOFIX®PLUS and MYKOGUARD®) in diets, containing small amounts of ochratoxin A and fusariotoxins, on some breeder hen clinical plasma parameters, body weight gain and egg quality

Poultry feeds, containing mycotoxins, can have adverse effects on clinical plasma parameters, feed intake, body weight gain and egg quality [1, 2]. The poultry industry demands the evaluation of different adsorbents, which could minimise adsorption of the toxins from the gastrointestinal tract [1].

37 weeks-old Plymouth Rock breeders were divided into 3 treatments (n=5700) and fed by mixed feed, containing small amounts of ochratoxin A (OA) and deoxynivalenol (DON), for 50 days. The feed for the 1st and the 2nd experimental treatments had additionally 0.1% of MYCOFIX® PLUS (MPL) and 1% of MYKOGUARD® (MKG), respectively. Clinical plasma parameters were evaluated after 1, 20 and 50 days (n=10). Egg quality was evaluated at the beginning and at the end of the trial.

After 50 days, the activities of the alanine and aspartate transaminases, alkaline phosphatase and lactate dehydrogenase did not differ significantly neither between the plasma of MPL, MKG and control hens nor within their activities determined at the start of the experiment. Gamma glutamyl transferase was, however, significantly lower (96%) within the MPL and MKG treatments. These data, except the last enzyme, are in accordance with the report of Kubena *et al*. [2]. No differences were found neither between nor within treatments in the levels of total protein, bilirubin, cholesterol and sodium. The decreases of 52-58% in uric acid, glucose and phosphorus within the control group were possibly caused by OA/DON. These data are in agreement with the effects of high OA/DON doses on chicks, reported by Kubena *et al*. [2]. However, in the present study the toxins might have been too low to cause similar effects on other parameters. The levels of vitamin A and E in hen plasma did not differ significantly neither between nor within the treatments. This indicates that the vitamins were neither altered nor adsorbed from the diets. The body weight gain was, however, 50% and 22% lower in the MPL and MKG treatments, respectively, and MPL eggs had 8% lower weight. The reduced feed consumption in the adsorbents groups might have been the reason of lower body weight gain and also lower egg weight in the MPL treatment.

The MPL and MKG treatments had, however, a significant increase in several clinical plasma parameters after 20 days. An increase of 37–772% was noted for alanine transaminase, alkaline phosphatase, lactate dehydrogenase and the concentrations of total protein, cholesterol, glucose and inorganic phosphorus. Bilirubin, sodium and uric acid were depressed within all treatments. These data are in the contrary to that the addition of the adsorbents to diets do not significantly alter the serum biochemical parameters, as reported by Kececi *et al*. [1]. These transient effects may indicate a temporary influence by adsorbents on the metabolism.

Literature

[1] Kececi, T., Oguz, H., Kurtoglu, V., Demet, O. 1998. Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br. Poult. Sci. 39, 452-458.

[2] Kubena, L.F., Huff, W.E., Harvey, R.B., Corrier, D.E. 1988. Influence of ochratoxin A and deoxynivalenol on growing broiler chicks. Poult. Sci. 67, 253-260.

Karlovsky, P.[1]; Maddox, J.[1]; Gilliam, G.[1]; Crasta, O.[2]; Folkerts, O.[2]; Duvick, J.[1]

[1] Pioneer Hi-Bred International, Johnston, Iowa, USA
[2] CuraGen Corporation, New Haven, Connecticut, USA

Enzymatic degradation of *Fusarium* mycotoxins

Fusarium species infecting cereal crops produce a number of secondary metabolites. Some of them are toxic to mammals and lead to a health risk to humans and livestock ingesting contaminated food and feed. Some *Fusarium* metabolites are involved in pathogenesis as virulence factors, facilitating the colonization of the host plant.

Crops can be engineered to enzymatically detoxify fungal secondary metabolites by expressing enzymes that modify or degrade these substances within the plant tissue where the toxins are secreted. Detoxifying activities can serve two purposes, depending on the secondary metabolite involved: increase the tolerance of the host plant to infection and protect humans and farm animals from health risk posed by mycotoxins [1].

We identified bacteria and fungi detoxifying the following mycotoxins of *Fusarium* spp.: beauvericin, fumonisins, fusaric acid, moniliformin and zearalenone. The mechanism of detoxification was studied and a functional genomic approach is being used to clone genes encoding enzymes involved in the process. The goal of this work is to reduce the contamination of agricultural commodities with mycotoxins by extending the enzymatic repertoire of cultured plants.

Literature

[1] Karlovsky, P. 1999. Biological detoxification of fungal toxins and its use in plant breeding, feed and food production. Natural Toxins 7, 1-23.

POSTERS

Taxonomy and Genetics

Munaut, F.[1]; Langue, C.[1]; Hove, F. Van [1]; Colson-Corbisier, A.-M.[1], Maraite, H.[2]

[1] Mycothèque de l'Université catholique de Louvain (MUCL)[1], Unité de Microbiologie, Louvain-la-Neuve, Belgium. Part of the Belgium Co-ordinated Collection of Micro-organisms (BCCM™) consortium.
[2] Unité de Phytopathologie, Université catholique de Louvain, Louvain-la-Neuve, Belgium

Taxonomy in an integrated approach of analysis of mycotoxins produced by *Fusarium* spp.

Among the 30,000 isolates of filamentous and yeast-like fungi stored at the Mycothèque de l'Université catholique de Louvain (MUCL), approximately 300 isolates were identified as *Fusarium*. These isolates originated from various countries worldwide and were obtained from different vegetal host species, from soils or from stocks of animal or human food. The research work undertaken on that genus at MUCL is based on three different approaches : taxonomic studies within the genus, detection and biochemical characterization of mycotoxins (more particularly fumonisins), and analysis of their biosynthetic pathways. The taxonomic approach proposed in this paper is based on the implementation of the morphological identification by the use of molecular biology tools. For this purpose, ribosomal DNA, mitochondrial DNA and several genes allowing the distinction between *Fusarium* species (β-tubulin, calmodulin, elongation factor) are analysed. DNA is amplified by the polymerase chain reaction (PCR) technique, using rDNA and mtDNA specific primers, or primers constructed from gene sequences already published. After sequencing, aligned sequences are analyzed together with those of *Fusarium* spp. available in the EMBL databank. Phylogenetic relationships between MUCL and reference isolates are then analyzed using PHYLIP and PAUP software. Integration of morphological and sequence-defined groups will help to clarify the taxonomic position of the isolates within the genus *Fusarium*. Furthermore, in the frame of the increasing importance of this mycotoxinogenic fungus in human and animal health, overlapping of the taxonomic data with those obtained for the mycotoxins analyses will serve to the construction of a database which will help to develop adequate strategies of management of mycotoxin contamination.

Klemsdal, S. S.[1]; Lund, G.[1]; Torp, M.[2]

[1] The Norwegian Crop Research Institute, Plant Protection Centre, Department of Plant Pathology, Høgskoleveien 7, N-1432 Ås, Norway
[2] National Veterinary Institute, Food and Feed Hygiene, Box 8156 Dep, N-0033 Oslo, Norway

Genetic diversity in the section *Sporotrichiella* as revealed by sequence analyses of the ITS regions of the rDNA

Recent observations regarding the occurrence of T-2 toxin and the deacetylated form HT-2 in Norwegian cereals, produced by isolates of *Fusarium* described as "powdery *F. poae*", have revealed the need for a thorough study of these isolates. Through the research network, available within COST action 835 "Agriculturally Important Toxigenic Fungi", a total of 109 strains of *Fusarium* were collected and selected for further studies. The "powdery *F. poae*" is morphologically similar to *F. poae*, but has a mycotoxin

profile similar to *F. sporotrichioides*. Representatives from these two species in addition to the "powdery *F. poae*" isolates and the closely related *F. kyushuense* were studied.

The strains were grown on disks of cellophane on Potato Dextrose Agar (PDA), harvested and DNA was extracted. The internal transcribed spacer (ITS) regions I and II of the ribosomal DNA (rDNA) were amplified by PCR and sequenced.

Preliminary results show that the "powdery *F. poae*" isolates clearly can be distinguished from the other species studied. These isolates seem to be more closely related to *F. sporotrichioides* than to *F. poae* and *F. kyushuense*. Of the 23 strain of "powdery *F. poae*" only one genotype of the ITS regions was represented. Neither did we observe any genetic variation within the species *F. sporotrichioides* and *F. kyushuense*. Four genotypes of *F. poae* could be found. Two of these genotypes were more frequently occurring than the other two. The four *F. poae* genotypes seemed to have the same geographic distribution. The impact of these results on the development of molecular methods for diagnosis of members of Section Sporotrichiella is discussed.

Corazza, L.[1]; Maccaroni, M.[1]; Luongo, L.[1]; Balmas, V.[2]

[1] Instituto Sperimentale per la Patologia Vegetale- Roma, Italy
[2] Dipartimento di Protezione delle Piante, Sez. Patologia Vegetale, Università degli Studi di Sassari, Italy

Characterization of *Fusarium culmorum*, the causal agent of head blight and foot rot of wheat, by RAPD-PCR

Head blight and foot rot are the most important diseases of durum wheat in Italy. *Fusarium* head blight and *Fusarium* foot rot reduce the quality of the grain.

Field surveys conducted over many years in Italy indicate that *Fusarium graminearum* Schwabe Group 2 *sensu* Francis and Burgess (1977) is the most frequent causal agent of head blight while *Fusarium culmorum* (W.G. Smith) Sacc. is the most common pathogen of foot rot of wheat. *F. culmorum*, even though less frequently, was also isolated from blighted heads in several localities. Thirty isolates of *F. culmorum* were morphologically identified on CLA (Carnation Leaf Agar) (Nelson *et al*. 1983) and their identification was confirmed by specific PCR marker (Schilling *et al*. 1996). *F. culmorum* isolates obtained from heads (11) and base stem (14) of wheat and 5 isolates from other hosts (melon, artichoke, hazel-nut, carrot, sugar-beet) were tested by RAPD-PCR to analyze the level of genetic diversity.

Thirty random deca-primer (Operon Technologies- Alameda, CA, Kit L and Kit AN) were tested on genomic DNA; eleven of them were used to analyse DNA profiles to determine differentiation between isolates. RAPD data obtained from amplifications were recorded by scoring DNA bands and compiled in a binomial matrix in which 1 indicates the presence and 0 the absence of a marker. The resulting matrix was used to compute a cluster analysis by the unweighted pair-group method of arithmetic averages (UPGMA: Sneath and Sokal, 1973). The cluster analysis was performed with the similarity values using the SAHN procedure of the program NTSYS-pc Version 2.0 (Rohlf, 1992). The preliminary phylogenetic tree constructed on RAPD data suggested that a diversity may be present among isolates of *F. culmorum* obtained from blighted heads and from foot rot of wheat. However, further analyses on DNA profiles with RAPDs and on the pathogenic and morphological aspect of the isolates have to be developed to confirm the diversity among isolates of different origin.

Literature

Francis, R.G. and Burgess, L.W., 1977. Characteristics of two populations of *Fusarium roseum* 'Graminearum' in eastern Australia. Trans. Br. Mycol. Soc. 68 (3): 421-427.

Nelson, P.E., T.A., Toussoun and W.F.O., Marasas, 1983. *Fusarium* Species. An Illustrated Manual for Identification. Pennsylvania State University Press. University Park, 193 pp.

Rohlf, F. J., 1992. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis, System Vers. 2.0, Exeter Software, Setauket, New York.
Schilling A.G., E.M.,Moller and H.H., Geiger, 1996. Polymerase Chain reaction-based assay for species specific detection of *F. culmorum*, *F. graminearum* and *F. avenaceum*. Phytopathology 86: 515-522.
Sneath, P.H.A. and R. R.,Sokal, 1973. Numerical Taxonomy-Freeman and Company, San Francisco.

Hering, O.[1]; Nirenberg, H. I.[2]

[1] Federal Biological Research Centre for Agriculture and Forestry, Information Centre for Phytomedicine and Library, Königin-Luise-Str. 19, D-14195 Berlin, Germany
[2] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, D-14195 Berlin, Germany

Studies on specialized forms of *Fusarium oxysporum* – Problems and opportunities

Different spezialized forms and races exist in *Fusarium oxysporum*. Specialized forms are distinguished by pathogenicity tests on plants. Often *Fusarium redolens* Wollenweber f. sp. *dianthi* Gerlach and *F. oxysporum* f. sp. *dianthi* (Prillieux & Delacroix) Snyd. & Hans. are synonymized because of identical infection symptoms on *Dianthus caryophyllus* L. (Booth 1971).

Isolates of different spezialized forms of *F. oxysporum* and of species within the section *Elegans* and *Liseola* such as *F. redolens*, *F. udum* E. Butler und *F. acutatum* Nirenberg & O'Donnell were investigated using ITS-RFLP and RAPD. ITS-RFLP results of all investigated isolates of *Fusarium oxysporum* show identical restriction patterns. Isolates of *Fusarium redolens*, *F. udum* und *F. acutatum* can clearly be distinguished. The results demonstrate that the ITS-RFLP technique is quite useful for differentiating fusaria at the species level.

The RAPD results (see fig. 1) also confirm the delimitation of *F. redolens* f. sp. *dianthi* from *F. oxysporum*.

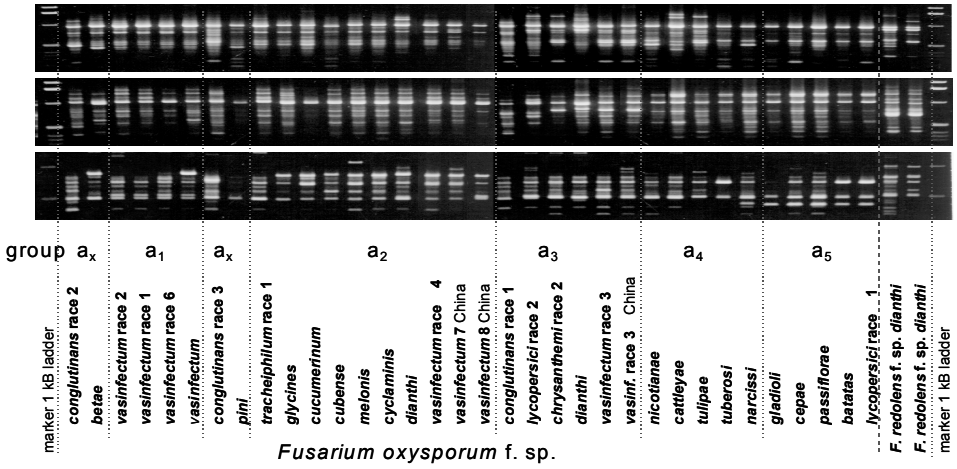


Figure: RAPD-banding patterns of *Fusarium redolens* and spezialized forms of *F. oxysporum* Primer: MB05 5'-gagggtggcggttct, MB15 5'-gtcgtcgtcgtcgtc, MB65 5'-gcgcatgactggcag

A high degree of similarity in the RAPD banding patterns of *Fusarium oxysporum* attracs attention. Some isolates can be grouped using some characteristic bands (see group a1-a5). Isolates of *F. oxysporum* f. sp. *vasinfectum* race 1, 2 and 6 within group a1 and isolates of the spezialized forms *gladioli*,

cepae, *passiflorae*, *batatas*, *lycopersici* race 1 within group a5 show homogeneous banding patterns. The variability of the RAPD patterns of the investigated races sometimes is comparable with the forma speciales level. The genetical similarity can sometimes be larger at the level of spezialized forms than it is at the level of races within one forma speciales. This means that the present concept of specialized forms and races within *Fusarium oxysporum* should be reconsidered.

Literature

Booth, C. 1971. The genus *Fusarium*. Commonwealth Agric. Bureaux, Farnham Royal: Bucks, UK; 237 p.

Golińska, B.[1] ; Narożna, D.[1]; Kwaśna, H.[2]; Króliczak, J.[1]; Kostecki, M.[3]; Goliński, P. K.[3]; Wakuliński, W.[4]; Mądrzak, C. J.[1]

[1] Dept. of Biochemistry and Biotechnology, A. Cieszkowski Agr. Univ., Wołyńska 35, 60-637 Poznań, Poland
[2] Dept. of Forest Pathology., A. Cieszkowski Agr. Univ., Wojska Polskiego 71 C, 60-625 Poznań, Poland
[3] Dept. of Chemistry, A. Cieszkowski Agr. Univ., Wojska Polskiego 75, 60-625 Poznań, Poland
[4] Dept. of Phytopathol., Warsaw Agricult. Univ. (SGGW), Nowoursynowska 166, 02-757 Warszawa, Poland

Analysis of *Fusarium avenaceum* diversity by RAPD-PCR and RFLP

A group of over 60 isolates of *Fusarium avenaceum* was analyzed with respect to their pathogenicity and the ability to synthesize mycotoxins (moniliformin). Isolates were dentatively divided into three groups based on their ability to produce MON. Based on this phenotypic feature, fifteen isolates were selected. Five isolates producing low amonts of MON (0-1 µg/g tissue), five belonging to the medium level group (10 - 1000 µg/g), and five highly productive (over 1000 µg MON/g of tissue) were taken for further studies. The taxonomical classification of isolates was confirmed by RFLP analysis of amplified rDNA sequences [1]. The physical structures of genomes of the selected group of isolates were analyzed by RAPD-PCR technique using 10 bp. long nucleotide primers [2, 3, 4]. The RAPD-PCR patterns revealed significant variability among analyzed isolates. This is in good agreement with the results of other authors [5, 6]. We have found no correlation between strain ability to produce MON and its pathogenicity. We suggest that extensive work is necessary to establish phylogenetic relations among isolates selected for further studies, followed by the analysis of their phenotypes, in order to correlate the pathogenic features of isolate with its genotype. The search for genes responsible for the ability to synthesize moniliformin and/or for pathogenicity of particular strains has to be performed via comparison of closely related isolates of well recognized phylogenetic origin.

Literature

[1] Bateman, G.L., Kwaśna, H., Ward, E., 1996. Relationships among *Fusarium* spp. estimated by comparing restriction fragment length polymorphism in polymerase chain reaction-amplified nuclear rDNA. Can. J. Microbiol. **42**, 1232-1240
[2] Schilling, A.G., Moller, E.M., Geiger, H.H., 1994. RAPDs of *Fusarium culmorum* and *Fusarium graminearum*: Application for genotyping and species identification. In: Schots, A., Dewey, F.M., Oliver, R., eds. Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification. Oxford, UK: CAB International, 47-56.
[3] Schilling, A.G., Moller, E.M., Geiger, H.H., 1996. Polymerase chain reaction based assays for species specific detection of *Fusarium culmorum, F. graminearum* and *F, avenaceum*. Phytopathology **86**, 515-522.
[4] Manulis, S., Kogan, N., Reuven, M., Ben-Yephet, Y. 1994. Use of the RAPD technique for identification of *Fusarium oxysporum* f. sp. *dianthi* from Carnation. Phytopathology **84**, 98-101.
[5] Bentley, S., Pegg, K.G., Dale, J.L., 1995. Genetic variation among a world-wide collection of isolates of *Fusarium oxysporum* f. sp. *cubense* analysed by RAPD-PCR fingerprinting. Mycol. Re. **99**, 1378-1384.
[6] Yli-Mattila, T., Paavanen, S., Hannukkala, A., Parikka, P., Tahvonen, R., Karjalainen, R., 1996. Isozyme and RAPD-PCR analyses of *Fusarium avenaceum* strains from Finland. Plant Pathology **45**, 126-134.

Hering, O.[1]; Nirenberg, H. I.[2]

[1] Federal Biological Research Centre for Agriculture and Forestry, Information Centre for Phytomedicine and Library, Königin-Luise-Str. 19, D-14195 Berlin, Germany
[2] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, D-14195 Berlin, Germany

Differentiation of *Plectosporium tabacinum* isolates by RAPD and ITS-RFLP

Plectosporium tabacinum (formerly *Fusarium tabacinum*) is known as a species with a high variability in morphological and phytopathological characters (Gams & Gerlagh 1968; Pascoe et al. 1984). 41 isolates obtained from different locations and hosts were examined using the molecular techniques ITS-RFLP and RAPD.

Five ITS-RFLP pattern groups using 9 restriction enzymes were found for isolates of *Plectosporium tabacinum*. As expected, a clear distinction of *P. tabacinum* isolates to other *Fusarium* species was observed (see fig. 1).

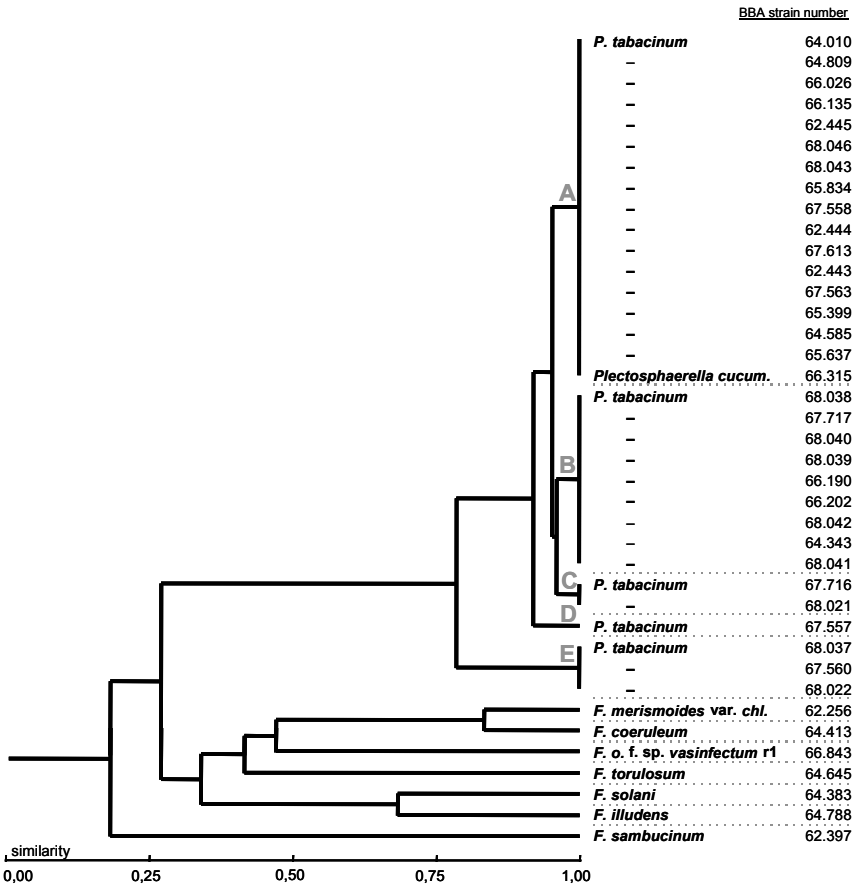


Figure: Cluster analytical evaluation of the ITS-RFLP results (*Plectosporium tabacinum*)

The RAPD banding patterns reflected the high intraspecific variability of this fungus. Considerations for further subdivisions within *P. tabacinum* (Palm et al. 1995) were supported by the results shown. Isolates of *F. tabacinum* pathogenic on *Cucurbita* could clearly be differentiated by both techniques. Therefore we suggest to delimit the isolates pathogenic to curcurbita as *F. tabacinum* f. sp. *cucurbitae*.

Literature

Gams, W. & Gerlagh, M. 1968. Beiträge zur Systematik und Biologie von *Plectosphaerella cucumeris* und der zugehörigen Konidienform. Persoonia **5**: 177-188.

Palm, M.E., Gams, W. & Nirenberg, H.I. 1995. *Plectosporium*, a new genus for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina*. Mycologia **87**(3): 397-406.

Pascoe, I.G., Nancarrow, R.J. & Copes, C.J. 1984. *Fusarium tabacinum* on tomato and other hosts in Australia. Trans. Brit. Mycol. Soc. **82**: 343-345.

Kosiak, B.; Holst-Jensen, A.; Torp, M.

National Veterinary Institute, Department of feed and food hygiene, 8156 Dep., 0033 OSLO, Norway

Molecular differentiation of *Fusarium* spp. isolated from Norwegian cereals

Restriction fragment length polymorphism (RFLP) of PCR amplified intergenic spacer (IGS) ribosomal DNA (rDNA) and translation elongation factor 1α (TEF-1α) introns, were applied to study genetic variability between and within *Fusarium* species isolated from wheat, barley and oats from four geographic regions of Norway. Altogether 81 isolates of *Fusarium* spp: 24 of *F.avenaceum*, 24 *F.culmorum*, 20 *F.graminearum*, 3 *F.tricinctum*, 3 *F.poae*, 4. *F.equiseti* and 3 *F.torulosum* were studied. Intra- and interspecific variation was observed. The RFLP patterns were compared with secondary metabolite profiles obtained from previous studies [1, 2, 3], in order to investigate if the phenotypic and/or genotypic variation corresponded with geographic origin. Geographic structuring of *Fusarium* spp. across continents has been reported [4]. One hypothesis we wanted to test was whether such structuring could also be observed at the national level. The application of the PCR-RFLP assay in combination with chemical analysis to study geographic structuring of isolates will be discussed.

Literature

[1] Langseth, W., Bernhoft, A., Rundberget. T., Kosiak, B., & Gareis 1999. Mycotoxin production and cytotoxicity of Fusarium strains isolated from Norwegian cereals. Mycopathologia **144**, 103-113.

[2] Langseth, A., Rundberget T., Kosiak, B., 1997. Production of fusarochromanone and trichothecenes by Norwegian isolates of Fusarium equiseti. The fifth European Fusarium Seminar Szeged August 1997.

[3] Langseth, W., Gehbremeskel, M., Kosiak, B., Miller, D., unpublished. Culmorin compounds and other metabolites produced by *Fusarium culmorum* and *F.graminearum* strains isolated from Norwegian cereals.

[4] ODonnell, K, Cigelnik, E, Nirenberg, HI Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex, Mycologia **90**, 32, 465-493.

Hering, O.[1]; Nirenberg, H. I.[2]

[1] Federal Biological Research Centre for Agriculture and Forestry, Information Centre for Phytomedicine and Library, Königin-Luise-Str. 19, D-14195 Berlin, Germany

[2] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, D-14195 Berlin, Germany

ITS-RFLP and RAPD investigations for differentiating fusaria within the section *Martiella*

Because of different interpretations of morphological and phytopathological characters various species systems exist within the section *Martiella*. 196 Strains of *Martiella* fusaria were tested by the internal transcribed spacer-restriction fragment polymorphism (ITS-RFLP) method using 9 different restriction

enzymes. This technique reveals banding patterns, which can be used as objective markers for characterizing isolates on different taxonomical levels.

Nineteen different banding patterns were obtained. Ten Taxa could be characterized: *Fusarium coeruleum*, *F. martiiphaseoli* (= *F. solani* f. sp. *phaseoli*), *F. javanicum*, *F. solani* f. sp. *cucurbitae* race 1, *F. solani* var. *petroliphilum* (= *F. solani* f. sp. *cucurbitae* race 2), *Fusarium* sp. nov. II, III and IV, *Nectria borneensis* and *Nectria plagianthi*. 21 taxa could be differentiated combining RAPD with ITS-RFLP: *F. ambrosium*, *F. carneolum* (= *F. solani* var. *minus*; = *F. caucasicum*), *F. epimyces*, *F. illudens*, *F. lathyri* (= *F. solani* f. sp. *pisi*), *F. pestis*, *F. radicicola*, *F. solani* var. *solani*, the specialized forms *batatas*, *mori*, *robiniae*, *xanthoxyli* of *Fusarium solani*, *F. striatum*, *Fusarium* sp. nov I, V, VI, *Nectria bolbophylli*, *Nectria haematococca* and *Nectria subsequens*. Unique ITS-RFLP and RAPD patterns were obtained for some additional unidentified isolates of the *Martiella* section pointing to the exsistence of even more taxa in this section.

The cluster analytical evaluations of the ITS-RFLP results (see fig. 1) set *Fusarium coeruleum* far away from the other *Martiella* fusaria. Therefore and due to greater ITS-RFLP-similarities to isolates of other sections, *F. coeruleum* should be removed from the section *Martiella*. This can be justified also on a morphological basis: The missing of "micro"conidia borne on long conidiophores in false heads of the aerial mycelium, features typical for the *Martiella* section.

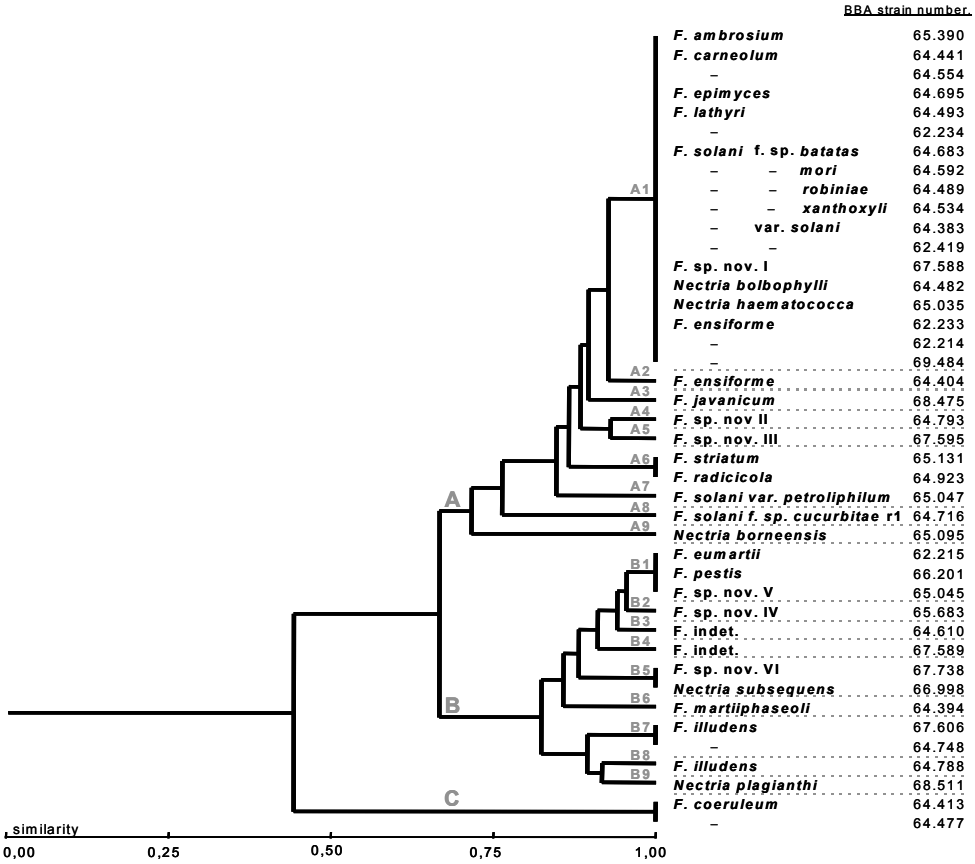


Figure: Cluster analytical evaluation of the ITS-RFLP results (*Martiella* fusaria)

Rogers, M.; Clapp, J. P.; Jeffries, P.

Research School of Biosciences, University of Kent at Canterbury, Canterbury, Kent CT2 7NJ, UK

Rapid typing of *Fusarium* spp. by PCR-SSCP analysis of the internal transcribed spacer 2 (ITS2) region

Identification of the ubiquitous fungal genus *Fusarium* has traditionally been performed using conidium morphology as the main distinguishing characteristic between species. Recent developments in the molecular systematics of the genus have allowed more accurate identification of fusaria isolated from the field. Thirty-nine isolates of *Fusarium* spp. were obtained from Samphire Hoe, a virgin soil site formed from spoil obtained during the construction of the Channel Tunnel. DNA was extracted from all isolates using a phenol/chloroform procedure, and the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA was amplified using the polymerase chain reaction. Polymorphisms in the ITS2 region between isolates were visualized by single strand conformational polymorphism (SSCP) analysis. SSCP patterns from the 39 unidentified isolates were compared with profiles from 14 named isolates from an international culture collection. Certain isolates could be given a putative identification to the species level, by comparison with SSCP patterns of type strains, while others were unique. SSCP analysis also indicated the possible presence of two nonorthologous ITS2 sequences in selected isolates. The technique thus has considerable potential for rapid typing of fresh isolates of *Fusarium*.

Klechkovskaya, H.

Plant Protection Institute, Kiev, Ukraine, Email: K_helena@mail.ru

Intraspecific variation in *Fusarium graminearum*

An analysis of different *Fusarium* spp., isolated from wheat in the Ukraine steppe and forest-steppe, has shown, that *Fusarium* strains within the species can possess different virulence levels. Among *F. graminearum* strains we have found a wide range of variations – from high virulence plants infected (95-100 %), to avirulent strains, (10-15%) infected wheat plants. PCR-analysis has shown, that differences in banding patterns between these strains were only quantitative. Virulent *Fusarium* strains Ao2, 5g2.11, K7 accumulated the highest concentration of RAPD–fragments, compared with avirulent strains, such as K5g, kar.11. At the same time the molecular weight of RAPD–fragments coincided (Figure)

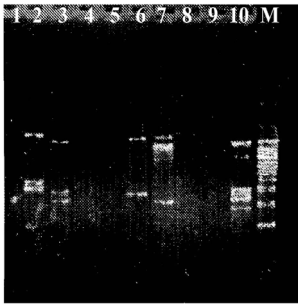


Figure: Electropherogram of DNA-amplification products of *F. graminearum* strains, which differ in their level of virulence, with the free primer UBC90 (5'-ggg ggt tag g-3'), M – marker of the molecular weight, restricted by Lambda Bs/Ell. The numbers above paths corresponded with number of strains: 1. - 3g1.17; 2. - Ao2; 3. - 7e3.5; 4. - K5g; 5. - Ov.9; 6. -6g4.36; 7. - 5g2.11; 8. - 11kar.; 9. - kar.17; 10. - K7.

It is possible to assume, that *F. graminearum* consists, generally, of three races – the first one with high virulence – race V, which comprises all virulent strains, such as Ao2, K7, 6g4.36, 5g2.11 and K7. The second one – race A, which comprises all avirulent strains, and, may be, race AV, with strains possessing a medium level of virulence.

Gaglaeva, T.[1)]; Koopmann, B.[2)]; Wolf, G. A.[2)]

[1)] All-Russian Institute of Plant Protection, 196608 St.-Petersburg-Pushkin, Russ
[2)] Institute of Plant Pathology and Plant Protection, University of Göttingen, D-37077 Göttingen, Germany

Biodiversity of *Fusarium graminearum* isolates from different geographical locations

The objectives of study were to investigate the biodiversity of *Fusarium graminearum* populations on the base of different markers relevant to a wide range of geographical occurrences. Fifty-two single-spore pathogen isolates originated from the Russian Far East, the South European part of Russia, China, Germany, and Finland (11, 16, 9, 12 and 4 isolates, respectively) were used. According to their geographical origin the isolates were divided into Asian and European subgroups.

Other pathogen properties were registered: 1) morphological-cultural characteristics (perithecium formation and radial growth rate of colonies on 6 different agar media), 2) pathogenicity to wheat seedlings, 3) enzymes activities (cellulase, chitinase, xylanase, 1,3-α-glucanase, amylase) in the tissue of inoculated wheat, 4) sensitivities to fungicides (Benomyl, Folicur, Sportak), 5) vegetative compatibility and 6) molecular genetic markers (PCR fingerprint with complex of ERIC primers).

It was shown that all isolates were homothallic. Mostly the growth rate of European isolates had a tendency to be lower on all used media. It was significantly lower when isolates were grown on PDA, Czapek-Dox, and carrot agar.

Pathogenicity varied in all geographical groups. On the whole the German and Chinese isolates were less aggressive than the rest group of isolates. At the same time these two groups were similar in low xylanase, cellulase and amilase activity.

It was shown *in vitro* that there is a variation in the sensitivity to fungicides among isolates. The Asian subgroup was significantly less sensitive than the European one.

Our study showed no clear clustering of isolates into Asian and European origin on the base of fingerprint patterns and VCGs.

The data have revealed, that all isolates can be grouped in 2 major molecular types closely associated with their aggressiveness. 32 *nit*-mutants were obtained belonging to 29 VCGs. The large number of non-complementary interactions demonstrates that vegetative incompatibility is widespread in *F. graminearum.*

Niere, B. I.[1)]; Sikora, R. A.[2)]; Speijer, P. R.[1)]; Nirenberg, H. I.[3)]

[1)] International Institute of Tropical Agriculture, Eastern and Southern Africa Regional Center, Namulonge, Uganda
[2)] Institute for Phytopathology, Soil Ecosystem Pathology and Nematology Section, University of Bonn, Germany
[3)] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Berlin, Germany

Vegetative compatibility grouping as a tool to differentiate between pathogenic and non-pathogenic isolates of *Fusarium oxysporum* targeted for the biological control of banana nematodes

Non-pathogenicity is considered crucial for the implementation of *Fusarium oxysporum* strains in biological control systems. At the International Institute of Tropical Agriculture (IITA) in Uganda, strains of *F. oxysporum* are being tested for the biological control of banana nematodes. Effective isolates originated from symptomless East African Highland Banana (*Musa* AAA) rhizomes. Their pathogenicity towards banana cultivars and other crops usually grown in the area needs to be tested. Pathogenicity testing on differential cultivars is space, labour, and due to extended latent periods often time consuming. Testing is further complicated by the fact that several specialised forms causing wilt diseases on many different crops exist and plants may serve as symptomless carriers. Consequently, the status of isolates used in biocontrol must be examined for possible effects on wilt susceptible crops grown in the region.

The accuracy of vegetative compatibility grouping (VCG) has been described as equal to pathogenicity tests. Therefore, biocontrol isolates were tested for vegetative compatibility with all known VCGs of the f. sp. *cubense* (courtesy R. Ploetz, Florida, USA), causing wilt in banana. None of the biocontrol isolates tested were vegetatively compatible with all known VCG testers of *F. oxysporum* f. sp. *cubense*, attacking banana. These results confirm that the strains of *F. oxysporum* being used in our biocontrol tests of plant parasitic nematodes are truly non-pathogenic to banana and support their important role in modern tissue culture propagation systems. At IITA, strains of *F. oxysporum* are used in protecting banana form nematodes, however, tomato and sweet potato, both susceptible to formae speciales *lycopersici* and *batatas*, respectively, are commonly grown in the vicinity of banana fields. Therefore, VCG testing will be expanded to these specialized forms.

Once, mutants for VCG have been selected and phenotyped, biocontrol strains can be tested in a short amount of time for compatibility with a set of testers for different *formae speciales*. VCG is a simple test and can be carried out in laboratories void of greenhouse space or experimental fields and, in contrast to molecular methods, no costly equipment is needed. This would be of special interest for developing countries such as Uganda lacking capital and expertise. To limit the number of tests and to avoid the importation of VCGs previously not reported in the country, testing should be restricted to VCGs present in the respective region. Vegetative compatibility grouping facilitates making qualified decisions on the use of *F. oxysporum* isolates for biocontrol purposes.

Nirenberg, H. I.[1]; Tiedemann, S. von[2]; Waalwijk, C.[3]; Seifert, K. A.[4]; Mouchacca, J.[5]; Niere, B. I.[6]; Westphal, A.[7]; Schütt, F.[1]

[1] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, D-14195 Berlin, Germany
[2] University of Göttingen, Institute for Forest Botany, D-37077 Göttingen, Germany
[3] Wageningen University and Research Centre, Plant Research International, Business Unit, Biointeractions and Plant Health, P.O.Box 16, NL-6700 AA Wageningen, The Netherlands
[4] Eastern Cereal & Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa ON K1A 0C6, Canada
[5] Muséum National d'Histoire Naturelle, Lab. de Cryptogamie, 12 Rue Buffon, 75005 Paris, France
[6] Institute for Phytopathology, Soil Ecosystem Pathology and Nematology Section, University of Bonn, Germany
[7] Department of Nematology, Riverside, California, USA

Three new *Fusarium* species from four continents

There are still new *Fusarium* species found soley on the basis of morphological features. This means that they are not cryptic species that only can be identified by sequencing certain regions of their DNA.

Fusarium sp. nov. 1 was first isolated from rootstocks of grafted grape vine in Germany. But no pathogenicity to grape vine plants (*Vitis* sp.) [1] could be found. Later it was isolated from a peaty orchid substrate and also from potatoes in Brazil. Therefore, the fungus is suspected to be a habitant of peaty soils. Morphologically it looks similar to *F. oxysporum*, but produces also pyriform conidia besides oval to allantoid ones which are borne once in a while on polyphialides. Therefore, the species is placed in section *Elegans*. Sequence data [2] and RAPD analysis support this grouping.

Fusarium sp. nov. 2 was first isolated from New Caledonian soil on which soja (*Glycine max*) had been grown. Later this fungus was recovered from *Abutilon theophasti* in Canada. In greenhouse experiments it proved to be pathogenic on both host plants producing a foot rot. Its sporodochial conidia resemble in shape and size somewhat *F. tumidum*. But its wild type produces vinaceous, short and powdery aerial mycelium as well as chlamydospores and grows somewhat restricted on PDA. Like *F.* sp. nov. 1 it generates moniliformin [3]. Therefore, this species does not belong to section *Discolor* but rather to the *Lateritium-Elegans-Liseola* complex. Since no (micro)conidia in the aerial mycelium are formed, it might be a member of section *Lateritium*.

The first isolate of *Fusarium* sp. nov. 3 was recovered from a banana rhizome grown in Thailand. Later cultures were isolated from *Heterodera schachtii* in California. Its pathogenicity is not proven yet. The fungus grows slowly with limited aerial mycelium. It produces small conidia on the substrate and sporodochial conidia which resemble those of *F. setosum*. Therefore, we place the species tentatively in the section *Setofusarium*.

Literature

[1] von Tiedemann, H., Brendel, G. and Fehrmann H. 1988. Untersuchungen über endophytische Pilze der Rebe unter besonderer Berücksichtigung des Gefäßsystems der Unterlage. J. Phytopthology **122**, 147-165**.**
[2] O'Donnell, Cigelnic, K., Nirenberg, H.I. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* complex of *Fusarium*. Mycologia **90**, 465-493.
[3] Schütt, F., Nirenberg, H. I., Deml, G. 1998. Moniliformin production in the genus *Fusarium*. Mycotoxin Research **14**, 35-40.

Summerell, B. A.[1)]; Burgess, L. W.[2)]; Bullock, S.[1)]; Benyon, F. H. E.[2)]; Nguyen, H. T.[3)]; Backhouse, D.[4)]

[1)] Royal Botanic Gardens, Mrs Macquaries Rd, Sydney, New South Wales 2000, Australia
[2)] Fusarium Research Lab., Dept. of Crop Sciences, University of Sydney, New South Wales 2006, Australia
[3)] Department of Plant Pathology, Hanoi Agricultural University, Gialam, Hanoi, Vietnam
[4)] University of New England, Armidale, New South Wales 2000, Australia

Natural occurrence of perithecia of *Gibberella* species in Australia and south-east Asia

During routine surveys of diseases in Australia and parts of south-east Asia (Indonesia and Vietnam) several species of *Gibberella* were recovered from wheat, sorghum and maize residues and from the residues of grass weeds growing within those crops. The species recovered were *Gibberella zeae*, *G. coronicola, G. fujikuroi* mating population A and *G. fujikuroi* mating population B.

Gibberella zeae (anamorph *Fusarium graminearum*) was recorded on wheat, sorghum, maize and barnyard grass (*Echinochloa crus-galli*) and wild oat (*Avena* sp.) residues in wheat crops in northern New South Wales, Australia. Its presence was associated with a localised epidemic of head blight in this region. The majority of the residue was infested with the fungus and perithecia were abundant. In northern Sulawesi, Indonesia and in northern Vietnam, *G. zeae* was discovered on residues of maize grown at higher altitudes of these countries.

Gibberella coronicola was recorded on mature wheat plants and on residues from some areas of the northern wheat belt in New South Wales, Australia. This fungus was associated with crops affected by crown rot caused by the anamorph of the fungus, *Fusarium pseudograminearum* (previously known as *F. graminearum* Group 1).

The perithecia of *Gibberella fujikuroi* mating population A (anamorph *Fusarium verticillioides*) were found on maize residues from various areas of northern Vietnam. *Gibberella fujikuroi* mating population B was recovered from maize residues from the Liverpool Plains, New South Wales, the anamorph of this fungus was *Fusarium subglutinans*. These findings suggest that perithecia of the *G. fujikuroi* complex may be more common than previously thought.

Waalwijk, C.; Hesselink, T.

Wageningen University and Research Centre, Plant Research International, Business Unit Biointeractions and Plant Health, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Mating type genes in the *Gibberella fujikuroi* species complex

The *Gibberella fujikuroi* species complex is composed of at least six mating populations. Each of these mating populations consists of two alleles that are arbitrarily named "+" and "-" , but cross-reference with mating type, *mat1-1* and *mat1-2*, was unknown. To facilitate mating type assignments within this species complex, two tester strains for each of the six mating populations have been analyzed with PCR primers designed for each of the two mating type genes. The *mat1-2* primers only produced amplicons in one partner of each set of tester strains. Amplifications with *mat 1-1* primers to detect the opposite mating type gene were not very robust. However, mating type probes derived from both idiomorphs of *Fusarium verticillioides* isolates A-0149 (*mat1-1*) and A-0999 (*mat1-2*) only hybridized to genomic DNA from these tester isolates, clearly demonstrating the heterothallic nature of the mating system in each of the tested species in the *Gibberella* complex.

Bragulat, M. R.; Martínez, E.; Rubiales, M. V.; Cabañes, F. J.

Departament de Patologia i Producció Animals. Unitat de Microbiologia. Facultat de Veterinària. Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Espanya.

Comparison of some selective culture media for *Fusarium* spp.

Some selective culture media had been developed for isolating and enumerating *Fusarium* spp. from natural samples. The Nash and Snyder medium (1962) (NS) with pentachloronitrobenzene (PCNB) as fungal inhibitor is one of the most widely employed. Several modifications of this media are also recommended for these purposes. However, PCNB have been reported to be carcinogenic (Sweet, 1986) and other antifungal compounds have been assayed like dichloran in DCPA (Andrews & Pitt, 1986) or in MCZ (Bullerman & West, 1992), iprodione in CZID (Abildgreen et al., 1987), or in PDID (Thrane et al., 1992), or malachite green in MGA 2.5 (Catellá et al., 1997). MGA 2.5 is a strong selective medium for *Fusarium* spp., whereas the other mentioned culture media allow the growth of many other different fungal species. The aim of this work was to compare different basal culture media (defined and undefined) emended with malachite green, together with other recommended media for *Fusarium* spp. isolation. In total, 30 strains belonging to the species: *F. anthophilum, F. culmorum, F. dlamini, F. graminearum, F. moniliforme, F. napiforme, F. nivale, F. nygamai, F. oxysporum, F. proliferatum, F. semitectum, F. solani,* and *F. subglutinans* were included in this study. Of the twelve different basal culture media assayed and emended with malachite green (2.5 ppm), MGA 2.5 and basal medium of MCZ amended with malachite green (2.5 ppm) showed both the highest colony diameters and the highest colony counts of the *Fusarium* spp. tested. Concernig the comparison of the recommended culture media, no statistical differences were detected in the colony counts of the different media used, although the colony diameters in MCz, DCPA, CZID and PDID were significantly higher than in NS and MGA media.

Plant Pathology

Mackinaite, R.

Institute of Botany, Zaliuju ezeru 49, LT-2021 Vilnius, Lithuania

Root rot agents at the border of natural and anthropogenic biotopes

The distribution of root rot agents of cultivated and wild plants was investigated at the border of anthropogenic and natural biotopes. The injured plants were collected from six localities in different regions of Northern Lithuania. A field of rye (*Secale cereale*) was chosen for an anthropogenic biotope. Root rot agents were isolated from the roots of rye and 18 wild plants (*Agropyron repens, Arthemisia vulgaris, Centaurea cyanus, Cirsium arvense, Dactylis glomerata, Erigeron annuus, Matricaria maritima, Medicago lupulina, Melilotus albus, Mentha arvensis, Myosotis arvensis, Phleum pratense, Rumex acetosella, Sonchus arvensis, Taraxacum officinale, Trifolium pratense, T. repens, Vicia angustifolia*) growing in the natural biotope next to the field.

Twentynine taxa of micromycetes, belonging to 15 genera, were identified in the roots of the investigated plants. Speices of the genus *Fusarium* predominated and constituted 44.8% of the total number of isolates. Fifteen taxa of *Fusarium* were determined (*F. acuminatum, F. avenaceum, F. chlamydosporum, F. culmorum, F. graminearum, F. graminum, F. heterosporum, F. oxysporum, F. poae, F. sambucinum, F. sambucinum* var. *minus, F. semitectum, F. solani, F. solani* var. *argillaceum, F. sporotrichiella*). *F. sambucinum* var. *minus*, *F. avenaceum, F. culmorum* and *F. graminearum* were the most widespread and made up 13.7, 7.5, 4.0 and 3.4% of the total number of isolates, respectively. The species of the genera *Phoma, Penicillium, Rhizoctonia* and *Alternaria* were the most common among other micromycetes (14.9, 10.3, 6.3 and 5.8% of the total number of isolates, respectively).

The same root rot agents were common in both anthropogenic and natural biotopes. However, in the natural biotopes they were less (Figure).

Most of the micromycetes detected in the roots of rye were isolated from the roots of wild plants. *F. sambucinum* var. *minus* was identified in 11, *F. avenaceum* and *Phoma* spp. in 7, *Rhizoctonia* spp. and *Cylindrocarpon* spp. in 5 plant species. Other fungi were conformed in roots of 2-4 plant species. *F. acuminatum*, *F. chlamydosporum* and *F. poae* were not detected in the roots of wild plants. However, frequency of distribution of these fungi in the roots of rye was not high either and was only 2.9%.

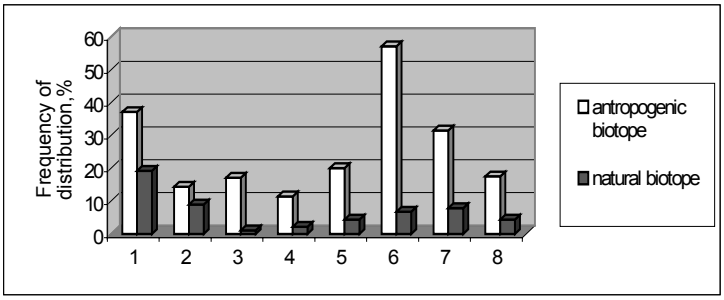


Figure: The frequency of distribution (%) of the most widespread root rot agents at the border of anthropogenic and natural biotopes (1 – *Fusarium sambucinum* var. *minus*, 2 – *F. avenaceum*, 3 – *F. culmorum*, 4 – *F. graminearum*, 5 – *Rhizoctonia* spp., 6 – *Phoma* spp., 7 – *Penicillium* spp., 8 – *Alternaria* spp.).

These investigations indicated, that the most widespread root rot agents have wide range of hosts and injure both cultivated and the wild plants. Although the distribution of these pathogens in natural biotopes is not very high and they do not cause severe damage for wild plants, but under favourable conditions they might become an important source of infection for cultivated plants.

Adugna, G.[1]; Hindorf, H.[2]

[1] Institute of Agricultural Research, Jimma, Ethiopia
[2] Univ. Bonn, Institut für Pflanzenkrankheiten, Nussallee 9, D-53115 Bonn, Germany, e-mail: h.hindorf@uni-bonn.de

Recent outbreaks and investigations on *Gibberella xylarioides* (*Fusarium xylarioides*) on coffee in East and Central Africa

Gibberella xylarioides causing tracheomycosis on coffee was first reported on *Coffea excelsa* in the Central African Republic [3]. However, STEYAERT [7] received infected *C. excelsa* material in 1939, isolated and identified *Fusarium xylarioides*.

The disease destroyed nearly all *C. excelsa* coffee in the Belgian Congo (Zaire, Congo) and affected Robusta (*C. canephora*) coffee, too. The vascular wilt became a serious problem in Robusta coffee of several countries in West and Central Africa. However, with the cultivation of resistant varieties and the eradication of diseased material, the impact of the disease was substantially reduced [2].

In a restricted area of Ethiopia in the west of the country the disease was known on Arabica coffee [5]. In the 70s the pathogen was confirmed by KRANZ and MOGK [4] as *Gibberella xylarioides*. The soil borne imperfect stage *Fusarium* of the pathogen penetrates into wounds and enters the vascular system, blocks the water transport and subsequently causes wilting of the whole bush. Finally the stem is covered by black-violet perithecia of the perfect stage *Gibberella* containing wind-borne ascospores. The disease causes a sudden death of the bushes and so far there exists no effective protection measure. A screening programme for resistant varieties in Arabica coffee by PIETERS and VAN DER GRAAFF [6] contributed towards overcoming this problem.

Beginning 1986 the disease started to manifest again in Haute Zaire and North Kivu on *C. canephora* and moved south, crossing the Uganda border and was first reported in 1993 in the Bundibugyo District [1,2]. In 1994 the disease was detected in the Rukungiri District and in 1995 in Mukono near Kampala, the area in which 40% of the Uganda coffee is grown. Today the disease is found in 12 districts. The newly sudden outbreak and the tremendously fast spread in very short intervals allows speculations of a mutation in the fungal population (FLOOD pers. comm.).

In a joint DAAD sponsored project at time we investigate on the fungal population of *G. xylarioides* in Ethiopia and will report on new results of the disease on Arabica coffee.

Literature

[1] Birikunzira, J.B. and G. Lukwago (1997): The status of coffee wilt disease (tracheomycosis) in Uganda. A Country report. Uganda Coffee Dev. Auth. Kampala (mimeographed).
[2] Flood, J. (1997): Tracheomycosis or vascular wilt disease of coffee in Uganda. A preliminary report. IMI Egham U.K.
[3] Guillemat, J. (1946): Quelques observations sur la tracheomycose du *Coffea excelsa.* Ev. Bot. Appl. Agr.Trop. **26**, 542-550.
[4] Kranz, J. and M. Mogk (1973): *Gibberella xylarioides* Heim et Saccas on Arabica coffee in Ethiopia. Phytopath. Z. 78, 365-366.
[5] Léjeune, P. (1958): Rapport au Gouvernement Impérial d'Ethiopie sur le production caféière. FAO, Rome, pp. 49.
[6] Pieters, R. and N.A. van der Graaff (1980): Resistance to *Gibberella xylarioides* in *Coffea arabica*: Evaluation of screening methods and evidence for the horizontal nature of the resistance. Neth. J. Pl. Path. **86**, 37-43.
[7] Steyaert, R.L. (1948): Contribution a l'étude des parasites des végétaux du Congo Belge. Bull. Soc. Royale Bot. Belgique **80**, 11-58.

Mielniczuk, E.[1)]; Kiecana, I.[1)]; Perkowski, J.[2)]

[1)] Department of Phytopathology, Agricultural University of Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland
[2)] Department of Chemistry, Agicultural University of Poznan, Wojska Polskiego 75, 60-625 Poznan, Poland

Reduction of yield and mycotoxin accumulation in oat cultivars and lines after *Fusarium culmorum* (W.G.Sm.) Sacc. and *F. sporotrichioides* Sherb. inoculation

Infection of oats with *Fusarium culmorum* and *F. sporotrichioides* may cause *Fusarium* head blight (scab), reduction of yield and accumulation of toxic metabolites in kernels.

The aim of this paper was to determine the demage of *F. culmorum* and *F. sporotrichioides* in relation to 14 genotypes of oats and accumulation of mycotoxins in kernels after artifical inoculation with these species.

The experiments were carried out in the fields in Zamość region in 1996-1998. The following 10 cultivars (Boryna, Borys, Dukat, Farys, German, Halny, Komes, Kwant, Santor and Sławko) and 4 lines of oats (CHD 1171, CHD 1236, STH 2594, STH 2795) were tested. All oat genotypes were inoculated with conidial suspension of the following two isolates *Fusarium culmorum* No. 37 and *Fusarium sporotrichioides* No. 161.

All examined oat cultivars and lines exhibited typical symptoms of *Fusarium* head blight when inoculated with *F. culmorum* and *F. sporotrichioides.* Kernels collected from infected heads were small, shrivelled and soft, while kernels from the control group did not exhibit symptoms of the disease.

The results of the field experiment showed that *F. culmorum* and *F. sporotrichioides* had an effect on yield reduction. The kernels yield have been calculated for each genotype and inoculated group compared with the non-inoculated control plots. Yield reduction of kernels after inoculation with *F. culmorum* ranged from 28% (STH 2594) to 66% (Santor), with *F. sporotrichioides* from 7% (Borys) to 40% (STH 2795).

Seed samples collected at harvest were analysed for several trichothecene mycotoxins. Chemical analysis revealed the presence (average concentration mg/kg) of deoxynivalenol (DON) – 0.23 in kernels of all genotypes inoculated with *F. culmorum,* 3-Acetylodeoxy-nivalenol (3-AcDON) – 0.02 in 9 samples and nivalenol (NIV) – 0.02 in three samples. After inoculation with *F. sporotrichioides* T-2 toxin (0.16) and HT-2 toxin (0.22) were found in kernels of all genotypes, while scirpentriol (STO) (0.06) were determined in three samples only.

Kiecana, I.; Mielniczuk, E.

Department of Phytopathology, Agricultural University of Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland

The occurrence of *Fusarium avenaceum* (Fr.) Sacc. and *Fusarium culmorum* (W.G.Sm.) Sacc. in oats (*Avena sativa* L.)

The aim of this work was to define the intensity of occurrence of *Fusarium avenaceum* and *Fusarium culmorum* on oats.

Investigations were carried out in 1996-1998 in Zamość region. The following 12 genotypes of oats were tested: cultivars Farys and Sławko, lines CHD 894, CHD 1095, CHD 1236, CHD 1607, CHD 1653, CHD 1692, STH 2293, STH 2393, STH 2494, STH 2694.

The occurrence of root necrosis and sheath necrosis in seedlings was observed 6 weeks after sowing every year in spring. Percentage of plants with disease symptoms ranged from 9 to 36. Results of myco-

logical analysis of seedlings showed that *Fusarium* spp. were strongly present on the infected parts (av. 30.5%), but their number and quality were different in every year. *Fusarium avenaceum* and *F. culmorum* were often isolated from examined plants, in the years 1997 and 1998. At all observed plants of estimated oat genotypes revealed necrotic stripes on the lower internodes. In 1996-1998 the percentage of necrotic stems on lower internodes ranged from 7 to 70. In the mycological examinations the colonies of *Fusarium* spp. increased up to 78% of all isolates and number and quality were different in each vegetation stage. *Fusarium culmorum* was isolated most frequently, the percentage of this fungus ranged from 33.7 in 1998 to 53.2 in 1997. *Fusarium culmorum* was isolated from stems with disease symptoms and from roots. Moreover, among *Fusarium* spp. pathogenic to cereals, *F. avenaceum* was isolated frequently every year (19-33.5% of all isolates *Fusarium* spp.).

Xu, X.-M.; Parry, D. W.

Entomology & Plant Pathology Department, Horticulture Research International, East Malling, West Malling, Kent, ME19 6BJ, UK

A new framework V project on epidemiology of *Fusarium* ear blight and accumulation of the associated mycotoxins

An application to the EU framework V on *Fusarium* ear blight of wheat (FEB) has been successful and is expected to start in this coming autumn. The overall project objective is to develop quantitative risk assessment models for *Fusarium* ear blight and production of associated mycotoxins on a pan-European scale. The project consortium consists of eight members of four countries, co-ordinated by Horticulture Research International, East Malling, Kent.

Studies will be carried out in controlled environments (CE) to determine relationships between development of the FEB complex, environmental variables and mycotoxin production on a single cultivar; models will be developed to describe such relationships. Further CE studies will be carried out to study the interactions between the most popular European cultivars and selected fungal isolates on FEB development and mycotoxin production. Quantitative models will be then developed to assess the risk of FEB and health risks due to the associated mycotoxins in grains, taking into account cultivar and climatic conditions. FEB development, mycotoxins and climatic conditions will be monitored over contrasting climatic sites in Europe; these observations will be used to improve the risk assessment models. Individual pathogen species and mycotoxins will be detected and quantified using the latest molecular and analytical techniques. The models will assist farmers to adopt more environmentally-friendly practices to produce high quality safe food. The models can be used to study the impact of climate change on the ecological interactions between FEB pathogens and risk of mycotoxin accumulation in grains.

Haikara, A.; Laitila, A.; Kleemola, T.

VTT Biotechnology, P.O.BOX 1500, FIN-02044 VTT, Finland

Effect of different *Fusarium* species and climatic conditions on the quality of barley and malt

During the summer of 1998 field trials were carried out at two experimental farms, Hahkiala and Jokioinen, in the southern part of Finland, in order to study the effects of different *Fusarium* species on the quality of two barley varieties and corresponding malts. The barley was artificially inoculated with *F. culmorum* VTT D-80148, *Gibberella zeae* (*F. graminearum*) VTT D-95470 and *F. poae* VTT D-82182,

during the ear emergence period. The *F. culmorum* strain had been isolated from Finnish barley and it was known to produce gushing factors and mycotoxins. The *G. zeae* strain was from the USA where it has been reported to produce deoxynivalenol, and to cause head blight and gushing of beer. A German *F. poae* strain has been isolated from oats. Ear samples were collected four times during the growing period for determination of *Fusarium* contamination and moisture content. Weather condition data were collected at both experimental sites. The *Fusarium*, and trichothecenes as well as zearalenone contents of harvested barley were analysed. The barley samples of 1 kg were malted and the overall quality of malt was analysed.

The summer of 1998 was exceptionally rainy in Finland resulting in the good proliferation of *Fusarium* strains used for contamination. The growth of *F. culmorum* and *G. zeae* was faster than *F. poae*. Also the *Fusarium* count and the amount of deoxynivalenol and zearalenone were higher in the barley samples with *F. culmorum*, and particularly more with *G. zeae* than with *F. poae* infested. Previous *in vitro* studies showed that the *G. zeae* strain was able to grow better and faster than the *F. culmorum* strain at 12°C. Both strains grew better at 12°C than at 30°C. The average temperature in August was about 13°C at both experimental sites. The *G. zeae* strain decreased the yield of barley. All three strains induced the gushing tendency of malt. Moreover they increased wort colour and the amount of wort- soluble nitrogen and free amino nitrogen (FAN). The activity of microbial β-glucanase (60°C) and xylanase were also higher in the malt samples produced from contaminated barley. The increased enzyme activity affected the content of β-glucans which was reduced by fusaria. The effect of *F. culmorum* and *G. zeae* was more pronounced on the quality of malt than that of *F. poae*.

Weather conditions at the experimental farms differed only slightly from each other. The barley was heavily lodged in Hahkiala but not in Jokioinen. However, the moisture content of ear samples throughout the growing period was higher in Jokioinen than in Hahkiala. In Jokioinen barley was harvested two weeks later than in Hahkiala. During this time the rainy weather continued which obviously, besides influencing the moisture content of ears, was a reason for higher microbial contamination, content of mycotoxins, and gushing tendencies of samples from Jokioinen experimental farm compared to barley samples cultivated at Hahkiala.

Rossi, V.; Ravanetti, A.; Pattori, E.

Istituto di Entomologia e Patologia vegetale, Università Cattolica S. Cuore, Via E. Parmense 84, I-29100 Piacenza, Italy

Influence of temperature and humidity on the infection of wheat spikes by *Fusarium* species causing head blight

Though *Fusarium* head blight (FHB) is a potentially destructive disease, its severity shows big differences in different years and locations, being strictly dependent on the epidemiological conditions: frequent rainfalls, high humidities, or heavy dews that coincide with the period of crop susceptibility favour infection. Environmental conditions favourable for the infection were not investigated exhaustively: some reports showed the dependence of *F. graminearum* on temperature and wetness, but less information are available for other important fungal species causing FHB .

The objective of this study was to determine the effect of environmental conditions on the infection of wheat spikes by fungal species causing FHB, with reference to *F. avenaceum*, *F. culmorum*, *F. graminearum*, and *F.* (*Microdochium*) *nivale*, which are the prevailing species infecting wheat in Italy.

In a first experiment, wheat spikes were inoculated at full flowering with a suspension ($2x10^4$ conidia/ml) of *in vitro*-borne conidia of the four fungi, and incubated at different temperatures (T, 10 to 35°C), wetted, and in saturated atmosphere. Infection frequency of glumes after different incubation times (4 to 72 hours) was determined by re-isolation of the fungi that had been inoculated.

Contour plots resulting from a regression analysis showed the different response of fungi to changing conditions following inoculation: *F. graminearum* and *F. avenaceum* showed similar patterns of the contour levels in the range of temperatures between 25 and 40°C, but the former fungus showed a higher ability in infecting wheat tissue below 25°C; for both fungi six contour levels were drawn to delimit the area between 60 and 90% of infected glumes. After 72 hours incubation, minimum T for infection was 10°C for *F. graminearum* and 14°C for *F. avenaceum*; optimum was 29 and 28°C, respectively, whereas maximum T was very similar (35 and 35.5°C, respectively). Only three contour levels (0.1 to 3% of infected glumes) were drawn for *F. nivale*; compared with the two previously described fungi, they were shifted over the lowest T, over a narrower range of T. Minimum and maximum T for infection were 10 and 28.5°C, respectively, whereas the optimum was 18°C. The contour plot for *F. culmorum* showed the limited ability of the fungus to infect wheat glumes under the environmental conditions of the present experiment: only two contour levels were drawn (0.1 and 1%), over a narrow range of T. Cardinal T were 16.5°C (min), 26.5°C (opt), and 33°C (max).

In a second experiment, spikes were inoculated as previously described, incubated for 72 hours at optimum T, either in the presence of a film of water, in saturated atmosphere, or without the film of water, under different levels of relative humidity (RH, 95 to 65%). When spikes were incubated wetted at 100% RH, frequency of infected glumes was very high for *F. graminearum* (74%) and *F. avenaceum* (65%), whereas for *F. nivale* (3%) and *F. culmorum* (1%) it did not significantly differ from the uninoculated test (0.5%). When spikes were incubated dry, the infection frequency was significantly influenced by RH. For *F. graminearum* and *F. nivale* the infected glumes occurred occasionally at any level of RH. For *F. aveanaceum* infection was erratic with a maximum of 7% glume infection at 65% RH. For *F. culmorum* the infection frequency remained as traces below 65% RH, then increased significantly at 75% and 85% RH (10 and 17% infected glumes, respectively).

The four *Fusarium* species considered in the present work showed different ecological requirements for infection. It explains changes in the infection frequency of kernels that can be obtained from wheat crops grown under changing environmental conditions.

Winson, S. J.; Hare, M. C.; Jenkinson, P.

Harper Adams University College, Newport, Shropshire. TF10 8NB, UK.

The relationship between point of inoculation of wheat ears and the infection of grain by *Fusarium culmorum* and *Microdochium nivale*

Several authors have identified in their work on factors affecting resistance to *Fusarium* ear blight that the disease characteristically spreads from the point of infection to neighbouring spikelets. This spread can continue until many or all spikelets on the ear are infected. Environmental factors such as the weather may help to accelerate the spread of infection and death of infected tissues. Despite optimum conditions, spread may be restricted to one or two spikelets per ear in some varieties. Adams [1] reported that under the conditions in Pennsylvania infection was usually restricted to one or two spikelets per ear, however, occasionally the upper half or the complete head may be infected. Atanasoff [2] thought the spread of infection from spikelet to spikelet or lack of spread to be due to environmental conditions alone. Pugh *et al*, [4] sectioned wheat ears infected with *Gibberella zeae* and reported evidence of hypal invasion up and down the rachis, but no evidence of hyphal invasion into the adjoining spikelets.

The movement of *M. nivale* and *F. culmorum* within the ear after inoculation at a given point was studied. In addition the hypothesis that pathogen infection affects the individual seed weight and infection according to the position within the ear. Wheat plants were grown in the glasshouse and 95 ears were individually inoculated with conidia of either *F. culmorum* or *M. nivale*, when 75% of the ears were at

decimal growth stage 65. 25µl of spore suspension (100,000 spores/ml) was placed using a pipette, between the lemma and palea of spikelet ten (Hilton, [3]). Four weeks after inoculation the number of spikelets showing necrosis and scalding in the distal and proximal parts of the ear were recorded. Necrosis ranging from one spikelet to the whole ear was seen in all ears that had been inoculated with *F. culmorum*, only 1% of ears inoculated with *M. nivale* showed necrosis and only of one spikelet. All ears were studied under a light microscope for evidence of external hyphal growth. External hyphal growth was found on 55% of ears that had been inoculated with *F. culmorum* and 28% of ears inoculated with *M. nivale.* Sections of the rachis were then studied under the scanning electron microscope for evidence of hyphal growth in the vascular tissue, this was then related to the position of symptoms on the ear. Grains and glumes from known points relating to the visible and light microscope symptoms were also studied. All grains were weighed individually and the position on the ear noted in relation to infection of the grain and the initial point of inoculation. Grain was plated out to potato dextrose agar (PDA) amended with streptomycin sulphate and carbendazim to assess the seed infection in relation to the point of inoculation.

Literature

[1] Adams, J.F . 1921. Observations on wheat scab in Pennsylvania and its pathological history. Phytopathology. **11**. 115-124.
[2] Atanasoff, D. 1920. Fusarium- blight (scab) of wheat and other cereals. Journal of Agricultural Research. **20**. 1-32.
[3] Hilton, A.J. 1999. Mechanisms of resistance to Fusarium ear blight in winter wheat. Ph.D. thesis, Open University.
[4] Pugh G.W., Johann, H, & Dickson, J.G. 1933. Relation of the semipermeable membranes of the wheat kernal to infection by *Gibberella saubinetii*. Journal of Agricultural Research. **45**. 609-626.

Cuel, C. E.[1]; De Las Heras, A.[1]; Patino, B.[2]; Gonzales-Jaén, M. T.[1]; Vázquez, C.[2]

[1] Departamento de Genética, Facultad de Biología, Universidad Complutense de Madrid. 28.040-Madrid, Spain
[2] Departamento de Microbiología III, Facultad de Biología, Universidad Complutense de Madrid. 28.040-Madrid, Spain

Differential regulation of polygalacturonases in isolates of *Fusarium*

Polygalacturonases (PGs, endo- and exoPGs) are important enzymes for both saprophytic and pathogenic fungal isolates. PG is a polymorphic system with complex isoform and endo/exoPG activity patterns. These patterns are variable depending on the isolate and the culture conditions considered, such as pH or the carbon source. This PG polymorphism is partly due to several PG coding genes, two exoPG and two endoPG coding genes described so far in the case of *Fusarium*. Previous analyses revealed substantial conservation of an *endopg* gene among distant *Fusarium* isolates suggesting that differences in the expression patterns of the several *pg* genes could be responsible for the variability mentioned above. This hypothesis has been tested in the present work. The expression patterns of the four *pg* genes were analysed in *Fusarium* isolates cultured in media with three different carbon sources (glucose, galacturonic acid and apple pectin). The results indicated that the four *pg* genes were independently regulated at transcriptional level showing both qualitative and quantitative differences in their expression patterns depending on the carbon source or on the isolate. *Endopg* genes were mostly expressed in the first days of cultures being later on replaced by *exopg* transcripts. These results would support the idea of different roles for the different PGs being all needed for a successful pectin degradation and probably also during host infection. Differences in regulation patterns of *pg* genes among isolates could have a functional significance conditioning the ability of a fungal isolate to infect successfully the host plant. Regulatory differences of *pg* genes could be explained by either distinct array of regulatory motifs or differences in the regulatory genes coding for transcriptional factors. In order to study the first hypothesis, promotor regions of *pg* genes of one isolate of *F. oxysporum* f. sp. *radicis lycopersici* have been analysed to identify putative regulatory motifs and to compare them in the different *pg* genes.

Conze, T.[1]; Klein, C.[2]; Moerschbacher, B. M.[2]

[1] Fraunhofer Institute for Environmental Chemistry and Ecotoxicology, Department Molecular Biotechnology, Auf dem Aberg 1, 57392 Schmallenberg, Germany, e-mail: conze@iuct.fhg.de
[2] Westfälische-Wilhelms-University of Münster, Institute of Plant Biochemistry and Biotechnology, Hindenburgplatz 55, 48143 Münster, Germany

Pectinolytic enzymes of *Fusarium graminearum*: Biochemical and molecular characterization

Fusarium graminearum is a pertotrophic pathogen that attacks almost all grasses and cereals. Recent studies [1] of the fungal growth during the first 76 hours showed that *F. graminearum* enters the host plant via stomata and invades the plant by subcuticular growth. After six hours a direct penetration of epidermal and parenchymatic cells was observed. The growth cycle ends after 2-3 days with the generation of asexual spores on the surface of the affected tissue. The observed fungal colonization of the host tissue implies the existence of pectinolytic enzymes enabling *F. graminearum* to macerate the host cell walls without causing severe tissue damage. In several other host/pathogen-interactions, these enzymes have been shown to play an important role in the infection cycle [2;3].

Our first goal was the creation of a reproducible *in vitro*-system for the fungal growth and the induction of pectinolytic enzymes. Enzyme activity was only detectable in a growth medium supplemented with pectin or polygalacturonic acid. The induced pectinolytic enzymes were characterized biochemically using a range of different methods. Starr et al. [4] described the spectrophotometric detection of the unsaturated double-bound between C4 and C5 at the newly formed non-reducing end as a result of trans-eliminative pectate/pectin degradation by lytic enzymes. The products formed were identified and quantified using high performance anion exchange chromatography (HPAEC) [5] to corroborate pectinolytic enzyme activity of *F. graminearum*. A specific method for activity staining of pectate/pectin degrading enzymes following isoenzyme separation by SDS-PAGE or isoelectric focussing was developed by Ried & Collmer [6]. All of these methods confirmed the existence of at least two different pectinolytic enzymes in *Fusarium graminearum*. We are currently separating these enzymes for further characterization of their substrate specificities and product patterns using anion exchange and cation exchange chromatography.

In addition to the biochemical characterization of the enzymes, we have initiated a molecular genetic approach to clone and characterize the corresponding fungal genes. Sequence comparisons of five different genes encoding for pectinolytic enzymes from other *Fusarium* species showed very high homology between these, but no homology to other known pectin or pectate lyase genes. Two heterologous primers were deduced from the Fusarium sequences and used for PCR-amplification of genomic sequences of *F. graminearum*. We have so far amplified two ~450 bp and ~300 bp genomic fragments showing high homology to the known pectinolytic enzymes from *Fusarium solani* and *Fusarium oxysporum*. The cloning of the complete genes encoding the pectinolytic enzymes in *Fusarium graminearum* will enable us to perform heterologous expression of the genes to further characterize the enzymes. After homologous transformation and gene knock out, the possible role of the pectinolytic enzymes from *Fusarium graminearum* as virulence or pathogenicity factors will be examined.

Literature

[1] Vance CP, Somers DA, Bushnell WR, Muehlbauer GJ, Pritsch C 2000: Fungal development and induction of defense response genes during early infection of wheat spike by *Fusarium graminearum*. MPMI **13**(2): 159-169.
[2] Barras F, Van Gijsegem F, Chaterjee AK 1994: Extracellular enzymes and pathogenesis of soft-rot *Erwinia*. Annu. Rev. Phytopathol. **32**: 201-234.
[3] Kolattukudy PE, Crawford MS 1987: Isolation and analysis of as novel inducible pectate lyase gene from the phytopathogenic fungus *Fusarium solan*i f. sp. *pisi* (*Nectria haematococca*, mating type IV) and characterization of the gene product expressed in *Pichia pastoris*. Arch. Biochem. Biophys. **323**(2): 352-360.
[4] Starr MP, Chaterjee AK, Starr PB, Buchanan GE 1977: Enzymatic degradation of polygalacturonic acid by *Yersinia* and *Klebsiella* species in relation to clinical laboratory procedures. J. Clin. Microbiol. **6**(4): 379-386.

[5] Hotchkiss AT, Hicks KB 1990: Analysis of oligomeric acids with fewer residues by high-performance anion-exchange chromatography and pulsed amperometric detection. Anal. Biochem. **184**: 200-206.
[6] Ried JL, Collmer A 1985: Activity stain for rapid characterization of pectic enzymes in isoelectric focussing and SDS-PAGE. Appl. Environmental Microbiol. **50**: 615-622.

Gilbert, J.; Morgan, K.

Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg MB, Canada

Field-based rating of spring wheat infected with *Fusarium graminearum*, cause of *Fusarium* head blight

Fusarium head blight (FHB), an important wheat disease in Manitoba, results in damaged kernels (FDK) which reduce yield and grain quality. Two methods of scoring FHB in spring wheat were evaluated: the FHB index and the visual rating index (VRI). Both methods measure percent incidence and percent severity of disease, but the FHB index requires that diseased spikelets be counted in the laboratory to give percent severity, while the VRI is estimated in the field. In 1998 and 1999, 433 rows in an inoculated field nursery were evaluated using both methods and the indices compared with percent FDK. The data were normalized using a square root transformation. Pearson correlation coefficients for FHB index and VRI, FHB index and FDK, and VRI and FDK, were all significant, ranging from 0.69 to 0.77. It was concluded that there was little loss of precision in moving to a more efficient field-based VRI rating system.

Wiwart, M.[1)]; Perkowski, J.[2)]; Godynicki, S.[3)]; Goliński, P. K.[2)]; Jackowiak, H.[3)]; Jelen, H.[4)]; Packa, D.[2)]; Stachowiak, J.[2)]

[1)] University of Warmia and Mazury, Department of Plant Breeding and Seed Production, Olsztyn, Poland
[2)] Agricultural University of Poznań, Institute of Chemistry, Poland
[3)] Agricultural University of Poznań, Department of Animal Anatomy, Poland
[4)] Agricultural University of Poznań, Institute of Food Technology, Poland

Morphological and chemical changes in spring wheat kernels after inoculation of spikes with *Fusarium culmorum* (W.G. Smith) Sacc.

The response of five Polish spring wheat cultivars (Torka, Broma, Kontesa, Hena and Helia) to spike infection of two pathogenic isolates of *Fusarium culmorum* (I_1, I_2) was investigated. Two different controls were used: spikes without any treatment and treatment with distilled water. Studies included: biometrical analyses of main yield components, analysis of content of deoxynivalenol (DON) and trichodien (Trich) in grain, digital image analysis of kernels as well as microscopic observations using SEM and light microscope. Inoculation resulted in a significant decrease in number of kernels per spike (23.9-48.5%), kernels weight per spike (29.1-43.6%) as well as one thousand kernels weight (59.7-69.4%). The cultivars differed in reaction to infection with *F. culmorum*. Kernels obtained from artificially inoculated spikes were characterised by different contents of the fungal metabolites. In all grain samples derived from inoculated spikes as well as from those treated with distilled water both compounds were detected. The highest contents of DON and Trich were found in grain of cv. BROMA, the lowest in grain of cv. TORKA. The findings indicated the correlation between contents of DON and amount of detected Trich and corresponded with the results of biometrical analyses of yield components. Regarding the result of digital colour image analysis of kernels, significant differences were observed between kernels obtained from inoculated and non-inoculated spikes, in the range of all three colour components, Hue, Saturation and Intensity. All kernels classified as FDK (*Fusarium* Damaged Kernels) were characterised by inten-

sive development of mycelium in the kernel furrow whereas infection process began in the embryonic part of the kernel.

Kryuchkova, L.

Plant Protection Institute UAAS, 33, Vasilkovskaya str., Kyiv-22, Ukraine, 03022

Fungi of the genus *Fusarium* associated with foot and root rot of wheat in the Ukraine

Plants of winter wheat from 7 regions of the Ukraine during 1996-1999 were analyzed for *Fusarium* species causing foot and root rot. 318 isolates of Fusarium spp. were collected. Seven species were identified: *F. oxysporum, F. culmorum, F. avenaceum, F. graminearum, F. sporotrichioides, F. solani, F. verticillioides*.

F. oxysporum and *F. culmorum* dominated. *F. oxysporum* prevailed among other species in all samples. The diversity of the isolates due to their morphological and pathogenic characteristics have been shown. About 60% of *F. oxysporum* isolates caused disease of winter wheat seedlings after artificial inoculation. *F. culmorum* was less frequently found. But all of its isolates were pathogenic to seedlings of winter wheat. The average severity of the disease of wheat seedlings after inoculation with this fungus was more intensive than after inoculation with *F. oxysporum*.

Chakaeva, S. A.

Head of the Scientific-Production, Center on Plant Protection, Bishkek, Kyrgyz Republic

Fusariosis of agricultural cereals in Kyrgyzstan

Fusariosis is the most wide spread agricultural disease in Chu Valley in Kyrgyzstan. The agents of *Fusarium* infect the stem vascular-transferring system, germs and young unprotected shoots inducing their death or make them to become rare crops. Wilting of the adult plants leads to quality decrease of the germs and reduces seed production.

Researchers: Alkhovskaya T., Dotsenko A., Zagurskyi A., Mamotina I., Yudanova A., Japarova G., under Chakaeva, S. A., found agricultural cereals affected by the following fungi of the genus *Fusarium* Link.

Table: *Fusarium* species of agricultural plants

Wheat, barley	*Fusarium avenaceum* Sacc.
	F. graminearum Schwabe
	F. nivale Ces.
	F. culmorum Sacc.
Corn	*F. moniliforme* Sheldon
	F. graminearum Schwabe
Alfalfa	*F. oxysporum* var. *medicaginis*
	F. solani
	F. culmorum,
	F. moniliforme
	F. heterosporum
	F. gibbosum

	F. sambucinum
	F. sporotrichiella
Soy-bean	*F. solani* Appel. & Wollenw.
	F. oxysporum Schl.
	F. avenaceum Sacc.
	F. gibbosum Appel. & Wollenw.
	F. heterosporum Fr.
Cotton plant	*F. oxysporum* Schl.
	F. vasinfectum
Potato	*F. oxysporum* Schl.
	F. solani Bilai
	F. culmorum Sacc.
	F. sambucinum Fckl.
Cabbage	*F. oxysporum* Schl. f. *conglutinans* Bilai
Melon	*F. oxysporum* Schl. f. *niveum* Wollenw.
Beet	*F. oxysporum* Schl.
	F. culmorum Sacc.

F. graminearum, F. avenaceum Sacc., *F. moniliforme* Sheldon were ascertained on grain, *F. oxysporum* var. *medicaginis, F. solani, F. gibbosum* Wr. on the perenial grasses, and *F. oxysporum* on vegetables.

The most aggressive species to alfalfa was *F. sporotrichiella*, to potato *F. sambucinum*, to soybean *F. heterosporum* and to wheat and barley *F. avenaceum* Sacc*., F. graminearum* Schwabe according observations in the fields and tests in the laboratory.

Microscopic analyses showed that there is a wide range of *Fusarium* species occurring on agricultural plants, which differ in number according to the host: Eight species were identified on alfalfa, 5 on soybean, ca. 4 on potato or wheat and 1-2 on the other cereal crops.

In this study about 11 *Fusarium* species were recorded to occur on agricultural cereals in Kyrgyzstan.

Literature

[1] Makarov K., Tiran T., Loginova A., Belyavskyi Yu. Study of the succumbing of the Alfalfa to fusariosys. Frunze 1990, 90-91.
[2] Chakaeva A., Turgunbekova R. Influence of the alfalfa fungus diseases agents on its fodder quality. Frunze 1987, 28-29.
[3] Desyatnikova G., Japarova T., Alfalfa sorts Selection on the succumbing to fusariosys. Frunze, 1987, 30-32.
[4] Desyatnikova G., Japarova T., To the question of the Alfalfa succumbing to fusariosys. Frunze, 1989, 108-110.

Battilani, P.; Chiusa, G.; Jimenez Sanchez, M. G.; Pietri, A.; Ravanetti, A.; Rossi, V.

Istituto di Entomologia e Patologia vegetale, Università Cattolica S. Cuore, Via E. Parmense, 84, 29100 Piacenza, Italy

Yield losses in wheat infected by *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium graminearum* and *Microdochium nivale*

Fusarium ear blight (FHB) induces damages of several types on wheat, including contamination with mycotoxins. Reduction in yield is often estimated between 10 and 30 %.

A study was carried out in the western Po Valley (North Italy) to investigate the effect of wheat species, fungal species and wheat growth stages on yield losses caused by FHB and on mycotoxin production. The research was carried out for a 3 year-period (1992-1993 to 1994-1995). Two wheat cultivars (Centauro, *Triticum aestivum* L. and Creso, *Triticum durum* Desf.), 3 growth stages (DC 65, DC 75 and DC 85, according to Zadoks) and 4 fungi (*F. avenaceum*, *F. culmorum, F. graminearum* and *M. nivale*) plus an uninoculated control were arranged in a completely randomized block design with split plots; plots

were artificially inoculated with 200 ml of a suspension with 1.5 x 10^6 conidia/ml of water, each one with a different fungal species, at the different growth stages.

Each plot was controlled for visible damages on kernels and the effect on yield and its components (number of kernels/head, 1000 kernels weight) was determined. Kernels were also incubated for reisolation and the *Fusarium* species were identified which had been inoculated. Ergosterol, deoxynivalenol (DON) and zearalenon (ZEA) were quantified in kernels inoculated with toxigenic species (*F. culmorum* and *F. graminearum*).

Results pointed out that 1994 was the most favourable year for *Fusarium* infection and confirmed that wheat plants are most susceptible at the flowering stage.

In all years *F. culmorum* had the highest incidence of infected kernels (27-46%), followed by *F. avenaceum* in 1993 (20%) and 1994 (42%). *F. graminearum* infected a high number of kernels in 1995 (37%). *M. nivale* was absent in 1993 and its incidence was about 6% in both 1994 and 1995.

Highest percentages of shrivelled kernels were associated to *F. culmorum* and *F. graminearum*. The other two fungi caused a lower level of visible symptoms (about 10% more than kernels collected from control plots).

Yield components were highly correlated to each other; they were strongly reduced by *F. culmorum* and *F. graminearum*, especially when the inoculum was applied at flowering.

Ergosterol and toxin content were also influenced by year and growth stage, while the fungus was irrelevant. Positive correlation was found between ranks of fungal incidence and ergosterol, ZEA and DON. Maximum content of ZEA and DON, obtained in the rank with more than 60% of infected kernels, was 0.7 ppm and 32 ppm respectively.

According to these results, *F. culmorum* and *F. graminearum* have a dominant role in FHB. In fact, they cause the higher yield reduction and they also produce mycotoxins. *F. avenaceum* can have high incidence of infected kernels, but it causes few visible damages to kernels and limited yield losses.

Strunnikova, O. K.; Shakhnasarova, V. Yu.; Vishnevskaya, N. A.

Research Institute for Agricultural Microbiology, Podbelsky Shosse 3, Pushkin 8, St. Petersburg, 196608 Russia

Influence of different soil conditions on *Fusarium culmorum*

Membrane filters and immunofluorescent staining techniques were used to study the dynamics of a *F. culmorum* population under several soil conditions. *F. culmorum* macroconidia were placed on membrane filters and introduced into the soil or into the rhizosphere of different crops. After definite time periods the filters were recovered and *F. culmorum* structures were identified using indirect immunofluorescence. Mycelia, macroconidia, chlamydospores and very seldom microsclerotia were observed both in the soil and in the rhizosphere. Soil conditions influenced *F. culmorum* development and amount of its structures formed in soil. Rhizosphere stimulated *F. culmorum* development. However the rhizosphere of barley (host plant) did not particularly affect *F. culmorum* behaviour in comparison with the rhizosphere of non-susceptible crops. Amount of *F. culmorum* mycelium and macroconidia were higher in soil with 20% moisture than in that with 60% moisture (-4,3 and -0,63 bar, respectively). Reduction in the amount of mycelia was caused by higher lytic bacterial activity in the moist soil (60%) than in dry soil (20%). Soils of different texture influenced the ratio of mycelia and macroconidia in the population of *F. culmorum*: abundance and the proportion of mycelia were higher in sand (light) soil, while the proportion of macroconidia was higher in clay (heavy) soil. Amount of *F. culmorum* mycelia and macroconidia varied significantly during the time of observation (100 days maximum) and under the influence of different

soil conditions. Amount of chlamydospores formed under the same conditions were insignificantly different. Increase in chlamydospore amount was observed under co-incubation of *F. culmorum* and *P. fluorescens* on the membrane filter in the soil. Very significant increase in chlamydospore amount was observed with the development *F. culmorum* in sterile soil.

Tvaruzek, L.[1]; Kraus, P.[2]

[1] Agricultural Research Institute Kromeriz, Ltd., Czech Republic
[2] Mendel University of Agriculture and Forestry, Brno, Czech Republic

A three years survey of toxigenic *Fusarium* spp. causing foot rot diseases of winter cereals

Foot-rot diseases are a serious problem on winter cereals in Central Europe. The role of *Fusarium* spp. and *Microdochium nivale* in this foot-rot complex has been assessed during the last three years.

About 400 samples of plants in 1998, 720 samples in 1999 and 1200 samples in 2000 were investigated for visual symptoms of infection. The pathogens were identified to species level. For each sample the sowing date, proceeding crop, cultivar of winter cereals and type of seed-dressing were recorded.

Microdochium nivale var. *nivale* was the dominant species. There was a significantly higher incidence of *Fusarium* spp. when cereals were grown after corn, wheat and winter rape. Young plants on untilled stands were more infected with these pathogens than those on ploughed stands.

There were significant differences between the years 1998, 1999 and 2000 in *Fusarium* spp. incidence as well as in the infection with other foot-rot diseases *(Pseudocercosporella herpotrichoides)*. Cool and wet weather during autumn promoted stronger infections with *Fusarium* spp. and snow mold in 1998 and 1999.

Reduction of radial mycelial growth of the *Fusarium* spp. and *Microdochium nivale* isolates using different DMI-fungicides was evaluated. In an *in vitro* assay, isolates were grown on agar plates containing different concentrations of fungicides. ED 50 values were calculated. There were highly significant differences between mean levels of ED 50 among the fungicides.

Munkvold, G. P.; Wilke, A.; Perry, J.; Bronson, C. R.

Department of Plant Pathology, Iowa State University, 351 Bessey Hall, Ames, IA 50011, USA

Use of green-fluorescent-protein transformation for epidemiological studies of *Fusarium verticillioides* and *Fusarium subglutinans*

Contemporary and future strategies for developing resistance to *Fusarium* infection in maize will include tissue-specific expression of genes with antifungal properties. Because *F. verticillioides* has multiple infection pathways, the optimal strategy for tissue-specific gene expression is not yet clear. Furthermore, the predominance of specific infection pathways can be influenced by environmental conditions, but the details of these interactions have not been described. Systemic, symptomless infection of maize has been demonstrated with *F. verticillioides*, but the evidence for this phenomenon in other *Fusarium* species is inconclusive. In order to address these questions, we have genetically transformed strains of *F. verticillioides* (*Gibberella fujikuroi* mating population A) and *F. subglutinans* (*G. fujikuroi* mating population E) with a green fluorescent protein (GFP) derived from the jellyfish *Aequorea victoria*. Expression of GFP facilitates the visualization of these specific *Fusarium* strains in infected tissue and in fungal cul-

tures isolated from infected tissue. Use of these marked strains in experiments also alleviates the difficulties associated with background infection of experimental plants by endemic *Fusarium* strains. We have selected transformed strains with high expression of GFP that demonstrate wild-type morphological, growth and sporulation characteristics *in vitro*. Expression of GFP in these transformants has been mitotically stable through five sequential transfers on artifical media. In seedling pathogenicity tests, the selected GFP strains were not significantly different from the wild-type strains. Transformed strains are being used to investigate the occurrence of symptomless, systemic infection of maize plants by *F. subglutinans* and the influence of temperature on systemic development of *F. verticillioides in planta*. Transmission of both fungi from inoculated seeds to seedlings was detected in a high percentage of plants grown in the greenhouse and growth chamber. The GFP strains also were recovered from stalks and kernels of mature plants. *Fusarium* strains expressing GFP promise to be useful tools for extending our understanding of host-pathogen interactions in this genus and for identifying specific plant tissues to target for expression of resistance genes.

Schjøth, J. E.[1]; Elen, O. N.[1]; Ristanovic, D.[2]; Sundheim, L.[1]

[1] The Norwegian Crop Research Institute (NCRI), Plant Protection Centre, 1430 Ås, Norway
[2] Golden Valley Regional Research Station, Chisamba, Zambia

The significance of hybrids, planting time, and climate on the incidence and levels of *Fusarium* spp. in maize from field experiments in Central and Northern Zambia

Maize diseases cause yearly yield losses of up to 30% in Zambia, and in some years and localized areas a considerably higher proportion of the crop is destroyed when one or more diseases become acute [1]. *Fusarium* ear rot is the most widespread disease of maize ears, besides rots caused by *Diplodia* spp. [2]. Several *Fusarium* species have the potential to contaminate grain with mycotoxins, that have been associated with human and animal health problems. The *Fusarium* situation of Zambian maize is cause for concern, because maize is a major source of human nutrition, and is also used for beer production and animal feed [3]. Most of the consumption is based on home grown maize. Little information is available on the relationship between *Fusarium* spp., mycotoxins and the consumption of home grown maize in Zambia.

Field experiments were conducted at two locations in Zambia to evaluate the significance of climate, maize hybrids and varying planting times on the incidence and levels of *Fusarium* spp. in maize. High yielding maize hybrids of different earliness and suitability for different geographical regions were selected from the Maize Pathology Breeding Program in Zambia. One experimental series compared 20 maize hybrids in the Central Province (Golden Valley Regional Research Station) in 1994 and 1995, and the other series 3 hybrids and 3 different planting dates in the Central Province and the Northern Province (Misamfu Regional Research Station) in 1993 and 1994. Average annual precipitation is approximately 800 mm at Golden Valley and 1300 mm at Misamfu.

Fusarium contamination of mature maize was investigated by culturing kernels on selective medium. Identification to species level was based on morphology. On average for two years and two regions, 31% of naturally infected seed was colonized by *Fusarium* species, ranging from 0 to 85% between individual samples. 98% of all samples contained *Fusarium* spp. In general, the samples looked healthy. The species composition and prevalence varied between years and locations. However, *F. moniliforme* was by far the most prevalent species, and it was present in 90% of all samples. A number of other species was also encountered, out of which *F. oxysporum*, *F. subglutinans* and *F. acuminatum* were the most numerous.

The significance of climate, Zambian maize hybrids and planting times on the incidence and levels of *Fusarium* spp. in maize will be discussed.

Literature
[1] Rao, K.N. and Ristanovic, L.D. 1986. The maize pathology program in Zambia. In: To feed ourselves. Proceedings of the first Eastern, Central and Southern Africa regional maize workshop, Lusaka, Zambia, March 10-17, 1985, B. Gelaw (ed.). CIMMYT, Mexico,. 259-264.
[2] Macdonald, I.A. and Raemaekers, R.H. 1974. Some results of feeding tests with *Fusarium* and *Diplodia* diseased maize. Farming in Zambia. **8**(4), 9-11.
[3] Siame, B.A. and Lovelace, C.E.A. 1989. Natural occurrence of zearalenone and trichothecene toxins in maize-based animal feeds in Zambia. J. Sci. Food Agric. **49**, 25-35.

Cosic, J.; Jurkovic, D.

Faculty of Agriculture, Trg sv. Trojstva 3, 31000 Osijek, Croatia

Biological characterization of *Fusarium graminearum* Schw. isolated from different host plants

A large number of *Fusarium* species with different frequencies in isolations, therefore of unequal significance for wheat, participate in diseases ethiology. According to our previous research [1, 2] and results obtained by other authors [6, 5, 3] *F. graminearum* is the most frequent species and strongly aggressive toward cereals, especially to wheat.

F. graminearum strains were isolated during the years 1996-1999 from different hosts: wheat (6), barley [1], maize seed [3], wheat root [1], maize stem [1], maize [5], wheat debris [4] and Capsella bursa-pastoris [2]. *F. graminearum* strains were isolated from plant tissues in moist chambers at 20ºC under 12 h dark/light regime. Developed fungal colonies were examined under a stereo microscope. Conidia or mycelium tips were transferred to PDA, CLA, Bilai's medium or water agar and subcultured at 5ºC, 15ºC, 20ºC, 25ºC and 30ºC. Colony diameters, presence of aerial mycelium, sporulation and size of macroconidia were measured after 3, 5 and 8 days. The fastest development of mycelium was recorded on all types of agars at both 20°C and 25°C. After an eight day period, sporulation of all isolates sub-cultured on PDA was very weak or there were no sporulating colonies at all. A larger number of sporulating colonies were obtained after 21-28 days (12h dark/light regime), which corresponds with the data provided by extensive literature published on this subject. With regard to the isolates sub-cultured on CLA, more sporulating colonies were obtained, despite the fact that the aerial mycelium was not particularly lush (the medium being water agar).

Pathogenicity of the strains was examined according to the methods described by Mesterhazy [4] on results two winter wheat cultivars. Residues showed that *F. graminearum* isolates had been strongly, even very strongly aggressive to wheat seedlings. Seed germination of wheat cv. 1 was 26.67% and there were 75% of seedlings with necrosis of radicle. On the other hand, seed germination of wheat cv. 2 was 53.33%, whereas there were 43.75% of seedlings with necrosis of radicle. It was established that all tested isolates from maise residues were weakly aggressive, the incidence of which was > (%) in germinating seeds and < (%) of seedlings with necrosis of radicle. The isolate 1Aw4 from grain of wheat may be described as extremly aggressive, while other isolates from grains of wheat as well as all other tested isolates may be said to be strongly aggressive to wheat seedlings.

The study has shown that there are no significant differences between strains in all examined characteristics except in aggressionen.

Literature
[1] Ćosić, J., Jurković, D. 1997. *Fusarium* species associated with winter wheat head blight in Croatia. 10th Congress of the MPU, Montpellier, France, Proceedings, 179-182.
[2] Jurković, J., Ćosić, J. 1999. *Fusarium* species isolated from weeds and their pathogenicity for wheat seedlings. 35th Croatian Symposium on Agriculture, Abstracts Book, 173-174.
[3] Mauler-Machnik, A., Suty, A. 1997. New findings on the epidemiology, importance and control of *Fusarium* ear blight. 5th European Fusarium Seminar, Szeged, Hungary, Cer. Res. Comm. 3/2, 705-709.
[4] Mesterházy, A. (1978.): Comparative Analysis of Artificial Inoculation Methods with *Fusarium spp.* on Winter Wheat Varieties. Phytopath. Z. **93**, 12-25.

[5] Tomasović, S., Vlahović-Lender, V. 1995. Moderna bolest pšenice - *Fusarium spp.* klasa. Sjemenarstvo **6**, 427-443.
[6] Wilcoxon, R. D., Kommedahl, T., Ozmon, E. A., Windels, C. E. 1988. Occurrence of *Fusarium* Species in Scaby Wheat from Minnesota and Their Pathogenicity to Wheat. Phytopathology **78**, 586-589.

Lević, J.; Petrović, T.

Maize Research Institute, Zemun Polje, Belgrade – Zemun, Yugoslavia

Pathogenicity of *Fusarium polyphialidicum* to maize seedlings

Fusarium polyphialidicum Marasas, Nelson, Toussoun & Van Wyk is known as a soil-borne fungus but not as a maize pathogen. Therefore, the test of pathogenicity was carried out with *F. polyphialidicum* isolates subtracted from the roots of young maize plants that had been grown under field conditions in Zemun Polje in 1996. The fungus was identified according to morphology of macroconidia (49.98-89.64 x 5.00-6.64 µm; 3-6 septa), microconidia (6.66-29.99 x 2.49-4.68 µm; 0-2 septa), polyphialides (many loci on conidogenious cell) and chlamydospores (in chains) on CLA and observing the moderately fast growing mycelium on PDA (abundant, cottony, white).

Assay of pathogenicity was conducted on slope Knop medium (1g KNO_3; 0.12g KCl; 0.25g KH_2PO_4; 0.25g $MgSO_4 \cdot H_2O$; in trace $FeCl \cdot 6\ H_2O$; 15g agar, and 1L distilled water) in glass tubes (16 x 2 cm) under laboratory conditions. Subcultures of the fungus were grown on PDA under ambient conditions for two weeks. Plugs of fungus-inoculated PDA (4x4 mm) were placed on sloped medium 2 cm apart from the buttom. Then the kernels were settled 2 cm above, with the embryo down. Previously, 100 kernels of each of the 5 the different maize genotypes were rinsed under tap water for 4 hours, then sterilized with 3% NaOCl, rinsed in distilled water and dried. Tubes were closed with a sterile cork borer and set into wire baskets. Checks were fungus-free. Root rot intensity and frequency in 21-day-old seedlings were evaluated as follows: 0 – no symptoms; 1 – dark tip of the small roots; 2 - small roots dark and development of necrotic lesion around the small roots on the primary root; 3 - numerous eyespots on the primary root, especially on the spots where small roots break out; 4 - numerous eyespots on primary and laterally roots; 5 – the lesions surround the roots and more than 50% of roots are dark.

Regarding the reaction of susceptible genotypes, *F. polyphialidicum* is pathogenic to maize. The average rates of root rot of 21-day-old seedlings varied from 1.4 (L_1) to 2.8 (L_3), while the percentage of infected plants ranged from 47 (L2) to 87 (L_2 x L_3). The highest rate was estimated in the inbred line (L_3) from which the fungus was isolated as well as in hybrid combinations (2.3 and 2.0) containing this inbred line (L_1 x L_3 and L_2 x L_3). The fungus seems to be is a weak pathogen, since the symptoms of the disease were more intensive and became more visible with the decrease in plants' vigour in the tubes.

Nagy, E.[1]; Bâgiu, L.[2]

[1] Agricultural Research Station Turda – România
[2] Research Institute for Cereals and Technique Plants Fundulea - România

Aggressiveness of *Fusarium graminearum* and *Fusarium moniliforme* isolates in maize

Among the pathogens on maize, *Fusarium* species are dominant under Transilvanian conditions. The most often occurring *Fusarium* species are *Fusarium graminearum* Schw., *Fusarium moniliforme* Sheldon., *Fusarium subglutinans* Woll. & Reink., Nelson, Toussoun & Marasas, which cause stalk and ear rot and breaking and lodging of plants at harvest time. The yield losses are 20% on average.

This paper presents the results regarding the aggressiveness of these *Fusarium* isolates tested on 10 maize hybrids for two years. Favorable temperature and rainfall during maize vegetation (April to September) influenced positively the pathogenesis of stalk and ear rot caused by *Fusarium* and allowed a good selection of the genotypes and of the aggressiveness of the *Fusarium* isolates.

The bifactorial experiments were designed by mid-divided plots in 3 blocks. Stalk disease severity was assessed by 3 criteria: breaking and lodging plants, plants with rotten basal internodes and the necrotic area on the second internod. Disease severity of the ear was based on percentage of diseased kernels at harvest. Impact of *Fusarium* attack was evaluated by the length and on the diameter of ear. The yield was expressed in grains (q/ha) with 85% dry matter.

The stalk rot severity caused by *Fusarium graminearum* infection was variable between 7,3-32,5% broken plants, and ear rot was between 22,6-44,6% diseased kernels. For *Fusarium moniliforme* between 6,4-28,7% of broken plants and between 11,5-27,0% of diseased kernels were recorded. For *Fusarium subglutinans*, broken plants were between 8,3-27,4% and diseased kernel percentages were between 10,4-23,3%, values that are statistically significant. From these data, it can be deduced that interactions between maize genotypes and *Fusarium* species are significant, which suggest that it could be a specific action of *Fusarium* species in maize.

Artificial infection of the ears led to a significant reduction of ear length with 3,2% and of ear diameter with 5,9%. Grain yield between 76,5-104,1 q/ha for 19 hybrids was recorded.

Artificial inoculation of the stalk resulted in yield losses between 1,1-1,9 q/ha and in the case of ear inoculation the yields were between 4,5-17,4 q/ha.

Among the *Fusarium* species tested, *F. graminearum* caused significant yield losses by 17,4 q/ha and *F. moniliforme* by 5,4 q/ha, compared with the uninoculated check. The most aggressive species on the ear proved to be *Fusarium graminearum* Schw. and in some cases *Fusarium subglutinans* on the stalk. The most resistant hybrids were: Saturn and HS 218.

The relationship between diseased kernels and yield has shown a negative correlation, $y = 105,81 - 0,73\ x$, the correlation coefficient being -0,805, very significant, and the determination coefficient was $d = 65\%$.

Besides these, the yield losses, food, feed, and seed quality of maize was also impaired by the mycotoxin content.

Lević, J.[1]; Petrović, T.[1]; Marjanović, S.[2]; Vukojević, J.[2]

[1] Maize Research Institute, Zemun Polje, Belgrade – Zemun, Yugoslavia
[2] University of Belgrade, Faculty of Biology, Belgrade, Yugoslavia

Late symptoms of *Fusarium subglutinans* on maize stalk

Fusarium subglutinans (Woll. & Reink.) Nelson, Toussoun & Marasas was often considered to belong to the *Fusarium* complex causing *Fusarium* or pinkish stalk rot. Our results, however, indicate the possibility of recognizing and separating symptoms of *F. subglutinans* from those of the other *Fusarium* species belonging to the section *Liseola*. *F. subglutinans* develops azure or bluish-green lesions on lower nodes and internodes of dried stalks at harvest.

One hundred plants were collected from each of three and five hybrids at the full maturity stage grown in 1998 and 1999, respectively. Tissues separated from the rind, beneath the rind, and core were sterilized, rinsed in distilled water, dried and cut up in 2-3 mm pieces which were then placed on PDA medium. Eight pieces of each tissue were analyzed. Part of the developing mycelium was transferred to PDA and CLA and incubated for 6-7 days in 12hr light / 12hr dark cycle.

Azure or bluish-green lesions (A) were dominant in 1998, while bluish-grey lesions (B) as well as lesions ranging from light to dark green (C) were noticed in 1999. In the first year, the symptom A was in low frequency and located only on stalk surface of H_1 hybrid. *F. subglutinans* was isolated in 28.6%, jointly with other fungi. However, on H_2 hybrid the fungus penetrated deeply into the core, followed by azure colour and was isolated in 100%, without the presence of other fungi. Similar results were obtained for H_3 hybrid, but in 20% of the samples *F. subglutinans* was associated with other fungi.

In 1999, the frequency of A, B and C symptoms varied from 22.2 (H_1) to 53.8% (H_3), 6.3 (H_4) to 66.7 (H_2) and 0 (H_5, H_2) to 50% (H_1), respectively. At the same time, the isolation of *F. subglutinans* was the most frequent in H_3 hybrid (7.7-58%), followed by H_2 (15.9-44.4%), H_4 (40.4-43.6%), H_5 (29-37.7%) and H_1 (7.7-25.6%). Generally, the pathogen was similarly present in the rind (35.7%) and core tissues (33.8%). The pathogen inducing lesions A was more present in the epidermis (56.3%), while in the core the one causing lesions B and C dominated (17.6-42.6%).

The results obtained indicated that the late symptoms of *F. subglutinans* were azure or bluish green lesions on infected parts of stalk, while symptoms intensity depended on maize genotype and environmental conditions/year. Furthermore, the pathogen directly infected stalk by hyphal penetration through the epidermal and sub-epidermal cells and developed in the core creating typical symptoms or remaining symptomless.

Burgess, L. W.[1)]; Summerell, B. A.[2)]; Giblett, G.[3)]; Backhouse, D.[4)]; Blake, M.[1)]; Smith-White, J.[1,2)].; Colville, M.[1)]

[1)] Fusarium Research Laboratory, School of Crop Science, University of Sydney, New South Wales 2006, Australia.
[2)] Royal Botanic Gardens, Mrs Macquaries Rd, Sydney, New South Wales 2000, Australia
[3)] "Lollybroch", RMB 583, Goonoo Goonoo Rd, Tamworth, New South Wales 2340, Australia
[4)] University of New England, Armidale, New South Wales 2000, Australia

Role of sorghum in overseasoning of *Gibberella zeae*

Gibberella zeae caused a localized epidemic of head blight in durum and bread wheats in the Liverpool Plains area of NSW Australia in October 1999. The epidemic caused significant losses in yield and downgrading of grain especially in durum wheat. It was the first significant outbreak of head blight caused by *Gibberella zeae* in Australia. The epidemic was favoured by wet conditions pre- and post-anthesis and high levels of inoculum of *G. zeae* in residues. The high levels of inoculum were, presumably a consequence of the recent significant increase in the acreage of durum wheat, which is very susceptible to *G. zeae*, and no-tillage cropping practices, which involve retention of crop residues. Head blight was particularly severe on farms where durum wheats were grown in rotations which included corn.

Sorghum is also grown in these rotations and its role as a potential carry-over host of *Gibberella zeae* was questioned. However, a survey of the literature revealed only one report of *G. zeae* being associated with sorghum. Therefore samples of 12 month-old sorghum stalks were collected at random from 22 fields in the northern grain belt of NSW including fields with a recent history of head blight. Thirty stalks from each sample were selected at random. A segment of stalk tissue from the first internode above the nodal roots was removed and plated on selective medium (peptone PCNB agar). *Fusarium* developing from these segments were subcultured on CLA for identification. The stalks were examined for the presence of perithecia of *G. zeae*.

Gibberella zeae was isolated from a portion of the samples while perithecia were also found on some of these samples. The fungus was only recovered from sorghum stalks from farms where there was a high level of inoculum of *G. zeae* on durum residues and where head blight was severe. It was not recovered

from sorghum stalks from farms where head blight was not reported or insignificant despite the long-term production of sorghum on these farms.

The results indicate that sorghum residues may act as a carry-over source of inoculum of *G. zeae.* Further studies are needed to clarify whether the fungus colonizes the sorghum stalk tissues before or after physiological maturity.

Balmas, V.[1]; Corda, P.[1]; Moretti, A.[2]; Ritieni, A.[3]; Logrieco, A.[2]; Bottalico, A.[1]

[1] Dipartimento di Protezione delle Piante-Sezione di Patologia Vegetale, Università di Sassari, Via E. De Nicola, 07100, Sassari, Italy.
[2] Istituto Tossine e Micotossine da Parassiti Vegetali, CNR, Viale Einaudi, 51, 70125, Bari, Italy.
[3] Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II" 80055, Portici, Italy.

Toxigenic *Fusarium* species associated with foot rot of rice in Sardinia

In a survey of *Fusarium* species causing seedling blight and foot rot of rice carried out in Sardinia (Oristano, S. Lucia), several fungal strains were isolated mainly belonging to *F. verticillioides* (Sacc.) Nirenberg (syn. *F moniliforme*, Sheldon), *F. proliferatum* (Matsushima) Nirenberg, *F. fujikuroi* Nirenberg, *F. nygamai* Burgess & Trimboli, *F. oxysporum* Schlecht. emend Snyd. & Hans., *F. solani* (Mart.) Appel & Wollenw. emend Snyd. & Hans. and *F. equiseti* (Corda) Sacc. Pathogenicity tests comparing one representative of each *Fusarium* species isolated were performed on rice cv. Arborio, sown in artificially infested soil in a greenhouse at 25° C with 8 hr photoperiod. The inoculum was prepared by growing *Fusarium* species in corn meal sand (1:30 wt/wt) at 25 °C for 3 weeks. The experimental layout was a completly randomized block design with three replicates. After 25 days, for each host-pathogen combination, the following parameters were taken into account: emergence, plant height and severity of infection.

Fusarium verticillioides, *F. nygamai*, *F. proliferatum* and *F. fujikuroi*, significantly reduced the emergenge of seedlings after 25 days, while the other species (*F. equiseti*, *F. oxysporum* and *F. solani*) did not show any significant difference in emergence as compared to the uninoculated control. *F. nygamai*, *F. proliferatum* and *F. fujikuroi* significantly reduced plant height while no or less reduction was observed on plants inoculated with *F. verticillioides, F. oxysporum, F. equiseti* and *F. solani* in comparison to the control. In particular, after 25 days, the seedlings grown in soil infested with *F. fujikuroi*, were mostly dead, while seedlings infected with *F. nygamai, F. verticillioides* and *F. proliferatum* exhibited a necrosis limitated to the lower leaf sheaths.

Investigations on the fertility of the strains belonging to *Liseola* section isolated from the rice plants, performed by mating tests on carrot agar medium, led to classify the strains to the mating populations A, C, D, and G, but also a high level of infertility was scored [1,2,3].

The strains belonging to *Liseola* section species, were then studied for their potential toxin production on rice. Single spore cultures were grown on 100 g autoclaved rice kernels, for 4 weeks in the dark at 25 °C. In particular, beauvericin, fumonisin B_1, fusaproliferin, and fusaric acid, were analyzed by HPLC or GC.

The results obtained showed: a) the occurrence of *F. nygamai*, a species described by Burgess and Trimboli in 1986 [4], on rice in Italy; b) the high pathogenicity of strains belonging to *F. fujikuroi* and, to a lesser extent, to *F. nygamai*, *F. proliferatum* and *F. verticillioides,* on rice plants cv. Arborio; c) the fertility of several strains which suggests a potential for a high recombination in rice fields. This aspect is important because a high possible genetic recombination could improve the genetic pool available for the pathogenic population of the different species; d) the high toxigenicity of *Liseola* section species that could be a further tool for increasing their aggressiveness on rice plants and being accumulated in infected tissues, representing a potential risk for rice consumption.

Literature

[1] Klittich C.J.R. and Leslie J.F., 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics, **118**, 417-423.
[2] Leslie J.F., Plattner R.D., Desjardins A.E., and Klittich C.J.R., 1992. Fumonisin B_1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phythopathology, **82**, 341-345.
[3] Moretti A., Logrieco A., Bottalico A., Ritieni A., Fogliano V., and Randazzo G. Diversiti in Beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Sidowia, **48**, 44-56.
[4] Burgess L.W., Trimboli D., 1986. Characterization and distribution of *Fusarium nygamai*, sp. nov. Mycologia, **78**, 223-229.

Hörberg, H.[1)]

[1)] Department of Ecology and Plant Production Sciences, Swedish University of Agricultural Sciences, Box 7043, S-750 07 Uppsala, Sweden

Comparison of three methods to evaluate pathogenicity of *Fusarium avenaceum, F. culmorum* and *F. poae* and their ability to infect different hosts

In Swedish cereals *Fusarium culmorum* is known to be responsible for the production of deoxynivalenol while *F. poae* has been shown to produce nivalenol [1]. *F. avenaceum* is considered a weak pathogen in cereals but is considered a strong pathogen of red clover *(Trifolium pratense* L.) producing a root rot and is the most frequently found species in Sweden [2]. *F. culmorum* and *F. poae* are considered weak pathogens in root rot of red clover [2]. In a Canadian cross inoculation survey isolates of *F. avenaceum* and *F. culmorum* from oats and barley were pathogenic on sweet clover, and the same fungi isolated from sweet clover were pathogenic on cereals [3]. In this trial the pathogenicity of *F. avenaceum,* isolated from red clover fields in Sweden, and *F. culmorum* and *F. poae,* isolated from oats cultivated in Sweden, were tested on different hosts. Also three different methods for evaluation of pathogenicity were compared.

In the first trial seeds of two cultivars of red clover (one more commonly used in the north of Sweden and the other in the south of Sweden) and one cultivar of spring wheat were planted in polyethylene plastic bags, 5 seeds in each bag. After 4 days spore suspensions of *F. culmorum, F. poae* and two isolates of *F. avenaceum* were added to the plastic bags. Samples were taken 2 wks, 4 wks and 6 wks after inoculation for comparison of sample data. The second trial was the same as the first, except that the source of inoculum was a mycelial plug placed under the seeds at the time of planting. In the third trial mycelial plugs of the fungi were placed on filter papers and seeds of the host plants on top of the mycelial plugs. The roots and coleoptiles were scanned for infections after 1 wk.

Infections were rated on a scale from 1-5, after McGee and Kellock, with the difference that the values 4 and 5 on their scale are put together into value 5 in my scale and all other values moved one step up so that value 0 is lacking [4]. All pathogens proved to be able to infect roots of both cultivars of red clover as well as stembases of spring wheat, but with somewhat different frequencies. *F. culmorum* was also able to infect the roots of spring wheat.

Three simple trials proved results for the evaluation of the pathogenicity of *Fusarium* spp. They show that Swedish isolates of *F. avenaceum, F. culmorum* and *F. poae* are able to infect different host plants in a different way . This may have effects on how crop rotations are to be considered in the future. Further studies are needed with more isolates of the respective fungi, collected from different hosts.

Literature

[1] Pettersson, H. 1991. Nivalenol production by *Fusarium poae*. Mycotoxin Res. **7**, 26-30.
[2] Rufelt, S. 1979. Klöverns rotröta: förekomst, orsaker och betydelse i Sverige. (Root rot of red clover: prevalence, cause and importance in Sweden.) Växtskyddsrapporter Jordbruk 9. Swedish University of Agricultural Sciences.
[3] Cormack, M W. 1937. *Fusarium* spp. as root parasites of alfalfa and sweet clover in Alberta. Can. J. Res. **11**, 493-510.
[4] McGee, D C and Kellock, A W. 1974. *Fusarium avenaceum*, a pathogen of subterranean clover rots. Aust. J. Agr. Res. **25**, 549-557.

Doohan, F. M.; Ferrer, N.; Egan, D.

Dept. of Environmental Resource Management, Faculty of Agriculture, University College Dublin, Belfield, Dublin 4, Ireland, Tel: +353-21-7067743, e-mail: fiona. Doohan@ucd.ie

Variation in the susceptibility of potato cultivars to *Fusarium* dry rot

This *in vitro* study investigated the susceptibility/resistance of potato tubers of the cultivars Barna, Cara, Rooster and Record towards *Fusarium sambucinum* and *F. culmorum* dry rot disease. *F. sambucinum* caused significantly larger dry rot disease lesions on all four potato cultivars than did *F. culmorum* ($p < 0.10$). The *F. sambucinum* dry rot lesions observed on cv. Rooster were significantly smaller than those observed on Barna, Cara and Record ($p < 0.01$). While the *F. culmorum* disease lesions observed on Rooster were significantly smaller than those observed on Barna ($p < 0.05$), they were not significantly different in size from those observed on Cara or Record ($p > 0.10$). There was no significant size differences between the *F. sambucinum* disease lesions observed on Barna, Cara and Record ($p > 0.01$). Similarly, there were no significant differences between the *F. culmorum* disease lesions observed on Barna, Cara and Record ($p > 0.10$). Future work will examine the relationship between potato cultivar resistance and mycotoxin contamination of potatoes.

Fleißner, A.; Sopalla, C.; Weltring, K.-M.

Inst. für Botanik, Westf. Wilhelms-Universität, Schlossgarten 3, 48149 Münster, Germany
e-mail: weltrin@uni-muenster.de

A multidrug-resistance ABC-transporter of *Fusarium sambucinum* is required for tolerance to phytoalexins and virulence on potato

Phytoalexins are low molecular weight antimicrobial compounds produced by plants in response to wounding and infection. They accumulate to high levels in the damaged tissue, why necrotrophic fungi like *Fusarium sambucinum* (teleomorph: *Gibberella pulicaris*), the causal agent of potato dry rot, must have developed mechanisms to overcome these chemical defense compounds. To identify such tolerance mechanisms we have looked for genes induced by the potato phytoalexin rishitin by differential cDNA screening. Among the isolated genes we found a multidrug-resistance ABC transporter (*Fsabc1*), which is induced by rishitin and lubimin. Although *F. sambucinum* strain R-6380 is able to metabolize both phytoalexins, disruption of the *Fsabc1* gene causes increased sensitivity to the phytoalexins and strongly reduced virulence on potato tubers.

Waalwijk, C.[1)]; Hesselink, T.[1)]; De Waard, M. A.[2)]; Kema, G. H. J.[1)]

Wageningen University and Research Centre
[1)] Plant Research International, Business Unit Biointeractions and Plant Health,
[2)] Department of Phytopathology, P.O. Box 16, 6700 AA Wageningen, The Netherlands

ABC transporters in *Fusarium*

The presence of ABC transporters in toxigenic Fusarium species and their involvement in the secretion of mycotoxins was studied using degenerated primers. These primers were based on conserved regions, in the ABC transporter genes from Saccharomyces cerevisiae, called Walker A and Walker B. The ABC transporters of yeast can be grouped into 6 clusters based on the topology of the encoded proteins [2].

Primers specific for clusters I and II were designed and PCR products of the expected size were sequenced. Some of these PCR products showed high homology with ABC transporters from Aspergillus nidulans, Botryotinia fuckeliana, Magnaporthe grisea and Mycosphaerella graminicola, while others showed homology with ABC transporters from Arabidopsis thaliana and Bos taurus.

A BAC library was constructed of F. proliferatum isolate ITEM 2287 because this strain produces a broad spectrum of mycotoxins: Fumonisin B1, moniliformin, beauvericin as well as fusaproliferin [1]. Primers specific for PCR fragments from F. proliferatum were used to screen this library, leading to contigs encompassing ABC transporter genes from both cluster I and cluster II.

Literature

[1] Decottignies, A. and Goffeau, A. (1997). Complete inventory of the yeast ABC proteins. Nature genetics **15**: 137-145.
[2] Munkvold, G., Stahr, H.M., Logrieco, A., Moretti, A. & Ritieni, A. (1998) Occurrence of fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. Applied Environ. Microbiol **64**: 3923-3926.

Toropova, E. U.[1)]; Greenberg, E. G.[2)]

[1)] Novosibirsk Agricultural University, Russia, e-mail: elena_toropova@pochta.org
[2)] Siberian Research Institute of Plant Growing and Breeding, Russia

Pathogenesis and control of *Fusarium* rot on shallot in Siberia

Shallot (*Allium ascalonicum* L.) is a rather popular crop in Siberia. *Fusarium* species, primarily *F. oxysporum* Schl. and *F. solani* Mart., cause foliage chlorosis, rotting of roots and basal rot of bulbs. *Fusarium* diseases intensify the effect of drought conditions typical for Western Siberia causing about 30% losses on shallot.

F. oxysporum and *F. solani* were isolated from rotting shallot roots on glucose agar and identified on rice agar and beer-wort agar [1, 2].

Field experiments, carried out in 1998-2000 on the local shallot cultivar Sprint in Novosibirsk region, showed that mineral fertiliser ($N_{60}P_{60}K_{60}$), humus (3-5 t/ha), and seed bulb treatment with Raxyl (BAYER) (0.5 l/t) may be used as control measures against this *Fusarium* disease (Tab.1).

Table: Influence of control measures on *Fusarium* disease and yield of shallot in the Novosibirsk region

Measure	Control	Fertiliser	Humus	Raxyl
Root rot severity, %	21.7	15.5	18.0	17.3
Chlorosis severity, %	14.9	11.9	13.9	14.8
Yield, t/ha	7.3	8.2	8.4	8.4

All measures were effective against this disease. Mineral fertiliser was most effective and helped reduce both root rot and chlorosis. Humus was effective against chlorosis by increasing resistance, probably because it helps soil to retain moisture during the drought time. Microorganisms antagonistic to *Fusarium* were increased, but their effect was low, because *Fusarium* competition was high. Treatment with the fungicide reduced root rot more effectively than chlorosis, but it increased seed germination up to 10-15% and provided significant yield increase like the other measures.

In laboratory experiments *Fusarium oxysporum* colonies showed slower growth on beer-wort agar with a trace of Zn SO_4 compared with the normal beer-wort agar. Zn salts can be used as microfertilisers together with N, P, K for the better control of this *Fusarium* disease. Zn salts can also be used in combination with fungicide seed bulb treatments.

Literature
[1] Booth, C (1975). The present status of *Fusarium* taxonomy. Annu. Rev. Phytopathology **13**, 83-93.
[2] Nelson, P.E., Toussoun,T.A., and Cook, R.J., eds. (1981). "*Fusarium:* Diseases, Biology and Taxonomy." Pennsylvania State Univ. Press, University Park.

Stojsin, V.[1)]; Balaz, F.[1)]; Lević, J.[2)]; Petrović, T.[2)]; Bagi, F.[1)]; Atlas, S.[1)]

[1)] University of Novi Sad, Faculty of Agriculture, Department for Environmental and Plant Protection, Yugoslavia
[2)] Maize Research Institute, Zemun Polje, Belgrade – Zemun, Yugoslavia

Fusarium root rot of sugar beet

Root rot of sugar beet caused by *Fusarium* species was described for the first time in Yugoslavia in the seventies, but the problem has not been investigated in greater details. To identify the causal agent, isolation on PCNB medium was made, from necrotic root tips, taken from stunted and chlorotic sugar beet plants. Sixtytwo isolates were pathogenic. Based on the severity of the disease and using Burgess (1994) and Nelson (1983) the isolates were ranked as: very highly pathogenic 10.1%, high 7.2%, medium 10.7%, low 39.9% and very low 32.1%.

In the high and medium pathogenic ranks we identified *Fusarium graminearum* Schwabe Group 2, *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. equiseti* Corda Sacc., while in the low and very low pathogenic ranks the dominant species were *F. oxysporum* Schlecht. emend. Snyd. & Hans. On the basis of these results, it can be concluded that *Fusarium* species have an important role in sugar beet root rot. *F. graminearum* Schwabe Group 2 and *F. equiseti* Corda Sacc. are reported as pathogens of sugar beet root for first time in FR Yugoslavia.

Ibrahim, G.[1)]; Nirenberg, H. I.[2)]

[1)] Agricultural Research Corporation, P.O.Box 126, Wad Medani, Sudan
[2)] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, D-14195 Berlin, Germany

Recent studies on *Fusarium* vascular wilt of cotton at the Federal Biological Research Centre for Agriculture and Forestry (BBA), Berlin[*]

Fusarium wilt of cotton is caused by *Fusarium oxysporum* Schlecht f. sp. *vasinfectum* (Atk.) Snyd. and Hans. (*F.o.v.*). Six races were known world-wide [1,2,5] until 1985. The assumed confined regional geographical distribution is eminently being replaced by many arrivals of those races to new areas.

Races in F.o.v. are basicly determined by their virulence on the appropriate differntial hosts. Other cultural, molecular (RAPD, DNA sequences) or genetical (VCG) results are, so far, either indicative or complementary. However, work at the BBA, Berlin, had shown that at least race 3 is culturally distinct from all other races [7]. But the cultural differences between other races are not quite distinct to warrant their value in that matter.

Race 5 in F.o.v. emerged from race 3 [5]. The validity of this race had been reviewed. Evidence, based on the re-examination of virulence on the different hosts suggested that race 3 and 5 are to remain as a single race [7]. Race 7 and 8 were recently reported in China as new races [4]. Work is currently in hand at the BBA on further verification of these findings with indications in favour.

[*] This work is partially funded by the Deutsche Akademische Austauschdienst (DAAD)

Techniques used in the infection tests varied from direct plant inoculation to infection through infested soil. The root dip method compared with stem puncture [3] has been found satisfactory. However, it can be tedious and laborious if a large number of isolates are to be tested simultaneously. Soil infestation and seed soaking are being evaluated.

Accurate interpretation of the symptoms exhibited by F.o.v. is particularly important in field diagnosis of the disease where more than one race is likely to be involved. Information in the literature seems to imply that the early important symptoms of the leaf namely vein discoloration is determined by the cotton species. But infection with race 3 results in vein yellowing regardless of the clearing and with races 1, 2 and 6 results in vein darkening [6]. Regarding race 4, symptoms are predominantly stunting and wilt with little vein clearing.

Literature

[1] Armstrong, J.K., Armstrong, G.M. 1958: A new race of cotton-wilt *Fusarium* causing wilt of Yelredo soybean and flue-cured tobacco. Plant Dis. Rep. **42**: 141-151.
[2] Armstrong, G.M., Armstrong, J.K. 1960: American, Egyptian and Indian cotton-wilt fusaria: their pathogenicity and relationship to other wilt fusaria. U.S. Dep. Agric. Tech. Bull. **1219**: p. 19.
[3] Bugbee, W.M., Sappenfield, W.P. 1968: Varietal reaction of cotton after stem puncture or root inoculation with *Fusarium oxysporum* f. sp. *vasinfectum*. Phytopathol. **58**: 212-214.
[4] Chen Qi Ying, Xiugen, J., Wenjl, S. 1985: Identification of races of cotton-wilt *Fusarium* in China. Plant Prot. Inst., Chinese Academy of Agric. Sci., Beijing.
[5] Ibrahim, F.M. 1966: A new race of cotton wilt *Fusarium* in the Sudan. Gezira – Empire Cotton Growing Rev. **43**: 296-299.
[6] Ibrahim, G., Nirenberg, H.I. 1993: Response of some Sudanese cotton cultivars to race 1 and 5 of *Fusarium oxysporum* f. sp. *vasinfectum*. J. Plant Dis. Prot. **100**: 645-651.
[7] Nirenberg, H.I., Ibrahim G., Michail, S.H. 1994: Race identity of three isolates of *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. and Hans. from Egypt and Sudan. J. Plant Dis. Prot. **101**: 594-597.

Zagurskiy, A.V.[1)]

[1)] Scientific-Production Center for Plant Protection, 75 Fyodorova St., Bishkek, 720000, Kyrgyz Republic

Influence of environmental conditions on growth, development, and pathogenic properties of *Fusarium oxysporum Schlecht.*

The development of fungal diseases depends on the environmental conditions and is determined by the interaction between the host plant and the causal organism. Under the influence of the environment, not only the susceptibility or resistance of plants, but also the pathogenic properties of fungi are changed.

An important environmental factor, which influences all the manifestations of activity of fungi is temperature. It stipulates the intensity of a disease and its rate of development. *Fusarium oxysporum* proved to be the most pathogenic fungus to sugar beet at 28 C, though it was already pathogenic enough at 15 C.

The penetration of certain nutritious substances into the cell and the fermentation activity depend on the pH level. In our research, the maximum growth and the most revealed pathogenic properties were observed at pH 7-8, which is most favourable for the development of *Fusarium* rot of agricultural plants.

Nitrogenous nutrition is of primary importance in the life activity of fungi. Usually microorganisms use both mineral and organic nitrogen compounds. Nitrogen of any form turns to ammonia, which is used for the synthesis of amino acids and proteins, composed of the fungal cell.

Our study of the influence of nitrogen nutrition on growth, development, and pathogenic properties of *Fusarium oxysporum*, the addition of equivalent quantities of different nitrogen sources (Chapek medium) showed that *Fusarium oxysporum* assimilated nitrogen better in organic and nitrate forms. It produced large dense colonies with a well-developed mycelium. And with the ammonium salts the colonies were small with fluffy, high, mycelium, weakly pigmented. The fungus, grown on media with ammonium salts, exhibited high pathogenic properties.

While studying the influence of temperature on growth and pathogenic properties of *Fusarium oxysporum*, we observed, that the highest pathogenic property of the fungus was manifested at the temperature optimum of its vegetative growth. In the experiment with nitrogen nutrition sources there was no correlation. Evidently, nitrogen nutrition influences mostly the fermentation activity, which determines the pathogenic properties of parasites in many ways.

The dependence of the pathogenic properties of fungi on the conditions of the environment can be used to enhance selection of plants resistant to dieseases.

Piwoni, A.

Agricultural University, Dept. of Plant Protection & Quarantine, Leszczynskiego str.7, 20 – 069 Lublin, Poland,
e-mail: piwonia@consus.ar.lublin.pl

Pathogenicity and mycelium growth at different temperatures of some *Fusarium solani* isolates obtained from crocus corms

The pathogenicity and growth of 22 *Fusarium solani* isolates obtained from crocus corms were investigated at different temperatures. Pathogenicity test was made in chamber on crocus corms cv. Flower Record planted in flower pots. Plugs of PDA medium with 12- day old fungal colonies, which were placed under the corms were used for inoculation. Five corms were used for testing each isolate. Degree of pathogenicity was indicated as percentage of necrosis on mother corms and cormels after flowering time. Variability of pathogenicity was obseved: one isolate S128 was strongly aggressive, isolates S108, S144, S78 and S52 were middle aggressive and isolates S35 and S132 were non-pathogenic, most of investigated isolates were only slightly aggressive to crocus corms under the experimental conditions. The rate of mycelium growth of investigated isolates after 7 days on PDA at temperature:s 8^{o}C, 12 ^{o}C, 20 ^{o}C and 25 ^{o}C were also studied. At the lowest temperature the growth of isolates: S55, S128, S132, S59, S6 was strongly inhibited (<3,5mm). Two groups of isolates with significant differences between them were observed: „slow-growing" at investigated temperatures: S44, S55, S6, S132, S52, S144 and „fast - growing": S99, S63, S15, S68, S69, S85, S138. No correlation between pathogenicity and mycelium growth rate of investigated isolates was observed.

Kurmut, A.[1)]; Nirenberg, H. I.[2)]; Bochow, H.[1)]

[1)] Institute of Horticultural Sciences, Department of Phytomedicine, Lentzeallee 55-57, D-14195 Berlin, Germany
[2)] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biological Safety, Königin-Luise-Str. 19, D-14195 Berlin, Germany

Fusarium acutatum Nirenberg and O'Donnell a causal agent of root rot of *Vicia faba* L. in Sudan

Several *Fusarium* spp. were isolated from wilted and rotted plants of *Vicia faba* received from two different localities in the Sudan. *Fusarium acutatum* Nirenberg and O'Donnell was recovered among other *Fusarium* species.

The symptoms exhibited were black root rot, which were associated with rot and death of the lateral root system. Severely infected plants showed black foot rot extending above the soil level. These symptoms were usually accompanied by yellowing of the margin of the lower leaves, these then turned brown and died. Death of the intact leaves also occured.

Most of the strains proved to be pathogenic on *Vicia faba*. The highest infection rate was produced by isolate BBA 71548. It caused complete death of all plants within 2 weeks. Isolates BBA 71542 and BBA 71544 produced 70% and 40% infection level, respectively. This is the first report of *Fusarium acutatum* as a pathogen on *Vicia faba*.

Aoki, T.[1]; Hirata, T.[2]; Kimishima, E.[2]; Nirenberg, H. I.[3]; O'Donnell, K.[4]

[1] National Institute of Agrobiological Resources, Department of Genetic Resources I, Ministry of Agriculture, Forestry and Fisheries, Kannondai, Tsukuba, Ibaraki 305-8602 Japan
[2] Research Division, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, 1-6-10 Shin-yamashita, Naka-ku, Yokohama, Kanagawa 231-0801 Japan
[3] Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virologie, Microbiology and Biological Safety, Königin-Luise-Straße 19, D-14195 Berlin-Dahlem, Germany
[4] Microbial Properties Research Unit, National Center for Agriculture Utilization Research, United States Department of Agriculture, Agriculture Research Service, Peoria, Illinois 61604, USA

Morphological and molecular identification of *Fusarium verticillioides* from rotten banana imported into Japan

During a routine plant quarantine inspection, many different size brownish spots were found on banana imported into Japan from Mexico. Of the seven strains of *Fusarium* isolated from rotten fruits, morphological features including white to purplish colonies on PDA, long chains of aerial conidia produced strictly on monophialides on SNA, and long, slightly curved, fusiform to falcate, septate sporodochial conidia induced by culturing under black light indicated that they were *F. verticillioides* (syn. = *F. moniliforme* s. str.). None of the strains formed chlamydospores. Frequent production of septate aerial conidia in chains in complete darkness and under black light, however, represented a difference from previous descriptions of this taxon. Pathogenisity to healthy banana fruits was also demonstrated by wound and non-wound inoculation. Nelson et al. [1] characterized *F. moniliforme* as having primarily single-celled microconidia (= aerial conidia) formed in long chains. Gerlach and Nirenberg [2], however, stated that *F. verticillioides* exceptionally forms 1- and 2-septate conidia, and Wollenweber and Reinking [3] characterized *F. moniliforme* (var. *moniliforme*) as producing 1- or 2-celled microconidia. Morphological studies were performed on the banana strains and the results were compared with previous descriptions, and four authentic strains of *F. verticillioides* (teleomorph = *G. moniliformis*, syn. = mating population A of the *G. fujikuroi* species complex): ATCC 38932 (A+), ATCC 38933 (A-), BBA 62264 and BBA 65898. The latter four strains formed 1- and 2-septate aerial conidia similar in size and shape to the banana strains in complete darkness and under black light, although septate conidia were formed with less frequency than in the banana strains.

Phylogenetic relationships of the banana isolates were investigated by maximum parsimony analysis of DNA sequences from the mitochondrial small subunit rDNA and translation elongation factor. The banana isolates and an authentic strain of *F. verticillioides* formed a highly supported, exclusive group in the molecular phylogeny, suggesting they are conspecific. Based on the morphological and molecular-phylogenetic analyses, the Mexican banana isolates were identified as *F. verticillioides*. These results suggest that morphological variability of *F. verticillioides* is somewhat larger than documented previously.

Literature

[1] Nelson, P.E., Toussoun, T.A., Marasas, W.F.O. 1983. *Fusarium* species, an illustrated manual for identification, Pennsylvania State University Press, University Park.
[2] Gerlach, W., Nirenberg, H. 1982. The genus *Fusarium*- A pictorial atras. Mitt. Biol. Bundesanst. Land- Forstw. Berlin-Dahlem **209**, 1-406.
[3] Wollenweber, H.W., Reinking, O.A. 1935. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. Paul Parley, Berlin.

Goßmann, M.

Humboldt-Universität zu Berlin, Fachgebiet Phytomedizin, Lentzeallee 55-57, D-14195 Berlin, Germany

Occurrence and pathogenicity of *Fusarium* spp. on *Miscanthus* x *giganteus* Greef & Deu. (China grass)

Miscanthus x giganteus (china grass) is a perennial grass and originated in East-Asia. As a regenerating plant it was cultivated intensively in the late eighties in Central Europe.

Apart from plant cultivation problems also phytoparasites were suspected to cause insufficient tillering capacity of predominantly one and two year old plants. There were no reports on soilborne fungal agents of the temperate region in Central Europe until the early ninties [1, 2]. Examinations of plant parts growing in and above the soil from localities in Brandenburg, Baden-Würtemberg and Mecklenburg-Vorpommern over several years revealed that especially rhizomes often were colonized by pathogenic *Fusarium* species like *F. acuminatum, F. avenaceum, F. cerealis, F. culmorum, F. graminearum, F. oxysporum, F. poae, F. proliferatum, F. subglutinans, F. sambucinum, F. sporotrichioides, F. sulphureum,* and *F. tricinctum*. At all stands evidence was found that the inability of *Miscanthus* plants to tiller can be attributed to potential root rot organisms. The following *Fusarium* species were assessed to be important pathogens of *Miscanthus*: *F. acuminatum, F. culmorum, F. graminearum, F. subglutinans,* and *F. proliferatum*.

Infection tests with selected isolates of *F. avenaceum, F. culmorum, F. graminearum, F. subglutinans,* and *F. proliferatum* on *Miscanthus* seedlings were carried out. The damage manifested, was mainly reduced tillering capacity. A reduced formation of rhizomes i.e. a lower bio-mass above as well as below the soil surface could also be demonstrated in a two year pot trial using *Miscanthus* plants inoculated with *F. culmorum* and *F. proliferatum*. Soil treatment with *Bacillus subtilis* showed positive results on growth of bio-mass and reduction of colonization of the rhizomes with fungal pathogens.

Literature

[1] Müller, R., Nirenberg, H.I. 1994. Erste Ergebnisse zum Auftreten von bodenbürtigen Pathogenen beim Anbau von Topinambur und *Miscanthus*. In: 49. Deutsche Pflanzenschutztagung. Mitt. Biol. Bundesanst. Land-Forstw. Heft 301, p. 51.

[2] Goßmann, M. 1994. Analyse der endogenen Pilzbesiedelung von *Miscanthus sinensis*, unter besonderer Berücksichtigung von *Fusarium*-Arten. In: 49. Deutsche Pflanzenschutztagung. Mitt. Biol. Bundesanst. Land-Forstw. Heft 301, p. 272.

Martins, M. L.[1)]; Peito, M. A. P.[2)]; Martins, H. M.[1)]; Távora, C. A.[2)]

[1)] Laboratório Nacional de Investigação Veterinária. LNIV. - Estada de Benfica ,701-1549-011 Lisboa, Portugal

[2)] Instituto Nacional de Engenharia e Tecnologia Industrial- DTIA-Estrada do Paço do Lumiar, 1699 Lisboa, Portugal

Contamination of mixed feed with *Fusarium* spp. in Portugal

Fusarium species are widely distributed in nature and cause deterioration of food and feeds. Their mycotoxins can adversely affect human and animal health. The predominant interest in the genus is plant pathogenicity of its species. Among these, *Fusarium moniliforme* is a common fungal contaminant of cereals and produces a variety of mycotoxins [1].

During the last four years (1996-1999) 464 samples of mixed feed (for poultry, swine and bovine) were analyzed in our laboratories, for the evaluation of the presence of *Fusarium moniliforme*. Ten grams of each sample were homogenized in 90 ml of peptone water for 3 min and surface plated immediately on DRBC(Oxoid). Plates were incubated for five days at 25ºC. The identification of *Fusaria* was performed according to the Pictorial Atlas - The genus *Fusarium* (1982) [2].

The results have shown a decreasing tendency of the incidence of *Fusarium moniliforme* in mixed feeds from 78.0 % in the period of 1985/1990 [3] to 40% from 1991/1995 [4] and to 22% in the period of this report (1996/1999). Bovine and poultry feedstuff showed similar occurrence (19.0%) and in the swine

feed it was higher (24.0%). This decrease may be due to a good practice of drying processing, packing and storage or/and the use of fungistatics.

Literature
[1] Bacon, C.W., Nelson, P.E. 1994. Fumonisin production in corn by toxigenic strains of *Fusarium moniliforme* and *F. proliferatum*. Food. Add. Contam **13**, 163-168.
[2] Gerlach, W., Nirenberg, H. 1982. The Genus *Fusarium* - A pictorial Atlas.
[3] Martins, H. 1987. Relatorio de Actividades Ed. LNIV. Lisboa, Portugal.
[4] Martins, L. 1989. Ocorrência de micotoxinas em materias primas e alimentos compostos para animais. Situação actual em Portugal. Rep. Trab. LNIV. XXI, 111-122.

Ittu, M.[1)]; Miedaner, T.[2)]

[1)] Research Institute for Cereals and Industrial Crops-Fundulea, 8264 Romania; e-mail: gittu@pcnet.pcnet.ro
[2)] University of Hohenheim (720), State Plant Breeding Institute, D-70593 Stuttgart, Germany

Effect of environmental conditions on the aggressiveness of German and Romanian *Fusarium graminearum* isolates towards wheat and on deoxynivalenol production

Under favourable conditions *Fusarium* head blight (FHB, scab) may lead to severe yield losses, reduced kernel quality and mycotoxin contamination in wheat crops worldwide. The control has been rather difficult, but genetic host resistance is considered the most promising component of a long-term management solution. Artificial inoculations, indispensable for characterisation and improvement of resistance to FHB in wheat, demand reliable assessment techniques and highly aggressive isolates. In naturally occurring populations and isolate collections of *F. graminearum* and *F. culmorum* large genetic variation of aggressiveness was reported. The main focus of this report was to investigate the environmental variation of aggressiveness of *Fusarium graminearum* isolates to common wheat and rye and the production of the mycotoxin deoxynivalenol (DON) on rye.

Thirty-three isolates with different geographic origins (18 German/15 Romanian) were tested on a susceptible winter wheat cultivar in Fundulea, Romania, for two years (1998, 1999) and Stuttgart-Hohenheim, Germany, for one year (1999). Additionally, isolates were inoculated on a susceptible winter rye cross (Lo7-PxLo6-N) in Hohenheim (1998). A spore suspension of each isolate increased on CMC medium by the "air-bubble" method was sprayed twice onto heads when 40% and 60% of the plots were flowering. Aggressiveness was assessed by mean head blight rating (1-9) and relative weight of inoculated spikes (% of control). From the rye samples, additionally DON content was analysed by high-pressure liquid chromatography (HPLC) in the lab of Dr. R. Krska, IFA, Tulln (A).

All 33 isolates of *Fusarium graminearum* significantly (P=0.01) varied in aggressiveness over three location year combinations irrespective of the trait assessed. Between mean head blight rating and relative weight of inoculated spikes high correlations were found in the results of two years from Romania (r=-0.825 at *P*=0.01) and over three environments as well (r=-0.863 at *P*=0.01). Coefficents of variation of the isolates were higher in head blight rating (26-38.4%) than in relative weight of inoculated spikes (12.6-22%) calculated for individual location year combinations. The variances of environment and isolate environment interaction were significant, too. However, no notable changes in the ranking of the isolates occurred indicating a high environmental stability in aggressiveness of *Fusarium* isolates. *F. graminearum* 108 was consistently least aggressive and *F. graminearum* 54 as well as *F. graminearum* 2311 highest aggressive for both traits.

DON production of the 33 isolates of *Fusarium graminearum,* measured as DON content of the grain at harvest, was strongly associated with head blight rating (r=0.878 at *P*=0.01). However, also the production of mycelium within the grains highly varied among isolates. The results of this report confirm the general assumption that aggressiveness of *Fusarium* isolates is a highly stable trait across environments. It could be measured as well in terms of head blight rating, weight of inoculated spikes relative to the control or DON production. The large genetic variation among isolates indicates a high plasticity of the pathogen that should be considered in resistance selection.

Breeding

Miedaner, T.[1)]; Moldovan, M.[2)]; Ittu, M.[3)]

[1)] University of Hohenheim (720), State Plant Breeding Institute, D-70593 Stuttgart, Germany
[2)] Agricultural Research Station, Str. Agriculturii 27, 3350 Turda, Romania
[3)] Research Institute for Cereals and Industrial Crops, Jud. Călărasi, 8264 Fundulea, Romania

The relative importance of type I and II resistance for assessing *Fusarium* head blight in wheat

Head blight caused by *Fusarium culmorum* and *F. graminearum* [telomorph: *Gibberella zeae*] is one of the most destructive diseases in cereals on a world-wide basis. Hence, a higher level of resistance is urgently needed. Different components of resistance have been described in the last decades [1, 2]. Among the most important are resistance to penetration (type I) and to spread of the pathogen within the host (type II). By spray-inoculation during anthesis both types of resistance are addressed, while a single-spikelet inoculation will result in type II-resistance only.

In order to investigate the relative importance of both resistance mechanisms, ten Romanian and ten German wheat cultivars have been artificially inoculated by both methods in a factorial design at two German and two Romanian locations with one isolate of *F. culmorum*. Disease severity was measured as head blight rating and counting of diseased spikelets, respectively, and head weight relative to the non-inoculated heads of the same genotype.

Single-spikelet inoculation showed a consistently higher disease severity than spray inoculation at three out of four locations. Genotypic differentiation was highly significant (P=0.01) for both methods combined across locations. The Romanian cultivars were similarly resistant as the German cultivars for counting/rating, but considerably more resistant to yield loss than those inoculated by single-spikelet inoculation. The most resistant genotype of all was the Romanian line F201R.

In the analysis of variance across all locations, the spray variant revealed for all traits higher genotypic variations, considerably lower genotype x location interaction and error variances resulting in medium to high heritabilities (0.6-0.8) compared to lower heritabilities for the single-spikelet inoculation (0.1-0.6). Correlations between single-spikelet and spray inoculation were non-significant. This was mainly caused by three German/Swiss genotypes that were highly (KIMON, ARINA) to medium (GREIF) resistant in the spray, but highly susceptible in the single-spikelet inoculation. The opposite reaction was not found, i.e. all genotypes being susceptible with single-spikelet inoculation were also susceptible with spray inoculation. Romanian cultivars preselected by single-spikelet inoculation showed, therefore, a higher correlation (r=0.6) of resistance traits between both methods.

Summing up, three out of twenty genotypes were differing highly in their resistance reaction due to the inoculation method. They seem to have a high type I resistance, but a low resistance level to the spread of the pathogen when it is injected. Some genotypes (e.g. PIKO, F201R) were highly resistant with both methods.

Literature

[1] Schroeder, H.W., Christensen, J.J. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology **53**, 831-838.
[2] Mesterházy, À. 1995. Types and components of resistance to *Fusarium* head blight of wheat. Plant Breed. **114**, 377-386.

Wisniewska, H.[1]; Chelkowski, J.[1]; Perkowski, J.[2]; Busko, M.[2]

[1] Institut of Plant Genetics, Polish Academy of Sciences, Poznań, Poland
[2] Departament of Chemistry, Agricultural University, Poznań, Poland

Resistance to *Fusarium* head blight and deoxynivalenol accumulation in spring wheat inoculated with *Fusarium culmorum*

Polish cultivars of spring wheat (12) and spring wheat lines (44) derived in CIMMYT from accessions resistant to *Fusarium* head blight (FHB) - Frontana, Sumai 3 and *Aegilops squarrosa* were inoculated with *Fusarium culmorum* isolate KF 846. The experiments were carried out in two years (1998 and 1999) under field conditions in one locality, Cerekwica, near Poznań.

In examined accessions differences within disease rating on heads (F_i), Fusarium damaged kernels (FDK) and deoxynivalenol (DON) accumulation were observed.

F_i ranged from 0-76% in 1998 and 2-66% in 1999 and FDK from 5-100% in the first year of experiments and from 0.3-23% in next year. Accumulation of DON in kernels ranged from 0-36mg DON/kg in 1998 and 0,27-65.77mg DON/kg in 1999. Three lines were found to be superior in their resistance to FHB in both seasons.

Three different resistance components were observed:

1. Resistance to the spread of the pathogen.
2. Resistance to kernel colonization.
3. Resistance to DON accumulation.

Only 6 accessions (No.5, 6, 11, 14, 16) have resistance to FDK and low DON accumulation in kernels. Accessions derived from *Aegilops squarrosa* were highly susceptible to FHB, but not affected by powdery mildew.

Miedaner, T.[1]; Schneider, B. G.[2]; Geiger, H. H.[2]

[1] University of Hohenheim (720), State Plant Breeding Institute, D-70593 Stuttgart, Germany;
e-mail: miedaner@uni-hohenheim.de
[2] University of Hohenheim (350b), Institute of Plant Breeding, Seed Science, and Population Genetics,
D-70593 Stuttgart, Germany

Trichothecene accumulation in wheat and rye genotypes differing in resistance to *Fusarium* head blight

Fusarium head blight (FHB) caused by *Fusarium graminearum* (teleomorph: *Gibberella zeae*) and *F. culmorum* may affect all cereals grown in Central Europe. Both species are potent mycotoxin producers with the trichothecenes deoxynivalenol (DON), 3-acetyl deoxynivalenol (3-ADON), and nivalenol (NIV) being the most prevalent toxins. In this study we analysed the inheritance of trichothecene accumulation in winter wheat, winter triticale, and winter rye.

In a first experiment the trichothecene accumulation of 8 wheat, 6 triticale, and 12 rye genotypes of different resistance levels was assessed across six environments (location-year combinations). In a second study the genetic variation among genotypes within the cereal species was analysed by inoculating 78 wheat, 100 triticale, and 394 rye lines across four environments. The wheat and rye lines represented segregating progenies derived from single crosses of parents differing in resistance. Inoculation was performed using either a highly aggressive DON- (exps. 1, 2) or a medium aggressive NIV-producing iso-

late (exp. 1) of *F. culmorum.* Trichothecenes were analysed either by gaschromatography-mass spectrometry or by a DON-specific ELISA. Head blight rating was assessed as resistance trait.

In all environments a medium to high disease severity occurred. Although rye genotypes were, on average, similarly affected by the pathogen as wheat genotypes, wheat accumulated twice as much DON than rye. Triticale was least affected and the grain contained only slightly more DON than rye. Significant (P=0.01) genotypic variation for DON accumulation existed in all three cereal species. Mean mycotoxin content of the grain could not be associated with specific weather conditions. This corresponded to strong genotype-by-environment interactions for resistance and mycotoxin contents. Correlation between resistance and DON content was tight among the pre-selected wheat varieties (r=0.8-0.9), but variable and on average only moderate in rye (r≈0.5). In both cereals, DON and 3-ADON contents of the genotypes were tightly correlated (r=0.87). All genotypes reacted similar to the DON- and NIV-producing isolate of *F. culmorum*.

In conclusion, the medium to large genotypic variation for disease severity offers good chances for resistance selection in all three cereal species. Strong dependence of the genotypic differentiation on the environment requires multiple locations and/or years for evaluating FHB resistance and trichothecene content. In view of the tight genetic correlation between resistance and mycotoxin content in wheat, selection for resistance alone can be expected to result in a satisfactory reduction of DON accumulation. In rye, genotypes visually selected for their resistance should additionally be analysed for their mycotoxin content to further reduce the DON accumulation rate. The tolerance of wheat and rye to the DON and NIV chemotype of *F. culmorum* seems to be most likely the same.

Chelkowski, J.[1]; Wisniewska, H.[1]; Golinski, T. A. P.[2]; Kaczmarek, Z.[1]; Kostecki, M.[2]; Perkowski, J.[2]; Surma, M.[1]

[1] Institute of Plant Genetics, Polish Academy of Sciences, ul. Strzeszynska 34, 60-479 Poznan, Poland
[2] Department of Chemistry, August Cieszkowski Agricultural University, ul. Wojska Polskiego 75, 60-625 Poznan, Poland

Effects of *Fusarium culmorum* head blight on mycotoxin accumulation and yield traits in barley doubled haploids

Susceptibility of barley doubled haploids (DH) to Fusarium head blight (FHB) has been studied. Heads of 24 DH lines (11 two-rowed and 13 six-rowed) derived from F_1 Maresi (two-rowed) x Pomo (six-rowed) hybrids were inoculated with a conidial suspension of isolate IPO348-01 of *Fusarium culmorum.* The experiment was carried out in three consecutive years (1996-1998) in one location. The number of kernels per ear, 1000-kernel weight and kernel weight per ear were recorded in inoculated and control plots. In the infected kernels nivalenol content (NIV) and deoxynivalenol content (DON) were determined. Effects of genotype, year and genotype x year interaction on reduction of yield traits were significant. For mycotoxin content only genotype and year effects were found to be important. The average nivalenol concentration in kernels of inoculated lines ranged from 0.15 mg/kg in the two-rowed line MP7 to 6.36 mg/kg in the six-rowed line MP113. A low accumulation of deoxynivalenol was observed in the studied population (from 0.01 to 0.20 mg/kg). Generally, no significant differences in mycotoxin content were found between 2-rowed and 6-rowed genotypes. Line MP7 was found to be superior – with the lowest mycotoxin accumulation, and reduction in yield traits. Environmental conditions (years) affected DON and NIV level in kernels, however, the tendency to a lower or higher accumulation of mycotoxins in individual lines was stable over the years.

Scholz, U.; Steffenson, B. J.

Department of Plant Pathology, North Dakota State University, Fargo, ND 58109, USA

Sources of *Fusarium* head blight resistance in six-rowed spring barleys

Fusarium head blight (FHB), caused by *Fusarium graminearum*, threatens the existence of the malting barley industry in the Upper Midwest region of the USA [1,2]. The deployment of resistant cultivars is the most effective and environmentally sound means of managing this disease. In the Upper Midwest, six-rowed cultivars are the preferred type for malting. Unfortunately, most of the resistant germplasm identified to date is in a two-rowed genetic background [3]. The most resistant six-rowed barley identified is Chevron, a Swiss landrace with poor agronomic performance and malting quality.

To identify additional sources of resistance in a six-rowed genetic background, 4035 spring barley accessions from the USDA germplasm collection were evaluated for FHB in North Dakota in August 1999. Accessions exhibiting low FHB severity ($\leq$20%) in North Dakota were subsequently evaluated for FHB resistance in 2000 in the field in China and also in the greenhouse in North Dakota. Field and greenhouse inoculation methods were modified from Prom et al. [3] and Salas et al [1] respectively.

Less than 1% (12 of 4035) of the accessions exhibited $\leq$20% FHB infection in North Dakota [4]. These resistant accessions originated from China (CIho 4530, CIho 5809), Serbia (CIho 9114), Romania (CIho 15258), Georgia (CIho 4095), Mongolia (CIho 4339), and USA (CIho 2236, CIho 6610, CIho 6611, CIho 6613, CIho 7163, CIho 11526). Ten of these 12 accessions also expressed FHB resistance in China, with the exception of CIho 4339 and CIho 5809, which gave relatively high FHB severities (40%).

In the greenhouse test, CIho 9114, CIho 11526, CIho 6613, CIho 4530 and CIho 4095 consistently showed FHB resistance with a maximum of 10% FHB infection regardless of their growth stage at the time of inoculation (i.e. early or mid dough). These accessions also had low deoxynivalenol (DON) levels (below 2ppm) compared with the susceptible six-rowed check malting barley cultivar Stander, which had DON values of 5 to 13ppm. By pooling the average data for all 12 accessions, a highly significant positive correlation (0.801) was found between *F. graminearum* infection (%) and DON concentration (ppm). The accessions CIho 15258, CIho 6610 and CIho 6611 exhibited relatively low DON concentrations despite having relatively high levels of FHB infection (10%-15%). These lines may be potential sources of resistance to DON accumulation for barley.

In conclusion, we identified several six-rowed accessions that possess a level of FHB resistance that is equal to that of Chevron. These accessions should be useful for increasing the genetic diversity for FHB resistance in barley improvement programs.

Literature

[1] Salas, B., Steffenson, B.J., Casper, H.H., Tacke, B., Prom, L.K., Fetch, Jr., T.G., and Schwarz, P.B. 1999. *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis. **83**: 667-674.

[2] Steffenson, B.J. 1998. *Fusarium* head blight of barley: Epidemics, impact, and breeding for resistance. MBAA Tech. Q. **35**: 177-184.

[3] Prom, L.K., Steffenson, B.J., Salas, B., Fetch, Jr., T.G., and Casper, H.H. 1997. Barley accessions resistant to *Fusarium* head blight and the accumulation of deoxynivalenol. Cereal Res. Commun. **25**: 807-808.

[4] Scholz, U., Steffenson, B.J., Urrea, C., and Horsley, R. 1999. Evaluation of six-rowed spring barley accessions for resistance to *Fusarium* head blight. In: Proceedings, National Fusarium Head Blight Forum. December 5-7, 1999, Sioux Falls, SD, USA.

Palaveršić, B.; Drašner, E.; Parlov, D.; Vragolović, A.

Bc Institute for Breeding and Production of Field Crops, Marulicev Trg 5, 10000 Zagreb, Croatia

Study of maize inbreds and hybrids for resistance to *Fusarium graminearum* Schw. infection via the silk

Within the maize breeding program, 63 inbreds and 62 maize hybrids were tested, using for the first time in 1998 a new method of artificial ear infection with *Fusarium graminearum* into the silk channel [1]. In 7 trials significant differences in resistance degree among the tested lines and hybrids were obtained. Estimates obtained by a 1-7 scale varied from 1.0 to 5.1 for the lines and from 1.4 to 4.4. for the hybrids. Mean values of 27 single cross hybrids to *Fusarium* ear rot were compared to mean values of parents resistance. Correlation coefficient of r=0.72 was obtained, which indicates that only approximate hybrid resistance can be predicted from line resistance. Therefore, testing for resistance needs to be conducted with both lines and hybrids.

In 1999, the second year of investigation, 37 inbred lines were tested. The ratings ranged from 1.6 to 6.5 and the differences were statistically significant. Also, screening of 1121 maize hybrids, mostly testcrosses, was made for resistance to *F. graminearum* ear rot and ratings ranged from 1.0 to 6.2. Susceptible hybrid combinations were identified, as well as inbred lines which need to be improved for their degree of resistance to *F. graminearum* ear rot.

Literature

[1] Reid, L.M., Hamilton, R.I., Mathev, D.E. 1996. Screening Maize for Resistance to *Gibberella* Ear Rot. Technical Bulletin 1996-5E. Research Branch, Agriculture and Agri-Food Canada.

Ledencan, T.; Simic, D.

Agricultural Institute Osijek, Maize Department, Juzno predgradje 17, 31000 OSIJEK, Croatia

Response of maize inbreds and hybrids to stalk rot naturally and artificially infested with *Fusarium* spp.

Due to considerable yield and quality reduction, stalk rot in maize is a very important issue in a resistance breeding program. The most frequent pathogens of stalk rot in maize in the region of Eastern Croatia are fungi from the genus *Fusarium* (*F. graminearum, F. moniliforme, F. subglutinans*) [1]. The selection of resistant material naturally infected is not reliable because the disease severity depends on environmental factors and differs between years and locations. Some of the investigations [2] proved high correlation between maize inbreds and resistance to stalk rot on artificially and naturally infected, and some did not [3,4,5]. The objective of the study was to test the resistance of 36 maize inbreds and 72 hybrids for stalk rot and to determine relationship between natural and artificial infection with a mixture of spores of *F. graminearum, F. moniliforme* and *F. subglutinans*. The trials were conducted in 1998 and 1999 according to a lattice design with three replications. Inbreds and hybrids were set in separate and adjacent trials. Each entry was planted in two rows, the first row for the artificial infection and the second row for the natural infection. Artificial infection was performed by injecting 1 ml inoculum, of spore concentration cca. 60000/ml, at the first elongated internode. Degree of stalk rot was estimated using a 1 - 5 scale [2] for artificial infection and FAO 1 - 9 scale for natural infection. Inbreds differed in their resistance to stalk rot significantly: from 1.0 to 4.8 for artificial and from 1.0 to 7.8 for natural infection. Correlation coefficients between inbreds resistance natural and artificial infections were highly significant (r = 0.87 and r = 0.80, in 1998 and 1999, respectively). There were significant differences between hybrids in several years and types of infections. Generally, scores for hybrids were lower than these in inbreds: for artificial infection between 1.0 and 3.0 and for natural infection between 1.0 and 2.4. Natural

and artificial infections in hybrids were weaker associated than in inbreds, with highly significant correlation coefficients (r = 0.45 and r = 0.55, in 1998 and 1999, respectively). Correlation coefficients among two years for artificial infection were highly significant (r = 0.67 for inbreds and r = 0.55 for hybrids). Our results suggest that artificial infection with *Fusarium spp*. can provide a satisfactory method of evaluating maize genotypes, particularly inbred lines, to insure a satisfactory level of stalk rot presence.

Literature

[1] Jurkovic. D. 1981. Proucavanje biologije i ekologije vaznijih *Fusarium* vrsta kao uzrocnika trulezi korijena i stabla kukuruza na podrucju Baranje. Dissertation, University of Osijek, Croatia
[2] Hooker. 1956. Association of resistance to several seedling, root, stalk and ear diseases in corn. Phytopathology **64**: 379-384.
[3] Zuber, M.S., C.O. Grogan, M.E. Michaelson , C.W. Gherke, J.F. Monge. 1957. Studies of the interrelation of field stalk rotting fungi, and chemical composition of corn. Agronomy Journal **49**: 328-331.
[4] Koehler, B. 1960. Corn stalk rots in Illinois. University of Illinois, Bulletin 658.
[5] Palaversic, B. 1983. Ispitivanje otpornosti kukuruza na trulezu stabljike u uslovima prirodne i umjetne infekcije s *Gibberella zeae* (Schw.) Petch s posebnim osvrtom na lom stabljike. Master thesis, University of Zagreb, Croatia

Diamond, H.; Cooke, B. M.

Department of Environmental Resource Management, University College Dublin, Belfield, Dublin 4, Ireland;
Fax: +353–1–7067010, e-mail: Mike.Cooke@ucd.ie

Towards the development of a novel in vitro strategy for early screening of *Fusarium* ear blight resistance in adult winter wheat plants

Seven winter wheat cultivars were assessed for components of partial disease resistance as 28 day-old detached leaf segments in the laboratory using isolates of *Microdochium nivale* var. *nivale* and *M. nivale* var. *majus*. Results were compared with disease data obtained at anthesis using the same cultivars as whole plants and the same isolates under glasshouse conditions. Significant cultivar differences were observed using detached leaves,with cv. Avalon (a *Fusarium culmorum* ear susceptible cultivar) having the shortest leaf incubation period, greatest leaf lesion development and shortest leaf latent period compared to cv. Spark (a *Fusarium culmorum* ear resistant cultivar), which had the longest leaf incubation period, least leaf lesion development and longest leaf latent period. Using whole plants, cv. Avalon had the shortest ear incubation period and greatest ear disease severity, whilst cv. Spark had the longest incubation period and least ear disease severity. Overall, cultivars of intermediate *F. culmorum* ear resistance expressed intermediate responses to *M. nivale* isolates, using both detached leaves and whole plants. Significant correlations were found with ear disease severity and ear incubation period in whole plants and components of partial disease resistance in detached leaves, with significant correlations obtained between leaf incubation period and ear disease parameters using the *M. nivale* var. *nivale* isolate. In addition, leaf latent period and leaf lesion size showed significant correlations with whole plant reactions using *M. nivale* var. *nivale* and var. *majus* isolates. The in vitro screening of cultivars as detached leaves using *M. nivale* isolates may offer a real possibility of a rapid bioassay for the early screening of FEB resistance in wheat and other cereals.

Šíp, V.; Stuchlíková, E.

Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

Response of winter wheat cultivars to artificial infection with *Fusarium culmorum* in 1992-1999 field trials

Response to artificial infection of ears with *Fusarium culmorum* was evaluated in winter wheat cultivars (lines) registered (yield tested) in the Czech Republic and resistance sources of different origin at the location Prague – Ruzyně in 1992-1999. Artificial inoculation of spikes with isolate 7710 was performed according to the methods developed by Mesterházy [1] at mid-anthesis of middle spikelets. The disease development was determined usually in one-week intervals from appearing of symptoms, depending on the disease progress. For the determination of resistance/ tolerance level in a cultivar visual symptom scores (VSS), AUDPC (area under the disease progress curve) values and data on yield characters were used.

The development of *Fusarium* head blight (FHB) apparently differed in the years of testing. Most favourable for the spread of the disease were the conditions of the years 1992, [*]1995 and 1996 with sufficient rainfall after inoculation, while a slow (delayed) progress in the years 1993, 1994 and 1997 mainly affected reductions of grain number per spike after infection. On average, pathogen-caused reductions of grain number per spike, thousand grain weight and grain weight per ear were 28.6%, 50.7% and 64.9%, respectively. The analyses of variance revealed significant differences for cultivars and years. Relatively lower, but significant in all traits, were cultivar x year interaction mean squares. The ranking of genotypes in different years was similar for VSS and AUDPC. VSS and AUDPC values were in every year significantly correlated with thousand grain weight and grain weight per spike after infection. For these yield traits highly significant correlations ($r>0.9$) were found between measurements in infected variants and reductions obtained from comparison with uninfected, control variants, which indicates the possibility to evaluate in these types of experiments the data from infected variants and lower the costs of tests. Because grain number per spike was highly influenced by development of FHB infection in certain years, it is recommended to combine this trait with thousand grain weight and evaluate infected or reduced grain weight per spike.

It was found that resistance/tolerance to FHB infection in certain years was influenced by genotypic differences in flowering date. Considering the performance in this trait as a covariate might increase the precision of infection tests. In spite of great differences between years, plant height did not apparently influence cultivar resistance to FHB.

On the basis of VSS and AUDPC values the highest resistance level was found in the cultivars (lines) Bizel, Arina, Praag 8, Kooperatorka, SG-U 466 (Bona) and SG-U-513. These materials also showed a relatively high tolerance to the infection. The detection of resistance in the Czech advanced breeding lines SG-U 466 (Bona) and SG-U 513, coming from the cross Brock/Hana, is important from breeding aspects, because these materials possess many other positive breeding characters.

The detailed results are presented in the publications [2], [3] and [4].

Literature

[1] Mesterházy, A. 1978. Comparative analysis of artificial inoculation methods with *Fusarium* ssp. on winter wheat varieties. Phytopath. Z. **93**, 12-25.

[2] Stuchlíková, E., Šíp, V. 1996. Rezistence českých a slovenských odrůd pšenice ozimé k fuzarióze klasu. /Resistance of Czech and Slovak winter wheat varieties to Fusarium head blight/. Genet. a Šlecht., Praha **32**, 79-94.

[3] Šíp, V. and Stuchlíková, E. 1997. Evaluation of the response of winter wheat varieties to artificial infection with *Fusarium culmorum* in field conditions. Cer. Res. Com. **25**, 977-983.

[*] The research was supported by the Ministry of Agriculture of the Czech Republic (Project EP 7239).

[4] Šíp, V. and Stuchlíková, E. 2000. Hodnocení reakce vybraných odrůd pšenice ozimé na infekci *Fusarium culmorum* v polních podmínkách. / Evaluation of the response of selected winter wheat cultivars to artificial infection with *Fusarium culmorum* in field conditions/. Czech J. Genet. Plant Breed. **36**, in press.

Góral, T.[1]; Perkowski, J.[2]; Stachowiak, J.[2]; Arseniuk, E.[1]

[1] Department of Plant Pathology, Plant Breeding and Acclimatization Institute, Radzików, 05-870, Błonie, Poland
[2] Department of Chemistry, Agricultural University, 60-625 Poznań, Poland

Application of deoxynivalenol for *in vitro* selection of winter triticale cultivars 'Moniko' and 'Presto' for *Fusarium* head blight resistance

The aim of this study was to find if selection of winter triticale (X *Triticosecale* Wittmack) calli with DON could improve *Fusarium* resistance of regenerated plants. Reaction of selected somaclones was compared to reaction of unselected somaclones and parental cultivars to identify types with improved resistance and tolerance.

In field experiments resistance to *Fusarium* head blight (*F. culmorum*) of somaclones and two parental cultivars (Moniko, Presto) of winter triticale was evaluated. Grain of somaclones and parental cultivars were analysed for the accumulation of trichotecene mycotoxins (DON and derivatives). A wide range of variability was found, in all parameters among selected somaclones. All Moniko-derived selected somaclones were more resistant than the donor cultivar. Somaclones with significantly less reduction of yield components and lower mycotoxin content of the grain were found. However, most somaclones accumulated more DON then cultivar Moniko. The Presto-derived selected somaclone was slightly more resistant than the donor cultivar, through accumulated more DON.

In laboratory experiments tolerance of somaclones seedlings and parental cvs to DON was evaluated. Somaclones more tolerant to DON than the parental cultivar were found. No correlation with field resistance to *Fusarium* head blight was found.

Presented results showed that *in vitro* selection with DON could improve type 1 and/or type 2 [1] resistance of studied cultivars to *Fusarium* head blight caused by DON-producing species. However, such selection did not improve type 5 resistance (decomposition of DON in kernels) and to some extent lead to increase in kernel accumulation of DON.

Literature

[1] Mesterhazy, A. 1995. Types and components of resistance to *Fusarium* head blight of wheat. Plant Breeding **114**, 377-386.

Plant Protection

Haigh, I; Hare, M. C.; Jenkinson, P.

Harper Adams University College, Edgmond, Newport, Shropshire, TF10 8NB, UK

Seed treatment efficacy under different field conditions

Seed-borne *Microdochium nivale* is frequently the major cause of seedling blight on UK winter wheat [1]. Seed treatments are used to control this commercially important pathogen. Controlled environment investigations have demonstrated that cold dry soils delay seedling emergence and increase disease severity [2]. However, this has not been investigated under field conditions, where soil moisture is often very high at drilling.

The aims of this investigation were to determine 1); the environmental conditions under which seedling blight is most severe and 2); the performance of seed treatments under different environmental conditions.

Winter wheat cv. Riband with 56 % *Microdochium nivale* infection was drilled at 400 seeds/m^2 on three different dates (18/10/1999, 23/11/1999 and 21/01/2000), with and without seed treatments at Harper-Adams. Soil moisture and temperature were measured and rate of emergence, stand establishment and disease severity were assessed during seedling growth.

Stand establishment was reduced with delayed drilling time, which appeared to be influenced most by soil moisture content. Rate of seedling emergence did not correlate with stand establishment numbers and appeared to be affected most by soil temperature.

Seed treatments tended to delay seedling emergence in comparison to untreated seeds. Seed treatments increased stand establishment and significantly reduced disease symptoms compared to untreated seed.

In conclusion, severity of *Microdochium nivale* and its control through seed treatments was dependent upon field conditions during and immediately after drilling.

Literature

[1] Colhoun, J. 1970. Epidemiology of seed-borne Fusarium diseases of cereals. Ann. Acad. Sci. Fenn. A. IV. Biologica. **168**, 31-36.

[2] Hare, M.C., Parry, D.W., Noon, R.A. 1995. Towards the prediction of Fusarium seedling blight of wheat. In: A vital role for fungicides in cereal production. Eds. Hewitt, H.G., Tyson, D., Hollomon, D.W., Smith, J.M., Davies, W.P., Dixon, K.R. pp. 211-220. Bios Scientific Publishers.

Ellner, F. M.; Schröer, R.

Federal Biological Research Centre for Agriculture and Forestry, Institute of Ecological Chemistry, 14195 Berlin, Königin-Luise Str. 19, Germany

Effects of fungicides containing strobilurin on mycotoxin production in wheat

Strobilurins, a novel group of fungicides, are derivatived of the naturally occurring strobilurin A that was first isolated from *Strobilurus tenacellus*. Generally, these compounds have an enol ether ester or an oxime ether ester toxophore and inhibit the mitochondrial respiration chain at the cytochrome b and c_1 site [1]. In addition, strobilurins cause physiological changes in plants: They shift the CO_2-compensation point in favor of CO_2 assimilation and mimic auxin effects, while actually being effective by reducing

the ethylene production. This may cause increased grain yield, dry matter, protein and the so-called greening effect with higher chlorophyll levels and delayed senescence [2, 3].

Although strobilurins have no or little antifungal effect on *Fusarium* spp. some field trails have occured to show that treated winter wheat plots have higher deoxynivalenol (DON) contaminations than untreated ones [4]. The question arose if strobilurin containing fungicides registered for use in cereal crops increase infection with pathogens causing Fusarium head blight, for instance *F. graminearum* and *F. culmorum*. Assumed reasons have been the physiological effects on the plants and the reduction of competitors.

The aim of the investigations presented was to find the effects of the two fungicides 'Juwel TOP' and 'Amistar' on the flowering process of wheat on the one hand and on the degree of infection and mycotoxin production after inoculation with *F. graminearum* and *F. culmorum* on the other. The performed greenhouse experiments included two varieties of summer wheat with different susceptibility towards fusaria and various application regimes: early application (BBCH-Code: 30/31), late application (51-59), and a combination of both. The inoculation with the pathogens was done by laying out rolled oats infected with *F. graminearum* and *F. culmorum*. The dispersion of conidia or ascospores was supported by ventilation and sprinkling.

The results emphasize that the strobilurin treatment may influence all parameters analysed.

With regard to the flowering process we discovered a shift to earlier blooming in several experimental plots, whereas no differences could be found concerning the duration and the progress of flowering. Ergosterol content as a parameter of fungal biomass indicated an increased degree of infection in some treatments, where DON levels rose up as well. Finally, a good correlation was found between ergosterol content and DON concentration within each variety, although revealing differences between them.

Literature

[1] Ammermann, E., Lorenz, G., Schelberger, K., Wenderoth, B., Sauter,H. and Rentzea, C. 1992, BAS 490F-A broad-spectrum fungicide with a new mode of action. Brighton Crop Prot. Conf. Pest Dis. 403-410.
[2] Grossman, K. and Retzlaff, G. 1997 Bioregulatory effects of the fungicidal strobilurin kresoxim-methyl in wheat. Pestic. Sci. **50**: 11-20.
[3] Gold, R.E. and Leinhos, G.M. 1995 Fungicidal effect of BAS 490F on the development and fine structure of plant pathogenic fungi. Pestic. Sci. **43**: 250-253.
[4] Nicholson, M. 1999 Recent developments in the study of Fusarium ear blight in the UK. Mycotoxines, IRTAC, Cycle de Formation 12.

Hope, R. J.; Colleate, A.; Baxter, E. S.; Magan, N.

Applied Mycology Group, Biotechnology Centre, Cranfield Univeristy, Cranfield Bedford MK43 0AL, UK

Interactions between *Fusarium culmorum,* other contaminant fungi, environmental stress and fungicides, affect growth and deoxynivalenol production in wheat grain

The objectives of this study were to determine the effect of (a) interspecific interactions between *F.culmorum* from different European countries and other grain fungi, (b) water availability and temperature and (c) cereal fungicide concentrations (azostrobin, propiconazole, epiconazole) on interactions, growth and deoxynivalenol (DON) production. Studies were carried out in vitro on milled wheat media, and on irradiated wheat grain with retained germinative capacity. There were both antagonistic and competitive interactions between *F.culmorum* and other grain fungi including *Alternaria alternata*, *Cladosporium herbarum*, *Aureobasidium pullulans* and *Penicillium verrucosum*. There were some intra and inter-isolate differences in growth in *F.culmorum* isolates from UK, Norway, Sweden and Italy. Regardless of water availability or temperature azostrobin was relatively ineffective against all isolates. Fungicides applied to wheat grain were less effective at the same concentrations when compared to those in vitro. DON production by the isolates was significantly stimulated by the fungicides, especially under water stress conditions on wheat grain, regardless of country of origin. The implications of competitive

interactions between *F.culmorum*, and other species and DON production under current fungicide applications will be discussed.

Pirgozliev, S.; Jenkinson, P.; Edwards, S. G.; Hare, M. C.

Crop and Environment Research Center, Harper Adams University College, Newport, Sropshire, TF10 8NB, UK

Effect of metconazole and azoxystrobin on the development of *Fusarium* ear blight and the accumulation of deoxynivalenol (DON) in winter wheat

Fusarium ear blight is an economically important disease in both temperate and semi-arid wheat growing areas in the world. Apart from causing significant reduction in grain yield, several of the casual organisms responsible for the disease, including *Fusarium culmorum* and *F. graminearum* can produce trichothecene mycotoxins. Harvested grain, contaminated with trichothecene mycotoxins, can cause both acute and chronic side effects in livestock and humans.

Chemical control of *Fusarium* ear blight has proved to be inconsistent under field condition. Results from *in vitro* studies have also indicated that the presence of certain fungicides can result in elevated concentrations of trichotecenes.

In this study, glass house experimente were carried out to investigate the effect of a range of concentrations of the fungicides metconazole and azoxystrobin on the infection of wheat ears (cv. Cadenza) by *F. culmorum* and *F. graminearum*. Concentrations of the trichothecene deoxynivalenol (DON) was also determined in harvested grain. Results showed that metconazole effectively reduced infection of wheat ears by both pathogens, and as dose rate was increased, severity of disease decreased. Azoxystrobin proved to be ineffective against the development of the ear blight irrespective of dose rate used.

Comparison of disease severity data and DON concentration revealed increasing disease severity resulted in a concurrent increase in mycotoxin contamination of harvested grain.

Kislykh, T.[1)]; Voloshchuk, S.[2)]

[1)] Plant Protection Institute, Kiev, Ukraine
[2)] Wheat Institute, Myronovka, Ukraine, e-mail: kolsi@mail.ru

Folicur BT influence on monosporous isolates of *Fusarium* spp. in vitro

Folicur 25% c.e. and Folicur BT 22,5% c.e. still are the most effective fungicides against *Fusarium* scab. The objectives of this work were: to study the reaction of different *Fusarium* species on the different concentration of Folicur BT and the possibilities of the appearance of new resistant strains.

Toxicogenic possibilities of Folicur BT evaluated in such ranges of concentrations: from 0,0001 till 0,1% on the monosporous isolates of *F. avenaceum* (14), *F. graminearum* (11) and *F. sporotrichiella* var. *poae* (8). Isolates have been collected from different zones of the Ukraine forest-steppe. The size of colonies was measured on the 3rd, 7th, 12th and 19th day.

It has been found that isolates of *F. sporotrichiella* var. *poae* are the most sensitive to the fungicide and those of *F. graminearum* the least sensitive. During the first estimation we registered the braking of the colonies growth from 85,9% to 100% in the concentration diapasons from 0,0001 to 0,1% a.i.. The number of *F. avenaceum* isolates, which grew on the media with 0,1% Folicur BT increased towards the end of the experiment. The inhibition of the colony growth was reduced to 7,9-98,7%. Four of the 11 *F.*

graminearum isolates demonstrated ability to grow on the media with 0,1% Folicur BT. The inhibition of the colony growth was about 3,0-99,2%. On average, the inhibition of *Fusarium* spp. colony growth was from 11,0 to 99,3%, when DC_{50} and DC_{95} were $2{,}8 \cdot 10^{-3}$ and $7{,}5 \cdot 10^{-2}$ respectively. The inhibition of the colony growth became smaller with the time period. Some isolates renewed growth in the second week after the experiment had started at the concentration of 0,1%.

Under field condition the fungicide was applied at 1,0 l/ha, the active ingrediant concentration was 0,075% (using 300 l/ha water as the standard amount). At this concentration under laboratory conditions according to the probit-analysis results, the inhibition of the colony growth on the 3rd day was 99,5-99,7%, on the 7th day – 99,5-99,7%, on the 12th day – 99,4-99,6%, on the 19th day – 86,8 – 99,6 % depending on the *Fusarium* species.

Therefore, it may be a real threat that *Fusarium* species will acquire resistance to high concentrations of Folicur BT in the nearest future.

Greenfield, J. E; Rossall, S.

School of Biological Sciences, Division of Plant Sciences, University of Nottingham, Sutton Bonington, Leicestershire LE12 5RD, UK

The effect of fungicides on productivity and toxin content of wheat inoculated with *Fusarium*

The problems caused by *Fusarium* Ear Blight are wide ranging, most notably yield loss and the potential for toxin production. In the UK *Microdochium nivale* is the species most often associated with the disease [1]. However *Fusarium poae, Fusarium avenaceum, Fusarium culmorum* and *Fusarium graminearum* are also found. The latter two species will be found particularly in areas where summer temperatures are warmer than in the rest of the UK, or when summer temperatures are higher than usual. A range of fungicides has been used over the years in an effort to combat *Fusarium* infection, but no product has yet been found to be widely efficacious. It has also been found in some studies [2, 3], that certain fungicides have increased toxin production. Whilst toxin levels found in UK grain tends to be low [1], the potential problem needs to be addressed given an increasing likelihood that maximum toxin levels in grain will be set by the EU.

This work aims to assess the effect of a range of fungicides on the visual disease severity, productivity and toxin content of wheat artificially inoculated with single and combined applications of *Fusarium*.

Wheat plants (cv. Cadenza) were grown, 10 per 30cm pot, in John Innes No. 2 compost. They were given a single spray of quinoxyfen to combat powdery mildew at growth stage 12, and fed weekly with a liquid fertiliser, Sangral 1:1:1, from 3 weeks post-germination.

At growth stages 39, 59 [4] or at both, the plants received treatment with one of 5 fungicides or with sterile distilled water. Fungicides used were tebuconazole, azoxystrobin, carbendazim, plus two new formulations, Compound A and Compound B, and were applied at field-rates. This was followed by inoculation with 10mls per spike of a suspension of *Fusarium poae, Fusarium culmorum, Fusarium culmorum* + *Fusarium poae,* all at 1×10^5 conidia ml^{-1}, or with sterile distilled water, at early anthesis (GS60) or mid anthesis (GS65).

Humidity was raised by covering the plants with plastic bags for 4 days. Spikes were then assessed for visual disease [5] on a weekly basis for 8 weeks. After harvesting, spikes were hand threshed and the chaff and seed separated. Seed and chaff weights were recorded to give levels of productivity. Toxin analysis was carried out using competitive ELISA kits, the Veratox® Quantitative T-2 and Vomitoxin Test Kits, used as per the manufacturer's instructions. Harvested seed was also plated on to antibiotic-

amended PDA following surface sterilisation and incubated at 20°C for 7 days to determine the presence of *Fusarium* within the harvested seed.

Results will be available and discussed on the poster.

Literature
[1] Turner JE, Jennings P & Nicholson P (1998*) Investigation of Fusarium infection and mycotoxin levels in harvested wheat grain (1998).* HGCA Project Report No. 207, HGCA, London
[2] Placinta CM, MacDonald AMC, D'Mello JPF & Harling R (1996) The influence of carbendazim on mycotoxin production in *Fusarium sporotrichioides*. In: Proceedings of the Brighton Crop Protection Conference, Pest & Diseases-1996, 415-416
[3] D'Mello JPF, MacDonald AMC & Placinta CM (1996) Production and control of mycotoxins from *Fusarium* species pathogenic on cereals. In: Proceedings of the Brighton Crop Protection Conference, Pest & Diseases-1996, 517-522
[4] Tottman DR & Broad H (1987) Decimal code for the growth stages of cereals. Annals of Appled Biology, **110**, 683-687
[5] Minnesota Assoc. Wheat Growers. www.smallgrains.org/Images/ScabIndex.gif

Elen, O. N.[1]; Abrahamsen, U.[2]; Øveri, A.[2]; Razzaghian, M. J.[1]

[1] The Norwegian Crop Research Institute, Plant Protection Centre, Høgskolevn 7, N-1432 Ås, Norway
[2] The Norwegian Crop Research Institute , Apelsvoll Research Centre, N-2858 Kapp, Norway

Effect of agricultural measures on the occurrence of *Fusarium* spp. in cereals in Norway

Mycotoxin contamination of grain is a serious problem in many countries. In Norway and other Northern European countries, grain is mainly contaminated with mycotoxins produced by fungal pathogens of the genus *Fusarium*. A field survey of barley, oats and wheat indicated that *Fusarium avenaceum, F. poae, F. culmorum* and *F. graminearum* are the most common species isolated from Norwegian cereals [1]. From 1988 to 1994, 66% of Norwegian wheat samples tested were contaminated with deoxynivalenol (DON) [2]. The prevalence of grain infected with *Fusarium* spp. is highly dependent on the environment, specially climatical factors. Wet conditions often promote the establishment of the fungus [3]. Agricultural measures may also have additional effects on *Fusarium* contamination in grain.

In 1996-1998 several field experiments were conducted to investigate the effect of either straw length, nitrogen fertilization or fungicides on the occurrence of *Fusarium* in wheat, barley and oats. To combine these factors, a factorial experiment with barley was carried out in 1999. To create variation in straw length, the plants were treated with growth regulators. The effect of the different treatments on *Fusarium* was highly affected by lodging, which seemed to be one of the major factors promoting a high level of infected seed. Lodging was positively correlated with both increasing N fertilization and increasing straw length, thus confounding the true effect of these two factors on *Fusarium*. Treatment with growth regulators shortened the straw with approximately 30 cm in oats and 15 cm in wheat, and prevented lodging. In most cases there was no correlation between straw length and the level of *Fusarium*, probably because of the strong effect of logding on *Fusarium*. In one oat trial without lodging, however, there was a negative correlation between the two factors; shortening the straw length by 1 cm increased the *Fusarium* level by 1%. N treatment was supplied at three levels; no N supply, 120 and 165 kg/ha, and the corresponding infection levels in the seeds were 54, 58 and 65%, respectively. Plots without N had thinner stands and shorter straw than other plots, which may partly explain the relatively high *Fusarium* level compared to plots with N supply. The effect of shorter straws and thinner stands will facilitate the splashing of spores from the soil surface to the spikes and thus give more infection. On the other hand these conditions will prevent lodging, which will give an effect in the opposite direction. In the fungicide experiments, 25% of the seeds in the control were infected with *Fusarium.* After the application of Tilt Top, Amistar and Stereo, 31%, 33% and 37% of the seeds were infected, respectively. The latter two were significantly more contaminated than the control. These results were supported in the factorial experiment. However, no cumulative effect of the individual factors could be found, probably due to the low contamination level

of 11% infected seeds. Combined effects of different agricultural measures on *Fusarium* will be investigated in further experiments.

Literature
[1] Kosiak, B., Torp, M. 1995. The occurrence of *Fusarium spp*. in Norwegian grain. In: Abstracts 7th Nordic Mycotoxin Meeting, P3.
[2] Langseth, W., Elen, O. 1996. Differences between barley, oats and wheat in the occurrence of deoxynivalenol and other trichothecenes in Norwegian grain. J. Phytopathology **144**, 113-118.
[3] Elen, O., W. Langseth, W. Liu, G. Haug , H. Skinnes, M. Gullord and L. Sundheim. 1997. The content of deoxynivalenol and occurrence of *Fusarium* spp. in cereals from field trials in Norway. Fifth European *Fusarium* Seminar, Szeged, Hungary, 1997. Cereal Research Communications **25** No. 3/2 1997, 585-586.

Vagelas, I. K.[1]; Andreoglou, F. I.[1]; Peters, J. C.[1]; Gravanis, F. T.[2]; Gowen, S. R.[1]

[1] Department of Agriculture, The University of Reading, Earley Gate, PO Box 236, Reading, RG6 6AT, Berkshire, UK.
[2] Department of Plant Production, Technological & Educational Institution of Larissa, GR-411 10 Larissa Greece.

Tests of the biological effect of the nematode symbiotic bacterium *Flavimonas oryzihabitans* (=*Pseudomonas oryzihabitans*) Holmes *et al.* on *Fusarium oxysporum* f. sp. *lycopersici*

Soil fumigants (e.g. methyl bromide) are used for control of *Fusarium oxysporum* f. sp. *lycopersici* (Fol) on tomato. However, with the increasing environmental awareness, biological methods, developed for an alternative environmentally friendly control method. *Flavimonas oryzihabitans* (=*Pseudomonas oryzihabitans*) Holmes et al., which is a bacterial symbiont of the entomopathogenic nematode *Steinernema abbasi* Elawad et al., was investigated as a potential biological control agent, acting as an antagonist of *Fol.* Agar plates were prepared with 20 g of nuntrient agar (NA) per l of deionised water (w/v). Three concentrations (10^7, 10^5 and 10^3 cells/ml) of a bacterial suspension of *P. oryzihabitans* were spread uniformly on the agar plate surface. A 5 mm diameter agar plug of *Fol* derived from a 7 day-old culture was placed in the centre of each plate. The mycelial growth of *Fol* was significantly inhibited (85%; $p<0.001$) by *P. oryzihabitans* on NA medium in all bacterial concentrations. Also, vacuolisation and disintegration of *Fol* hyphae were observed. Assays for the ability of the bacteria to suppress *Fol* were conducted in plastic trays. Inocula of *Fol* (two isolates) were grown in 5% Cornmeal-Sand Medium (Tuite, 1969). Four week-old pathogen inocula were added to a sandy-loam (1:3, w/w) soil mixture. *P. oryzihabitans* bacteria cells from *S. abbasi*, were isolated from the oozing haemolymph of infected *Galleria mellonella* larvae and were plated onto NA. After incubation at 28 ^oC in the dark for 24h, they were placed in a shaking incubator (150 rpm, 28 ^oC) for 2 days. Five concentrations of the bacteria (10^6, 10^5, 10^4, 10^3, 10^2 cells/ml) were prepared and these were tested for their ability to control *Fol*, by applying 100ml from each concentration onto the soil surface. The bacteria concentration of 10^4 cells/ml proved most promising in prevention of the infection of *Fol.*

Toxicology

Sýkorová, S.; Papoušková, L.; Stuchlíková, E.; Šíp, V.

Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

The influence of *Fusarium culmorum* infection on mycotoxin content in grain of selected wheat varieties *

Bread wheat cultivars are mostly susceptible to *Fusarium* head blight (FHB), which indicates the necessity to breed for resistance. The only effective way to reduce or even eliminate losses due to infection and contamination of grain with harmful toxins is to obtain the combined resistance to FHB and accumulation of toxins in grain. Genetic variation for resistance to head blight was found to be very large and valuable sources of resistance have been detected among spring and winter cereal cultivars in various research programs. Differences in resistance to FHB were also determined in modern Czech and Slovak winter wheat cultivars [1, 2]

Head blight of 10 selected winter wheat varieties [see 1, 2] (ARINA, BONA, SPARTA, SAMANTA,SIRIA, ŠÁRKA, HANA ,VLADA ,DANUBIA, BRUTA) was evaluated after inoculation [3] with 2 isolates (A – 7710, B –Stupice) of *Fusarium culmorum* at 3 experimental sites - Ruzyne, Uhretice and Stupice. The disease development was determined according to standard methods [1, 2] . The percent reduction of thousand grain weight (TGW), visual symptom score (VSS) and area under the disease progress curve (AUDPC) were determined. Obtained seed was used for the assessment of effect of various genotype and environmental conditions on the accumulation of mycotoxins in grain. The content of deoxynivalenol (DON) was determined by ELISA on RIDASCREEN FAST DON kits from R- Biopharm GmbH, Darmstadt, Germany according to the manufacturer´s instructions.

It was found that isolate A manifested itself as less virulent (especially in Ruzyne) than isolate B . Wheat cultivars ARINA, BONA and SPARTA were highly resistant to *Fusarium* head blight at all experimental sites, while HANA, DANUBIA, VLADA , SAMANTA and SIRIA were susceptible. The resistant cultivars showed both a lower TGW reduction and AUDPC values and also lower content of DON. The DON content ranged from 0,1 to 125 ppm. The highest DON content had susceptible cv. SIRIA, the lowest content the resistant cv. ARINA. It is evident that resistant wheat cultivars tend to lower mycotoxin accumulation. The determination of DON content was compatible with the determination by gas chromatography when using the new RIDASCREEN FAST DON kits with lower sensitivity (in ppm) than original kits (in ppt). Due to differences in the resistance levels obtained at the test sites, further examination is needed to specify the relations between head blight infetion, TGW reduction and mycotoxin content. Several methods of mycotoxin determination will be studied and compared.

Literature

[1] Stuchlíková, E., Šíp, V. 1996. Rezistence českých a slovenských odrůd pšenice ozimé k fuzarióze klasu. /Resistance of Czech and Slovak winter wheat varieties to Fusarium head blight/. Genet. a Šlecht., Praha **32**, 79-94.

[2] Šíp, V. and Stuchlíková, E. 1997. Evaluation of the response of winter wheat varieties to artificial infection with *Fusarium culmorum* in field conditions. Cer. Res. Com. **25**, 977-983.

[3] Mesterházy, A. 1978. Comparative analysis of artificial inoculation methods with *Fusarium* spp. on winter wheat varieties. Phytopath. Z. **93**, 12-25.

* This project was supported by GA CR No. 521/98/1019

Tunali, B.; Aktas, H.; Araz, A.

Plant Protection Central Research Institute, 06170 Yenimahalle, Ankara, Turkey

Determination of microflora in infested seed lots with head blight and detection of deoxynivalenol production by ELISA

A very severe head blight or scab of wheat (*Triticum aestivum* L.) observed in Western Anatolia, Turkey, in 1997 and 1998. It occurred during wintertime when rainfall and warm temperatures prevailed at anthesis stage. Wheat samples were sent in by T.M.O (Turkish grain board) and farmers for determination of the disease. The seeds were plated on PDA and the recovered microflora was identified as *Fusarium graminearum, F. poae, F. culmorum, F. heterosporium* and *F. sporotrichoides*. Besides *Fusarium* species *Alternaria alternata* was found at a very high level (94.7%), but the germination of kernels was not affected by the fungus. However the germination rate was significantly reduced in seed lots infested with *Fusarium*. In addition all seed samples were examined for deoxynivalenol (DON) by ELISA. The results revealed that the concentration was below 2ppm/g. This level is accepted as a tolerance limit for human consumption in Canada. Therefore DON levels reported in this work may be considered unharmful for adults.

Henriksen, B.[1]; Langseth, W.[2] ; Sundheim, L.[1]

[1] The Norwegian Crop Research Institute, Plant Protection Centre, N-1432, Ås, Norway
[2] National Veterinary Institute, Department of Chemistry, N-0033 Oslo, Norway

Factors affecting *Fusarium* infection and mycotoxin content in Norwegian cereal grains

Fusarium infection levels, mycotoxin content and prevalence of different *Fusarium* species were studied in cereal grains from field experiments at five Norwegian localities from 1994 to 1997. The effect of tillage, fungicide application, location and climatic parameters were investigated.

The most frequently isolated *Fusarium* species in the study, were *Fusarium avenaceum, F. tricinctum, F. poae and F. culmorum*. *F. avenaceum* dominated the grain infection in the fields under humid climatic conditions (silty soil type combined with frequent rainfall). *F. tricinctum* and *F. poae* were detected more often under relatively dry and warm conditions.

The total *Fusarium* infection level increased significantly after reduced tillage in two of the five fields during the investigation. When all years were analysed together for each of the two fields, autumn plowing gave significantly lower *Fusarium* infection than no-tilled, harrowed, or spring plowed plots.

The detected level of the mycotoxin deoxynivalenol (DON) was generally low, with no differences between tillage treatments. Also, the DON-producing species, *F. culmorum* and *F. graminearum* were detected in low frequencies. HT-2 toxin was detected at various levels in four of the five fields in 1997, the only year in which this toxin was analyzed. The level of HT-2 toxin was consequently higher in reduced tillage plots compared to autumn plowed plots. T-2 toxin was detected in the fields with the highest levels of HT-2 toxin.

Strong and significant correlations were found for total *Fusarium* infection level versus days with simultaneous occurrence more than 2 mm rainfall and mean temperatures above 12°C, in a two week period covering the plants flowering stage. Also, during the late maturing stages, rainfall correlated significantly with *Fusarium* infection level in the grains. Similar correlations were detected when infection levels of

F. avenaceum were studied. *F. poae* infection levels correlated negatively with rainfall in the late maturing stages.

In the present study, application of different fungicides in experimental plots significantly increased the infection level of *Fusarium* in the grain compared to untreated plots.

Papoušková, L.[1]; Sýkorová, S.[1]; Hubík, K.[2]

[1] Research Institute of Crop Production, Prague – Ruzyne, Czech Republic
[2] Agriculture Research Institute, Ltd., Kromeriz, Czech Republic

Survey of mycotoxin content in wheat samples obtained from different locations in the Czech Republic*

One of the most widespread genera of moulds infecting cereals especially winter wheat is *Fusarium* that occurs in both vegetative and generative states. High sensitivity to infection at anthesis stage causes considerable problems and economic loss during the harvest of matured grain. Infected grain is of a "shrivelled small" shape with a lower weight and changed colour.

At the same time, there are problems with grain quality. Dough prepared from the flour from wheat grain damaged by *Fusarium* infection exhibits low elasticity and higher extensibility that result in a lower loaf volume [1, 2, 3]. It is evident that *Fusarium* infection in small grain cereals causes both lowering of technological quality and through production of secondary metabolites (mycotoxins) remarkable veterinary and health risks followed by high economic losses, which is immense in the case of human health.

The presented study contains the information about levels of DON in winter wheat samples collected at the harvest in 1999 from several regions of the Czech Republic.

Wheat for human consumption (46 samples) was collected from several parts of the country. The main quality characters (volume weight, SDS-sedimentation value, Falling number, protein content, fraction under 2,5 and 2,2 mm) were determined .The proportion of shrivelled small and discoloured kernels was assessed. The DON content of all samples was tested by ELISA with commercially test kits and standards (RIDASCREEN DON, R-Biopharm, GmbH, Darmstadt, Gemany). DON extraction and tests were performed according to manufacturer´s instructions. All standard and sample solutions were analysed in duplicate wells.

DON was found in all 46 tested samples. The hygienic limit for cereals in the Czech Republic is controlled by Law on Foods No. 110/1997 and associated instruction 298/1997 that gives the highest acceptable concentration of DON in grain and flour as follows: - grain: 2 $mg.kg^{-1}$ = 2 ppm; - flour 1 $mg.kg^{-1}$ = 1 ppm . The content of DON exeeded in 15 samples the acceptable level (ranging from 3,1 to 7,0 ppm), remaining samples contained on average 0,8 ppm of DON.

The differences between the other quality characters were not found significant in samples of the same variety from different localities and they can not be attributed to different DON contents. Nevertheless the samples with an increased DON content had also a relatively high proportion of both „shrivelled small" and discoloured grain fractions (up to 13,1 %).

Literature

[1] Clarke, J.M., Clear ,R.M., Dexter, J.E., Marchylo, B.A. 1997. Effect of *Fusarium* head blight on semolina milling and pasta-making of durum wheat . Cereal chem. **74**(5): 519-525.
[2] Clear, R.M., Dexter ,J.E., Preston, K.R. 1996. *Fusarium* head blight: effect on the milling and baking of some Canadian wheats. Cereal chem. **73**(6): 695-701.
[3] Clear, R.M., Dexter ,J.E., Marchylo, B.A., Nightingale, M.J., Preston, K.R. 1999.*Fusarium* head blight: effect of fungal proteases on wheat storage proteins – Cereal chem. **76**(1): 150-158.

* This project is supported by COST action 835.

Obst, A.; Lepschy, J.; Beck, R.; Bauer, G.

Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, München und Freising, Germany

Risk factors for the head infection of wheat and the production of deoxynivalenol by *Fusarium graminearum*, derived from the *Fusarium* monitoring in Bavaria

In the year 1989 a *Fusarium* monitoring for small grains was established in Bavaria. For wheat every year 200-400 samples are analysed for their content of *Fusarium* propagules and the main toxin, i.e. deoxynivalenol (DON); in the eleven years 3478 wheat samples have been investigated in this way. In a questionnaire the farmers describe the agronomic conditions for their wheat production. Additionally the weather conditions during the critical growth stages of wheat can be obtained from nearly 120 agrarian meteorological stations in Bavaria.

It is the aim of this *Fusarium* monitoring:

- to get a survey on the health situation of the grain harvested every year
- to investigate the relationships between agronomical factors and the DON content and
- to develop a forecast of the toxin risk for wheat crops.

The following contribution refers to the DON content only, the major *Fusarium* species in Bavaria being *F. graminearum.*

There are big differences in the DON content of wheat from year to year, between different regions within one year, and even from field to field. These differences at present can be related to the following 5 risk factors:

- maize as the preceding crop,
- non-soil-turning tillage practice after prededing maize crop,
- medium or highly susceptible wheat cultivars,
- application of the strobilurin fungicides azoxystrobin or kresoxim-methyl + epoxiconazol (+ fenpropimorph),

weather conditions supporting *Fusarium* head infections after a monocyclic or bicyclic spread of the pathogen.

Having analysed the weather conditions for *Fusarium* head infection of more than 2000 wheat crops during the years 1993-98, in 1999 for the first time our preliminary weather model did not explain the differences in the DON content of the grain. We know that an ascospore infection requires warm weather spells with limited precipitation. In 1999 after the settlement of the ascospores on the upper leave blades before heading of the wheat cool and moist conditions obviously have induced the development of a second (conidio-)spore generation, which could infect the wheat heads even at low temperatures.

Each risk factor has nearly the same weight; it increases the toxin risk by the factor 2; solely the tillage practice non-turning the soil after a maize crop raises the DON risk by the factor 4-5. When several risk factors are accumulated in a wheat crop, their effects do not add to each other, but they do multiply. This means that an accumulation of 4 or 5 risk factors results in an exponential growth of the toxin concentration in the wheat grain.

Surely there are more *Fusarium* risk factors, which up to now have not been included in these investigations.

Chelkowski, J.[1]; Tomczak, M.[2]; Basinski, T.[2]; Perkowski, J.[2]; Kostecki, M.[2]; Stachowiak, J.[2]; Kaptur, P.[2]; Goliński, P. K.[2]

[1] Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland
[2] Department of Chemistry, August Cieszkowski Agricultural University, Poland

Contamination of cereals in Poland with *Fusarium* toxins

During 1996 – 1999 the *Fusarium* mycoflora, as well as the contamination level of *Fusarium* toxins in cereals from different regions of Poland was investigated.

In 1998 heads with scab symptoms were below 1 %, however, in some parts of the country the incidence was higher –near Lublin (South–East) (4 %) and Żuławy (North) (10 – 20 %). Microscopic analysis revealed that distribution of different fungi changed during the period of investigation. For example in Wielkopolska (central west) heads of wheat were contaminated with *F. graminearum* (0 – 23 %); *F. culmorum* (4 – 25 %); *F. avenaceum* (25 – 38 %) and *M. nivale* (16 – 55 %) while in Żuławy during 1998 and 1999 the same species were found, respectively, at a level of 20 – 26 %; 10 – 16 %; 29 - 31 % and 33 - 35 %.

In the western part of Poland secondary toxic metabolites produced by *Fusarium* were analyzed during 1996 and 1997, respectively, with the following average concentrations (mg/kg) DON 0,06 and 1,53; NIV 0,61 and 0,32; MON 0.11 and 0.28. In 1997 higher contamination levels of kernels with DON and MON were probably due to high humidity after a flood we experienced in the region of screening analyses. Much higher concentrations of the analyzed toxic secondary metabolites were found in kernels with scab symptoms than in healthy kernels. In contrast, in Żuławy, we did not observe such phenomenon – the higher percentage of *Fusarium* damaged kernels (12 – 25) was not correlated with mycotoxin accumulation. Samples of oat kernels collected at different locations of Poland, were contaminated with group A (DAS, T-2 toxin, HT-2 toxin) and group B (DON, NIV, AcDON) trichothecenes with the following concentration range (mg/kg): DON 0.01; NIV 0.05; Ac-DON 0,01, DAS 0.02; T-2 toxin 0.06 and HT-2 toxin 0.02.

Nedelnik, J.

Research Institute for Fodder Crops, Ltd., 664 41 Troubsko, Czech Republic

Spectrum of *Fusarium* species and contamination with mycotoxins of corn in the Czech Republic

Several *Fusarium* species are important pathogens of corn, causing severe crop yield reduction. In addition, some isolates are able to produce mycotoxins which are responsible for some diseases of farm animals and man. The formation and accumulation of the mycotoxins can occur both in infected plants standing in the field or in stored products colonized by the toxigenic isolates. *Fusarium* mycotoxins on corn were described from many world regions. The main groups of mycotoxins are trichothenenes, zearalenone, moniliformin and fumonisins.

Surveys of corn (infected plants and commercial kernels) for *Fusarium* species and their mycotoxins were carried out on samples collected from more than 50 localities within southern and middle Moravian regions in the years 1998 and 1999. Corn kernels were plated on selective *Fusarium* medium and after purification of the isolates, single-spore cultures were obtained for identification. ELISA was used for mycotoxin analysis (deoxynivalenol, zearalenone, T-2 toxin and fumonisin). Eight different *Fusarium* species were isolated from corn kernels. The most frequent species in case of the Czech republic were *Fusarium graminearum, F. culmorum, F. avenaceum* and *F. moniliforme.* Increased levels of deoxyniva-

lenol (max. level 550 ng/kg) and zearalenone (max. level 430 ng/kg) were detected in some samples mainly with pink and reddish pigmentation. Detection of other mycotoxins was not so often. Detailed results will be published in some separate papers. The incidence of *Fusarium* mycotoxins in corn kernels processed as feedstuffs might present a real hazard to livestock.

The research project was suported by the Grant Agency of the Czech Republic, project number 521/98/1019.

Sanchis, V.; Velluti, A.; Ramos, A. J.; Marín, S.

Food Technology Department. Lleida University. CeRTA. Rovira Roure, 177, 25198 Lleida, Spain

Fumonisin B_1, zearalenone and deoxynivalenol production by *Fusarium verticillioides, F. proliferatum* and *F. graminearum* in mixed cultures on irradiated corn kernels

The impact of interactions between fumonisin-producing isolates of *F. verticillioides* and *F. proliferatum*, and a zearalenone (ZEA) and deoxynivalenol (DON) producing isolate of *F. graminearum* inoculated together on irradiated maize, on their growth and mycotoxin formation at 15 and 25ºC and at 0.98, 0.95 or 0.93 a_w was studied.

Rehydrated maize was placed in sterile Petri plates. For pure culture inoculation, four agar discs (5 mm diameter) were used, while for mixed cultures, four similar discs for each species were distributed on the grain in a fixed pattern. Then, plates containing grain of the same a_w were placed in sealed containers and incubated for 4 weeks at 15 and 25ºC. All treatments were repeated twice. After incubation, plates were analysed for fungal populations (CFU g^{-1}) by dilution plating using MEA (malt extract agar) as enumerating media. Fumonisin B_1 (FB_1), DON and ZEA were quantified by using HPLC.

The presence of *F. graminearum* made the number of fungal populations (CFUs) per gram grain of *F. verticillioides* and *F. proliferatum* decrease under almost all conditions tested. In presence of *F. verticillioides*, CFUs of *F. graminearum* raised significantly at 25ºC, while the presence of *F. proliferatum* made them increase at 15ºC. The presence of *F. graminearum* had no significant effect on FB_1 production by *F. verticillioides* neither had *F. proliferatum*. However, the production was in general inhibited under all conditions except at 25ºC and 0.98 a_w. There was no effect of fungal interaction on ZEA production by *F. graminearum*, however, when paired with *F. verticillioides* and *F. proliferatum*, DON production by *F. graminearum* was significantly stimulated mainly at 0.98 a_w.

Clear, R. M.[1]; Abramson, D.[2]; Gaba, D.[1]

[1] Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main St., Winnipeg, Manitoba, Canada, R3C 3G8
[2] Agriculture Canada Cereal Research Centre, 195 Dafoe Rd., Winnipeg Manitoba, Canada, R3T 2M9

Trichothecene and moniliformin production by *Fusarium* isolates from Western Canadian wheat

Fusarium graminearum, F. culmorum and *F. avenaceum*, isolated from *Fusarium*-damaged kernels of wheat harvested in Alberta, Saskatchewan and Manitoba, Canada, were assessed for mycotoxin production in rice cultures. Extracts from the culture media were assayed for trichothecenes by gas chromatography-mass spectrometry, and for moniliformin by liquid chromatography. Deoxynivalenol (DON) was present in 28/42 isolates of *F. graminearum* and 42/42 isolates of *F. culmorum* at levels ranging from 0.5

to 25.0 µg/g, with a mean value of 6.4 µg/g. 15-AcetylDON was present in 28/42 isolates of *F. graminearum* at levels ranging from 1.0 to 7.1 µg/g, with a mean value of 3.6 µg/g. 3-AcetylDON was present in 41/42 isolates of *F. culmorum* at levels ranging from 0.8 to 13.0 µg/g, with a mean value of 3.8 µg/g. Several other trichothecenes were assayed but not detected in the isolates. Moniliformin was found in 40/42 isolates of *F. avenaceum* at levels ranging from 1.3 to 138.1 µg/g, with a mean value of 26.7 µg/g, but was not present in any of the isolates of *F. graminearum* or *F. culmorum*.

Matthies, A.; Menck, B.-H.; Bleiholder, H.

BASF-AG Agrarzentrum Limburgerhof, Germany

Fusarium-toxins in winter wheat and possibilities for fungicide treatments

Field trials were conducted in the 1999 season at different locations within Germany in order to determine the effects of strobilurin containing fungicides (both products which only contained a strobilurin as well as combination products with azoles) compared with azole solo products on the deoxynivalenol (DON) content of wheat samples. In general, this season was characterized by notably lower DON contents than the previous season. The 1999 median of 0.4 mg/kg (n = 280 samples from untreated, control plots, natural infection as well as inoculation with infected oat grains and maize) is clearly well below the currently proposed and discussed maximum tolerated DON level of 1 mg/kg. In contrast to this, the median value in 1998 was 1.7 mg DON/kg (n = 144 samples from untreated, control plots, natural infection as well as inoculation with infected oat grains and maize). The results indicate that both the location as well as the prevailing weather conditions have a significant influence on the degree of toxin contamination. A fungicide application, irrespective of the type of active ingredient and also independant of the level of toxin contamination, resulted in a significant reduction in the level of DON. The degree of toxin reduction following treatment with Strobilurin 2 was always weaker than either Strobilurin 1 (which had an effect similar to the azole fungicides) or the other fungicides used. This indicates, that strobilurin fungicides can be clearly differentiated. Not all strobilurins can be put into the same basket. Furthermore, it could be shown that the effectiveness of a fungicide in reducing the DON level was strongly dependant on the time of its application. In this report, the strobilurin containing fungicides (mixtures 1 & 3) were comparable to Azole 1 within the application period „beginning of flowering“ to „end of flowering“.

Martins, M. L.[1)]; Martins, H. M.[1)]; Dias, I.[1)]; Bernardo, F.[2)]

[1)] Laboratorio Nacional Investigação Veterinaria- Estrada de Benfica, 701-1549-011-Lisboa, Portugal
[2)] Faculdade de Medicina Veterinaria, Polo Univers. da Ajuda, R. Prof. Cid Santos 1300-417 Lisboa, Portugal

Fumonisin in black tea and medicinal plants

Fumonisins are a group of mycotoxins recently discovered and produced by *Fusarium moniliforme*, toxic ability of which was shown experimentally in rats and birds as causing hepatocarcinoms. This mycotoxin has been recognized as a natural cause of leukoencephalomalacia (horses), pulmonary edema (porcine) and esofagic cancer (humans) [1].

In this study, eighteen samples of black tea (n=18), and sixty nine samples (n=69) of four different types of medicinal plants, chamomile (n= 18), leaves of orange tree (n= 18),leaves and flowers of tilia (n=18) and corn silk (n= 15), totalizing 87 samples were analyzed for fumonisins B_1 (FB_1) and B_2 (FB_2). The samples were purchased from the local markets in Lisbon, Portugal. The fumonisins were quantified by high performance liquid chromatography (HPLC) according to Shephard et al 1990 [2].

Fumonisin B1 was detected in fifty five samples (63.2 %). The higher incidence of positive samples and also the higher FB1 concentration were found in black tea (88.8%, with levels from 80 to 280 µg/kg), followed by leaves of orange tree and leaves and flowers of linden tree(66.6% with levels ranging from 350-700 µg/kg and 10-200 µg/kg, respectively).Of the fifteen samples of corn silk, nine were positive (60.0% with levels from 50 to 150 µg/kg) while only eight (44.4%) of eighteen samples of chamomile were contaminated at levels of 20-70 µg/kg. Neither one of the samples tested had contamination levels of fumonisin B_2.

This is the first report of the natural occurrence of fumonisins in black tea and medicinal plants in Portugal and therefore the authors reinforce the necessity to implement more effective measurements of safety control to this kind of products.

Literature

[1] Dutton M.F. 1996. Fumonisins, Mycotoxins of increasing importance: their nature and their efects. Pharmacol. Ther., **70**, 137-161.

[2] Chephard G.S., Sydenhman, E.W., Thiel, P.G. and Gelderblom, W.C.A. 1990. Quantitative determination of fumonisins B_1 and B_2 by HPLC with fluorescence detection. J. Liq. Chromatography, **13**, 2077–2087.

Langseth, W.; Rundberget, T.; Kosiak, B.

National Veterinary Institute, Dep. Feed and Food hygiene, P. Box 8156 Dep, N-0033 Oslo, Norway

Production of fusarochromanone and trichothecenes by Norwegian isolates of *Fusarium equiseti*

Twenty-eight *Fusarium equiseti* strains isolated from Norwegian cereals were studied for their ability to produce mycotoxins and other secondary metabolites. The strains were cultivated on rice. Methanol-water-ammonia (cons.) extract of the cultures were analysed for fusarochromanone by liquid chromatography (HPLC) with fluorescence detection after extraction into dichlorometane and purification on a Bond Elute silica column. Acetonitril-water extract was analysed for trichothecenes by gas chromatography mass spectrometry (GC-MS) after purification on Mycosep™#225 columns and derivatization with pentafluoropropionic (PFP) reagent.

Morphologically all isolates produced dense light mycelium on PSA, and macroconidia with short apical cells. All isolates produced chlamydospores.

Fusarochromanone was produced by all isolates, except two, in large quantities of mg/g level. All isolates were found to produce at least small quantities of trichothecenes. The trichothecene profile of *F. equiseti* was similar to that of *F. poae* isolates. Significant amounts of nivalenol, 4-acetylnivalenol (fusarenon-X), 15acetylnivlenol or diacetylnivalenol (mg/kg level) were produced by all isolates except four, while only small quantities were detected in three of the isolates. None of the nivalenol-derivatives were detected in one of the isolates. The other main group of trichothecenes detected were the scirpenol derivatives; 4,15-diacetoxyscirpentol (DAS), 15-monoacetoxyscirpenol (MAS) and scirpentriol. The two groups of trichothecenes were formed in approximately the same amounts. Neither deoxynivalenol (DON), acetyl-DON, HT-2 toxin or T-2 toxin were detected in any of the cultures.

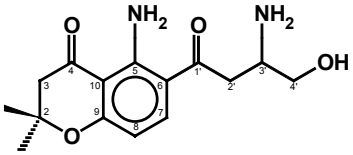


Figure: Fusarochromanone

Altomare, C.[1]; Favilla, M.[1]; Evidente, A.[2]; Amalfitano, C.[2]

[1] Istituto Tossine e Micotossine da Parassiti Vegetali, CNR, 70125 Bari, Italy
[2] Dipartimento Scienze Chimico-Agrarie, Università di Napoli "Federico II", 80055 Portici , Italy

Screening F*usarium semitectum* strains from different origins for production of the bioactive metabolites fusapyrone and deoxyfusapyrone

Fusapyrone (FP, $C_{34}H_{54}O_9$, MW 606) and deoxyfusapyrone (DFP, $C_{34}H_{54}O_8$, MW 590) are two bioactive metabolites produced by *Fusarium semitectum* Berk. & Rav. Structurally, FP and DFP are 3-substituted-4-hydroxy-6-alkyl-2-pyrones that consist of a highly functionalized aliphatic chain and a 4-deoxy-β-*xylo*-hexopyranosyl C-glycosyl residue bound respectively to the C-6 and C-3 of the 2-pyrone ring [2]. These molecules show dramatic differences in their biological activities, in spite of the minor structural difference in the alkyl side chain bound to the C-6 [1]. In particular, FP displays a selective antifungal activity against filamentous fungi and some agents of human mycoses. On the other hand, DFP shows noteworthy zootoxic activity in *Artemia salina* bioassays, its LC_{50} (21.8 µg/mL) being comparable to other *Fusarium* mycotoxins such as beauvericin and fusaproliferin [3]. In the present study we report the production of FP and DFP by 46 strains of *F. semitectum* complex (H. Nirenberg, personal communication) with different origins. The aims of this project were i) investigate the suitability of α-pyrones production as a chemotaxonomic tool for discriminating taxa within the *F. semitectum* complex; ii) select high producers to be utilized for purification of large quantities of α-pyrones for further studies; iii) examine the production of DFP in order to evaluate its possible mycotoxicological relevance. For this purpose, a simple, sensitive and rapid HPLC method for the simultaneous quantitative analysis of FP and DFP was developed. Such method was optimized on C-18 reverse phase column with a sequence of linear elution steps with MeOH-H_2O mixture and using an UV detector fixed at 285 nm. Twelve out of 46 examined strains produced detectable amounts of both FP and DFP on PDA, while 5 strains produced only DFP. FP was found in concentrations ranging from 6.8 to 155 ppm and DFP from 4.2 to 227 ppm. On the basis of the above data, namely DFP toxicity and amounts produced, and considering the wide occurrence of *F. semitectum* in foodstuffs [4, 5, 6], DFP may cause some mycotoxicological concerns. However, in order to evaluate the actual mycotoxicological relevance of DFP, further investigations on the production of this toxin by other *Fusarium* species, its occurrence in naturally infested agricultural commodities, and its zootoxic activity in more specific and complex systems are needed.

Literature

[1] Altomare C., Perrone G., Zonno M. C., Evidente A., Pengue R., Fanti F., Polonelli L. 2000. Biological characterization of fusapyrone and deoxyfusapyrone, two bioactive secondary metabolites of *Fusarium semitectum*. J. Nat. Prod. (in press).
[2] Evidente A., Conti L., Altomare C., Bottalico A., Sindona G., Segre A., Logrieco A. 1994. Fusapyrone and deoxyfusapyrone, two antifungal α-pyrones from *Fusarium semitectum*. Natural Toxins, **2**, 4-13.
[3] Logrieco, A., Moretti, A., Fornelli, F., Fogliano, V., Ritieni, A., Caiaffa, M.F., Randazzo, G., Bottalico, A., Macchia, L. 1996. Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina*, SF-9 insect cells, and IARC/LCL 171 human B lymphocytes. Appl. Environ. Microbiol., **62**, 3378-3384.
[4] Marin, D.H., Sutton, T.B., Blankenship, S.M., Swallow, W.H. 1996. Pathogenicity of fungi associated with crown rot of bananas in Latin America on Grande Naine and disease-resistant hybrid bananas. Plant Dis. **80**, 526-528.
[5] Munimbazi, C., Bullerman, L.B. 1996. Molds and mycotoxins in foods from Burundi. J. Food Prot. **59**, 869-875.
[6] Roy, K.W., Ratnayake, S. 1997. Frequency of occurrence of *Fusarium pallidoroseum*, effects on seeds and seedlings, and associations with other fungi in soybean seeds and pods. Can. J. Plant Pathol. **19**, 188-192.

Logrieco, A.[1]; Rizzo, A.[2]; Ferracane, L.[3]; Ritieni, A.[3]

[1] Istituto Tossine e Micotosine da parassiti vegetali, CNR, 70125, Bari, Italy
[2] National Veterinary and Food Research Institute, Department of Chemistry, Helsinki, Finland
[3]Dipartimento di Scienza degli Aliment, Università degli Studi di Napoli "Federico II" 80055, Portici, Italy

Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight

Head blight of small grains is the most important phase of cereal fusariosis for the potential accumulation of toxins in scabby grains. Among *Fusarium* species causing this disease, *F. avenaceum* (Fr.)Sacc, the anamorphic state of *G. avenacea* R.J. Cooke, is one of the most common in Europe, including Finland [1]. This species can produce significant quantities of secondary metabolites, for example antibiotic Y [2] and mycotoxins such as fusarin C [3], moniliformin [4] and enniatins [5]. Recently, Logrieco et al. [6] found beauvericin (BEA) to be produced by many species in the genus *Fusarium*, including *F. avenaceum,* and suspected that beauvericin could be a common cereal contaminant. Beauvericin, as well as enniatins, is a well-known cyclic hexadepsipe with a specific cholesterol acyltransferase inhibitor activity [7]. Beauvericin is toxic to several human cell lines inducing programmed cell death similar to apoptosis and causing cytolysis accompanied by internucleosomal DNA fragmentation into multiples of 200 bp [8]. In the present study we investigated the occurrence of BEA and enniatins in cereal grains mainly infected by *F. avenaceum* in Finland and the BEA production capabilities of isolated *Fusarium* strains. Beauvericin was commonly detected as natural contaminant of wheat kernels (up 3.5 µg/g) and most strains of *F. avenaceum* and *F. poae* isolated from these samples produced BEA and other esadepsipeptides in high amounts (up to 3,703 µg/g). This is the first report on the natural occurrence of BEA in *Fusarium* infected wheat.

Literature

[1] Bottalico, A. 1998. *Fusarium* diseases of cereals: species complex and related mycotoxin profiles in Europe. J. Planth Pathol, **80**(2) 85-103.
[2] Golinski, P., S. Wnuk, J. Chelkowski, A. Visconti, and M. Schollenberger. 1986. Antibiotic Y: biosynthesis by *Fusarium avenaceum* (Corda ex Fries) Sacc., isolation, and some physiochemical and biological properties. Appl. Environ. Microbiol **51**: 743-745.
[3] Farber, J. M. and G.W. Sanders. 1986. Production of fusarin C by *Fusarium* spp. J. Agric. Food Chem **34**: 963-966.
[4] Marasas, W.F.O., P.E. Nelson and T.A. Toussoun. 1984. Toxigenic *Fusarium* species: identity and mycotoxicology. The Pennsylvania State University Press. University Park, Pa.
[5] Cook, A.H., S.F. Cox, T.H. Farmer and M.S. Lacey. 1947. Production of antibiotics by Fusaria. Nature **160**: 31-32.
[6] Logrieco, A., A. Moretti, G. Castella, M. Kostecki, P. Golinski, A. Ritieni and J. Chelkowski. 1998. Beauvericin production by *Fusarium* species. Appl. Environ. Microbiol. **64**(8) 3084-3088.
[7] Tomoda, H., X.H., Huang, H. Nishida, R. Nagao, S. Okuda, H. Tanaka, S. Omura, H. Arai, and K. Inoue. 1992. Inhibition of acyl-CoA:cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. J. Antibiot. **45**: 1626-1632.
[8] Macchia, L., R. di Paola, F. Fornelli, S. Nenna, A. Moretti, R. Napoletano, A. Logrieco, M.F. Caiffa, and A. Botalico. 1995. Cytotoxicity of beauvericin to mammalian cells, p.72-73. In: Abstract of the International Seminar on *Fusarium*: Mycotoxins, Taxonomy and Pathogenicity, 9 to 13 May 1995, Martina Franca, Italy. Stampasud, Mottola, Italy.

Pettersson, H.[1]; Langseth, W.[2]

[1] Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7024, SE-750 07 Uppsala, Sweden
[2] Central Veterinary Laboratory, PB 8156 Dep, N-0033 Oslo, Norway

Methods for trichothecene analysis – A status report

The trichothecenes deoxynivalenol, nivalenol, HT-2 and T-2 toxins have been found frequently in European cereals. During the last two years also high quantities of deoxynivalenol have been detected in certain European regions. Food authorities and the European Commission look seriously on the contamination of cereals by trichothecenes and prepare for introduction of maximum tolerance levels. For control

purposes there is an urgent need for a good and validated method. There are today no reliable standardized methods for the trichothecenes, CEN, the European Standardization Body, requests at least to get a standardized method for evaluation of deoxynivalenol.

Several collaborative studies on trichothecene analysis have revealed high variations in the analytical results both between and within laboratories. A SMT-project "Intercomparison of Trichothecene Analysis" started about four years ago with the main objective to improve trichothecene analysis and to investigate the feasibility to produce certified reference material. Twenty laboratories from 12 countries using mainly gas chromatographic methods have participated. The first intercomparison of trichothecenes in solution and recovery studies resulted in relatively high within- and between-laboratory variation. They were, however, comparable to those in earlier studies. Method problems such as trichothecene response enhancement by matrix, non-linear calibration curves, carry-over or drifting response were identified in most laboratories. They have been studied and recommendations have been given how to eliminate or minimize the problems. Most laboratories seem still to have problems with trichothecene response enhancement by matrix. A gas chromatographic method to be standardized has to take the identified problems and recommendations into consideration in order to be robust enough. Matrix assisted calibration curves and GC-internal standards will probably be needed to correct for both the enhancement and the drift in trichothecene response. They will probably, although not desirable, be used in the projects final intercomparison study for evaluation of the feasability to certify reference material.

Beside the gas chromatographic methods, HPLC and ELISA, methods are also used for deoxynivalenol analysis. A popular HPLC method is using an immunoaffinity column for clean-up. Water with PEG is used for extraction and may not be sufficient for extraction of naturally contaminated samples and reference material, as many laboratories have got too low values for these materials. An interfering contaminant has also been a hopefully transient problem. An ELISA method used in many European laboratories has shown to be good for survey purposes. A relatively good agreement with a special HPLC-method has been obtained for naturally contaminated samples.

Widestrand, J.; Pettersson, H.

Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7024, S-75007 Uppsala, Sweden

Cytotoxicity screening of trichothecenes using the BrdU colorimetric bioassay

As a complement to the traditional chemical analysis we have studied the use of cell culture technique for screening of trichothecenes in cereal samples.

The aim of this study was 1) to evaluate the use of MycoSep #225 column for cleanup of cereal samples for cytotoxicity screening, 2) to compare the cytotoxicity of wheat and oat extracts spiked with T-2 toxin (T-2), HT-2 toxin (HT-2), deoxynivalenol (DON) and nivalenol (NIV) with the cytotoxicity of pure toxins and 3) to investigate the cytotoxicity of naturally contaminated wheat and oat samples.

After extraction with acetonitrile/water, blank, spiked (at concentrations of T-2, HT-2, DON and NIV frequently found in cereal samples) and naturally contaminated wheat and oat extracts were purified using the MycoSep #225 cleanup column. The clear extracts were evaporated and the residues were dissolved in cell culture medium and incubated in triplicates as two-fold dilution series with 3T3 mouse fibroblasts on a 96-well microtiter plate for 24 h. The cytotoxicity was determined using the colorimetric BrdU cell proliferation bioassay measuring DNA synthesis.

The use of MycoSep #225 column as the only cleanup step was sufficient to eliminate the cytotoxic matrix effect of blank wheat and oats extracts at concentrations of 400 mg/ml (Fig. 1). The dose response

curves of wheat and oat extracts spiked with T-2, HT-2 DON and NIV correlated very well to the curves obtained using pure toxins (Fig. 2). Cytotoxicity screening of naturally contaminated wheat and oat samples showed good correlation to trichothecene analysis performed on GC with electron-capture detection.

In conclusion, the combination of the fast and selective cleanup of wheat and oat extracts using the MycoSep #225 column, the sensitive 3T3 cells and the BrdU colorimetric bioassay provides a suitable tool for screening of trichothecenes in cereal samples.

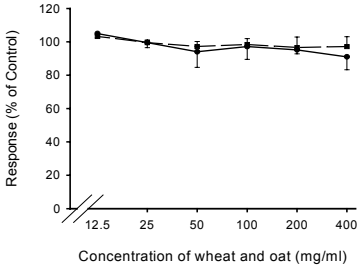


Figure 1. The effect on 3T3 cells exposed to blank wheat (----) and oat (—) extracts. The values are expressed as percent of control response and are means of at least three experiments.

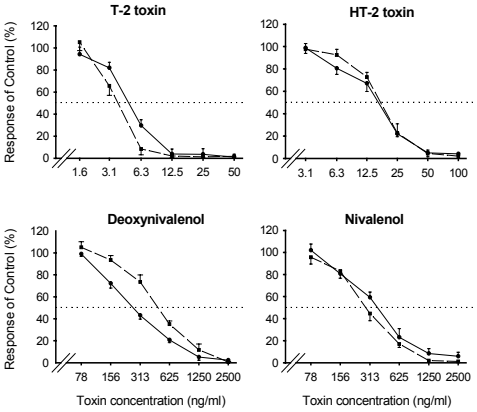


Figure 2. The effect on 3T3 cells exposed to T-2, HT-2, DON and NIV spiked to blank wheat extracts (----) or pure toxins (—). The values are expressed as percent of control response and are means of at least three experiments.

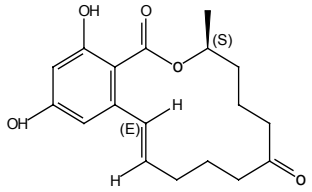
Radová, Z.; Hajšlová, J.; Králová, J.

Department of food Chemistry and Analysis, Institute of Chemical Technology, Technická 3, 166 28 Prague 6 – Dejvice, Czech Republic, e-mail: zuzana.radova @vscht.cz

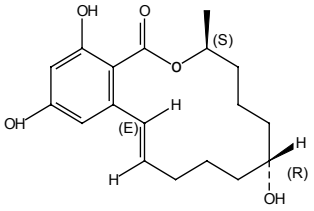
Optimization of a method to determine zearalenone, α-zearalenol and β-zearalenol in oil seeds*

Zearalenone, a derivative of β-resorcyclic lactone, is a natural toxicant occurring worldwide mainly in corn, cereals and other agricultural commodities contaminated with some *Fusarium* species. This mycotoxin often accompanies trichothecenes, of which deoxynivalenol (DON) is the dominating compound. Zearalenone and its metabolites α-zearalenol and β-zearalenol demonstrate estrogenic activities and due to this and other adverse effects they cause mycotoxicoses in animals. For instance in swine, zearalenone has been associated with abortion, mummified fetues, reduced size and incoordination of the hind limbs. Considering these facts, concern regarding similar human health risks, has been raised.

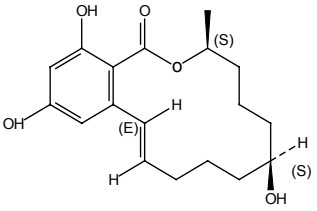
Among different types of chromatographic techniques (TLC, GC/MS, ELISA) applied in various studies for determinating zearalenone in plant crops, the use of high-performance liquid chromatography with fluorescence detection (HPLC/FLD) is dominating. As long as a large number of samples is to be analyzed, the HPLC method is rather laborious and time consuming and therefore the enzyme-linked immunosorbent assay (ELISA) method is often used for routine screening.



ZEARALENONE



α- ZEARALENOL



β- ZEARALENOL

This study describes an analytical procedure applicable for the determination of zearalenone, α-zearalenol and β-zearalenol in oil seeds by using HPLC/FLD. The objective was to develop a rapid method that could be used for less frequently examined commodities represented by poppy and rape seeds. Due to relating high lipid content intensive sample clean-up of the crude extract (for extraction solvent mixture acetonitrile-water (84:16, v/v) was used) compared with cereals is needed. Main part of the presentation is focused on comparison of two clean-up techniques:

i] liquid-liquid partition (dichlormethane)

ii] gel permeation chromatography (GPC). For GPC purification two different systems, gel Bio-Beads S-X3 with mobile phase chloroform or cyclohexane-ethylacetate (1:1, v/v) solvent mixture and PL gel with mobile phase cyclohexane-ethylacetate (1:1, v/v) were tested.

* This study represents a contribution to the establishment of a project within COST Action 835 „Agriculturally important toxi genic fungi“ in the authors' department.

Bagnara, A.; Färber, P. F.; Geisen, R.

Federal Research Center for Nutrition, Institute of Hygiene and Toxicology, Haid-und-Neu-Str. 9,
D-76131 Karlsruhe, Germany

Quantification of mycotoxin producing fungi by Real Time PCR

Several conventional PCR methods for the detection of mycotoxinogenic fungi in food samples have been described. These methods have the drawback, that only qualitative results are obtained and nothing can be stated about the intensity of the contamination. For aflatoxinogenic fungi a quantitative Real Time PCR (Q-PCR) has been developed. As a prerequisite for this development conservative target sequences must be available, as variations in the target sequence can influence the quantification. Based on these facts two genes of the aflatoxin biosynthetic pathway, the *afl*R gene and the *nor*1 gene have been analysed in more detail. It could be shown by PCR-RFLP and Southern blotting that the *afl*R gene showes high sequence variability whereas the *nor*1 gene is conserved. For that reason the *nor*1 gene was chosen. By using this system the copy number of the *nor*1 gene could be determined in pure DNA solutions and in food samples. The copy numbers of the *nor*1 gene was compared to the cfu number obtained by conventional methods.

Lauber, U.; Dillenburger, T.; Drochner, W.

Hohenheim University, Institute of animal nutrition, Emil-Wolff-Straße 10, 70599 Stuttgart, Germany

Effects of feeding diets containing *Fusarium* naturally contaminated wheat or pure deoxynivalenol in growing pigs

Numerous feeding experiments examined the effect of deoxynivalenol (DON) fed to pigs on feed consumption and weight gain. Although the biochemical influence of this trichothecene mycotoxin on cells is well known (PBS-inhibition, enhanced cytokine production, modulation of immunological response etc.), there exists no clear information about the minimum effective dose in pigs. All experiments described in literature used diets containing cereals. Therefore the absence of other mycotoxins (known or even unknown) could not be guaranteed. After a certain time a remarkable recovery of treated animals could be observed. In connection with these findings DON-de-epoxidation through intestinal microflora is discussed to be responsible for adaptation mechanisms. Less is known about the irritant effect of DON on the gastrointestinal tract especially in swine. Due to the slight macroscopic lesions found in the tissues usually no histological examinations have been carried out or provided no results.

To evaluate the minimum effective dose of pure DON leading to measurable losses in weight gain and feed consumption a special feeding experiment was created to compare the effect of DON in natural contaminated wheat and - for the first time - a non-cereal diet (potato) spiked with pure DON (tab. 1). The most interesting parameters were examined: weight gain, feed consumption and blood parameters.

Table: Experimental design

Trial	Groups	Pigs/group	Diet groups and feeding	DON µg/kg feed
A	4	5	Contr. (wheat and potato), Exp. (wheat and potato) restricted feeding	0, 4000
B	3	5	Control, “Low dose”, High dose (wheat) ad lib.	0, 4000, 6000
C	3	5	Control, “Low dose”, High dose (potato) ad lib.	0, 4000, 6000

In addition we were interested in DON-de-epoxidation through the large intestine microbial flora depending on DON-concentration in the feed and on the source (nat. contam. material and pure DON, respectively). Furthermore, the tissue damaging effects were inspected in trial B by histological examination of two animals of the control and the high exposed group, respectively.

Severe effects on weight gain and feed consumption were found in trial B; the differences in body weight were significant in the high exposed group compared to the control. By contrast no differences in any parameter were found in trial A and C and further no significant changes in blood parameters were found in any trial. Differences were seen between naturally contaminated material and pure DON. Regarding, DON-de-epoxidation through intestinal microflora, also a relationship between concentration and time of adaptation could be observed. The result in trial B could not clearly be supported by the histopathological findings, because only one pig of the high exposed group showed serious lesions in the gastrointestinal tissues. More detailed results are presented in the poster.

Eriksen, G.S.; Pettersson, H.

Department of animal nutrition and management, Swedish University of Agricultural Sciences,
P.O Box 7024, 75007 Uppsala, Sweden

Transformation of trichothecenes in incubations with pig faeces

Some studies have shown that trichothecenes are de-epoxidated in incubations with faeces from pigs, while no such de-epoxidation activity was found in other studies. In a previous study in our laboratory, the de-epoxy-metabolite was found in faeces incubates from pigs exposed to the trichothecene nivalenol in the diet for 1 or 3 weeks, while no such metabolite could be detected before exposing the pigs to the toxin. It was assumed that the microbial de-epoxidation capacity is achieved after exposure of the pigs to low levels of the toxins for a certain periode. The de-epoxidation capacity of the intestinal microbes was suggested to be used as an indicator of recent exposure to trichothecenes.

Faeces were collected from 8 different pig farms and from 2 different pig experiments. Faeces from 5 different pigs were used from each farm and incubated with either 3-acetylDON (3-acDON) or nivalenol (NIV) for 48 hours. The toxins were extracted with ethylacetate, washed with hexane, derivatisized with trisil and analysed as previously described. The chromatograms were compared with a control sample (3-acDON and nivalenol added after incubation) and a blank sample from the same pig.

The de-epoxide metabolites of DON and NIV were detected in incubates from all 5 pigs from the 8 pig producers. In samples collected from 5 experimental pigs, de-epoxides of the 2 toxins were also detected, but a significant amount of unmetabolised toxins were also present in incubates from 2 of the 5 pigs. In incubates from 4 pigs from another experiment, no de-epoxides at all were detected in incubates from 2 of the pigs, while more than 90% of the toxins were de-epoxidated in the other 2 incubates.

Less than 5% of the 3-acDON that had been added to the incubates was found as 3-acDON after 48 hours (except of 1 incubate from 1 pig, were 31% was found as 3-acDON and 69% as DON de-epoxide). Most of the added toxin had been transformed to de-epoxide DON, but significant proportions of DON were found in a few incubates.

Faeces incubates from all pigs from 8 different pig producers had the capacity to de-epoxidate the added trichothecenes. This may be because the de-epoxidation capability, in contrast to what have been found in some studies, is normal in pigs. It may eventually have been caused by low levels of trichothecenes in the feed. The farmers in the study produce their own feed. The feed or cereals produced in this region last year has not been analysed. Low levels of de-epoxy-NIV were, however, found in the control incubates from 2 pig farms, indicating a presence of nivalenol in the feed.

In incubates from experimental pigs, the de-epoxidation activity was lower or not present at all compared to in incubates from pig farms. The pigs were normally obtained from specific pathogen free breeders. It is possible that the hygienic conditions for such pigs are different from normal producers, and that this may affect the de-epoxidation capacity.

Miscellaneous

Färber, P. F.; Geisen, R.

Federal Research Center for Nutrition, Institute of Hygiene and Toxicology, Haid-und-Neu-Str. 9, D-76131 Karlsruhe, Germany

Penicillin biosynthesis in *Penicillium nalgiovense* a fungal starter culture for the production of mold fermented food products.

Penicillium nalgiovense a terverticillate species of the genus *Penicillium* is a frequently used starter culture for mold ripened foods. This fungal species can preferably be isolated from fermented meat products e.g. salami. From the physiological as well as the genetical point of view *P. nalgiovense* seems to be related very closely to *P. chrysogenum* a well known producer of the β-lactam antibiotic penicillin. It was possible to prove the complete set of genes δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine synthetase (*acvA*), isopenicillase N synthase (*ipnA*) and acyl-CoA:6-aminopenicillanic acid acyltransferase (*aat*) necessary for biosynthesis of penicillin in *P. nalgiovense* as well by gene specific polymerase chain reactions using oliginucleotide primers derived from sequences of *P. chrysogenum*, as in southern hybridization experiments using DIG labeled DNA probes. For further judging the degree of relation between these two species cluster analysis of penicillin biosynthetic genes in *P. nalgiovense* have been made and the results of these experiments have been compared to the penicillin gene cluster in *P. chrysogenum*. These investigations showed that penicillin genes in *P. nalgiovense* are clustered in a similar way as in *P. chrysogenum*. In contrast to this similarity the location of the penicillin gene cluster is completely different in *P. nalgiovense* compared to *P. chrysogenum*. Experiments in which the location of the penicillin cluster was investigated, showed that this cluster in *P. chrysogenum* is, in accordance to the literature, located on the chromosome I, the biggest chromosome in *P. chrysogenum*. In *P. nalgiovense* the penicillin gene cluster is located on chromosome IV, the smallest chromosome of this fungus. In addition to these differences the sequence homolgy of the nucleotides as well as the amino acids were determined. There is a homology between the nucleotide sequences of the IPNS genes of *P. nalgiovense* and *P. chrysogenum* of 94%, the homology of the amino acids sequences is 89%. In contrast to other penicillin producing microorganisms like *Aspergillus nidulans*, *Cephalosporium acremonium*, *Streptomyces griseus* or *Nocardia lactamdurans* these are very slight but remarkable differences in the sequence homology between *P. nalgiovense* and *P. chrysogenum*.

List of Authors

I

J

K

L

M

N

O

Ø

P

R

S

T

V

W

X

Y

Z