



17th

European Fusarium Seminar

October 21-24, 2025 Bordeaux France

Book of Abstracts

Organisation :



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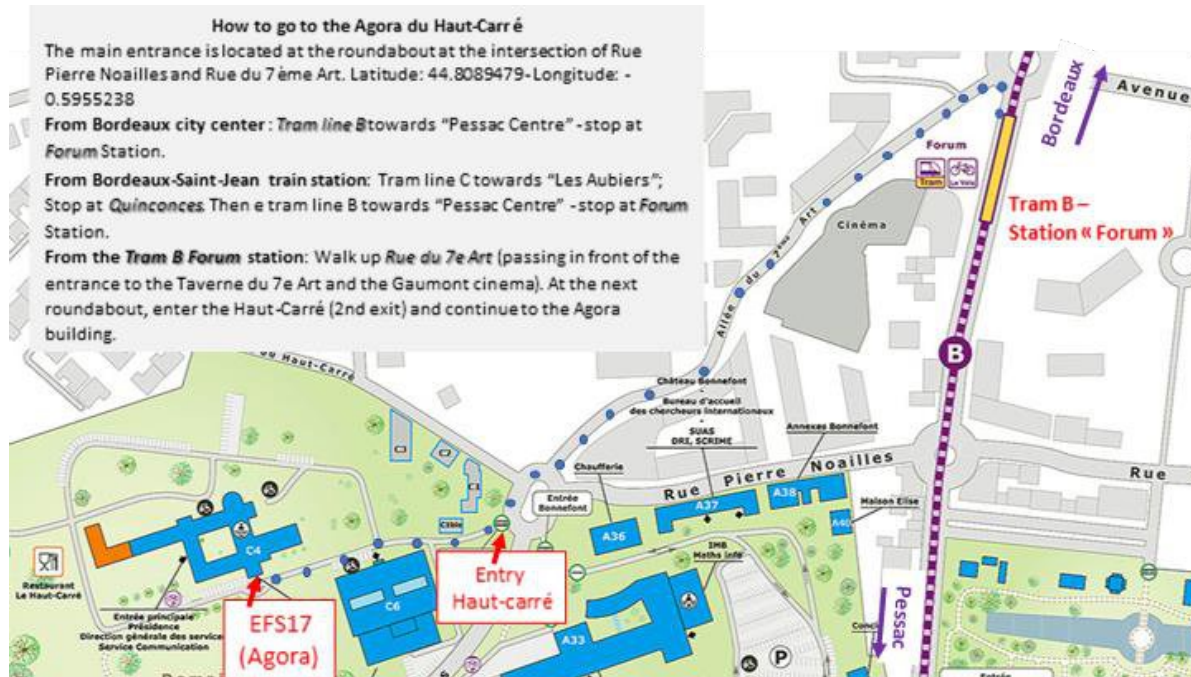
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Domaine du Haut Carré, Université de Bordeaux, 43 rue Pierre Noailles. 33400 TALENCE



PROGRAM Auditorium Agora

Tuesday 21

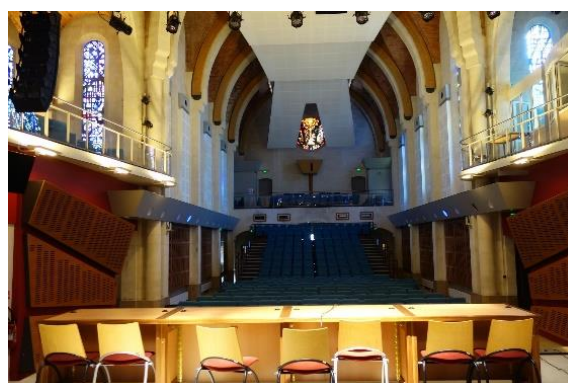
Welcoming	13h00-14h00
Introduction by the local scientific committee	14h00-15h00
Opening Lecture: Bart Thomma , - How fungal pathogens manipulate host microbiota to establish infection.	
Session 1: <i>Fusarium</i> genetics & physiology Chairs: - Dr Kim Hammond-Kosack, Rothamsted Research, England - Dr Nadia Ponts, INRAE MycSA, France	15h00-18h00
Matias Pasquali - How does <i>Fusarium musae</i> adapt to human and banana hosts?	15h00-15h30
Terance Mobarak -Deciphering trophic shifts in <i>Fusarium verticillioides</i> : A mycotoxin story	15h30-15h50
Chris Blackman - The pan-genomic effectome of the <i>Fusarium sambucinum</i> species complex reveals novel virulence factors	15h50-16h10
coffee Break (Badiane Room)	
Marie Foulongne-Oriol - Quantitative genetics approach to decipher life history traits in <i>Fusarium graminearum</i>	16h40-17h00
Tapani Yli-Mattila - Phylogenetic analysis of a 139.9-kb 52-gene dataset to reassess diversity within <i>Avenaceum</i> clade of <i>Fusarium tricinctum</i> species complex	17h00-17h20
Adeline Picot - Intra- and interspecific diversity of <i>Fusarium</i> spp. responsible for <i>Fusarium</i> Head Blight based on metabarcoding and population genetics	17h20-17h40
John F. Leslie - Nit Mutants in <i>Fusarium</i>	17h40-18h00
Program announcements	18h00-18h05
POSTER SESSION & Social Mixer (Cloister Gallery)	18h05-19h30

Wednesday 22

Session 1: <i>Fusarium</i> genetics & physiology (continue) Chairs: - Dr Marie Foulongne-Oriol, INRAE MycSA, France - Pr John F. Leslie, Kansas State University, USA	09h00-12h20
Jade Smith - Epigenetic control of effector proteins secreted by <i>Fusarium graminearum</i>	09h00-09h30
Jens Laurids Sørensen - Identification of the phenalenone biosynthetic pathway for perithecial pigmentation in the <i>Fusarium solani</i> species complex	09h30-09h50
Luca Degradi - Chromosome-level genome assembly and functional annotation of <i>Fusarium musae</i> from a human isolate: insights into host-specific infection mechanisms	09h50-10h10
Aurélie Touya - Intraspecific variation in <i>Fusarium avenaceum</i> response to Hydrogen Peroxide-induced oxidative stress	10h10-10h30
Refreshment Break (Badiane Room)	
Kim Hammond-Kosack - Exploration of <i>Fusarium</i> interactions using the Pathogen-Host Interactions database (PHI-base)	11h00-11h20
Paulo Cezar Vieira - Metabolic profiling and chemodiversity of <i>Fusarium</i> species isolated from edible fruits using LC-MS/MS and molecular networking	11h20-11h40
Armelle Marais-Colombel - The virome of <i>Fusarium graminearum</i>	11h40-12h00
Christof Rampitsch - Identifying hubs in the wheat immune system as it reacts to <i>Fusarium graminearum</i> effector protein challenge using TurboID	12h00-12h20
Lunch (Badiane Room)	
Session 2: <i>Fusarium</i>, interactions with hosts and microbiota Chairs: -Dr David Overy, Agriculture & Agri-Food Canada, Ottawa, Canada - Dr Louis Carles, INRAE MycSA, France	13h40-17h40
Elizabeth K. Brauer - Plant sensing of fungal secondary metabolites – immunogenicity versus toxicity	13h40-14h10
Gerhard Adam - NX-producing <i>Fusarium</i> may have a selective advantage as the NX-3 toxin can escape two plant detoxification mechanisms	14h10-14h30
Lidija Bitz - Early gene response of moderately resistant oat (<i>Avena sativa</i> L. cv. <i>Akseli</i>) to <i>Fusarium culmorum</i> artificially inoculated in controlled condition	14h30-14h50
Giovanni Beccari - Deciphering the “priming” effect of enniatin B, deoxynivalenol and their combination on different wheat tissues toward <i>Fusarium</i> infection	14h50-15h10
Elsie Ayamoh Enow - Host-specific response of two <i>Asparagus</i> cultivars to <i>Fusarium proliferatum</i> strains	15h10-15h30
Refreshment Break (Badiane Room)	
Luca Sella - Endo-xylanases in <i>Fusarium graminearum</i> : Unraveling the complex interplay between virulence and genetic background	16h00-16h20
Trang Tran Minh - Endolevanase: a host-specific hidden weapon of <i>Fusarium graminearum</i> provoking Fusarium Head Blight in wheat	16h20-16h40
Clara Sanchez-Rodriguez - Pectin acetylsterases are key virulence factors in <i>Fusarium oxysporum</i> root colonization and disease progression	16h40-17h00
POSTER SESSION (Cloister Gallery)	17h00-19h00

Thursday 23

Session 2: <i>Fusarium</i>, interactions with hosts and microbiota (continue) Chairs: - Dr Anne Van Diepeningen, WUR, Netherlands - Dr Jean-Michel Savoie, INRAE MycSA, France	09h00-12h20
Marine Navarro - Fungal microRNAs: key players in interaction	09h00-09h30
David Overy - Recruiting the host to reshape the microbiome: Immune priming by <i>Fusarium avenaceum</i> during asymptomatic colonizatio	09h30-09h50
Louis Carles - Co-occurrence between <i>Fusarium</i> and bacteria in wheat grains – towards a better understanding of microbial interactions	09h50-10h10
Eleonora Cappelletti - Biological durum wheat seed priming for <i>Fusarium</i> root and crown rot management through the application of Bacillus and LAB strains	10h10-10h30
Refreshment Break (Badiane Room)	
Sophia Hein - Multiomics of <i>Fusarium</i> Head Blight disease in spring barley	11h00-11h20
Juho Hautsalo - Investigating oat resistance against <i>F. langsethiae</i> in Finland	11h20-11h40
James Tucker - Evaluation of two-row barley germplasms reveals accessions resistant to <i>Fusarium</i> head blight with low levels of deoxynivalenol	11h40-12h00
Sylvia Salamon - Cross-kingdom RNA interference in the wheat– <i>Fusarium culmorum</i> interaction: evidence from small RNA and degradome analysis	12h00-12h20
Lunch (Badiane Room)	
Session 2: <i>Fusarium</i>, interactions with hosts and microbiota (continue) Chairs: - Dr Florence Richard-Forget, INRAE MycSA, France - Dr Jens Laurids Sørensen, Aalborg University, Denmark	14h10-15h30
Thomas Svoboda - Cell cycle controls pathogenic processes and mycotoxin production in <i>Fusarium graminearum</i>	14h10-14h30
Gerlinde Wiesenberger - Culmorin inhibits detoxification of the mycotoxin deoxynivalenol by plant UDP-glucosyltransferases	14h30-14h50
Valentin Fiévet - Comparative analysis of the metabolomic profiles of seven <i>Fusarium</i> strains causing head blight, cultivated alone or together as a synthetic community called Meta- <i>Fusarium</i>	14h50-15h10
Wanxin Chen - Mutation of sucrose: fructan 6-fructosyltransferase (6-sft) in hexaploid wheat reduces susceptibility to fungal disease	15h10-15h30
Refreshment Break (Badiane Room)	



Thursday 23

Session 3: <i>Fusarium</i>, interactions with the changing environment Chairs: - Dr Ingerd Skow Hofgaard, NIBIO, Norway - Dr Adeline Picot, Brest University, France	16h15-17h45
Emmanuel Wicker - Reconstructing the worldwide emergence of the Tropical Race 4 (TR4) of the banana pathogen <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> using population genomics	16h15-16h45
Leonardo Lascala - Discovering of emerging pathogens belonging to <i>Fusarium</i> spp. in Italian wheat kernels	16h45-17h05
Shimosh Kurera - <i>Fusarium</i> identification, emerging pathogens and host wheat resistance in Canada	17h05-17h35
Sean Walkowiak - 30 Years of <i>Fusarium</i> damaged kernel incidence, severity, and pathogen diversity in Canada	17h35-17h45

20h00-00h00: Gala dinner Boarding for the dinner cruise at:

24 Quai des Chartrons, Bordeaux – face to Ibaïa Café / 44.84 87 58 N
| -0.57 00 15 W. Back to the pier at 22h30. Appointment recommended 15 minutes before the departure of the boat.



Friday 24

Session 3: <i>Fusarium</i>, interactions with the changing environment (continue) Chairs: - Dr Marie-Anne Garcia, INRAE EcoSys, France - Dr Matias Pasquali, University of Milan, Italy	09h00-13h00
Ludovic Bonhomme - Wheat responses to FHB under pre-anthesis drought: How extensively is the molecular dialogue reshaped?	09h00-09h30
Felix Hoheneder - Drought stress modulates <i>Fusarium</i> Head Blight severity in barley	09h30-09h50
Safa Oufensou - Distribution and prevalence of <i>Fusarium</i> crown rot pathogens of Durum wheat in Southern Italy	09h50-10h10
Ingerd Skow Hofgaard - Mathematical models to predict the risk of DON-contamination in Norwegian spring oats based on weather data	10h10-10h30
Elise Forgues - Impact of agroforestry systems on fungal populations in wheat crops	10h30-10h50
Refreshment Break (Badiane Room)	
Linda Felici - Chitosan-cellulose nanocrystals based formulation for <i>Fusarium</i> Head Blight protection: from lab to open field evaluation	11h20-11h40
Simon Edwards - Global assessment of HT2 and T2 occurrence	11h40-12h00
Ida Čurtović - Clinical and environmental sources of <i>Fusarium fujikuroi</i> species complex: Antifungal susceptibility and proteomic insights	12h00-12h20
Matthew G. Bakker - Investigating resistance to azole fungicides in <i>Fusarium graminearum</i>	12h20-12h40
Conclusion	12h40-13h00

Posters

The posters (A0, Portrait) will be exposed from Tuesday to Friday in the Cloister Gallery



Poster sessions on Tuesday 21 – 18h05-19h30, and on Wednesday 22 – 17h40-19h30.
The posters can also be viewed during the breaks and lunches

	Session 1: <i>Fusarium</i> genetics & physiology	
P1.1	Portable e-nose: an innovative tool for <i>Fusarium globosum</i> identification	Virgilio Balmas
P1.2	Emerging members of the <i>Fusarium tricinctum</i> species complex associated with <i>buxus sempervirens</i> decline in Northern Italy	Eleonora Cappelletti
P1.3	Phenotypic and genetic characterization of <i>Fusarium oxysporum</i> f. sp. <i>pisi</i> infecting <i>Pisum sativum</i> in Europe	Fredrik Dölfors
P1.4	In the genomes we trust: unveiling the genomic landscape within the Nectriaceae family	Veronica Ghionna
P1.5	Investigating the pathogenicity, population structure and morphology of <i>Fusarium oxysporum</i> strains infecting pea (<i>Pisum sativum</i> L.)	Tomas Hoogendam
P1.6	Characterization of <i>Fusarium graminearum</i> and <i>Fusarium boothii</i> originated from wheat and maize in Serbia	Ana Obradovic
P1.7	Prevalence and contribution of <i>Fusarium juglandicola</i> , a newly-described species, to walnut dieback	Adeline Picot
P1.8	<i>Fusarium graminearum</i> populations in Europe and globally	Tapani Yli-Mattila
P1.9	Investigating conserved D effector activity in <i>Verticillium dahliae</i> and <i>Fusarium oxysporum</i>	Ilaria Zannini
P1.10	Portable Real-Time PCR for Field-Based Monitoring of Mycotoxin-Producing Fungi in Cereals	Monika Szymańska-Czerwińska
P1.11	Development of a tool for <i>Fusarium</i> species identification isolated from cereal based on DNA barcoding	Mylène Ruh
P1.12	Association between chromatin accessibility and gene expression: machine learning approach. A whole-genome study on <i>Fusarium graminearum</i>	Mathilde Bruguet
P1.13	Critical opinion on the molecular approaches for the mycotoxigenic <i>Fusarium</i> species identification in wheat	Carol Verheeecke-Vaessen

	Session 2: <i>Fusarium</i> , interactions with hosts and microbiota	
P2.1	Interaction between wheat-associated bacteria and enniatin B: growth inhibition and biodegradation	Fojan Agahi
P2.2	First report of crown and foot rot on durum wheat caused by <i>Fusarium algeriense</i> in Europe	Daria Carella
P2.3	<i>Fusarium oxysporum</i> : evaluation of intraspecific molecular biodiversity among the different formae speciales	Daria Carella
P2.4	Associations between germination capacity and fungal infestation of wheat seeds	Birgitte Henriksen
P2.5	The Journey of Neocosmospora species – from Pipes to Patients	Marta Kozarić
P2.6	Targeting Fusarium Head Blight with synthetic peptaibol analogs: Efficacy and mode of action	Arianna Panichi
P2.7	Mycotoxins accumulated in <i>Asparagus officinalis</i> L. plants infected with <i>Fusarium oxysporum</i>	Łukasz Stępień
P2.8	Genotype-dependent <i>Fusarium</i> mycotoxin accumulation and its impact on early and late <i>Asparagus</i> infection stages	Łukasz Stępień
P2.9	Dissection of Fusarium Head Blight (FHB) resistance in spring wheat genotypes	Shayan Syed
P2.11	Effects of enniatin B as emerging mycotoxin and its association with deoxynivalenol on wheat microbiota	Matias Pasquali
P2.12	<i>Streptomyces coelicoflavus</i> BM40: A promising biocontrol agent against chickpea Fusarium wilt	Matias Pasquali
P2.13	Which <i>Fusarium</i> species cause crown rot on banana fruit in the Hawaiian Islands?	Matias Pasquali
P2.14	Antifungal and anti-mycotoxin capacity of extracts and compounds from selected strains of <i>Lentinula edodes</i> against <i>Fusarium verticillioides</i>	Jean-Michel Savoie
P2.15	Exploring new potential biological control agents (BCAs) for the management of Fusarium head blight in durum wheat: <i>in vivo</i> screening of bacterial strains	Giovanni Beccari

	Session 3: <i>Fusarium</i> interactions with the changing environment	
P3.1	The EvolTox project wrap-up: how global change is reshaping fusarium and mycotoxin risks in wheat	Marie Foulongne-Oriol
P3.2	Effects of seed treatment, sowing time, and cultivar on <i>Microdochium nivale</i> infection and in winter wheat	Aurimas Sabeckis
P3.3	Geographic distribution of trichothecene chemotypes of the <i>Fusarium graminearum</i> species complex from maize kernels in Serbia	Milica Lucev
P3.4	Impact of cob orientation and husk coverage on field-level mycotoxin accumulation in maize	John F. Leslie
P3.5	Incidence of Fumonisin B1 in small grains in Serbia	Slavica Stankovic
P3.6	Surveillance of emerging fusariotoxins in the food chain in France: presentation of FUSÉ, a working group of the SCA Platform	Margot Bärenstrauch
P3.7	Diversity of <i>Fusarium</i> species and their mycotoxins in major cereals	Manuela Zadavec
P3.8	Non-host colonization by <i>Fusarium oxysporum</i> : common weeds as potential disease reservoirs	Alarik A.D. van Diepeningen
P3.9	Ambrosia (2024-2027): Bridging Knowledge, Communication, and Action for Food Safety in a Changing Climate	Carol Verheecke-Vaessen
P3.10	Non-destructive detection of deoxynivalenol and zearalenone in individual oat grains using spectroscopy	Carol Verheecke-Vaessen

Oral presentations abstracts

How fungal pathogens manipulate host microbiota to establish infection

Bart Thomma

University of Cologne, Cologne, Germany



Fungal effectors evolved from ancient antimicrobials to suppress plant immunity and shape microbiota for infection.

Abstract

The evolutionary history of fungal effector proteins -molecules secreted by pathogens to help them colonize plants- is still not well understood. Traditionally, most known effectors have been studied for their ability to interfere with plant immune systems, allowing the fungus to avoid detection or suppress plant defenses. However, more recent research shows that some effectors also act in a very different way: they can influence or attack the community of microbes living on and inside plants, the so-called plant microbiota.

We recently developed a computational tool called Antimicrobial Activity Predictor for Effector Candidates (AMAPEC) to search for effector proteins with antimicrobial properties. Using this approach, we discovered a surprisingly large number of effectors that can inhibit or kill other microbes. Many of these antimicrobial effectors are highly conserved across fungal species, suggesting they have very ancient evolutionary origins. Even more intriguingly, we found that several effectors previously known only for their role in suppressing plant immunity also possess antimicrobial activity. This points to the possibility that such proteins originally evolved as antimicrobials and later acquired the additional ability to manipulate host immune responses, while still retaining their ancestral function.

Taken together, our findings support the idea that antagonism toward microbes is not just an occasional feature, but a fundamental function of fungal effectors. We propose that during the long evolutionary arms race between plants, their pathogens, and the surrounding microbiota, fungi may have repurposed ancient antimicrobial proteins into versatile effectors that can simultaneously weaken plant defenses and shape the plant's microbial community to favor infection.

How does *Fusarium musae* adapt to human and banana hosts?



Matias Pasquali¹, Luca Degradi^{1*}, Valeria Tava^{1,3*}, Daniela Bulgari¹,

Andrea Kunova¹, Marco Saracchi¹, Paolo Cortesi¹, Matthias Brock², Greetje Vande Velde³

¹ Defens- University of Milan, Milan, Italy

² School of Life Sciences, University of Nottingham, Nottingham, UK

³ Department of Imaging and Pathology, KU Leuven, Leuven, Belgium

*equal contribution

***Fusarium musae* is a cross-kingdom pathogen that infects both fruits and humans; genomic and transcriptomic data help decipher its adaptability to diverse hosts.**

Abstract

Fusarium musae Van Hove is a fungal pathogen responsible for crown rot in bananas and clinical infections in humans. Genome analysis of 18 strains from diverse geographic and host origins (banana and human patients) revealed gene and genome variations. Two reference genomes from strains isolated from human and banana were assembled and annotated by implementing RNAseq data from different growth conditions.

Mitochondrial haplotype analysis revealed genetic homogeneity among plant and human isolates, supporting potential cross-host transmission. Infection studies demonstrated the ability of *F. musae* to grow at both 24°C and 37°C, while strain-dependent pathogenicity was observed on banana fruits and in *Galleria mellonella* larvae used as a surrogate model for animal infection.

RNA sequencing of a virulent strain on both hosts, grown on blood and banana peel media, identified a diverse effector repertoire, whereas RNAseq performed from *G. mellonella* infection highlighted specific effectors associated with animal colonisation by the fungal species. These findings underscore the trans-kingdom pathogenic potential of *F. musae* and its genomic adaptability. This adaptability is likely driven by active transposable elements, which drive overexpression of some key infection-related genes. This study offers a comprehensive framework for understanding novel fungal threats to human health and food security by integrating genomic, transcriptomic, and infection biology approaches, stressing the value of the One Health paradigm in framing research questions.

References

- Tava, et al. (2025). *Fusarium musae* Infection in Animal and Plant Hosts Confirms Its Cross-Kingdom Pathogenicity. *Journal of Fungi*, 11(2), 90.
- Degrad, et al. (2022). Exploring mitogenomes diversity of *Fusarium musae* from banana fruits and human patients. *Microorganisms*, 10(6), 1115.
- Degrad, L. et al. (2021). Telomere to telomere genome assembly of *Fusarium musae* F31, causal agent of crown rot disease of banana. *Molecular Plant-Microbe Interactions*, 34(12), 1455-1457.

Deciphering trophic shifts in *Fusarium verticillioides*: A mycotoxin story

MOBARAK Térance¹, GROPPI Emie¹, GADEA Alice¹, VANSTEELAND

Marieke¹, CRISTOFOLI Valérie¹, HADDAD Mohamed¹



¹ UMR 152, PharmaDev, IRD/Université de Toulouse, France

This study, which forms the basis of the DYNAMycs project, highlights how cereal-based substrates shape mycotoxin production by *Fusarium verticillioides*, with important implications for food safety and the molecular understanding of its trophic transitions.

Abstract

Fusarium verticillioides (Fv) is a major phytopathogenic fungus of cereals, particularly maize, its main host, and can cause substantial economic losses. This fungus has the ability to live as an endophyte and, at specific stage of its or its host's life cycle, shift to a necrotrophic pathogen. It also represents a serious health risk to humans and animals due to its ability to produce mycotoxins, such as fumonisins, which are harmful even at low doses when chronically ingested [1]. This fungus is commonly found in soil but can also occur in infected seeds, plant debris, food and feed [2].

To explore the influence of plant substrate on mycotoxin production by Fv, we performed combined metabolomic and transcriptomic analyses on fungal cultures grown on Poaceae-based media (corn, millet, rice, and sorghum flours mixed with agar) [3]. These experiments revealed that the nature and relative abundance of produced mycotoxins, as well as the regulation of their biosynthetic pathways, differ significantly depending on the nutritional source. Molecular networking also suggested the presence of non-regulated mycotoxin derivative potentially contributing to the toxic potential. This work lays the foundation for the DYNAMycs project (ANR-24-CE34-4957, 2025–2027), which aims to deepen our understanding of substrate-dependent mycotoxin biosynthesis. Beyond risk assessment for food safety, this project also seeks to elucidate the molecular determinants of trophic transitions in this pathogen, from endophytism to necrotrophy.

References

- [1] K. A. Voss, G. W. Smith, and W. M. Haschek, 'Fumonisin: Toxicokinetics, mechanism of action and toxicity', *Anim Feed Sci Technol*, vol. 137, no. 3–4, pp. 299–325, 2007, doi: 10.1016/j.anifeedsci.2007.06.007.
- [2] A. A. Blacutt, S. E. Gold, K. A. Voss, M. Gao, and A. E. Glenn, 'Fusarium verticillioides: Advancements in understanding the toxicity, virulence, and niche adaptations of a model mycotoxigenic pathogen of maize', *Phytopathology*, vol. 108, no. 3, pp. 312–326, 2018, doi: 10.1094/PHYTO-06-17-0203-RVW.
- [3] E. Groppi, M. Haddad, V. Cristofoli, M. Vansteelandt, and A. Gadea, 'Unveiling the substrate-dependent dynamics of mycotoxin production in *Fusarium verticillioides* using an OSMAC- metabolomics approach', *Chem Bio Divers*, vol. 202401747, pp. 1–9, 2024, doi: 10.1002/cbdv.202401747.

The pan-genomic effectome of the *Fusarium sambucinum* species complex reveals novel virulence factors

Chris Blackman^{1,3}, Tian Lei^{2,3}, Margaret Balcerzak³, Alexia

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Rajagopal Subramaniam^{1,2,3}



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³ Ottawa Research and Development Centre, Agriculture and AgriFood Canada, 960 Carling Ave., Ottawa, ON, K1A 0C6

Novel, core effectors of the *Fusarium sambucinum* species complex contribute to virulence and modulate host immunity in a cultivar-specific fashion.

Abstract

The *Fusarium sambucinum* species complex (FSAMSC) comprises prominent phytopathogenic fungi that collectively place a severe burden on global agriculture¹. Understanding the interaction between host plants and fungi is crucial to delineate mechanisms of plant defence and mitigate agricultural losses. Many phytopathogens deploy suites of secreted proteins, called effectors, to modulate host immunity and promote colonization; however, fungal phytopathogenic effectors are poorly understood in comparison to those of bacterial phytopathogens. To promote characterization of FSAMSC effectors, we developed a pan-genomic effectome for this species complex using a combination of bioinformatics tools. Our pipeline led to the prediction of 125,777 candidate-secreted effector proteins (CSEPs) from 195 isolates, including 235 conserved (“core”) effector families. A subset of core families demonstrated diversifying selection, and we hypothesized that these epitopes may be critical to host recognition and are thus likely to interact with host defence factors². We developed structural comparisons to known fungal effectors using AlphaFold and Foldseek, and employed multiple virulence assays to demonstrate effector-like, and immune-eliciting function for a subset of conserved effectors. Host interaction partners of fungal effectors will be identified via proximity-based labelling with TurboID, to ultimately resolve a plant-pathogen interaction network.

References

1. Laraba, I., McCormick, S. P., Vaughan, M. M., Geiser, D. M., & O'Donnell, K. (2021). Phylogenetic diversity, trichothecene potential, and pathogenicity within *Fusarium sambucinum* species complex. *PLoS ONE*, 16(1 January). <https://doi.org/10.1371/journal.pone.0245037>
2. McCann, H. C., Nahal, H., Thakur, S., & Guttman, D. S. (2012). Identification of innate immunity elicitors using molecular signatures of natural selection. *Proceedings of the National Academy of Sciences of the United States of America*, 109(11), 4215–4220. <https://doi.org/10.1073/pnas.111389310>

Quantitative genetics approach to decipher life history traits in *Fusarium graminearum*

Marie Foulongne-Oriol¹, Antoine Vajou¹, Nils Hocquemiller¹, Thomas Dumartinet¹, Benoit Laurent¹

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Quantitative genetics, through QTL mapping or GWAs, offers powerful insights into the genetic basis of *Fusarium graminearum* key complex traits, from metabolism to pathogenicity.

Abstract

In *Fusarium graminearum* (Fg), numerous phenotypic traits exhibit a quantitative variation within populations, suggesting a polygenic genetic determinism. Understanding such complex traits is essential for elucidating fungal biology. Notably, we need to consider how genetic influences a broad spectrum of traits across its entire life cycle and in response to diverse environmental interactions, at various scale, from cells to fields. Among others, metabolism efficiency, resource allocation, fitness, growth, sporulation, mycotoxin production, stress tolerance, aggressiveness should be prioritized for study as they directly underpin the pathogen's survival, reproduction, and capacity to cause disease in crops. Beyond deciphering the molecular mechanisms underlying these traits, unravelling the genetic architecture, in terms of the number of involved loci, their genomic location, their relative effects, and their possible interactions is essential to anticipate the pathogen evolvability. All these questions are addressed through quantitative genetic approaches based on association between genotype and phenotype variation, measured in recombinant population. Two major strategies have been developed: classical quantitative trait locus (QTL) mapping based on experimental population derived from crosses and genome wide association studies (GWAS) using natural populations. We will draw an overview of the quantitative genetics frameworks we have implemented in our team over the past decade to investigate Fg biology, and point out successes and failures through concrete examples ^[1,2]. We will particularly emphasize on the added value of the genomics and the deployment of other omics phenotyping in the renewal of such classical genetic approach. Finally, we will explore further prospective research avenues to advance our understanding of this pathogen and ultimately develop more effective management strategies.

References

1. Laurent et al. (2021) QTL mapping in *Fusarium graminearum* identified an allele of FgVe1 involved in reduced aggressiveness. *Fungal Genet Biol.* 153:103566. doi: 10.1016/j.fgb.2021.103566.
2. Benoit Laurent, et al. (2021). QTL mapping in *Fusarium graminearum* identified an allele of FgVe1 involved in reduced aggressiveness. *Fungal Genetics and Biology*, 153
3. Vajou et al. Phenotypic plasticity in the fungal pathogen *Fusarium graminearum* is involved in the adaptive response to changing environments. *In prep.*

Phylogenetic analysis of a 139.9-K B 52-gene dataset to reassess diversity within *Avenaceum* clade of *Fusarium tricinctum* species complex



Tapani Yli-Mattila¹, Tatiana Gagkaeva², Imane Laraba³ and Robert Proctor⁴

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Phylogenetic analysis of a 52-gene dataset from multiple members of the *Fusarium tricinctum* species complex (FTSC) resolves the *Avenaceum* clade into seven phylogenetically distinct species, including two species within *F. avenaceum sensu stricto* (= FTSC 4)

Abstract

Within the *Fusarium tricinctum* species complex (FTSC), *Fusarium avenaceum* occurs on diverse plant species and can contribute to *Fusarium* head blight of cereal crops. In addition *F. avenaceum* isolates from one plant species can be pathogenic on other distantly related plants (Nalim et al. 2009). Using partial sequences of TEF1 and TUB2, Yli-Mattila et al. (2022) resolved multiple isolates of *F. avenaceum* and morphologically similar isolates into the *Avenaceum* clade, which included four subclades designated Main Groups I, II, III and IV as well as multiple isolates that did not group with any of these Main Groups. More recently, Laraba et al. (2022) proposed that FTSC consists of 36 species and gave the species the ad hoc designations FTSC 1 – FTSC 36. Here, we used phylogenetic analyses of full-length sequences of 52 housekeeping genes to match Main Groups and FTSC species. The results indicate that FTSC 4 corresponds to Main Groups I and II, FTSC 11 and 27 correspond to Main Group III, and FTSC 5 corresponds to Main Group IV. In addition, the results indicate that the *Avenaceum* clade includes at least six phylogenetically distinct species: FTSC 4 (*F. avenaceum sensu stricto*), FTSC 5 (*F. paeoniae*), FTSC 11, FTSC 22, FTSC 30, and FTSC 34, but also that the FTSC4 was resolved into two phylogenetically distinct species. One of these phylopecies consisted of two Main Group II isolates, while the other consisted of Main Group I isolates and one Main Group II isolate from the Yli-Mattila et al. (2022) study. These seven species formed a well-supported clade that was distinct from *F. acuminatum*, *F. tricinctum*, *F. torulosum*, and *F. flocciferum*. These results clarify species diversity within the *Avenaceum* clade

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Intra- and interspecific diversity of *Fusarium* spp. responsible for Fusarium Head Blight based on metabarcoding and population genetics

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By studying *Fusarium* diversity at both intra- and interspecific levels in maize residues and wheat heads throughout the wheat cycle, we found that *F. graminearum* was the predominant species associated with FHB and displayed very high levels of genetic diversity, with high gene flow and low genetic differentiation across substrates and geographic locations in Brittany, suggesting the existence of a single metapopulation.

Abstract

Fusarium Head Blight is a devastating disease of cereals, mainly caused by a complex of *Fusarium* species (Fsp). Despite being primary inoculum sources, Fsp diversity and dynamics in soils and residues have been less studied than in grains, while the importance of long-distance airborne spores vs. residue-borne spores in inoculum dispersal is still under debate. The objectives of this study were to i) decipher Fsp diversity and dynamics in maize residues, soil and wheat heads over the wheat cycle; and ii) determine the extent to which strains isolated from residues contribute to head contamination. Six minimal tillage wheat fields, with maize as previous crop, were monitored over two consecutive years. Soils, maize residues, and wheat grains were collected at four stages throughout the wheat cycles. Among the 31 species identified through metabarcoding sequencing of the EF1 α region, grains were dominated by *F. poae* (Fp), *F. graminearum* (Fg) and *F. avenaceum* (Fa), with the former being associated with very low disease pressure and mycotoxin levels. Residues were mainly contaminated by Fg and Fa, with low presence of Fp. To further investigate inoculum transfer from residues to heads, the diversity and structure of the Fg population were then studied on 442 and 146 isolates from residues and grains, respectively, using a sequence-based microsatellite genotyping method targeting 34 markers. We found high genetic diversity, with 453 distinct multilocus genotypes, and no significant genetic differentiation by geographic location or substrate, suggesting the presence of a single, regionally-distributed metapopulation. The absence of substrate genetic differentiation supports the role of residue-borne inoculum in FHB infection. However, 60-97% of grain-associated genotypes were not found in residues, suggesting that our sampling depth was insufficient and/or that airborne inoculum also contributed to disease dissemination. Altogether, our approach provided insights into FHB epidemiology at community and population levels, taking into account intra- and interspecies diversity, while investigating inoculum source and monitoring pathogens at the field scale.

Nit Mutants in *Fusarium*

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Abstract

Nit (nitrate non-utilizing) mutants can be generated spontaneously in many strains of *Fusarium* and have been used for genetic mapping and forcing heterokaryon formation since the 1980s. Non-functional mutants are generated by culturing on minimal medium + KClO₃ + minimal levels of an amino acid that will support growth of mutant strains. Most of the work has been done in *F. verticillioides*, but the genetic basis of these mutants was never investigated. We evaluated 120 spontaneous mutations that inactivate the nitrate reductase (*nit-1*) gene of *Fusarium verticillioides* that occurred while the fungus was growing on media containing 1.5% KClO₃. We sequenced the gene from 116 mutant strains and found that 102 sites were altered. There were four mutants with mutations at two different sites. Of the mutants with changes at a single site, 69% had a single nucleotide polymorphism (SNP) change, 11% had small insertions, and 19% had small deletions. Most of the insertion and deletion events resulted in frame-shifts and presumptive premature translation termination. Of the SNP mutants, 38% resulted in a premature stop codon, 26% resulted in an amino acid change near a site known to be essential for enzyme activity, and 36% resulted in an amino acid change at a site not previously known to be essential for enzyme activity. The distribution of the mutations within the *nit-1* gene and the limitation of most of the mutations to SNPs or to small insertions or deletions are both consistent with errors in DNA replication and/or repair serving as the causal agent. Such errors could enable *F. verticillioides* to quickly and permanently respond to environmental stresses and help explain how these plant pathogenic fungi can evolve, adapt and survive in rapidly changing and challenging environments. We expanded our studies beyond *F. verticillioides* to include 179 strains from 68 different *Fusarium* species to determine if the mutagenesis process observed in *F. verticillioides* was common across the genus. We tested strains for ability to form *nit* mutants on PDA and minimal media amended with one of four amino acids – arginine, asparagine (used for *F. verticillioides*), glutamine, and proline. There were no general patterns observed. Ten percent of the strains produced no mutants on any media, and others produced a mutant in every sub-cultured colony. Strains within the same species could differ significantly from one another. Mutations are the driving force for adaptation to changing or toxic environments and create the genetic diversity required for species evolution. Our results suggest that some species and strains mutate more readily than others and could help explain the variation and degeneration of some strains of these species when repeatedly sub-cultured under laboratory conditions or when they encounter novel environmental challenges under field conditions.

Epigenetic control of effector proteins secreted by *Fusarium graminearum*

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***Fusarium graminearum* effector proteins exhibit dynamic epigenetic patterns during infection.**

Abstract

Fusarium graminearum (*Fg*) is a highly destructive fungal pathogen of wheat, responsible for annual losses of 28 million tonnes of wheat grain globally [1]. A key factor in its pathogenicity lies in the action of *effectors*; small, secreted proteins that suppress host plant immune responses and facilitate infection. Advancing our understanding of effector expression and function is critical for developing effective and sustainable control strategies.

In this study, we curated a database of predicted *Fg* effector proteins, annotating their conservation across strains and predicted subcellular localisations. To investigate the role of epigenetic regulation of these effectors during infection, we integrated published datasets on the histone modification landscape of *Fg* under infection-like conditions [2, 3]. Our analysis revealed that predicted effector-encoding genes are enriched in H3K27me3, an epigenetic marker associated with gene repression.

To validate this finding under more infection-relevant conditions, we generated the first CUT&Tag dataset for *Fg* during wheat infection, confirming the enrichment of H3K27me3 at effector loci during host infection. This dataset covered a time course of infection and shows dynamic changes in those genes marked by H3K4me3 and H3K27me3 at different infection timepoints. These results highlight the role of epigenetic regulation in controlling effector expression and suggest that histone modifications may influence infection dynamics. In summary, our work provides valuable insights into the regulation and function of fungal effectors, while establishing a novel approach for epigenetic analysis in *Fg*-infected wheat.

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Identification of the phenalenone biosynthetic pathway for perithecial pigmentation in the *Fusarium solani* species complex



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Perithecial pigmentation in the *Fusarium solani* species complex is mediated by phenalenones and corymbiferan lactones.

Abstract

The *Fusarium solani* species complex (FSSC) comprises some of the most important pathogens of plants and humans. The taxonomic status of the FSSC has been highly discussed in recent years where a segregation out of the genus *Fusarium* and into *Neocosmospora* has been argued (Crous et al., 2021) and subsequently counterargued (Geiser et al., 2021). One of the features that separates the FSSC from the remaining members of the *Fusarium* genus is pigmentation. Aurofusarin and bikaverin are the primary mycelium pigments in the genus, while fusarubins and the nitrogen-derived bostrycoidin are produced in perithecia. However, in FSSC species fusarubins and bostrycoidins are used for mycelial pigmentation and the perithecial pigments have remained unidentified.

The responsible gene cluster behind perithecial pigmentation is present in all FSSC members and to unravel the puzzle we used a combination of heterologous expression in baker's yeast *Saccharomyces cerevisiae* and deletion/overexpression in *Fusarium vanettenii* (Nielsen et al., 2024). With this approach we identified prephenalenone as first step in the biosynthetic pathway, which is produced by the polyketide synthase *fsnI* (PKS35). We next identified a conserved cluster of 10 genes flanking *fsnI* in *F. vanettenii* controlled by an internal transcription factor (*fsnC*). Overexpression of *fsnC* induced production of several known compounds, including phenalenone and corymbiferan lactone E. We also isolated a new compound that we named corymbiferan lactone F, which we propose is the final pigment of the biosynthetic pathway for perithecial pigmentation in the FSSC.

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Chromosome-Level Genome Assembly and Functional Annotation of *Fusarium musae* from a Human Isolate: Insights into Host-Specific Infection Mechanisms

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The first chromosome-level genome assembly of *Fusarium musae* reveals condition-specific gene expression and potential horizontally transferred elements, offering new insights into its host-specific infection strategies.

Abstract

A comprehensive genome assembly, annotation, and expression analysis of a *Fusarium musae* IUM11-0508 clinical isolate was conducted to better understand the genomic basis of host specificity and pathogenicity. Hybrid assembly produced a 45 Mb genome organized into 12 chromosomes and two additional unplaced contigs, putatively representing minichromosomes. In addition, the mitochondrial genome was assembled and annotated.

Fusarium musae IUM11-0508 was grown on three different media: Blood (Blood Sheep Medium to simulate human bloodstream conditions), FDM (Fusarium Defined Medium), and BPE (Banana Peel Extract to mimic the natural environment). RNA-Seq data obtained from these conditions improved the initial nuclear annotation, increasing the number of predicted coding sequences (CDS) from 13,875 to 15,703 (a 13% increase), and highlighted condition-specific gene expression patterns potentially contributing to environmental adaptation.

Moreover, to investigate virulence genes, gene expression was analyzed during *F. musae* infection of *Galleria mellonella* larvae, a widely used in vivo model for human fungal infections. This infection-based transcriptomic profiling confirmed the expression of 754 previously annotated genes and identified approximately 117 additional putative CDS uniquely expressed during infection.

The genome was also examined for transposable elements, revealing a specific element present at four distinct loci. Notably, several genes adjacent to these elements showed high sequence similarity to genes from non-*Fusarium* species, suggesting possible horizontal gene transfer events that may have introduced novel functions related to pathogenicity or adaptation.

This work presents the first comprehensive genomic resource for *F. musae*, revealing key features of its genome structure, gene expression, and host-specific adaptations. It provides a valuable foundation for future research into fungal pathogenesis and the evolution of this emerging pathogen.

Intraspecific variation in *Fusarium avenaceum* response to Hydrogen Peroxide-Induced Oxidative Stress

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This work highlights the importance of phenotypic diversity in *Fusarium avenaceum* regarding the response to H₂O₂-induced oxidative stress, as well as emphasizes the need to work on a panel of strains and not a model strain to better account for this variability.

Abstract

Oxidative stress, primarily triggered by host-generated reactive oxygen species (ROS), is a pivotal factor shaping plant-pathogen interactions. As such, pathogens must effectively counteract ROS to ensure their survival and pathogenicity. *Fusarium avenaceum*, a notorious fungal pathogen of cereal crops, is one of the main producers of enniatins, a class of emerging mycotoxins posing threat to human and animal health. In the recent years, several studies have pointed out the considerable intraspecific diversity in *Fusarium avenaceum* both genetically and phenotypically, influencing its adaptability and pathogenic potential.

In this study, we investigate the response of multiple *F. avenaceum* isolates to hydrogen peroxide (H₂O₂), a key ROS molecule involved in host defence signalling. 12 *F. avenaceum* strains were grown on both liquid and solid media supplemented or not with H₂O₂, and were assessed for growth and mycotoxin production. *F. avenaceum* sensitivity to H₂O₂ was found to be highly strain-specific. Exposure to H₂O₂ led to a reduction in growth consistent with a delay in spores' germination, and enniatin production was either enhanced or reduced depending on the isolate. These data were also supported by a transcriptomics analysis on enniatins biosynthetic genes. Moreover, oxidative stress-related genes were also found to be poorly activated by H₂O₂, consistent with the high sensitivity of *Fusarium avenaceum* isolates to oxidative stress in regard to other *Fusarium* species. Comparison of enzymatic equipment related to oxidative stress response is ongoing, and could help better understanding these differences in H₂O₂-response between *F. avenaceum* strains.

These findings highlight the complex interplay between genotype and stress response in *F. avenaceum*. Understanding the oxidative stress response of *F. avenaceum* contributes to deciphering its pathogenic mechanisms, and could potentially lead to targeting specific vulnerabilities in integrated disease management strategies.

Exploration of *Fusarium* interactions using the Pathogen-Host Interactions database (PHI-base)

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Speed up your *Fusarium* research by using PHI-base. Increase the visibility and FAIRness of your *Fusarium* peer reviewed data by author curation into PHI-base .

Abstract

PHI-base is a multispecies gene to phenotype database that contains curated information from peer-reviewed research articles investigating host–pathogen interactions, 1st host targets and fungicide targets. PHI-base is freely accessible at www.phi-base.org and phi5.phi-base.org. PHI-base provides comprehensive pathogen-host interaction and FAIR data (Findable, Accessible, Interoperable, Reusable) (Wilkinson et al., 2016) for both wet lab and computational biologists. PHI-base details the phenotypic effects on hosts and pathogens when genes are deleted or mutated.

Information on phenotypes, protein function, resistance to chemicals, 1st host targets and other data types are extracted by biocurators and authors using our community curation tool, PHI-Canto (canto.phi-base.org; Cuzick et al., 2023), or added using our bulk data upload tool.

Our latest PHI-base 5 release (version 5.0, as of May 2025) provides information on ~275 pathogens, ~220 hosts covering > 28,000 interactions, > 8,600 genes curated from > 4,700 peer reviewed articles. PHI-base 5 displays detailed phenotype data and ontologies such as GO and PHIPO in a gene-centric manner, i.e. all information on a pathogen or host gene are located on a single page for easy of comparison and use.

All *Fusarium* species explored using molecular genetic approaches are in PHI-base - total 1798 interactions, and 653 genes from 10 species. These have diverse genomes, lifestyles and host interactions (Armer et al., 2024) The highest entries are for *F. graminearum* (1045 interactions, 389 genes), *F. oxysporum* (8 *forma specialis*) (515 interactions, 168 genes), and *F. verticillioides* (129 interactions, 53 genes).

Here we describe the different functionalities of our new web portal and its integration with other databases such as Ensembl, FungiDB and UniProt. We also show various PHI-base use cases for *Fusarium* research include shortening the research discovery process, annotation of RNAseq datasets and new genomes/pangenomes, generating graphical knowledge networks, and identifying new intervention control points.

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Metabolic Profiling and Chemodiversity of *Fusarium* Species Isolated from Edible Fruits Using LC-MS/MS and Molecular Networking

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Integrated LC-MS/MS, NMR, and molecular networking analyses highlight the metabolic diversity of *Fusarium* complexes isolated from papaya, pineapple, and persimmon, emphasizing the importance of underexplored ecological niches in fungal metabolomics.

Abstract

The exploration of phytopathogenic fungi from edible fruits represents a promising strategy for discovering novel sources of bioactive metabolites. In this study, fungi were isolated from papaya (*Carica papaya*), pineapple (*Ananas comosus*), and persimmon (*Diospyros kaki*) fruits. Among the isolates, six *Fusarium* strains were identified through genetic sequencing and phylogenetic analysis, belonging to the *F. solani*, *F. fujikuroi*, and *F. incarnatum-equiseti* species complexes.

Fungal cultures were grown on autoclaved rice for 21 days, followed by extraction with ethyl acetate. The chemical profiles of the extracts were comprehensively analyzed using ¹H NMR and LC-MS/MS, with molecular networking performed via the GNPS platform. Our analyses revealed characteristic *Fusarium* metabolites, including fusaric acid and its derivatives, hymeglusin, fusaridic acid A and its derivatives, fumonisins, beauvericins, bostrycoidin, and equisetin. Molecular networking revealed distinct chemical profiles among the various *Fusarium* complexes, highlighting both unique metabolites specific to certain groups and shared compounds among them. These observations were supported by principal component analysis (PCA), which confirmed chemical separation between the analyzed complexes.

The *Fusarium* genus is renowned for its wide distribution, adaptability, and production of mycotoxins that negatively impact various crops, causing significant losses in food production. Notably, our NMR and LC-MS/MS analyses provided complementary data, with NMR revealing major constituents and LC-MS/MS enabling detection of trace secondary metabolites. This multi-platform approach allowed for comprehensive metabolic profiling of the *Fusarium* strains.

Investigating *Fusarium* chemodiversity from edible fruit sources contributes to understanding the genus's metabolic diversity and reveals its biotechnological potential. The identification of both known phytotoxins and potentially novel metabolites underscores the importance of studying fungal chemical ecology in under-explored niches. These findings provide a basis for future studies on fungal secondary metabolism and its applications in agriculture and drug discovery.

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The virome of *Fusarium graminearum*

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***Fusarium graminearum* hosts mycoviruses. When these viruses are cured, biological traits are affected.**

Abstract

Fusarium head blight (FHB) of wheat is a particularly worrying disease in Europe, caused mainly by the filamentous fungus *Fusarium graminearum*. In addition to potentially dramatic yield losses associated with bleached and shriveled grains, the situation is aggravated by the capacity of *F. graminearum* to produce the mycotoxins type B trichothecenes, which are secondary metabolites with proven toxic effects for humans and animals when ingested. These mycotoxins are produced in the developing grains and persist until the finished cereal product. It is therefore imperative to prevent contamination to ensure the sanitary quality of cereals. Different strains of *F. graminearum* differ in their abilities to produce toxins, and their aggressiveness in plant. Recently, the presence of mycoviruses has been proposed as a factor that can modulate these traits. Using a metagenomic approach, 20 *F. graminearum* strains were screened for the presence of mycoviruses. Globally, six mycoviruses were identified, two of which are potentially novel viruses, belonging to the *Botourmiaviridae* and *Mitoviridae* families. The majority of strains included in the study were found to be virus-free, whereas six were infected by at least one mycovirus. Multi-infection situations were relatively rare, with only 30% of the infected strains harboring two or three mycoviruses in co-infection. In order to evaluate the impact of mycoviruses on the fungal host biology, efforts have been undertaken to cure the infected strains. The comparison of biological traits such as growth rate, sporulation rate, germination rate and mycotoxin production between infected and cured *F. graminearum* strains will be presented and discussed.

Identifying hubs in the wheat immune system as it reacts to *Fusarium graminearum* effector protein challenge using TurboID.



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TurboID is emerging as a powerful technique for indentifying protein-protein interactions. We are using it to find host immune proteins critical in host-pathogen interactions.

Abstract

The overall aim of this project is to identify hub proteins in the wheat immune system. Hubs represent important components of host immunity and are therefore ideal targets for pathogen effectors. To define these hubs, we first identified core candidate effector proteins from *Fusarium graminearum* (FG). Next we tagged a subset of these effector candidates with the modified biotin ligase, TurboID, by creating in-frame fusion protein constructs. We are using a heterologous system to deliver these tagged effector candidates to susceptible wheat to identify interacting host proteins. TurboID exploits a promiscuous biotin ligase which, when coupled to bait proteins (the FG effectors), labels interacting proteins by covalently attaching a biotin residue on available free amines (i.e. Lys, N-terminus). The biotinylated targets are recovered by affinity chromatography and subsequently identified by mass spectrometry in standard LC-MS runs. Proteins are quantified based on peptide precursor ion intensity with potential interactors showing significantly greater abundance. We have been optimizing both the biological and the mass spectrometry aspects of this experiment and as proof of concept, we have identified a set of interacting proteins targeted by two effectors localized to the nucleus. The putative interactions have been corroborated with Alpha-Fold 3. Our most recent findings will be presented.

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Plant sensing of fungal secondary metabolites

– immunogenicity versus toxicity.

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We used a systems approach to evaluate plant responsiveness to microbial secondary metabolites including Fusarium mycotoxins.

Abstract

Fusarium species deploy secondary metabolite to acquire nutrients, compete with other microbes and influence their plant host. Plants can detect structurally diverse microbial secondary metabolites to activate stress responses through unknown molecular mechanisms. For example, gramillin induces ROS production, transcriptional changes and callose production at non-toxic doses (Brauer et al., 2024). These metabolite-induced plant responses have recently been shown to contribute to microbial resistance or susceptibility during infection indicating that a better understanding of the phenomenon is warranted (Brauer et al., 2024, Thoms et al., 2023). To characterize the potential breadth of these responses, we screened a set of over a hundred microbial secondary metabolites to determine which compounds induced ion leakage, peroxidase activity and callose production in Arabidopsis leaves. We also tested the compounds for their ability to suppress flagellin-induced immunity to find potential suppressors of plant defense. The trichothecenes suppressed inducible immunity as well as peroxidase activity due to their toxic effect on plant cells. The cyclic lipopeptides surfactin and gramillin induced all of the stress responses and enhanced some flagellin-induced responses. The remaining metabolites could be grouped by their impact on plant cellular responses accordingly, having an stimulatory or inhibitory effect on inducible stress responses. Interestingly, the metabolites' impact on each of the stress responses did not predict impact on the other stress responses indicating independence of the associated pathways. Overall, we present an overview of the complex interactions between plants and microbial secondary metabolites, and identify a novel function for several of these metabolites in plants.

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NX-producing *Fusarium* may have a selective advantage as the NX-3 toxin can escape two plant detoxification mechanisms

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DON but not NX-3 can be detoxified by glutathione-S-transferases by formation of the DON-10-GSH adduct, and by novel plant UDP-glucosyltransferases forming DON-8,15-hemiketal-7-glucoside.

Abstract

The spread of emerging *Fusarium graminearum* populations and NX mycotoxins poses significant risks to agriculture, food supply, and economic stability in Canada and globally” was recently claimed by Canadian group [1]. A study on *F. graminearum* strains from wheat in Argentina reported the first occurrence of NX-producers in South America, stating that “From the total evaluated strains, 82% produced NX-2 and 54.5%, NX-3.” [2]. We propose that release of intermediates of DON biosynthesis is not a novel phenomenon, but is mainly an *in vitro* artefact occurring when *TRI1* is expressed at low levels so that C8-hydroxylation is partly skipped. While true NX-producers, so far endemic in North America, produce no DON, the “leaky” strains produce mostly 3-ADON/DON in culture and in addition up to 10% NX-2/NX-3. In order to produce reference materials in liquid culture we have engineered NX-3 production in *F. sulphureum* by knocking out *TRI13* and knocking in of different *TRI1* alleles which determine DON or NX-production. It is still an open question whether host plants exist, where NX-production provides a selective advantage, allowing this chemotype to increase in frequency. NX-3 lacking the keto group cannot form the Michael adduct observed in a GST-catalyzed reaction between DON and glutathione. Recently discovered plant UGTs can detoxify DON [3] but produce neither DON-3-glucoside nor DON-15-glucoside, but DON-8,15-hemiketal-7-glucoside [4]. Since NX-3 lacks the keto group, a hemiketal cannot be formed, and this toxin escapes detoxification, although it possesses a C7-OH. It is still unknown whether plants exist where these mechanisms are major detoxification reactions.

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Early gene response of moderately resistant oat (*Avena sativa* L. cv. Akseli) to *Fusarium culmorum* artificially inoculated in controlled conditions



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Moderately *Fusarium*-resistant oat cultivars exhibit an early gene expression response to inoculation, characterized by initial downregulation followed by upregulation of plant defense-related genes, and subsequently, activation of detoxification genes - highlighting potential molecular targets for unrevealing and thus improving resistance.

Abstract

Fusarium culmorum is a major producer of deoxynivalenol (DON) in oats, infecting spikelets. DON poses serious health risks to humans and livestock. Predicted climate changes in Finland may favor infection, while EU regulations are tightening mycotoxin limits and restricting fungicide use.

To address the growing *Fusarium* challenge in oats, we conducted a differential gene expression analysis in the oat cultivar Akseli, selected for its moderate resistance and previously demonstrated uniform response to infection across diverse environments (field and greenhouse). Spikelet tissues (developing caryopses) were sampled immediately following point inoculation and at 12, 24, and 72 hours post-inoculation (hpi) to capture the early transcriptional response to infection.

RNA was extracted from all samples and sequenced using Illumina RNA-seq. Differential gene expression between infected and control plants was analyzed using DESeq2 and/or edgeR. We examined the most significantly differentially expressed genes, characterizing their molecular functions, biological processes, and cellular components based on annotations in oats, other cereals, and model species. Additionally, we identified enriched pathways and key genes within those pathways.

In terms of temporal expression patterns, the early response of the moderately resistant cultivar Akseli was characterized by an initial down-regulation of gene expression at 12 hours post-inoculation, followed by up-regulation of plant defence related genes at 24 hours (including multiple pathogenesis-related (PR) protein genes), and a marked up-regulation of detoxification-related genes at 72 hours, such as glutathione S-transferase. The most extensive transcriptional changes occurred at 72 hours post-inoculation, with over 7,000 genes up- or down-regulated, compared to 421 and 30 genes at 12 hours, respectively. Among the genes in the plant–pathogen interaction pathway, the most significantly differentially expressed KEGG ortholog was RPM1-interacting protein 4 (RIN4), a key regulator of plant immunity known to be essential for RPM1-mediated resistance in *Arabidopsis thaliana*.

Deciphering the “priming” effect of enniatin B, deoxynivalenol and their combination on different wheat tissues toward *Fusarium* infection

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***Fusarium* secondary metabolites can enhance wheat defense response and impact on *Fusarium* development in a tissue-specific manner.**

Abstract

Fusarium graminearum (FG) and *Fusarium avenaceum* (FA) are among the species involved in Fusarium Head Blight (FHB) of wheat and, above all, produce the secondary metabolites deoxynivalenol (DON) and enniatins (ENNs), respectively. Enniatin B (ENB) induces oxidative stress and cell death in wheat (1), but it is not such a strong virulence factor for FA as DON is for FG (2). The present study investigated a possible “priming” effect of ENB, DON, and ENB+DON on common wheat coleoptiles and spikes toward FG or FA infections. Pre-germinated seeds or spikes were subject to 48 h treatments with ENB, DON or ENB+DON and the tissues collected for expression analysis of seven defense-related genes. Coleoptiles and spikes were then either inoculated with FG or FA and fungal biomass was estimated by qPCR. ENB-treated coleoptiles triggered the highest expression levels of all investigated defense-related genes. While DON increased coleoptile susceptibility to FG, ENB, both alone and in combination with DON reduced susceptibility. Regarding FA, only the ENB treatment caused a decrease in coleoptile susceptibility. In spikes, ENB+DON induced the highest expression of some defense-related genes. ENB did not appear to have significant effects on FG colonization, whereas both DON alone and in combination with ENB caused a decrease in FG biomass. All three treatments showed a negative impact on FA development. These results suggest that, in the coleoptile, ENB-induced activation of wheat defense-related genes can decrease susceptibility to both FG and FA. In the spike, the signaling responses triggered by the treatments were more complex and, despite some indications of a possible link between defense gene induction and pathogen suppression, the relationship was less clear. It is likely that additional factors, potentially including direct effects on FG and FA, also contribute to the observed changes in susceptibility.

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Host-Specific Response of Two Asparagus Cultivars to *Fusarium proliferatum* Strains

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Genotype-specific responses of asparagus cultivars to *Fusarium proliferatum* infection reveal differences in pathogen colonisation, mycotoxin accumulation, and disease tolerance, highlighting the need for targeted screening in breeding programs.

Abstract

Asparagus (*Asparagus officinalis* L.) is a valuable perennial vegetable crop globally cultivated for its high nutritional and economic value. However, its productivity is severely affected by root and crown rot diseases caused by *Fusarium* species. Although several *Fusarium* species are known to infect asparagus, there is limited information on how different cultivars respond to individual *Fusarium* pathogens. Therefore, in this study we investigated host-specific responses of two important asparagus cultivars, 'Mary Washington' and 'Apollo F1', to infection caused by two *F. proliferatum* strains. Seedlings of both cultivars were inoculated with spore suspension of each strain and monitored over a 28-day time course (48 hours, 7, 14, 21, and 28-day post-inoculation) under controlled environment. Inoculated plants were assessed for disease development, biomass reduction, mycotoxin accumulation, and fungal colonisation. Fungal colonisation was quantified by qPCR, and mycotoxin production were detected using Ultra-High Performance Liquid Chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Results revealed genotype-specific differences in biomass production, pathogen colonisation, and mycotoxin accumulation. Both *F. proliferatum* strains were pathogenic but varied in aggressiveness and mycotoxin production. Mycotoxin profiling of infected plant tissue detected differential accumulation of key toxins, including fumonisins, with higher concentrations at the later time points. Additionally, the differences in disease tolerance correlated with reduced fungal biomass and toxin load. These findings emphasize the complexity of asparagus-*Fusarium* interactions and suggest the need for incorporating more detailed screening in breeding programs for the development of resistant varieties. This study contributes to a better understanding of host-pathogen specificity in perennial crops and provides a foundation for improving disease resistance in asparagus cultivation.

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Endo-xylanases in *Fusarium graminearum*: Unraveling the complex interplay between virulence and genetic background



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***F. graminearum* endo-xylanases play distinct virulence roles, with contrasting mutant phenotypes revealing complex genetic interactions and compensatory mechanisms.**

Abstract

Fusarium graminearum, the causal agent of Fusarium Head Blight, employs an arsenal of cell wall-degrading enzymes (CWDEs) and effectors to colonize wheat spikes. Among CWDEs, endo-xylanases play a pivotal role by hydrolyzing xylan, a major component of plant cell walls. Despite their apparent functional redundancy, the endo-xylanase genes FGSG_03624 and FGSG_10999 exhibit distinct contributions to *F. graminearum* virulence and pathogenicity. By generating single and double knockout mutants in different genetic backgrounds, we dissected the roles of these two major endo-xylanases. Deletion of FGSG_10999 consistently led to severe virulence attenuation on wheat and soybean, reduced biomass accumulation, impaired DON production, and downregulation of other endo-xylanases and effector genes. Conversely, the FGSG_03624 mutant retained pathogenicity but exhibited reduced fungal growth and DON levels in planta, suggesting a role in host colonization. Strikingly, the double mutant lacking both genes displayed contrasting phenotypes depending on the order of gene deletions. When FGSG_10999 was disrupted in the FGSG_03624 background, the resulting strain maintained wild-type virulence levels, indicating potential compensatory mechanisms. However, deleting FGSG_03624 in the FGSG_10999 background recapitulated the severe virulence defects of the single FGSG_10999 mutant. This study provides insights into the complex virulence networks of *F. graminearum*. Our findings highlight the intricate interplay between endo-xylanases and the genetic background in shaping *F. graminearum* pathogenicity. The asymmetric outcomes observed in double mutants underscore the importance of considering pleiotropic effects and regulatory adaptations when targeting virulence factors.

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Endolevanase: A Host-Specific Hidden Weapon of *Fusarium graminearum* Provoking Fusarium Head Blight in Wheat



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Abstract

Graminan- and levan-type fructans are primarily stored within specialized cellular organelles known as vacuoles in wheat. These fructans serve diverse functions, including osmotic regulation, serving as a reservoir for energy, and possibly acting as signaling molecules to elicit the plant's immune response upon subsequent pathogen challenges. We propose that upon breaching the initial physical barrier - the plant cell wall, *Fusarium graminearum* may invade the underlying plant tissues through the secretion of a cocktail of enzymes, notably endolevanase. This enzyme likely plays a pivotal role by degrading gramINAN-type fructans, potentially utilizing them as a carbon source to support the proliferation of the pathogen within the host. Despite its critical implications, the presence and functional significance of endolevanase in the pathogenicity of *F. graminearum* remain understudied.

Through protein-carbohydrate docking, we identified a gene that encodes a novel endolevanase in *F. graminearum*. Intriguingly, this gene remained dormant when *F. graminearum* PH-1 was cultured in a minimal medium supplemented with high DP levan as an inducer. However, the gene was activated by a combination of high DP levan and wheat plant extract, while lettuce extract failed to induce its expression. This observation suggests the involvement of specific host-derived metabolic compounds in endolevanase activation in *F. graminearum*. Further, we successfully heterologously expressed the endolevanase in *Pichia pastoris*, where it exhibited high activity, efficiently degrading high DP levan into levan-derived oligosaccharides (LOS). Moreover, we demonstrated the synergistic action of endo and exolevanases in levan degradation by the pathogen. Next, we aim to employ CRISPR-Cas technology to knockdown this gene, thus elucidating its role in the pathogenicity. These findings hold promise for controlling *Fusarium* head blight by targeting such crucial genes involved in pathogenicity.

Pectin acetylsterases are key virulence factors in *Fusarium oxysporum* root colonization and disease progression



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Pectin acetylsterases FoPAE1 and FoPAE2 are essential for *Fusarium oxysporum* to metabolize pectin, penetrate host roots, and cause disease, making them potential targets for controlling Fusarium wilt.

Abstract

The soilborne fungal pathogen *Fusarium oxysporum* (Fo) causes vascular wilt diseases in a wide range of economically important crops by colonizing plant roots and directly interacting with host cell walls (CWs). Pectin—the most abundant and dynamic CW component—is highly decorated with acetyl- and methyl-esters. During plant-microbe interactions, these modifications can be removed *in muro* by both plant- and microbe-derived enzymes, influencing the outcome of infection.

Pectin acetylsterases (PAEs) are hypothesized, though not yet confirmed, to facilitate microbial colonization by modifying CW integrity and composition. Using the model *Arabidopsis*-Fo5176 pathosystem, we identified two putative Fo PAEs, FoPAE1 and FoPAE2, as potential contributors to fungal pathogenicity. After confirming their acetylsterase activity *in vitro*, we generated single knock-out mutants for each gene to assess their role in *Arabidopsis* root colonization. In *in vitro* growth assays under various stress conditions, mutant strains (FoΔPAEs) displayed growth comparable to the wild-type (WT) strain, except when pectin was used as the sole carbon source. Under these conditions, both mutants exhibited significantly reduced growth, suggesting that FoPAEs are crucial for utilizing this plant CW carbohydrate. Functionally, both FoΔPAEs showed a marked reduction in root vascular penetration and disease progression in *Arabidopsis* compared to the WT Fo5176 strain. These findings strongly support a role for PAEs in pathogenicity.

In summary, our data identify PAEs as key determinants of *F. oxysporum* infection success, highlight their involvement in host-pathogen interactions, and suggest their potential as molecular targets for controlling Fusarium wilt diseases. We are currently extending this work to assess the role of FoPAE orthologs in the *F. oxysporum* f. sp. *lycopersici*-tomato pathosystem and to further characterize their molecular functions in pathogenicity.

Mutation of Sucrose: Fructan 6-fructosyl-transferase (6-SFT) in hexaploid wheat reduces susceptibility to fungal disease



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Genome editing of the key fructan biosynthesis enzyme 6-SFT in hexaploid wheat reduced susceptibility to both biotrophic and necrotrophic fungi without compromising plant growth or yield.

Abstract

Fructans are important storage carbohydrates synthesised from sucrose in many grass species, including wheat, via the action of three enzymes: 6-SFT, 1-SST and 1-FFT. These compounds accumulate in stems and are remobilised to spikes and grains post-anthesis. Our previous studies suggested that fructans may act as host susceptibility factors contributing to Fusarium head blight (FHB) disease severity. Using genome editing (GE), we generated knock-out mutants of 6-SFT and 1-SST in the wheat cultivar Cadenza. Triple homozygous mutants with 61–70 bp deletions in the three 6-SFT homoeologues (on chromosomes 4AL, 7AS, and 7DS), resulting in frameshifts and premature stop codons, were identified. Phenotyping of T1–T3 GE lines under controlled conditions revealed that most lines exhibited normal growth compared to control lines, with some lines producing significantly larger grains. In T1 and T2 grains of the triple GE lines, fructan content was significantly reduced compared to non-GE controls and wild-type Cadenza, while starch content remained similar across all T2 lines. Inoculation of T2 seedlings with yellow rust or powdery mildew identified five and one moderately resistant lines, respectively, from two independent events. FHB phenotyping of T3 adult plants showed that two lines had significantly reduced infection levels at 7 to 14-days post-inoculation. Further phenotyping is ongoing. In parallel, triple 6-SFT TILLING mutants and Cadenza backcross lines are being developed. These GE and TILLING mutants represent promising resources for future breeding strategies aimed at enhancing resistance to multiple fungal pathogens.

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Fungal microRNAs: key players in interaction

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miRNAs produced by *Fusarium* during interaction affect genes regulation and molecular dialogue.

Abstract

The *Fusarium* genus comprises over 80 phytopathogenic species causing Fusarium Head Blight, or FHB, a devastating cereal disease worldwide. FHB causes significant economic losses and causes health problems due to the production of mycotoxins by fungi, thermostable molecules dangerous to humans and animals, if ingested. FHB results from the interaction of several *Fusarium* species, including up to sixteen different species (1). Recent studies have shown that the infection stage and mycotoxin production are influenced by intra-microbial interactions mediated by molecular dialogue (2). We hypothesized that this molecular dialogue is mediated by fungal microRNAs (miRNAs). MiRNAs are small non-coding RNAs, of 18–25 nucleotides, that induce post-transcriptional gene silencing (3). The abundance and nature of miRNAs are influenced by various factors such as stress, developmental stages, and microbial interactions. The aim of our research is to explore miRNAs produced by *Fusaria* during interactions.

To achieve this, confrontation tests between *Fusarium graminearum* and other *Fusaria* were set up to investigate interaction zones. Morphological modifications were initially observed using confocal microscopy, which raises questions about the mechanisms behind these morphological changes. Multi-omics approach was realized, including comparative transcriptomic analyses to identify miRNAs and their potential targets during *Fusaria* interactions. In addition, metabolomic analyses using HPLC-MS complemented this approach to detect signature metabolites of the interaction.

These results will give us keys to the diagnosis and prevention of FHB and mycotoxin accumulation in the plant system.

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Recruiting the host to reshape the microbiome: Immune priming by *Fusarium avenaceum* during asymptomatic colonization.



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**Discovery of a novel *Fusarium* metabolite that reprograms plant immunity—
enhancing defense against microbial competitors through manipulation of the host-
microbiome.**

Abstract

Plant–microbe interactions are dynamic, spanning a continuum from mutualism to pathogenicity, with microbial roles often shifting in response to host genotype, environmental conditions, and molecular context. Emerging evidence will be presented supporting the hypothesis that latent *Fusarium* pathogens manipulate host immunity to reshape microbial communities and exclude competitors. Transcriptomic analysis of asymptomatic wheat colonized by *Fusarium avenaceum* revealed active expression of multiple fungal secondary metabolite biosynthetic gene clusters. Focused investigation of one such cluster led to the discovery of a novel molecule, immunomodulide. In *Arabidopsis* leaf disk assays, immunomodulide selectively modulates host pattern-triggered immunity (PTI) by amplifying PAMP (pathogen associated molecular pattern) receptor triggered ROS expression by bacterial PAMPs flg22 and elf18. The observed activity suggests that *F. avenaceum* may potentiate the plant's ability to detect microbes thereby excluding competitors or manipulating the microbiome. In planta bacterial growth assays confirmed a host-mediated immune effect: immunomodulide and its synthetic derivatives suppressed *Pseudomonas syringae* proliferation in wheat and *Arabidopsis*, with no antimicrobial effect observed in culture. The biosynthetic gene cluster responsible for immunomodulide is conserved in several *Fusarium* species and shares homology with clusters in *Colletotrichum*, suggesting a broader ecological strategy among latent phytopathogens. These findings highlight a novel mode of inter-microbial competition mediated through selective manipulation of host immunity.

Co-occurrence between *Fusarium* and bacteria in wheat grains – towards a better understanding of microbial interactions

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The composition of *Fusarium* species and the diversity of bacteria in wheat grains are influenced by each other, as well as by environmental factors.

Abstract

Fusarium head blight (FHB) poses a significant threat to wheat production worldwide, resulting in substantial yield losses and deterioration in grain quality due to mycotoxin contamination. Although the impact of climatic conditions, agricultural practices and host genotype on the incidence of FHB and the composition of *Fusarium* species is well understood, the role of wheat microbiota in modulating *Fusarium* infection is not.

This study aims to determine whether wheat-associated bacteria influence the species composition of *Fusarium* in wheat grains, and vice versa. Bacterial diversity has been investigated via sequencing of the 16S rRNA gene in over 500 wheat grain samples of known *Fusarium* species composition, collected from various French agricultural regions. The influence of abiotic parameters such as wheat variety, agricultural practices, climatic conditions, and mycotoxin contamination levels on bacterial diversity and community composition was evaluated. Co-occurrence network analysis was conducted to identify significant positive and negative associations between bacteria, *Fusarium* and/or mycotoxin levels in grains. These associations were tested *in vitro* via co-cultures of bacteria (single strains and synthetic communities) and *Fusarium*.

The results revealed significant differences in bacterial community structure between samples collected in 2020, 2021, and 2022, as well as between soft and durum wheat. Co-occurrence network analyses revealed distinct clustering patterns within the bacterial and *Fusarium* communities. Furthermore, the observed associations between climatic variables, mycotoxin levels and microbiome composition mirrored those identified between *Fusarium* and specific bacterial taxa. For example, negative correlations were observed between *Pseudomonas* spp. and both *Fusarium graminearum* and the mycotoxin deoxynivalenol.

This study shed light on the ecological interactions between wheat-associated bacteria and *Fusarium* pathogens. This could lead to new biological strategies for managing FHB and mitigating mycotoxins in wheat production systems.

Biological durum wheat seed priming for Fusarium Root and Crown Rot management through the application of *Bacillus* and LAB strains



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Biological seed priming with *Bacillus* and LAB strains effectively reduces Fusarium Root and Crown Rot in durum wheat, providing a sustainable solution to enhance yield and quality in organic farming systems.

Abstract:

Fusarium Foot Rot (FFR) and Fusarium Crown Rot (FCR), mainly caused by *Fusarium culmorum* and *Fusarium graminearum* pathogens, severely threaten durum wheat, an essential crop in global and European agriculture, by reducing yield and quality through mycotoxin contamination. As chemical fungicides often fail to control FFR/FCR, biological seed treatments with natural antagonists offer a promising alternative, suppressing pathogens and enhancing the seed–plant–soil system via biopriming. Within Spoke 2 of the Agritech National Centre (WP2.3, Task 2.3.3) and the PNRR Prin 2022 BICONTRARIUM project, biological strategies were explored to enhance the resilience of durum wheat. From a pool of 89 beneficial bacterial strains, promising candidates, primarily *Bacillus* and Lactic Acid Bacteria (LAB), were identified through *in vitro* screening. Seed priming methods were optimized, enabling selection of four top-performing strains for *in vivo* trials. LAB strains reduced disease severity caused by *F. culmorum* (strain Fc1126) by 44.4% and *F. graminearum* (strain Fg566) by 49.2% compared to the control in growth chamber tests. Open-field trials (2023–2025) in Northern Italy evaluated bacterial treatments under natural conditions. San Carlo durum wheat seeds, pretreated with bacterial inocula, were sown alongside barley seeds that had been double-autoclaved and pre-inoculated with strain Fc1126. Seedling emergence was initially monitored manually (plants/m²) and later using drone technology at BBCH stages 30–31, employing image thresholding and area analysis via ImageJ software. First-year results showed that one *Bacillus* strain improved germination by 50%, and LAB treatments by up to 37.3%. Drone evaluations highlighted that, despite lower disease pressure in the second year, the *Bacillus* strain improved seedling emergence by over 15% in both years. The results suggest that seed priming with beneficial bacteria can be a promising tool for crop production and protection under field conditions due to both direct antagonistic activity and indirect growth promotion.

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Multionics of Fusarium Head Blight disease in spring barley

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Multi-omics analysis of Fusarium Head Blight in spring barley reveals the activation of defence pathways, highlighting pathogen-triggered biosynthesis of hydroxycinnamic acid derivatives and other key metabolites involved in pathogen response.

Abstract

Fusarium Head Blight (FHB) is a devastating fungal disease of small grain cereals like wheat and barley, causing substantial yield losses each year worldwide. Climate change and a growing global population make fungal diseases like FHB a more pressing issue. FHB is caused by *Fusarium* species that produce mycotoxins like deoxynivalenol (DON), which impairs protein biosynthesis and induces ribotoxic stress in plants. Although transcriptional defence responses in barley to *Fusarium* infection have been described, it remains unclear to what extent these responses are translated into functional changes at the protein and metabolite levels. To date, no comprehensive study has investigated the molecular response to FHB in barley using a multiomics approach.

In this study, we employed transcriptomics, proteomics, and metabolomics to dissect the defence responses of barley during infection with *Fusarium culmorum*. Our analyses revealed a set of significantly regulated gene-protein pairs linked to biosynthetic pathways that correspond to upregulated defence-related metabolites. These include well-known defence compounds such as tryptophan and serotonin, as well as barley-specific metabolites like hordatines and their biosynthetic precursors. By integrating multiple layers of molecular data, our findings provide novel insights into the complex regulatory networks underpinning barley's defence against FHB, highlighting key pathways involved in pathogen response.

Investigating oat responses against *Fusarium langsethiae* in Finland



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Abstract

Fusarium head blight is a serious challenge for cereal farmers in Northern Europe. After several years with very low mycotoxin contents, the oat and also wheat growers in Finland have faced severe challenges due to high mycotoxin accumulation in years 2023 and 2024. Both DON and T2/HT2-mycotoxins are found in Finnish cereals and several research projects are ongoing to find solutions for keeping the accumulation of these toxins below the food safety limits set by EU. Cultivar resistance would be a sustainable tool for managing the problem and several research projects have been conducted also in Finland in order to improve especially the resistance of oats against deoxynivalenol accumulation. Unfortunately, the resistance to DON producing *Fusarium* species *F. graminearum* and *F. culmorum* is not necessarily working against T2-producing fungi such as *F. langsethiae* and *F. sporotrichioides* (Hofgaard et al. 2022). Therefore, we in an ongoing research project we have established methods for phenotyping cultivar resistance against *Fusarium langsethiae* in semi-greenhouse and in field conditions and simultaneously we are conducting a RNA sequencing study to detect how *Fusarium langsethiae* interacts with oat host. Within this presentation we introduce how we have inoculated *Fusarium langsethiae* in field conditions, how we have conducted RNA sequencing study and give insight to preliminary results from the past two years. Also other ongoing work with resistance and other management strategies against DON producing fungi are mentioned.

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Evaluation of two-row barley germplasm reveals accessions resistant to Fusarium head blight with low levels of deoxynivalenol.



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Breeding barley resistant to Fusarium head blight is difficult due to lack of resistance sources.

Investment in screening diverse genebank accessions has identified resistant, two-row barley germplasm with low deoxynivalenol accumulation.

Abstract

Barley (*Hordeum vulgare* L.) is an important crop globally, which is used both as animal feed and in malting and brewing industries. Severe Fusarium head blight (FHB) epidemics occurring in Canada during the mid-1990s led to large investments in barley breeding for development of varieties with low deoxynivalenol (DON) accumulation. While breeding resistant varieties remains the most sustainable approach to disease management, that has been a huge undertaking due to fact that barley resistance is quantitative with single resistance loci contributing minor additive effects. Due to incorporation of resistance from exotic sources, several moderately-resistant varieties are now commercially available to Canadian barley producers. However, these still accumulate DON under epidemic conditions. A large FHB nursery at Agriculture and Agri-Food Canada (AAFC), Brandon Research and Development Centre has been used to screen two-row barley accessions from the national seed gene bank of AAFC, Plant Gene Resources of Canada, and other sources for FHB resistance. Since 2018, five hundred accessions were grown annually with selections made based upon FHB reaction and acceptable agronomics. Promising lines were harvested and tested for DON content via enzyme-linked immunosorbent assay (ELISA). Lines which continued to display low DON levels relative to checks, were re-tested annually under replication. Several accessions have demonstrated resistance levels equal to commonly used resistance sources. The panel of accessions identified through this study may help diversify the genetic base of resistance for combating this devastating disease by breeding resistant varieties. Noteworthy accessions include: CN 72032 (2314), CN 72222 (2534) and CN 68864 (HOR 929) from Turkey; CN 72606 (E 104/8); CN 72656 (E 161/4) and CN 76863 (GAW 36-6) from Ethiopia; CN 68460 (Cluj 33/139) and CN 68463 (Thigina 2621) from Romania; CN 68472 (HOR 203) from Ukraine; CN 73442 (Pollesskij) from Belarus; CN 68570 (Asiatische Nackt) from the Soviet Union.

Cross-kingdom RNA interference in the wheat–*Fusarium culmorum* interaction: evidence from small RNA and degradome analysis

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Abstract

Cross-kingdom RNA interference (ckRNAi) represents a recently characterised form of molecular dialogue between plants and fungi, in which small RNAs from one organism can modulate gene expression in another. In the context of crop protection, host-derived microRNAs (miRNAs) are emerging as potential regulators of fungal virulence through targeted gene silencing.

In this study, we investigated the possible involvement of wheat (*Triticum aestivum*) miRNAs in ckRNAi during the early stages of infection by *Fusarium culmorum*, a significant necrotrophic pathogen responsible for root and head blight. Small RNA and degradome sequencing were performed on wheat roots and leaves from two genotypes differing in susceptibility. This approach enabled the identification of wheat miRNAs predicted to target *F. culmorum* transcripts, with several interactions supported by degradome evidence indicative of canonical miRNA-mediated cleavage.

Temporal expression analysis revealed dynamic regulation of selected miRNAs, particularly an early induction (within 24 hours post-inoculation) in the more susceptible wheat genotype. These findings suggest that miRNAs may contribute to early-stage defence signalling by engaging in cross-kingdom regulatory interactions with the pathogen.

While the specific miRNAs and fungal targets are the subject of ongoing validation, the evidence presented here suggests a role for wheat-derived miRNAs in ckRNAi-mediated defence during *F. culmorum* infection. This study contributes to a deeper understanding of the molecular interplay within the wheat–*Fusarium* pathosystem and may inform future RNA-based strategies for crop protection.

Cell cycle controls pathogenic processes and mycotoxin production in *Fusarium graminearum*



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Downregulation of the cell cycle is essential for the expression of pathogenicity related genes and full virulence of *Fusarium graminearum*.

Abstract

RAS proteins control the cell cycle in all eukaryotes and lead to cancer in mammals when mutated to permanent activity. We previously isolated a spontaneous, highly proliferative, fluffy mutant of the major cereal pathogen *Fusarium graminearum* that turned out to carry a loss of function mutation in the Ras-GTPase activating protein (Ras-GAP) presumably leading to permanently active RAS allele. In this study we validated this loss of function mutation in a *F. graminearum* PH-1 background and evaluated the impact of an impaired Ras-GAP function on axenic growth phenotypes and pathogenicity. The mutant showed an altered secondary metabolite profile and significantly reduced virulence on wheat. We analyzed the transcriptomes of the wild type and the Ras-GAP mutant during infection and found that the continuously proliferating, but hardly virulent mutant strain is not able to switch to the genetic program necessary for pathogenicity and is consequently unable to spread throughout the wheat ear. While the wild type reprogrammed the expression of 953 genes within two to four days post infection (dpi), only six genes were significantly altered in the mutant strain. In the early infection phase at 2 dpi, genes involved in cell cycle control, response to nitrogen limitation and pathogenesis were predominantly different between wild type and mutant. We also analyzed the plant transcriptomes during this infection process and saw that plants showed a drastically different response to the presence of the Ras-GAP mutant and only mildly in comparison to the wild type infection. Our data for the first time demonstrates that downregulation of the cell cycle is necessary for various pathogenicity related processes in *F. graminearum*.

Culmorin inhibits detoxification of the mycotoxin deoxynivalenol by plant UDP-glucosyltransferases

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Culmorin interferes with DON detoxification by competitive or non-competitive inhibition of glucosyltransferases of small grain cereals.

Abstract

The trichothecene mycotoxin deoxynivalenol (DON) is an important virulence factor of *Fusarium* species infecting cereal crops. Culmorin (CUL), another *Fusarium* metabolite, frequently co-occurs with DON, but its role in virulence on plants has so far been unclear. CUL has low phytotoxicity by itself but increases the toxicity of DON in wheat, barley and corn synergistically. Due to the structural similarity between DON and CUL it was hypothesized that CUL might compete with DON for detoxification enzymes.

CUL, while non-toxic on its own to wheat in root elongation assays, significantly increased the toxicity of DON, this was accompanied by reduced DON-3-glucoside (DON3Glc) levels in CUL treated wheat roots. Furthermore, *cul1* deletion strains of *F. graminearum* producing no CUL were less virulent on Apogee wheat compared to the isogenic wild-type strain PH1. The DON3Glc/DON was significantly higher in wheat ears treated with *cul1* strains than in those treated with PH-1, supporting the hypothesis that CUL interferes with DON-detoxification by UDP-glucosyltransferases (UGT). To further investigate this effect, inhibition kinetics of CUL were investigated with three recombinant plant UGTs known to efficiently glucosylate DON. This revealed that CUL in fact may interfere with DON-detoxification either by serving as competitive substrate (barley HvUGT13248) or by unproductive binding causing a moderate inhibition of the rice OsUGT79 or a drastic reduction of enzyme activity in case of *Brachypodium* BdUGT5g03300. Our results indicate a possibly complex synergy of culmorin with DON, as culmorin itself was also glycosylated in wheat, but even the glucosides were inhibitory to BdUGT5g03300. Our data indicate that culmorin may allow the fungal pathogen to at least partly counteract or overcome breeding efforts or transgenic approaches aiming to increase *Fusarium* resistance by increasing UGT-mediated detoxification of DON.

Comparative analysis of the metabolomic profiles of seven *Fusarium* strains causing head blight, cultivated alone or together as a synthetic community called *Meta-Fusarium*

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A synthetic *Fusarium* community for the mechanistic understanding of *Fusarium* interactions and their impact on disease outcomes.

Abstract

Fusarium species are causative agents of *Fusarium* head blight (FHB), a devastating fungal disease affecting cereal crops worldwide. FHB causes yield losses and grain contamination with mycotoxins (type A and B trichothecenes, zearalenone, enniatins, beauvericin), posing significant health and food safety concerns¹. While several *Fusarium* species likely interact during infection, most studies have focused on single species, particularly *F. graminearum*, which is insufficient for a comprehensive understanding of the disease outcomes².

This study aims to compare the metabolomic profiles among seven *Fusarium* strains causing FHB, cultivated alone or together as a synthetic community (syncom) called *Meta-Fusarium*. This community was built with one strain of each of the seven major FHB species encountered in Europe (*F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum*, *F. sporotrichioides*, *F. langsethiae*, *F. tricinctum*)¹. The metabolomic data of both mycelium methanolic extracts and diluted culture supernatants were acquired using a Q Exactive Focus Orbitrap LC-HRMS/MS system. First results indicate differences in the metabolomic profiles of each strain, with species from the *F. sambucinum* species complex including *F. culmorum*, *F. graminearum*, and *F. sporotrichioides* and species from the *F. tricinctum* species complex including *F. tricinctum* and *F. avenaceum* being grouped together, respectively. In addition, discriminant as well as common metabolites have been identified, including putatively annotated mycotoxins. By examining the *Meta-Fusarium* metabolome, only specific metabolites of *F. culmorum* and *F. graminearum* were retrieved, indicating that they were the major contributors to the syncom metabolome. This observation was supported by the biomass composition obtained by qPCR, which showed that *F. culmorum*, followed by *F. graminearum* and *F. poae* were predominant in the syncom.

These results offer the first comparative metabolomic profiling of FHB strains, providing insights into the behaviour of a complex *Fusarium* syncom. This research advances the mechanistic understanding of *Fusarium* interactions and their impact on disease outcomes.

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Reconstructing the worldwide emergence of the Tropical Race 4 (TR4) of the banana pathogen *Fusarium oxysporum f.sp. cubense* using population genomics



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Foc-TR4 is less clonal than previously considered, and subdivided in four sublineages.

Abstract

Fusarium oxysporum f. sp. cubense (Foc) is a soilborne fungus that causes banana Fusarium wilt, a destructive plant disease that has resulted in devastating economic losses to banana production worldwide. The major concern for the future of dessert banana is related to the spread of the Foc « tropical race 4 » (Foc-TR4), which is virulent to Cavendish and most banana cultivars. FocTR4 has been long considered a clonal lineage, but its actual diversity has remained obscure.

For a global understanding of the Foc TR4 epidemic over time and space, we sequenced the complete genomes of 119 Foc TR4 isolates from 20 countries sampled over the three periods of the worldwide emergence, and analysed the whole-genome SNPs following a population genomics approach.

We established that Foc-TR4 is a monophyletic lineage, yet subdivided into several sublineages, two being responsible for the pandemic expansion of this plant pathogen.

Indonesia was identified as the mostly likely unique centre of diversity and origin of Foc TR4, as it harboured the highest genetic and genotypic diversity, and highest richness in private alleles ; ancestral state reconstruction analysis further supported this assumption.

We also investigated the evolutionary and demographic history of this emergence, considering the genetic differences between the 1990s-Asian populations and the out-of-Asia-2010s populations. We identified the main migration routes of TR4 from its Indonesian cradle to the non-Asian world. Furthermore, we established that the Foc-TR4 reproductive mode was less clonal than previously considered.

Collectively these results constitute the most comprehensive phylogenomic study on FocTR4 emergence to date, and can be useful to predict future dissemination of the fungus. These also pave the way to future studies ranging from Foc virulence on banana to genome evolution.

Discovering of emerging pathogens belonging to *Fusarium* spp. in Italian wheat kernels

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This study highlights emerging *Fusarium* spp. in Italian wheat kernels and supports the advantage of combining culture-dependent and culture-independent methods for pathogen surveillance.

Abstract

Wheat is one of the most cultivated cereal crops in the Mediterranean basin area, with both durum and soft wheat representing strategic resources for food security (1). As consequence of climate change, globalization and trade, adaptation, resistance to pesticides, monocultures and migration of insect vectors, the emergence of phytopathogenic fungal pathogens has been increasing for several years (2), posing a potentially devastating threat to critical crops to food safety and security. In this context, being Italy a geographical quadrivium and one of the most important European countries for durum wheat production it is essential to monitor the spread of wheat pathogens to find possible strategies in preventing their spread. Two parallel methods for fungal identification have been explored in this work, one by fungal metabarcoding, using the MinION sequencing platform (ONT), and one exploiting the in vitro cultivation of endophytic fungi from superficially sterilized kernels. The two methodologies showed complementary results, and although the in vitro isolation was much more time consuming, it allowed the isolation species that could not be identified by metabarcoding, including potential pathogens emerging in Europe such as *Fusarium bothii* and *Fusarium elaeagni*. *Fusarium bothii* is a phytopathogenic fungal species that is part of the *Fusarium graminearum* species complex (FGSC), normally restricted to other geographical areas in the world. It has been previously reported in the USA, Mexico and South Africa (3) in different agriproducts. Recent works from 2025 reports its emergence also in Ethiopia (3) and Serbia (4). *Fusarium elaeagni* is part of the *F. fujikuroi* species complex and there are currently no reports of its detection in Italy. These two species were preliminarily identified by Sanger sequencing of the ITS1-4 regions and subsequently performed whole genome sequencing on both. The isolates were also tested for their ability to produce mycotoxins on wheat matrices. This work highlights the importance of monitoring crop microbiomes in a changing environment to support early management strategies.

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***Fusarium* identification, emerging pathogens and host wheat resistance in Canada**

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Continuous surveillance of pathogens is vital to keep the grains safe for consumers; MALDI-TOF mass spectrometry is a rapid and cost-effective tool for identifying environmental isolates of *Fusarium*. Studying the interaction between emerging pathogen genotypes and the wheat host is important when examining genes involved in the pathogenicity and resistance of the host. The project delivers valuable new insights about host resistance and emerging pathogens, providing benefits to wheat breeding programs.

Abstract

In the grain value chain, fungal pathogens and the occurrence of fungal mycotoxins are among the key factors affecting grain processing, production, quality, and safety. There are three project objectives for this research. The first is to develop a rapid method for identifying *Fusarium* species causing Fusarium Head Blight (FHB) in grains, as accurate identification of these fungal pathogens is crucial for effective disease management 1. The second objective is to investigate reasons for population shifts in FHB pathogens within Canada, i.e., the dominance of the NA2 population of *F. graminearum* (historically, associated with production of 3ADON) over the NA1 population (historically, associated with 15ADON production) 2–5. The final objective is to investigate host resistance to different *F. graminearum* isolates in wheat. The rapid identification of *Fusarium* isolates was successfully achieved using MALDI-TOF mass spectrometry, a rapid and cost-effective method for microbial identification, via protein-based species-specific biochemical profiles. This method was validated with identity confirmed (PCR) *Fusarium* species isolated from infected grains 6. The second objective is currently being addressed by investigating the factors contributing to the dominance and abundance of the *F. graminearum* NA2 population. Comparative genomics analysis of two representative *F. graminearum* isolates, which belong to NA1 and NA2, has identified a set of genes that might be associated with the competitive advantage of the NA2 population. CRISPR-Cas9 gene editing is being used to create targeted mutations within these genes, and the resulting mutants are being compared to wild-type isolates in vitro and in vivo. The final objective involves testing the resistance of five wheat varieties (AAC-Tenacious, AAC-Brandon, CDC-Landmark, CDC-Stanley, and CDC-Teal) to the same two *F. graminearum* isolates, both as sole-inoculations and co- inoculations. The results of this study will contribute to improving grain processing, production, quality, and safety, which will benefit the entire grain value chain.

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30 Years of Fusarium damaged kernel incidence, severity, and pathogen diversity in Canada

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Abstract

Many biotic factors can negatively affect cereal grain quality and safety, including fungal damage due to Fusarium Head Blight (FHB). FHB can be caused by many different species of Fusarium, some of which produce trichothecenes, such as deoxynivalenol or DON. Trichothecenes are of particular concern due to their regulation and toxicity to humans and livestock. However, Canada has a robust grain grading and monitoring system to ensure fungal and toxin contamination remains within acceptable levels and that Canadian wheat meets the quality and safety requirements of domestic and export markets. Since 1995, our harvest monitoring program has assessed the occurrence and severity of Fusarium Damaged Kernels (FDK) in wheat for over 200,000 harvest samples. The results of our monitoring identified regional and temporal differences in the fungi, with the occurrence of FDK having increased over time, particularly in the last two decades. Traditional methods of Fusarium species identification involved culturing and manual inspection through microscopy, which is low throughput, laborious, and can not provide information on the toxin potential of the fungi. Using high-throughput DNA testing, we have assessed the Fusarium species and toxin chemotypes for fungi in tens of thousands FDK, allowing us to increase our testing capacity and provide robust data on shifts in the pathogen populations and risks for trichothecene occurrence. To complement our DNA testing, we have also developed new biochemical fingerprinting tools and databases, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), that can be used for fungal identification and biotyping. Our monitoring dataset is one of the largest of its kind, and provides valuable information on trends on FDK spanning the last 30 years.

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Wheat responses to FHB under pre-anthesis drought: How extensively is the molecular dialogue reshaped?

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This study demonstrates that pre-anthesis water stress in wheat significantly alters metabolic and immune-related molecular responses, leading to reduced susceptibility to Fusarium Head Blight (FHB). The findings highlight shared regulatory mechanisms underlying plant adaptation to combined abiotic and biotic stresses, offering potential targets for enhancing FHB resistance under adverse environmental conditions.

Abstract

The impact of abiotic stresses on plant physiology significantly reshapes plant-pathogen interactions by modulating immune responses. In wheat, the development of Fusarium Head Blight (FHB) is highly influenced by environmental conditions, particularly during the pre-anthesis stage, just before fungal infection occurs. This suggests that the initial phases of infection may be conditioned by prior environmental changes, with consequences on disease progression that remain to be fully characterized. In this study, we investigated how pre-anthesis water depletion affects the expression of molecular determinants, with a specific focus on FHB susceptibility factors and metabolism-related processes. Different water regimes were applied, producing mild to moderate physiological impacts, which persisted for at least three days post-rehydration. Notably, longer durations of water stress led to a significant reduction in FHB symptoms (Adamik et al., 2025). Dual-transcriptomics, combined with untargeted metabolomics, yielded two major findings: (i) profound metabolic alterations specific to prior water stress, and (ii) robust conservation in the regulation of previously identified candidate susceptibility genes and pivotal fungal effectors (Rocher et al., 2022; Rocher et al., 2024). Taken together, these results reveal a distinct molecular response signature under combined stress, emphasizing the deep integration between immune responses and physiological adaptation. Moreover, a shared component of these specific responses was observed across the different stress conditions, suggesting the presence of common regulatory mechanisms that may facilitate plant adaptation to complex environmental challenges. Overall, our findings provide new insights into the trade-offs plants make under multiple stress conditions and identify wheat metabolic determinants that could contribute to enhanced FHB resistance, even under suboptimal physiological states.

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Drought stress modulates Fusarium Head Blight severity in barley

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The combination of *Fusarium* infection and drought stress modulates disease severity and leads to additive stress responses in barley.

Abstract

The incidence and severity of Fusarium Head Blight (FHB) in barley are influenced by weather conditions and depend on the genotype (Hoheneder et al., 2022). However, the effects of drought and heat, in particular when these stresses occur around the critical stage of anthesis, on barley's quantitative resistance and successful fungal infection remain poorly understood. We hypothesise that the interaction of abiotic (e.g., drought, heat) and biotic (e.g., *Fusarium* spp.) stressors can alter the plant's stress response mechanisms, potentially affecting its ability to defend against *Fusarium* infections.

Our results show that drought stress, whether applied before or simultaneously with *Fusarium culmorum* inoculation, significantly influences FHB severity under controlled greenhouse conditions (Hoheneder & Steidele et al., 2023; and unpublished data). The timing of drought in relation to flowering and pathogen inoculation plays a critical role, either reducing or enhancing disease severity. Analysis of stress-related plant hormones and metabolites suggests a link between specific hormone levels and susceptibility to FHB. The global gene expression revealed that genes associated with specific stress responses are co-expressed in stress-associated clusters. Under combined stress, gene expression patterns reflected additive effects of individual stress responses rather than a unique response specific to combined stress. Additionally, the enhancing or reducing effect of drought stress on FHB severity is dependent on the barley genotype.

Similarly, genotype-dependent effects of abiotic stress on FHB severity were also found when elevated temperatures during anthesis were applied (Hoheneder et al., unpublished), which predominantly increased disease severity and lowered yield and grain quality.

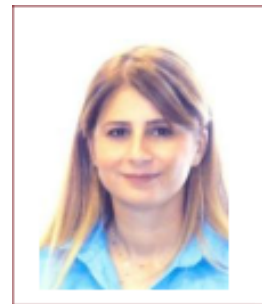
Overall, our data highlight potential physiological trade-offs and the modular response of barley to combined abiotic and biotic stresses. These findings suggest important challenges and considerations for breeding barley cultivars which are robust against *Fusarium* infections and abiotic stress.

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Distribution and prevalence of Fusarium crown rot pathogens of Durum wheat in Southern Italy



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This is the first comprehensive survey carried out on the epidemiology and distribution of FCR causal agents in Southern Italy space for author's picture.

Abstract

Fusarium Crown Rot (FCR) is an important worldwide disease on durum wheat. *Fusarium pseudograminearum*, *F. culmorum* and *F. graminearum* are the most important causal agents. They have the common ability to biosynthesize harmful secondary metabolites. In addition, the fungal species distribution undergoes a continuous evolution influenced by environmental factors. This study aims to investigate FCR epidemiology, distribution and pathogenicity of FCR-causing *Fusarium* species in durum wheat, in Southern Italy. The monitoring of FCR symptoms in 60 wheat fields located in 6 different regions (Sardinia, Sicily, Calabria, Apulia, Basilicata and Campania) has been carried out in 2024. A total of 10 symptomatic wheat plants in each field investigated were considered to assess FCR incidence and severity. In order to evaluate *Fusarium* species distribution occurring on durum wheat in Southern Italy, 500 *Fusarium* strains isolated from crowns and stems were molecularly identified. A great biodiversity of *Fusarium* species was observed with 18 species identified: *F. culmorum*; members of *Fusarium incarnatum-equiseti* species complex (FIESC); *F. avenaceum* and *F. pseudograminearum* were the most prevalent, accounting for 68, 8, 6 and 5% of the total identified species, respectively. Some of the less frequent *Fusarium* species detected have been isolated from FCR symptoms for the first time in Italy and even in Europe such as *F. algeriense* detected in Sicily, Apulia, Campania and Basilicata regions, representing (1%) of the total identified species. In pathogenicity trials carried in glasshouse conditions, *F. pseudograminearum* was the most aggressive (severity: 89%), followed by *F. culmorum* (62%), *F. graminearum* (55%) and *F. avenaceum* (50%). *F. algeriense* was the least pathogenic (16%) and FIESC species have been shown to be non-pathogenic. Sensitivity testing to three representative fungicides with different modes of action (fluazinam, prothioconazole and cyclobutrifluram, the latter not yet registered in the EU) is ongoing.

This research was supported by the PNRR-

PRIN project 2022L5Y28K – Fusarium Crown Rot on durum wheat in Southern Italy: epidemiology, evolution and toxigenic potential in a climate change scenario.

Mathematical models to predict the risk of DON-contamination in Norwegian spring oats based on weather data

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By using Random Forest machine learning algorithms we have developed mathematical models to predict the risk of elevated DON-concentrations in Norwegian oats based on weather data. The model outputs are available online and can be used as a decision support tool for managing Fusarium/DON in oats.

Abstract

The fungal plant pathogen *Fusarium graminearum* is the main producer of deoxynivalenol (DON) in Norwegian oats. Weather conditions significantly impact *Fusarium*-infection and DON concentrations in oats (Hjelkrem et al. 2022) and may also lay ground for the following years inoculum potential of *Fusarium* on straw residues. The DON-levels in harvested oat grains vary significantly between years and locations within years. To reduce the risk of elevated DON-concentrations in oats, farmers can plant cultivars with a moderate resistance to *F. graminearum*, bury/remove straw residues or avoid continuous cereals, and spray with a fungicide at flowering. We have developed mathematical models for the prediction of DON-contamination in oats based on previous and present years' weather data. The models were developed based on datasets from the Norwegian grain industry including DON-concentrations in a total of almost 279 000 grain lots of oats harvested at 932 different locations in the cereal district in Norway within a fourteen-year period (2011 – 2024). The dataset was evenly distributed between years, with 4.7 to 9.5% of the total dataset each year. Weather data was collected from the nearest weather station. Two classification models were developed by using Random Forest machine learning algorithms: A model that predicts the inoculum potential of DON-producers in spring, prior to sowing, based on previous years' weather data (model 1), and a model that, prior to flowering, predicts the potential risk of elevated DON-concentrations in oats based on the previous and present years' weather data (model 2). Each model consists of sub-models to identify the year-location combinations with DON concentrations greater than 750 µg kg⁻¹ in a defined proportion of the grain lots. Model output is freely available at vips-landbruk.no for farmers and advisers to use as a decision support tool when planning eventual measures to reduce the risk of elevated DON-concentrations in oats.

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Impact of agroforestry systems on fungal populations in wheat crops

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Investigating the pathogen risk in agroforestry: we are using metabarcoding to qualify the impact of agroforestry on fungal populations, specifically Fusarium Head Blight (FHB) in wheat, to assess the potential disease risks of this sustainable practice.

Abstract

Agroforestry is an emerging agricultural practice that makes use of the complementarity between trees and crops, so that the available resources can be more effectively exploited. However, the issue of biological regulation within these systems is poorly documented, particularly regarding plant pathogens. It is essential to gain knowledge of the benefits and potential risks associated with the deployment of such a practice applied to field crops. Fusarium Head Blight (FHB), one of the major diseases affecting wheat is caused by a cohort of *Fusarium* spp., often dominated by *Fusarium graminearum* and strongly influenced by the environment. The presence of hedges or trees in intra-parcel wheat fields may modify fungal populations, by acting as pathogens reservoirs, or by modifying the microclimatic conditions favorable to their development.

The aim of this study is to investigate the impact of the agroforestry system associated with wheat on fungal populations and in particular on the pathogens responsible for FHB. By using metabarcoding approaches targeting either ITS and EF1alpha genes, we investigate the representativeness of fungal genera and the occurrence of *Fusarium* species in samples collected in the wild compartment (leaves, wild grasses) and in wheat fields (heads) during the growing season.

Our study will contribute to fuel scientific knowledge on FHB epidemiology of FHB in agroforestry landscapes and will also provide broader insights into potential non-intended effects relevant to disease management strategies in diverse cropping environments.

Chitosan-cellulose nanocrystals based formulation for Fusarium Head Blight protection: from lab to open field evaluation



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The use of wheat bran-derived cellulose nanocrystals in a chitosan-based nano-formulation offer a sustainable and effective strategy to combat Fusarium Head Blight, thereby reducing agricultural waste and the reliance on conventional pesticides.

Abstract:

Circular agriculture aims to reuse crop waste to obtain products with high added value, such as innovative crop protection products. In particular, nano-functionalized carriers could enhance antimicrobial properties of pesticides thanks to the controlled release of active ingredients. Fusarium head blight (FHB), among the main wheat diseases, is caused by different Fusarium species, especially *F. graminearum*. In this work, a nanostructured particle formulation (NPF) was studied for wheat protection against FHB. Wheat bran was used for cellulose extraction and synthesis of cellulose nanocrystals (CNC) by using an optimized chemical protocol. The protocol allowed the production of CNC (18% yield), having a mean size of 70.53 nm, a negative charge and good stability in solution. CNC obtained from the bran showed features similar to industrially produced CNC, and it was used for the preparation of a chitosan hydrochloride-CNC based formulation (ratio CNC- chitosan 40:60), obtained through the spray-drying technique. In vitro assays revealed that this NPF used at 0.005% w/v allowed a 30% reduction of *F. graminearum* conidial germination while a concentration of 0.01% w/v led to complete growth inhibition. The efficacy against FHB (in terms of disease severity and mycotoxins accumulation), and the biocompatibility on wheat plants were also evaluated using two in vivo trials: FHB assay, through the treatment of artificially inoculated wheat head in controlled environment, and in open field, comparing 0.5% NPF-treated, tebuconazole- treated and mock-treated naturally infected experimental parcels. To date this study is among the first evaluations of chitosan-based nano-formulation in open field against FHB.

Global assessment of HT2 and T2 occurrence

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Fusarium mycotoxins HT2 and T2 are largely restricted to European cereals, in particular oats.

Abstract

The fusarium mycotoxin type A trichothecenes, HT2 and T2 are produced on small grain cereals predominantly by *Fusarium langsethiae*, they are produced on the same metabolic pathway, and as T2 is rapidly metabolized into HT2 after ingestion the combined concentration (HT2+T2) is assessed with regards exposure. HT2 and T2 were first identified at high concentrations in cereal grains in the late 1990s during studies on Norwegian oat crops. A global survey of occurrence was conducted by JECFA (Joint WHO/FAO Expert Committee on Food Additives and contaminants) in 2020 (JECFA, 2023). The survey considered submissions to the GEMS/Food database for HT2 and T2 from 2000 to 2019. After data cleaning and calculation of the combined concentration of HT2+T2 there were ca. 50,000 samples analysed from 2000 to 2019. Comparison of analyses for HT2 and T2 across global regions identified stark differences in the number of tests reported, the distribution of foodstuffs analysed and the analytical results. Most of the analytical records were submitted by the European Region, with limited numbers submitted by a few countries within the other regions. Outside of Europe, the concentrations of HT2 and T2 reported were consistently low. HT2 and T2 levels reported in Europe were much higher in cereals and any food category that may contain cereals. More detailed analysis of the European dataset showed that the highest levels were detected in oat, maize, barley and wheat grain (LB mean concentrations of 241, 24, 17 and 5 µg/kg, respectively). In a subsequent meeting, the JECFA Committee set a group Tolerable Daily Intake for HT2, T2 and diacetoxyscirpenol of 25 ng/kg body weight/day and estimated lower bound mean range of exposure of 0.3 – 53 ng/kg body weight/day indicating a possible health concern (JECFA, 2024).

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Clinical and Environmental Sources of *Fusarium fujikuroi* Species Complex: Antifungal Susceptibility and Proteomic Insights



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The *Fusarium fujikuroi* species complex (FFSC), comprising significant plant and human pathogens, exhibits broad resistance to azole antifungals but displays sensitivity to the novel agent olorofim. Proteomic analyses reveal temperature- and media-dependent alterations in protein expression, potentially reflecting adaptive mechanisms relevant to pathogenicity.

Abstract

Fusarium fujikuroi species complex (FFSC) comprises mycotoxin-producing phytopathogenic fungi and opportunistic human pathogens, causing infections from superficial to invasive.

This study investigated the biodiversity and antifungal susceptibility of FFSC isolates from clinical samples obtained from infected patients (N=19) and environmental samples derived from maize, banana peels and water drains (N=14). Genus-level identification relied on macro- and micromorphology, while species-level identification used MALDI-TOF/MS and TEF-1 α and RPB2 phylogenetic analysis, referencing the FUSARIOID-ID database. Proteomic profiling of *Fusarium verticillioides* DSM 62264 was performed after culturing for 24–72 h under varied conditions (25 °C or 37 °C, +/-CO₂, in Sabouraud broth, RPMI + 10% human or calf serum). Proteins were extracted, digested, and analysed via high-resolution mass spectrometry (TripleTOF 6600+). Antifungal susceptibility (CLSI guidelines) was tested for amphotericin B, voriconazole, posaconazole, isavuconazole, and olorofim.

Identified species included *F. annulatum* (11/33), *F. verticillioides* (21/33), and *F. fujikuroi* (1/33). *F. annulatum* and *F. fujikuroi* were mostly azole-resistant except for some voriconazole susceptibility, moderately susceptible to amphotericin B, and highly susceptible to olorofim (MIC <0.03–0.125 mg/L), with no source differences. *F. verticillioides* from corn showed high amphotericin B resistance, while clinical strains were more susceptible; azole and olorofim susceptibility varied but was source-independent. Over 400 proteins were detected. Media composition significantly affected expression of 150 proteins (P<0.05), while temperature affected 4 (P<0.05). Activator of Hsp90 ATPase (AHSA1) was prominent. Preliminary observations suggest increased iron transport protein expression in RPMI + 10% HS at 25 °C after 48–72 hours, warranting further study.

In conclusion, FFSC isolates were broadly resistant to azoles but susceptible to olorofim. Environmental *F. verticillioides* strains showed greater amphotericin B resistance than clinical ones, while resistance to other antifungals was source-independent. Proteomic analysis of DSM 62264 identified over 400 proteins, with expression significantly affected by temperature and media, notably involving AHSA1 and iron transport proteins.

Investigating resistance to azole fungicides in *Fusarium graminearum*

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Understanding how populations of fungal pathogens like *Fusarium graminearum* respond to the selective pressures imposed by widespread fungicide use is critical for maintaining the efficacy of fungicides. We document variation in the sensitivity of field-collected isolates from Canada toward two different demethylation inhibitor fungicide active ingredients. In a laboratory directed evolution experiment, we observed linkages between fungicide sensitivity and spore germination.

Abstract

Members of the *Fusarium graminearum* species complex are major pathogens of cereal crops and contaminate grain with trichothecene mycotoxins [1]. Demethylation inhibitor (DMI) fungicides like prothioconazole and tebuconazole are critical tools in managing fusarium head blight [2], but will lose their efficacy if fungicide resistance develops. We are testing contemporary *Fusarium* populations in the Canadian prairies for fungicide sensitivity, using isolates collected from harvested grains from commercial farms. We determined the effective concentration of prothioconazole and tebuconazole required to inhibit growth by 50% (EC₅₀) when grown from macroconidia in RMPI-1640 liquid medium for 48 hours. Among the 44 isolates screened to date, EC₅₀ values for prothioconazole averaged 0.032 ± 0.013 µg/mL and ranged up to a maximum of 0.067 µg/mL, while EC₅₀ values for tebuconazole averaged 0.40 ± 0.11 µg/mL and ranged up to 0.96 µg/mL. Thus, there is existing variation for sensitivity toward fungicides within contemporary *Fusarium* populations in Canada. To understand how *F. graminearum* responds to persistent selective pressure from DMI fungicides, we performed a laboratory directed evolution experiment. *Fusarium graminearum* strain DAOMC 233423 (synonym GZ3639) was exposed to increasing concentrations of tebuconazole, prothioconazole, or a combination of both fungicides, until strain extinction. We demonstrated that spore germination is less sensitive to DMI fungicides than is mycelial growth. One evolved lineage acquired resistance to tebuconazole and also showed a phenotype of early germination of macroconidia. Genomic analysis identified a single base insertion causing a premature stop codon in the *smt3* gene, which has been linked to conidiation in other fungi [3]. Knocking out the *smt3* gene re-created the abnormal spore production phenotype in *F. graminearum*, and we are currently testing the impacts on fungicide resistance. In the presence of strong selection pressure from fungicide use, populations of *F. graminearum* in Canada could evolve increased resistance to azole fungicides.

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Posters abstracts

Portable e-nose: an innovative tool for *Fusarium globosum* identification

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VOCs allow discrimination between *F. proliferatum* and *F. globosum*.

Abstract

Fusarium globosum was described for the first time in 1992 on maize in South Africa (Rheeder et al., 1996). In 1999, fungal strains belonging to this species were found on wheat seeds in Japan (Aoki and Nirenberg, 1999). In Italy, some fungal strains have been isolated from tomato presenting the same morphological characteristics (Balmas et al., 2008). *Fusarium globosum* can be usually morphologically distinguished from *F. proliferatum* and *F. fujikuroi* by the presence of globose microconidia ($\geq 10 \mu\text{m}$). Globose microconidia are lately produced in culture under specific conditions. Furthermore, molecular identification of *F. globosum* using TEF 1-alpha often misidentifies specimens as *F. proliferatum*. Recently, only 2 out of more than 100 isolates resembling *F. globosum* obtained from durum wheat kernels and basal portions in Italy were correctly identified. Fungi produce several volatile organic compounds (VOCs), comprising aliphatic and aromatic hydrocarbons, esters, ketones, aldehydes, alcohols. They have been used, among others, as markers for species identification. In this work, a portable e-nose (Smell Inspector, Freital, Germany), manufactured by smart-nanotubes, was used to discriminate between 32 *F. proliferatum* isolates (21 from garlic and 11 from other crops) and 33 *F. globosum*: 19 strains from wheat kernels from 7 different regions of Italy; 5 from basal stem of wheat, 2 from a tomato and 1 quinoa plant grown in Sardinia, including 3 from SouthAfrica and 3 from Japan. Principal Component Analysis clearly differentiates the two taxa, while the model based on Partial Least Squares Discriminant Analysis (PLSDA) linear approach exhibited excellent performance, with a 100%-correct classification rate and low errors (classification error and RMSEC). To better understand the differences between *F. proliferatum* and strains morphologically resembling *F. globosum* but not based on *tef* sequence, genome comparison for strains belonging to the different species categories is in progress.

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Emerging Members of the *Fusarium* *tricinctum* Species Complex Associated with *Buxus sempervirens* Decline in Northern Italy

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A previously uncharacterized *Fusarium* lineage within the FTSC complex has been identified as a cause of reddening and decline in historic *Buxus sempervirens* plants in Verona, underscoring the importance of genomic surveillance in ornamental plant health.

Abstract

Buxus sempervirens (boxwood) is a widely cultivated ornamental shrub, particularly valued in historical gardens for its aesthetic and cultural significance. In a prestigious historic garden in the province of Verona, Italy, century-old boxwood specimens have shown increasing signs of decline, prompting urgent phytopathological investigation. This study aimed to isolate and characterize fungal pathogens associated with defoliation, reddening, and dieback in these plants, with a focus on identifying potential emerging species. Surveys were conducted during July 2022 and May 2023. In both years, symptomatic tissues exhibiting leaf yellowing, reddened spots, vascular necrosis, and complete desiccation were sampled. Diverse fungal isolates were obtained and single-spore culture were characterized based on morphological traits. Multilocus phylogenetic analyses using partial sequences of the internal transcribed spacer (ITS) region of rDNA and translation elongation factor 1- α (TEF-1 α). Consistently, *Diaporthe stictica* was isolated from desiccated branches and necrotic vascular tissues, confirming its association with severe dieback. Instead, colonies belonging to the *Fusarium tricinctum* species complex (FTSC), specifically FTSC14, were identified from yellowing leaves. These species are already known pathogens of boxwood and have been reported in Italy, France, Germany, and parts of East Asia. However, in 2023, an increased frequency of leaf and branch reddening led to the isolation of novel fungal strains with characteristics resembling members of the Nectriaceae family. Phylogenetic analysis revealed that these isolates formed a distinct clade within the FTSC complex but did not cluster with any known *Fusarium* species, suggesting the possible presence of an undescribed taxon. To confirm species novelty, whole genome sequencing was conducted on the NovaSeq 6000 platform (Illumina) in the paired-end mode. The identification of potentially new fungal pathogens on historic *Buxus sempervirens* highlights the need for continued monitoring and advanced molecular diagnostics to protect valuable plant heritage.

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Phenotypic and genetic characterization of *Fusarium oxysporum* f. sp. *pisi* infecting *Pisum sativum* in Europe

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Abstract

Fusarium oxysporum f. sp. *pisi* (Fop), the causal organism of Fusarium wilt in peas, causes significant crop losses and is becoming a major obstacle to pea production. Despite its agronomic importance, comprehensive understanding of the population structure, genetic variation, virulence determining factors and infection biology of Fop isolates in European soils remain limited. Here, we present an integrative investigation of the Fop–pea interaction based on a collection of 48 Fop isolates collected across Europe with special emphasis on southern Sweden. The genetic diversity within the collection will be assessed by comparative genomic analysis to generate SNP markers for isolate identification and detection of extrachromosomal elements. To quantify isolate-specific virulence, we established a reproducible and scalable disease screening protocol using the soil drench inoculation method of vermiculite grown peas. In parallel, a hydroponic infection system is developed that reduces assay duration while enabling precise environmental control and real-time live-cell imaging of fungal colonization and host responses. Furthermore, the hydroponic system enables rapid pre-screening of plant and fungal genotypes, the development of early disease detection tools based on transpiration and photosynthetic rate and the evaluation of fungal nutrient-dependencies. Initial screening results indicate the presence Fop strains with contrasting virulence profiles and differing disease symptoms. To elucidate molecular mechanisms underlying pathogenicity, transcriptomic data from isolates of high and low virulence will be compared to identify candidate effector genes. Functional characterization of effector candidates will be pursued through transient expression of GFP-tagged proteins and subcellular localization via confocal microscopy. Furthermore, protoplast-based GFP transformation will enable detailed visualization fungal colonisation process and cell type specific host interactions *in planta*. Our aim is to establish foundational resources for molecular diagnostics of *Fusarium oxysporum*, effector-based monitoring, and resistance breeding strategies, while advancing our understanding of the infection biology, diversity and pathogenesis of the Fop-pea interaction.

In the genomes we trust: unveiling the genomic landscape within the Nectriaceae family



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Overall, our analysis demonstrates a reliable standard of genomic quality, with the potential to categorize genomes based on a statistical quality metric to be defined.

Abstract

The Nectriaceae family encompasses a wide array of fungal taxa including saprobes, plant pathogens, fungicolous, and insecticolous species. Advancements in genome sequencing have significantly contributed to our understanding of fungal biology, taxonomy, and pathogenicity. However, the presence of several low-quality genomes underscores the need for high-quality genome sequencing to refine species boundaries, enhance taxonomic stability, and ensure the reliability of comparative and functional genomic studies.

This study evaluates the genomic quality of 1,530 Nectriaceae genomes deposited in GenBank, examining metadata completeness and assembly metrics. Our results highlighted that some complexes such as *Fusarium oxysporum*, *F. sambucinum*, and *F. fujikuroi* are overrepresented, while some genera or species are represented by only one or two genomes. Metadata completeness is inconsistent: only 67% of genomes include full information (host, country, and collection date). According to BUSCO results, 277 genomes were classified as outliers, mostly due to a combination of high fragmentation and missing genes. N50 values were used to categorize assemblies into three quality groups: Good, Moderate, and Poor. A total of 577 genomes were categorized as Good, indicating relatively contiguous assemblies, 589 as Moderate, and 364 as Poor. To assess taxonomic consistency, we compared MLST-based and phylogenomic trees. Overall, a strong topological congruence was observed, confirming MLST's reliability in the absence of representative genomes. However, discrepancies were revealed in the *F. oxysporum* and *F. sambucinum* species complexes. The comparative metrics between the MLST and phylogenomic alignments clearly highlight the superiority of genome-scale data in resolving evolutionary relationships. While both alignments exhibited a comparable proportion of variable and conserved sites, the phylogenomic dataset, derived from 763 orthologous proteins, contained nearly three times as many parsimony-informative characters and a substantially greater number of singletons. Overall, this work underscores the importance of assuring genome quality and metadata annotation to enhance phylogenetic accuracy and support robust comparative genomics.

Investigating the pathogenicity, population structure and morphology of *Fusarium oxysporum* strains infecting pea (*Pisum sativum* L.)



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Our research will investigate the variation present in a Swedish *Fusarium oxysporum* f.sp. pisi strain collection with regards to their pathogenicity on pea, their genetic relatedness and population structure, and their morphological differences.

Abstract

Fusarium wilt in pea caused by *Fusarium oxysporum* f.sp. pisi (FOP) is a devastating disease that has the potential to cause complete yield losses. While integrated control methods exist¹, the occurrence of *Fusarium* wilt in Sweden is rising². Partly due to the highly diverse nature of *Fusarium oxysporum* and its evolutionary potential⁴. Our research aims to investigate pathogenicity factors, population structure and morphological diversity of *Fusarium oxysporum* strains that infect pea. Our current strain collection of 48 FOP strains will be morphologically characterized and evaluated for their virulence on pea. High and low virulence strains will then be used for inoculations on a diverse pea germplasm collection of 500 genotypes under semi-controlled conditions. The resulting data will be used in combination with previously generated genotypic data to identify genetic markers segregating for disease resistance against FOP using GWAS. Marker efficacy for disease resistance will be analysed in independent pea populations, so that they may be used for Marker Assisted Selection programmes in the future. Data on the pathogenicity and degree of virulence from our characterization will be combined with the genetic data on our FOP collection to identify SNPs related to both traits and to investigate population structure. In this assessment we will consider the presence/absence of newly discovered evolutionary components related to effector content, such as mini-chromosomes and Starship transposable elements⁵. To conclude our investigation into the FOP-pea interaction we will analyse transcriptomes of both FOP and pea varieties during infection of resistant and susceptible plants with high and low virulence strains. This will provide us with insight into differentially expressed genes between the two groups and allow us to infer functions related to resistance and virulence respectively. Ultimately, this will contribute to a greater understanding of interactions between *Fusarium oxysporum* and pea.

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Characterization of *Fusarium graminearum* and *Fusarium boothii* originated from wheat and maize in Serbia

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Continuous monitoring of *F. boothii* on maize and wheat is necessary.

Abstract

Agroecological conditions prevailing in Serbia favor the development of numerous pathogenic and toxigenic *Fusarium* species out of which *Fusarium graminearum* is the most important pathogen of maize and small grains. At least 15 phylogenetic species within the *Fusarium graminearum* species complex (FGSC) were discovered using phylogenetic analysis and the GCPSR method (Genealogical Concordant Phylogenetic Species Recognition) (Sarver et al. 2011). The aim of this work was to characterize *F. graminearum* and *F. boothii* originated from wheat and maize in Serbia

In this research, 79 isolates were studied, isolated from wheat (42) and maize (37) grains from 12 different locations in Serbia. Using the TEF-1a genomic region, all isolated isolates (79) were sent for service sequencing, and then 9 representative isolates were selected and further sequenced based on two more genomic regions β -tubulin and histone H3. Phylogenetic analysis of nine selected sequences of three gene regions of TEF-1a (MF974399, MF974400, MF974402, MF974403, MF974404, MF974405, MF974406, MF974407, MF974408, MF974409, MF974410), β -tubulin (MG063783, MG063784, MG063786, MG063787, MG063788, MG063789, MG063790, MG063791, MG063792, MG063793, MG063794) and histone H3 (MF999139, MF999140, MF999142, MF999143, MF999144, MF999145, MF999146, MF999147, MF999148, MF999149, MF9991450), revealed that seven isolates were identified as *F. graminearum* sensu stricto and two isolates as *F. boothii*, one of maize origin (MF974409, MG063793, MF999149) and one isolate of wheat origin (MF974410, MG063794, MF999150).

Genes TEF-1a, β -tubulin and histone H3 were found to be informative enough to separate the *F. boothii* species within FGSC. Species within the FGSC synthesize a variety of mycotoxins that affect human and animal health. Based on chemical and molecular analyses, it was confirmed that all isolates belonged to chemotype 15ADON. Since previous research has shown that climate change is the main cause of the appearance of new, potentially more toxic species, future research must pay special attention to changes in the population of the FG complex.

Prevalence and contribution of *Fusarium juglandicola*, a newly-described species, to walnut dieback

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***Fusarium juglandicola* is a newly-described species. Despite its low aggressiveness, its high prevalence and association with *Diaporthe eres* in symptomatic walnut twigs questions its role in disease development leading to walnut dieback.**

Abstract

English walnut (*Juglans regia* L.) is an economically-important fruit crop worldwide, particularly in France, where it is the second most important fruit crop after apples in terms of cultivated area. Walnut orchards are targeted by numerous diseases, and new symptoms have been widely observed since 2015 in France, consisting in typical branch dieback and fruit blight and necrosis, as commonly reported in California since the early 2000s (Chen et al., 2014), and more recently in Europe (López-Moral et al., 2020; Gusella et al., 2021). Based on a survey of 12 commercial French walnut orchards during 3 years in which symptomatic and asymptomatic twigs and husks were collected, we found that *Neofusicoccum*, *Botryosphaeria* and *Diaporthe* were consistently isolated from husks and twigs, which are pathogens commonly associated with this disease. Surprisingly, symptomatic twig samples were also largely contaminated with *Fusarium juglandicola*, a recently-identified species isolated from nut and plant samples (Crous et al., 2021), with prevalence yielding 60% (out of 288 twigs). Unlike *Botryosphaeriaceae* and *Diaporthe* species, pathogenicity tests revealed that, when inoculated alone, *F. juglandicola* isolates were non-pathogenic, raising questions about its contribution to the disease. In particular, *F. juglandicola* and *D. eres* were the most frequent dual association in symptomatic twigs and *F. juglandicola* contaminated twigs had 73% of probability to be also contaminated with *D. eres*. To go further, sequential inoculations, performed on detached twigs, showed that the first pathogen to arrive significantly limited the establishment of the second, regardless of its aggressiveness (that is, *F. juglandicola* restricted *D. eres* development and vice-versa). In mixed inoculations, however, the most aggressive species dominated, with *D. eres* suppressing *F. juglandicola*, indicating antagonistic rather than synergistic interactions. Genome analysis of *F. juglandicola* is currently underway and will provide additional insights into its role in disease onset and development. Potential to produce toxic secondary metabolites will also be scrutinized.

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Phylogenetic analysis of a 139.9-Kb 52-gene dataset to reassess diversity within *avenaceum* clade of *Fusarium tricinctum* species complex



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Phylogenetic FTSC species of *Avenaceum* clade are supported by the phylogenetic analysis of a 52-gene dataset, which also divides *Fusarium avenaceum sensu stricto* (FTSC 4) into two phylogenetic species.

Abstract

The *Fusarium tricinctum* species complex (FTSC) includes *F. avenaceum*, which occurs on different plants and also involved in Fusarium head blight development of cereal crops. *Fusarium avenaceum* isolates from one host can often be pathogenic also on other distantly related plants (Nalim et al. 2009). Yli-Mattila et al. (2022) assessed the diversity of *F. avenaceum* and related species (*Avenaceum* clade) of the FTSC based on partial *TEF1* and *TUB2* sequences, and further divided the isolates into Main Groups I, II, III and IV or as not belonging to any of these Main Groups. Recently, Laraba et al. (2022) proposed that the FTSC consists of 36 species and gave the species the *ad hoc* designations FTSC 1 – FTSC 36. Comparison of data from these studies indicate that FTSC 4 includes Main Groups I and II, Main Group III includes FTSC 11 and 27, and Main Group IV corresponds to FTSC 5. Here, we used full-length DNA sequences of 52 protein-coding genes to reassess species identity and relationships of a subset of isolates from the Yli-Mattila et al. studies. The analysis included isolates of six species, which belong to the *Avenaceum* clade within FTSC: FTSC 4 (*F. avenaceum sensu stricto*), FTSC 5 (*F. paeoniae*), FTSC 11, FTSC 22, FTSC 30, and FTSC 34. The results indicate that FTSC4 can be resolved into two distinct clades, which correspond to two phylogenetically distinct species. One of these phylopecies consisted of two Main Group II isolates, and the other consisted of Main Group I isolates and one Main Group II isolate of Yli-Mattila et al. (2022). Together, these seven species formed a well-supported clade that was distinct from *F. acuminatum*, *F. tricinctum*, *F. torulosum*, and *F. flocciferum*. These results help clarify the species diversity among FTSC isolates of *Avenaceum* clade from Europe and worldwide.

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Investigating conserved D effector activity in *Verticillium dahliae* and *Fusarium oxysporum*



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The conserved fungal D effector, shared by *Verticillium dahliae* and *Fusarium oxysporum*, induces host-specific developmental responses in *Arabidopsis thaliana*, highlighting its functional conservation and potential host-dependent activity.

Abstract

Fungal pathogens secrete effector proteins to interfere with host immunity and facilitate colonization (Rovenich et al., 2014; Cook et al., 2015). *Verticillium dahliae* (Vd) and *Fusarium oxysporum* (Fo) are soil-borne fungi with similar infection strategies but distinct host ranges. While Vd can infect numerous plant species, Fo strains are typically host-specific and classified into formae speciales (Fradin & Thomma, 2006; Di Pietro et al., 2003). Some effector genes are conserved across these plant pathogens and contribute to virulence (De Jonge et al., 2011; Derbyshire & Raffaele, 2023). One of them is the D effector, originally identified in defoliating Vd strains infecting cotton. Homologous genes have also been found in Fo, including *F. oxysporum* f. sp. *vasinfectum* (Fov) and *F. oxysporum* f. sp. *radicis-cucumerinum* (Forc), as well as in non-defoliating Vd strains. Previous functional assays using purified D protein homologs from these strains showed their ability to induce wilting symptoms in cotton, suggesting a conserved functionality.

We investigated whether *Fusarium oxysporum* and *Verticillium dahliae* D effector homologs induce similar effects on *Arabidopsis thaliana* as previously observed in cotton. To test this, we performed protein assays in 96-well plates, where *Arabidopsis* seedlings were grown on liquid medium supplemented with purified effector proteins. This setup allowed us to monitor protein-induced phenotypic changes during early seedling development. The observed effects were quantitatively measured and statistically analysed to assess their significance. Interestingly, while the D effector from non-defoliating *V. dahliae* strains did not induce symptoms in cotton, it triggered a distinct phenotypic response in *Arabidopsis thaliana*, highlighting a potential host-dependent functionality of this conserved effector.

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Portable Real-Time PCR for Field-Based Monitoring of Mycotoxin-Producing Fungi in Cereals



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A portable PCR system offers laboratory-grade accuracy for on-site mycotoxin detection, ensuring early intervention and safer food chains.

Abstract

Monitoring fungi capable of producing mycotoxins remains a major challenge in agriculture. Mycotoxin contamination affects an estimated 60–80% of agricultural products worldwide, posing serious health and economic risks. Given these threats, the development of rapid detection systems for early risk assessment is critical.

The Polish Academy of Sciences (PAS) is developing innovative technologies to address food contamination challenges. In response to growing market needs, Bioaccure Ltd. (Poland) has created a portable, field-deployable system for the rapid detection of fungi capable of mycotoxin biosynthesis. The system consists of a four-channel real-time thermal cycler (PCR:smart®), a dedicated mobile application for system operation, lyophilized qPCR assays, and a simplified DNA extraction.

This research focuses on integrating mobile molecular diagnostics into routine mycotoxin monitoring workflows. Bioaccure has designed qPCR assays that enable broad-spectrum detection of toxigenic fungi by targeting conserved biosynthetic genes rather than species-specific markers. For instance, the detection of aflatoxin producers is based on *norA*, *norB*, and *aflK* genes, while *tri101* is used for trichothecene producers. This strategy allows the identification of multiple fungi that share the same biosynthetic pathways, thereby improving detection sensitivity and broadening applicability across diverse agricultural safety settings.

The smart® platform, combined with direct PCR reagents, also facilitates a rapid DNA extraction workflow that bypasses the need for laboratory-based sample preparation, making it well-suited for field use. PAS is conducting comprehensive research to validate the system under non-laboratory conditions and is optimizing analytical protocols for in-field deployment. Initial validation studies have shown 100% specificity in detecting genetic markers of mycotoxin biosynthesis, with over 98% reproducibility compared to conventional laboratory qPCR systems. These promising results highlight the potential of the portable PCR:smart® system as a reliable, on-site tool for rapid monitoring of mycotoxin contamination, offering a significant advancement in food safety management.

Development of a tool for *Fusarium* species identification isolated from cereal based on DNA barcoding.



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A DNA barcoding approach provides a reliable and complementary method to identify *Fusarium* species isolated from cereal, supporting improved phytosanitary diagnostics.

Abstract

Fusarium is among the most economically important fungal genera affecting cereals worldwide. One major disease, *Fusarium* Head Blight (FHB), can cause yield losses of up to 70% and is caused by several species, in mono- or co-infection, varying according to crop, region and environmental conditions. Many *Fusarium* species also produce harmful mycotoxins. Due to the withdrawal of chemical treatments, seed testing laboratories are receiving increasingly requests to detect and identify *Fusarium* species on cereal for phytosanitary certification, which requires reliable methods. An ongoing ISTA project aims to expand the current *Microdochium* detection method on wheat seeds⁽¹⁾ to include *Fusarium* species. This method relies on strain isolation and species identification based on morphological criteria, which requires high expertise. As a complement, we propose a DNA barcoding approach to identify fungal species isolated from seeds.

A collection of some 100 strains isolated from around the world, including target species and closely related non-target strains isolated from cereals, was established. These strains were characterised both morphologically and by PCR. After a literature review^(2,3,4,5,6,7,8) and *in silico* testing, three primer pairs targeting the *ef1*⁽⁹⁾, *rpb1*⁽¹⁰⁾, and *cal*⁽¹¹⁾ genes were selected for *in vitro* trials. For each strain, all genes were amplified and sequenced using Sanger method, and all sequences were analysed using 'Nucleotide BLAST' from NCBI⁽¹²⁾, 'Polyphasic identification' from Fusarioid-ID Database⁽¹³⁾ and by MLSA with a home-made sequence database.

All *Fusarium* species were successfully identified using *ef1* and *rpb1* genes, either alone or in combination, depending on the analysis method. An inter-laboratory test will be organised under ISTA rules to validate this method.

Once validated, this DNA-based identification method will complement the existing protocol based on morphological criteria. In addition, the results obtained support the potential development of a new metabarcoding method using high throughput sequencing directly on seeds.

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Association between chromatin accessibility and gene expression: machine learning approach.



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This study aims at describing the efficiency of machine learning methods to study the association between chromatin accessibility and gene expression in the phytopathogen fungus *Fusarium graminearum* genomes.

Abstract

The filamentous fungus *Fusarium graminearum* is a highly resilient clonal plant pathogen that produces mycotoxins responsible for heavy damages in wheat crops. These mycotoxins, and notably deoxynivalenol, are elements of the fungal stress-response system and act as virulence factors [1], considering that climate change is expected to intensify environmental stress, virulence in crop infections is likely to increase [2]. Epigenetic mechanisms, particularly variations in chromatin accessibility driven by changes in nucleosome positioning, play a key role in regulating gene expression in response to environmental changes. High-throughput sequencing-based methods such as MAINE-seq [3] enable genome-wide measurement of these epigenetic variations [4].

We developed statistical methods to characterize biological variation in epigenetic regulation of gene expression across the *F. graminearum* genome. As a first step, we identified a key genomic region around the START codon of genes, where chromatin accessibility signals were highly correlated to gene expression. Building on this, we design a mixture model framework that accounts for potential clustering of genes sharing similar chromatin-expression association patterns within this region. This approach not only identifies cluster-specific association signals that can be biologically interpreted but also provides a predictive framework showing robustness regarding other machine learning approaches. Importantly, it allows detection of changes in chromatin-expression associations across variable conditions, offering new insights into epigenetic regulation of fungal pathogenicity under climate change. Specifically, we focus on *F.graminearum* genomes where the gene *Fglae1* , controlling virulence [5] and potentially a chormatidian remodeler, is mutated.

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Critical opinion on the molecular approaches for the mycotoxigenic *Fusarium* species identification in wheat



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Many earlier published *Fusarium* species-specific primers lack reliability when evaluated against the recently developed high-quality genomic resources, highlighting the urgent need for more robust diagnostic assays to ensure accurate detection in wheat.

Abstract

Accurate *Fusarium* species diagnostics is crucial for mitigating mycotoxin-related food safety concerns in wheat. Over the years, numerous molecular tools have been published to distinguish species of this taxonomically complex genus. The rapid growth in genome sequencing has now made it possible to systematically verify whether these widely used primers remain taxonomically accurate. In this study, published *Fusarium* species-specific primer sets were critically evaluated for their performance against a curated genome library representing wheat-associated *Fusarium* spp. [1]. Genome assemblies were checked for reliable strain provenance and reported chemotype records and further assessed with BUSCO [2] and QUAST [3] to ensure completeness and contiguity, providing a high-quality basis for downstream analyses. A Python-based script was developed to screen the genomes with each primer pair and identify potential amplicons across different orientations and mismatch thresholds. In addition, NCBI Primer-BLAST searches were conducted to assess possible cross-reactivity with non-target fungi and wheat sequences.

From the 48 primer pairs tested, only 10 were found to be highly specific in both the curated genome library screen and Primer-BLAST searches. The remaining primer pairs exhibited varying levels of cross-reactivity, most frequently with closely related *Fusarium* species, but also occasionally with unrelated fungal taxa, while five primer pairs failed to yield any amplicon hits or Primer-BLAST matches. The preliminary findings highlight significant gaps in the molecular tools currently used for *Fusarium* detection and suggest that several primer pairs that are widely cited in the literature may lack sufficient accuracy for reliable species identification, when evaluated against the current *Fusarium* genomic resources. Future work will extend this evaluation to primers targeting biosynthetic gene clusters associated with trichothecene (Type A and Type B) and zearalenone production, as well as qPCR and metabarcoding, ultimately guiding the design of more robust diagnostic assays.

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Interaction between wheat-associated bacteria and enniatin

B: growth inhibition and biodegradation

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Fusarium mycotoxin, wheat microbiota, enniatin B, biodegradation.

Abstract

Emerging mycotoxins such as enniatins, particularly enniatin B (ENNB), are frequently detected in cereal grains, especially wheat. While the wheat-associated microbiome is of major agricultural importance, limited information exists on how microbial community composition is affected by exposure to *Fusarium*-produced enniatins. To investigate this, a preselection of wheat-associated bacteria was conducted using isolates from various sources and representing different parts of the wheat plant. Of these, 12 strains were selected to evaluate their sensitivity to ENNB and their capacity for its degradation. Bioassays were performed to assess the impact of ENNB on microbial growth, and to quantify the extent of ENNB degradation.

To evaluate sensitivity, each strain was exposed to a range of ENNB concentrations (50, 15.81, 5, 1.58, 0.5, 0.15, and 0 μ M) in tryptone soy broth, and growth was monitored hourly by measuring optical density at 600 nm over 48 hours. Bacterial growth was fitted to logistic equation whose parameters were used to derive effective concentration 50 % (EC₅₀) by applying a dose-response model. ENNB degradation was assessed by measuring the mycotoxin concentration in the culture medium containing an initial concentration of 15.81 μ M, at the beginning (T₀) and after 48 hours of exposure (T₄₈), using high-performance liquid chromatography with diode-array detection (HPLC-DAD).

The results showed that *Pseudarthrobacter niigatensis*, *Microbacterium phyllosphaerae*, *Pseudomonas graminis*, and *Stenotrophomonas rhizophila* exhibited a clear growth delay at all tested ENNB concentrations (0-50 μ M). According to ENNB-degrading capacity of studied strains, *Bacillus pumilus* and *Bacillus atrophaeus* reduced ENNB concentration in the culture medium to below the limit of detection. Additionally, *Bacillus amyloliquefaciens* was able to reduce ENNB concentration by up to 18 % after 48 hours of exposure. Hence, these findings highlight the potential role of specific wheat-associated bacteria in mitigating enniatin B contamination through natural degradation mechanisms.

First report of crown and foot rot on durum wheat caused by *Fusarium algeriense* in Europe



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FCR/FRR are major threat for durum wheat in Italy with *F. culmorum* as primary causal agent. During a 2024 field survey in southern Italy, *F. algeriense* has been reported for the first time in Apulia, Basilicata, Campania and Sicily. Pathogenicity tests on susceptible durum wheat showed *F. algeriense* to be a weak pathogen compared to *F. culmorum*.

Abstract

Crown and Root Rot (FCR; FRR) is an economically important disease on durum wheat (*Triticum turgidum* ssp. *durum*) in Italy, particularly in the Mediterranean region. Several fungi may cause FCR or FRR leading to significant yield and quality losses. The most common causal agent in Italy is *Fusarium culmorum* (W.G. Smith). During an extensive survey on fields affected by FCR or FRR in Southern Italy, in 2024, besides confirming the presence of *F. culmorum* and *Fusarium pseudograminearum*, *Fusarium*-like colonies were also isolated from symptomatic plants sampled in 4 regions: Apulia, Basilicata, Campania and Sicily. After 6 days of incubation at 25°C, the developing colonies were removed, and single-spore cultures were obtained. On PDA, the pigmentation varied from light orange to yellowish-white. Morphological characteristics observed under microscope matched the first description of *Fusarium algeriense* made by Laraba et al. (2017).

Sequence analyses were performed for four genes (*tef1*-α, ITS, *CaM*, *βT*), showing high identity with sequences available in GenBank for the *F. algeriense* type strain, isolate NRRL 66647 (Laraba & O'Donnell): *tef1*-α showed 99.68–99.82% and ITS showed 99.81–100% identity. A pathogenicity assay was carried out in greenhouse conditions using the susceptible durum wheat cv Iride. The tested isolates of *F. algeriense* varied in virulence and generally proved weak pathogens, causing a disease index ranging from a 4.4% to 38.0% with an average of 15.0% compared to 100% scored for a highly virulent isolate of *F. culmorum*.

F. algeriense was successfully reisolated from the symptomatic tissues of the inoculated seedlings, thereby fulfilling Koch's postulates. To the best of our knowledge, this is the first report of FRR or FCR caused by *F. algeriense* in Europe.

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Fusarium oxysporum: evaluation of intraspecific molecular biodiversity among the different formae speciales



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This project aims to fill the knowledge gap between most and least studied *Fusarium oxysporum* formae speciales and to identify effective molecular markers to discriminate among different formae speciales. This research will integrate comparative genomics, effector biology, and metabolomics to distinguish the different formae speciales, predict pathogenicity and host specificity, improve the agronomic and health management of crops.

Abstract

Fusarium oxysporum species complex (FOSC) includes the most widespread soil-borne fungal pathogens that pose a threat to food quality and safety due to their ability to produce toxic secondary metabolites, such as mycotoxins. In FOSC, species belonging to this complex have been reported as able to produce the so-called emerging mycotoxins, such as enniatins and beauvericin, which exhibit phytotoxic effects and contribute to the virulence of the producing strains, together with fusaric acid, another phytotoxic metabolite produced by this species.

A distinctive feature of FOSC is the classification of strains into formae speciales based on host specificity, but there is still a knowledge gap between most and less studied. This new project aims to investigate the currently uncharacterized formae speciales from both genomic and metabolic point of view, since these traits have been shown to be the most informative in explaining variability among formae speciales.

Genomic analysis will be focused on effectors, small proteins produced by fungi during host-plant interaction, which represent major pathogenicity determinants across different *F. oxysporum* formae speciales. The literature shows that strains belonging to the same forma specialis encode similar repertoires of effectors, suggesting that they can be used for more rapid discrimination of the different formae speciales.

In addition, we will characterize the metabolic profiles of a selected group of strains using analysis of both targeted and untargeted metabolites, to explore potential correlations between host specificity, geographic origin, and metabolite production. The results obtained through different approaches will provide new genomic and metabolomic data, improving our understanding of toxigenic strain distribution within FOSC. This knowledge will enhance the resolution of evolutionary relationships among formae speciales and their association with host and environmental factors. Additionally, the identification of more reliable molecular markers for discrimination may lead to innovative strategies for crop protection, sustainable agriculture and food-safety.

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Associations between germination capacity and fungal infestation of wheat seeds

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Low germination capacity in Norwegian spring wheat seeds is linked to higher infestation by specific fungal species, notably *Microdochium majus* and *Fusarium graminearum*, with fungal community composition influencing seed viability.

Abstract

To identify possible associations between germination capacity and fungal infestation of wheat seeds, we used seed lots of two spring wheat varieties (Mirakel and Zebra) grown in Norway in 2016 and 2017. The percentage of seed germination (determined by the ISTA germination method) differed between seed lots and they were grouped accordingly (<90% germinated seeds = low germination capacity, ≥90% germinated seeds = high germination capacity). For each seed lot the percentage of seeds infested by important wheat pathogenic fungi in Norway, namely *Microdochium* spp., *Fusarium* spp., and *Parastagonospora nodorum*, was determined using a plate-out test on PDA. In addition, the DNA-concentration of *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *M. majus*, *M. nivale*, and *P. nodorum* was determined by species-specific qPCR, and the mycobiome was analysed by metabarcoding of ITS 1 and ITS 2.

Seed lots with a low germination capacity were highly infested with *Microdochium* spp. and had a high DNA-concentration of *M. majus*. Germination capacity was also negatively associated with the DNA concentration of *F. graminearum*, despite the low concentrations of *Fusarium* species observed in general. Metabarcoding additionally revealed a negative association between germination capacity and the relative presence of fungi within the genus *Neosascochyta*. *Parastagonospora nodorum* was present at high levels (% of seeds infested and fungal DNA) but seemed not to be associated with seed germination capacity. Our results also indicated some co-existence patterns between fungal species, including both pathogenic and non-pathogenic species, with some species combinations associated with the germination capacity of wheat seed.

The Journey of *Neocosmospora* species – from Pipes to Patients

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***Neocosmospora petroliphila*, is found in both clinical and household drain settings and exhibits resistance to conventional and novel antifungals, highlighting its potential as a persistent opportunistic pathogen.**

Abstract

Species of the *Neocosmospora* (*Fusarium solani* complex-FSSC), are frequently found in soil and plant debris and are also associated with invasive mycoses in immunocompromised individuals. Potential household reservoirs of the *Neocosmospora* include water drains, which promote biofilm formation, increasing the risk of their transmission in the indoor environment. Thus, the aim of this study was to investigate the biodiversity and antifungal susceptibility of clinical and water drain *Neocosmospora* isolates.

Clinical strains (N=15) were obtained from the University Hospital Zagreb, from stool (7) minilavate (3), blood cultures (2), urine (2) and sinus secretions (1). Environmental isolates (N=20) originated from household water drains collected in Zagreb. Identification of the isolates was performed using MALDI-TOF/MS in combination with sequencing of the ITS and TEF-1 α regions and phylogenetic analysis (Fusarioid-ID, <https://www.fusarium.org>). Susceptibility of isolates to amphotericin B, voriconazole, posaconazole, isavuconazole and two novel antimycotics olorofim and manogepix was tested according to CLSI guidelines.

MALDI-TOF identification was consistent with sequence-based identification at the species complex level; however, discrepancies were observed at the species level within *Neocosmospora*/FSSC. Clinical isolates were represented with two species *N. petroliphila* (12/15) and *N. metavorans* (3/15), while environmental isolates showed greater diversity including *N. keratoplastica* (5/21), *N. petroliphila* (4/21), *N. solani* (3/21), two strains of *N. tonkinensis*, *N. stercicola* and *N. crasa*, and one strain of *N. falciformis* and *N. quercicola*.

All isolates were resistant to posaconazole, isavuconazole, olorofim, and manogepix, with 28/36 also resistant to voriconazole. Manogepix showed an MIC₉₀ for most isolates. All but one clinical *N. petroliphila* isolate remained susceptible to amphotericin B.

MALDI-TOF identification of *Neocosmospora*/*Fusarium* remains challenging within species-rich complexes. Detection of *N. petroliphila* in clinical and environmental sources, along with broad antifungal resistance, highlights its adaptability and clinical persistence.

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Targeting Fusarium Head Blight with Synthetic Peptaibol Analogs: Efficacy and Mode of Action



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Naturally-inspired eco-friendly peptides show promising antifungal activity against *Fusarium graminearum*, reducing FHB severity and DON accumulation in wheat, with an apoptotic-like mode of action.

Abstract

Fusarium graminearum is one of the most destructive fungal pathogens affecting wheat, causing the Fusarium Head Blight (FHB) disease. *F. graminearum* also contaminates grain with deoxynivalenol (DON), posing risks to food safety. Growing concerns over fungicide resistance and environmental impact have increased interest in eco-friendly disease control strategies. This study investigated the antifungal efficacy on *F. graminearum* of synthetic peptides derived from trichogin GA IV, a peptaibol naturally produced by *Trichoderma longibrachiatum*, elucidating their mode of action through optical, fluorescence, and transmission electron microscopy. Twenty-four trichogin analogs were screened in vitro for their ability to inhibit conidial germination, with selected candidates tested in planta. Pep 5 and Pep 4r emerged as the most effective peptides, significantly reducing both FHB disease severity and DON accumulation in wheat spikes. Treatments with Pep 5 and Pep 4r induced morphological alterations consistent with an apoptotic-like mechanism, including cytoplasmic condensation, nuclear fragmentation, mitochondrial membrane disruption, and vesicle-like structure formation. No detectable production of reactive oxygen species (ROS) was observed, suggesting a ROS-independent cell death mechanism. Treatment with the inactive Pep 3 led to the formation of large, non-viable, round structures surrounded by viable hyphae, indicating a localized cytotoxic response not affecting fungal proliferation. This study highlights the potential of trichogin-derived synthetic peptides as effective antifungal agents against *F. graminearum*, supporting their further development as sustainable alternatives to conventional fungicides in integrated disease management strategies.

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Mycotoxins Accumulated in *Asparagus officinalis* L. Plants Infected with *Fusarium oxysporum*



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Both *F. oxysporum* strains showed progressive mycotoxins biosynthesis and disease symptoms (root lesions and reduced biomass). The amounts of toxins correlated with the disease level observed for roots.

Abstract

Fusarium oxysporum is a major pathogen of *Asparagus officinalis* L., contributing to vascular wilt and yield decline in commercial fields. These pathogens are known to produce mycotoxins which aid in the colonization of their host and are detrimental to food safety. While mycotoxin production by *Fusarium* species is well known in cereals, limited information exists regarding the specific mycotoxins produced in asparagus tissues, particularly during the later stages of infection. This study aimed to evaluate *in planta* mycotoxin accumulation in the widely cultivated 'Mary Washington' asparagus genotype following infection with two genetically distinct *F. oxysporum* strains. Seedlings were inoculated with spore suspensions of two *F. oxysporum* isolates under greenhouse conditions. Symptom severity, plant biomass, were recorded on 21-day post-inoculation and the plant material were separated into root and shoot tissues. Homogenized samples were subjected to targeted mycotoxin analysis using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Our findings demonstrated that all strains reduced root and shoot weights of the infected plants as compared to the uninfected plants. Mycotoxin profiling revealed that both isolates induced detectable mycotoxin accumulation *in planta*, with strain-dependent differences in type and concentration. Specifically, one of the strains produced markedly higher toxin concentrations, particularly in root tissue which correlated with more severe visible symptoms of root browning. Mycotoxins were either absent or present at trace levels in non-inoculated controls. This study demonstrated the importance of monitoring the presence of mycotoxins in edible crops and highlights the need for strain-level characterization in pathogen risk assessments.

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Genotype-Dependent *Fusarium* Mycotoxin Accumulation and Its Impact on Early and Late Asparagus Infection Stages



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Both genotypes showed progressive disease symptoms (root lesions and reduced biomass). Susceptible cultivar accumulated higher amounts of mycotoxins than the tolerant cultivar.

Abstract

Fusarium species not only cause root and crown rot in asparagus (*Asparagus officinalis* L), but also produce harmful mycotoxins that accumulate in plant tissues. These toxins may influence infection dynamics and pose risks to food safety. While *Fusarium* pathogenicity is well studied, the relationship between host genotype and mycotoxin accumulation remains poorly defined in asparagus. This study investigated the impact of genotype-dependent mycotoxin accumulation on disease expression during early and late *F. oxysporum* infection stages in two asparagus cultivars (susceptible and tolerant). Seedlings of both cultivars were inoculated with fungal spore suspensions under greenhouse conditions. Infection was monitored at early (7–14 days) and late (21–28 days) stages. Growth parameters (root/shoot) were monitored for symptom production, while key mycotoxins in infected plant tissues were analysed using Ultra-High Performance Liquid Chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Results demonstrated a clear genotype-dependent pattern of mycotoxin accumulation at both infection stages. Mycotoxin levels and root necrosis increased significantly over time in both cultivars. Toxin production at the late infection stages (21d, 28d) were strongly associated with biomass suppression in both tested cultivar. Comparatively, the susceptible cultivar accumulated higher levels of toxins during the late infection stage, which coincided with more severe root damage and biomass loss. These findings highlight the role of genotype-driven mycotoxin regulation in disease progression and provides a foundation for selecting cultivars with both agronomic and food safety advantages.

Research was supported by the National Science Centre OPUS 22 grant: NCN 2021/43/B/NZ9/02701

Dissection of Fusarium Head Blight (FHB) Resistance in Spring Wheat Genotypes

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Abstract:

Fusarium head blight (FHB) caused by *Fusarium spp.*, is a devastating wheat disease that causes significant yield losses, posing serious threats to food safety and the economy worldwide. Advancement in breeding for disease resistance is hindered by complex genetic inheritance and limited availability of well-adapted resistant germplasm. In this study, 335 NOBAL wheat genotypes (from Nordic, Baltic, and exotic origin) were investigated in two field trials using spray inoculation, 2 spawn grain inoculation trials in nursery and 2 greenhouse trials i.e., one spray and one point inoculation method under controlled conditions. The aim of the study was to assess the association between morphological traits and Type I, Type II and overall FHB resistance. Best linear unbiased estimates (BLUEs) were calculated to reduce biasness in data. Besides, genome-wide association study (GWAS) was done utilizing a 25K SNP chip array, to identify significant marker-trait associations (MTAs) associated to FHB resistance, helping define key quantitative trait loci (QTL) regions. Genotypes having low FHB severity were identified, which could serve as valuable germplasm resource for the development of FHB-resistant wheat varieties in the Baltic region.

Effects of enniatin B as emerging mycotoxin and its association with deoxynivalenol on wheat microbiota

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The combination of enniatin B and deoxynivalenol has minimal impact on wheat microbiota, but *F. avenaceum* secondary metabolites likely drive the performance of applied biocontrol strains, so exploring the role of other enniatins is warranted.

Abstract

Agricultural products are commonly contaminated by *Fusarium* mycotoxins, including the emerging enniatin B (ENB), which has shown a consistent increase in occurrence. This mycotoxin is frequently co-detected with deoxynivalenol (DON) in wheat crops, posing an important risk for animal and human health [1]. This work aims to investigate the role of ENB in association with DON on (i) selected biocontrol agents (BCAs) and on (ii) the composition of wheat microbiota, which is considered a key factor in plant health and resilience.

In this context, the growth of selected BCAs was not affected by exposure to ENB (50 µg/ml) and DON (88 µg/ml) *in vitro* assay. ENB treatment resulted in minor variations in the metabolomic profile of the most promising strain BCA2, which preserved its ability to inhibit DON biosynthesis in *Fusarium graminearum* *TRI5::GFP* mutant *in vitro*. On the other hand, the presence of *F. avenaceum*, as the main producer of enniatins, may interfere with the efficacy of our BCAs in field conditions.

Furthermore, PacBio full-length 16S sequencing was employed to analyse possible changes in the composition of microbial communities in wheat spikes after mycotoxin exposure. The experiments were carried out using both mycotoxin-producing fungi and direct injection of mycotoxins into the spikelets. According to the results, the composition, richness and relative abundance in taxa of wheat microbiota were not significantly influenced by ENB and DON compared to the control. However, the microbial community seems to differ among samples, probably depending on the physiological characteristics of each spike.

In conclusion, the ENB-DON combination appears to have minor and non-significant effects on the wheat microbiota, however *F. avenaceum*, including its secondary metabolites, may play an important role in modulating biocontrol mechanisms of BCAs. For this reason, it would be interesting to further investigate the role of other enniatins besides ENB.

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***Streptomyces coelicoflavus* BM40: A promising biocontrol agent against chickpea Fusarium wilt**



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***Streptomyces coelicoflavus* BM40 demonstrates strong antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris*, highlighting its potential as an effective and sustainable biocontrol agent for managing Fusarium wilt in chickpea cultivation.**

Abstract

Fusarium oxysporum f. sp. *ciceris* is the causal agent of Fusarium wilt in chickpea (*Cicer arietinum*), posing a major threat in regions such as the Mediterranean, India, California, and Ethiopia. Environmentally friendly biological control agents play a crucial role in managing this disease. In Algeria, *Streptomyces* species have shown promising potential as biocontrol agents against this pathogen.

Four *Streptomyces* strains, isolated from vineyard rhizospheres in different regions of Algeria (Mascara, Tipaza, and Boumerdes), were selected for in planta biocontrol tests against *Fusarium oxysporum* FP3. Preliminary screening of these strains was based on in vitro assays, including plant growth-promoting (PGP) traits, antifungal dual culture tests, and enzymatic activity assays. Strains MS18 and TZ2 exhibited strong antifungal activity, while MS22 and BM40 demonstrated significant PGP and enzymatic activities. In vivo, disease severity, shoot weight, and root weight were measured to evaluate each strain's performance in promoting plant growth and suppressing disease.

The results showed that strain BM40 significantly reduced disease incidence in plants infected with *F. oxysporum* FP3 and enhanced both shoot and root biomass. Based on these promising *in vivo* results, the draft genome of strain BM40 was sequenced and analyzed. Genome sequencing identified BM40 as *Streptomyces coelicoflavus*, with a genome size of 8,786,797 bp and a GC content of 72%. antiSMASH analysis revealed a high number of biosynthetic gene clusters associated with the production of antimicrobial compounds, including antibacterial and antifungal agents, as well as siderophores. These findings highlight the potential of BM40 as a promising biological control agent with plant growth-promoting capabilities for sustainable chickpea cultivation.

Which *Fusarium* species cause crown rot on banana fruit in the Hawaiian Islands?



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The main *Fusarium* species causing crown rot of banana in Hawaii plantations and local markets include the *Fusarium incarnatum equiseti* species complex, *Fusarium petrophillum* and *F. verticillioides*. No *F. musae* was detected.

Abstract

The Hawaiian archipelago represents a unique environment due to its geographic isolation and, for banana cultivation, the unique use of a diverse set of local varieties. The study aimed to assess which *Fusarium* species are associated with Fusarium crown rot disease of banana fruit in Hawaii by sampling local and international varieties both in production areas as well as in local markets. A total of 41 banana fruits belonging to 10 varieties showing typical crown rot symptoms were collected during June/July 2024 in plantations of Oahu and Hawaii Island, as well as in supermarkets and local farmers' markets of the two islands. A total of 18 *Fusarium* colonies, isolated using Komada medium, were obtained. Single spore isolation and RPB2 and EF sequencing led to the identification of the main *Fusarium* species/species complexes. The most abundant group of isolates belonged to FIESC (n=12), followed by *F. petrophillum* (n=2), FOOSC (n=1) and *F. verticillioides* (n=1). Three whole genomes were obtained to serve as references for the islands' local *Fusarium* populations. No *F. musae* was detected on Oahu or Hawaii Island during this survey.

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Antifungal and anti-mycotoxin capacity of extracts and compounds from selected strains of *Lentinula edodes* against *Fusarium verticillioides*



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Extracts and pure compounds from *Lentinula edodes* could offer a natural, and a lower environmental impact alternative for controlling *Fusarium verticillioides* and its mycotoxin contamination in corn.

Abstract

Corn (*Zea mays* L.) is an essential crop in Mexico due to its economic, nutritional, and cultural importance. However, contamination of its grains by filamentous fungi of the *Fusarium* genus during all the production process is a major challenge. *Fusarium verticillioides* produces mycotoxins such as fumonisins, deoxynivalenol, fusaric acid, and zearalenone that constitutes a risk to human and animal health, as they can cause chronic non-infectious diseases (mycotoxicosis) leading to death in severe cases. Various studies have shown that some edible mushrooms, such as *Lentinula edodes* (shiitake), generate bioactive compounds with antifungal, antioxidant, and antimycotoxin properties. It has been reported that crude methanolic extracts (CEs) of *L. edodes* can reduce the production of type B fumonisins by *F. verticillioides* up to 80%.^{1, 2} However, there are still few studies that propose these fungal substances for biological control in agriculture, particularly from strains belonging to Mexican biological collections. So, we aimed to explore the antifungal and antimycotoxin potential of four strains of *L. edodes* grown in three different native common plant substrates from cloud forest resulting in 12 *L. edodes* CEs. We have analyzed and compared the chemical profiles of these 12 CEs by metabolomics approaches and our preliminary results exhibited a highly dynamic metabolism that strongly depend on the plant substrate (unpublished data). Among the main biochemical pathways are those related to the metabolism of carbohydrates and amino acids. The results of the antifungal and antimycotoxin activities of *L. edodes* CEs against *F. verticillioides* will be correlated with the chemical composition, and the bioactive compounds in the ECs will be identified. The present project could contribute to the development of natural control agents with low environmental impact.

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Exploring new potential biological control agents (BCAs) for the management of Fusarium head blight in durum wheat: *in vivo* screening of bacterial strains

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Selected bacterial strains showed promising *in vivo* activity against Fusarium head blight in durum wheat, with a *Lactobacillus kunkeei* strain emerging as the most effective, highlighting its potential as a sustainable biological tool for FHB management in wheat production.

Abstract

Fusarium head blight (FHB) is a major wheat disease caused by several *Fusarium* species capable of producing harmful mycotoxins. Integrated management relies on agronomic practices, resistant cultivars, and chemical control. In sustainable agriculture, biological control agents (BCAs) are gaining attention as eco-friendly alternatives, although only a limited number of solutions are currently applicable under field conditions. This study evaluated, under both controlled environment and in the field, the efficacy of bacterial strains belonging to the genera *Lactobacillus*, *Bacillus*, and *Pseudomonas* for FHB management. Preliminary controlled environment and field screenings in 2023/2024 allowed to select the ten most effective strains and the optimal starter solution (10 mM MgCl₂ + 5 g/L glucose) to promote bacterial colonization of durum wheat heads. In 2024/2025, these strains were compared to chemical and microbiological products in a controlled environment experiment on durum wheat (cv. San Carlo). Bacterial suspensions (2.5×10⁷ CFU/mL), applied with the best starter at BBCH65, were followed, after 24 h, by inoculation with the deoxynivalenol (DON)-producing *F. culmorum* strain FC74 (2.5×10⁵ conidia/mL). Efficacy was assessed by FHB symptom scoring and quantification of pathogen DNA by qPCR. The same experiment was conducted in the field where, in addition to the previous parameters, DON (by ELISA test) and grain yield (t/ha) were also determined. In the controlled environment experiment, the *Lactobacillus kunkeei* strain delayed FHB symptoms and reduced pathogen DNA. In the field, all ten bacterial strains showed at least a minimal ability to reduce FHB severity, increase yield, and limit pathogen DNA and DON but the *L. kunkeei* strain was the most effective. Overall, results indicate that the selected bacterial strains, particularly the *L. kunkeei* strain, can reduce FHB severity, pathogen DNA, and mycotoxin accumulation, highlighting their potential as sustainable biocontrol tools in durum wheat production.

This research was supported by “PRIN_PNRR-2022” project “Filling gaps in the biological control of Fusarium diseases of durum wheat and of related mycotoxins – BICONTRARIUM” (P2022HAF42) funded by the European Union (NextGeneration EU) and Italian Ministry of University and Research.

The EvolTox Project Wrap-Up: How Global Change is Reshaping *Fusarium* and Mycotoxin Risks in Wheat

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The ANR-funded EvolTox project (2021-2026) is taking a multi-scale approach, combining long-term field data, ecophysiological studies, and evolutionary genomics, to understand how climate and agricultural practices drive shifts in *Fusarium* species and mycotoxin contamination in French wheat.

Abstract

Studying the evolution of the risk associated with *Fusarium* spp mycotoxins contamination in wheat under global changes, is indeed crucial to ensure the safety of future cereal-derived food products.

The EvolTox project, funded by the French National Research Agency (ANR-20-CE32-0011-01, 2021-2026), proposes to tackle this challenge using a multi-scale approach, combining field surveys and laboratory studies to feed models. First, the representativeness of the *Fusarium* species/toxins over a 15-years-period was investigated in the light of climatic and/or agronomic factors to identify the drivers that shape their distribution: about 1200 samples collected from wheats kernels harvested from 2007 to 2021 in France were analyzed by metabarcoding and mycotoxin profiling. Even though *F. graminearum* (Fg) was usually the most common species, some shift in favor of other species likely to be due to more conducive environmental conditions were occasionally observed^[1]. Second, ecophysiological responses under various combination of temperature (θ) and water availability (aw), in terms of growth and toxin production, were evaluated for five main species alone or in competition^[2]. Our findings clearly demonstrated that each species has a contrasted and unique θ /aw requirements which may contribute to shape *Fusarium* communities equilibrium. Third, we focused on how fungi may adapt under changing conditions. Through an Evolve and Resequencing approach, we demonstrated a strong stability of Fg genome under moderate stressful temperature condition^[3]. If mutations seem to play a minor role in Fg evolvability, we observed a wide range of phenotypic responses across genetic diversity^[2] that could significantly contribute to shape fungal populations. The findings of this study will contribute to improve mycotoxin risk prediction models, thereby supporting the development of effective strategies for managing future mycotoxin threats in wheat production.

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Effects of Seed Treatment, Sowing Time, and Cultivar on *Microdochium nivale* Infection and in Winter Wheat

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This study confirms that seed treatment, cultivar selection and sowing time affect *Microdochium nivale* infection in winter wheat, highlighting the need for integrated management under changing conditions.

Abstract

Microdochium nivale is a significant pathogen in winter wheat and traditionally associated with snow mould in temperate climates. However, increasing evidence highlights its significant role in Fusarium pathogen complex as a causing agent of seedling blights and FHB. With recent shifts in winter weather patterns, particularly, milder conditions and prolonged low positive temperatures—*M. nivale* may become an even more damaging pathogen, extending its impact beyond snow mould related diseases.

Field experiments were conducted from 2022 to 2024 in Lithuania to assess the effects of cultivar, sowing time, and seed treatment on *M. nivale* infection. Four winter wheat cultivars were sown at two timings (no earlier than the second decade of September and the first decade of October) and treated with four fungicide formulations: Fludioxonil (25 g/L); Fludioxonil + Prothioconazole + Tebuconazole (37.5/50/10 g/L); Fludioxonil + Fluxapyroxad + Triticonazole (33.3/33.3/33.3 g/L); and Sedaxane + Fludioxonil + Triticonazole (25/25/20 g/L), alongside untreated controls. Visual assessments and stem base sampling for qPCR-based quantification of *M. nivale* DNA were conducted each spring.

Overall, most samples from seed-treated plots showed reduced *Microdochium nivale* DNA levels, with this effect being most highlighted in 2022 and 2023, regardless of the fungicide used. The data also indicated that the cultivar 'Ada' samples had more *M. Nivale* DNA, particularly in 2022 and 2023, while in 2024, higher DNA levels were detected in 'Skagen' samples. Molecular analysis further revealed that sowing time influenced infection levels: with late-sown wheat generally showing lower DNA concentrations. These findings in part coincided with visual assessments, which indicated lower disease severity in treated plots compared to untreated controls.

These results highlight the importance of an integrated disease management strategy—combining resistant cultivars, considered sowing time, and effective seed treatment fungicides—to reduce *M. nivale* pressure in winter wheat under changing climatic conditions.

Geographic Distribution of Trichothecene Chemotypes of the *Fusarium graminearum* Species Complex from Maize kernels in Serbia

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Continuous monitoring of trichothecenes synthesis on maize is necessary.

Abstract

Since *Fusarium* species are toxic to humans and animals, a greater attention is paid to certain species as potentially more toxigenic than pathogenic species. Results of studies of synthesis of various mycotoxins indicate the diversity of *Fusarium graminearum* species complex. In view of the synthesis of trichothecenes (chemical structures, including the position of acetyl ester derivatives) the following two chemotypes have been described: 1) Chemotype I that synthesises deoxynivalenol (DON) and its acetyl ester derivatives, and 2) Chemotype II that synthesises nivalenol and fusarenon-X. Recognising chemotypes is an important information for establishing a risk assessment strategy for the protection of human and animal health. The aim of this study was to observe distribution of trichothecene chemotypes of *F. graminearum* species originating from maize kernels in Serbia.

The belonging to trichothecene chemotypes was determined by the High-Performance Liquid Chromatograph method (HPLC). Thirty-seven isolates of *F. graminearum* was collected from maize kernels from 15 different locations in Serbia. Trichothecenes were extracted from samples by a mixture of acetonitrile and water (21:4, v/v). Samples were purified with MycoSep 113 Trich and MycoSep 230 Niv cleanup columns (Romer Labs).

Obtained results point out to a great variability in the biosynthesis of DON derivatives. It was determined that the highest percentages of observed isolates belonged to the 15ADON chemotype (28/37). The 3ADON chemotype was detected in nine isolates. The concentration ranged from 8.97 to 154.97 µg/g, i.e. from 3.18 to 159.25 µg/g in 15ADON, i.e. 3ADON isolates, respectively. According to achieved results, there is a great diversity in the production of DON derivatives, while none of observed isolates from Serbia belonged to the NIV chemotype.

Impact of Cob Orientation and Husk Coverage on Field-Level Mycotoxin Accumulation in Maize

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Abstract

Mycotoxin contamination, particularly by aflatoxins and fumonisins, poses a major threat to food safety in sub-Saharan Africa. We assessed the influence of maize cob orientation, husk coverage, mold severity, and insect damage on mycotoxin accumulation in maize in 583 fields across five agroecological zones during the 2021 and 2022 cropping seasons. In each field, cobs on the plant were categorized by orientation, husk tightness and mold and insect damage into 36 different categories. A composite sample of 25 cobs collected in a transect of the field was analyzed for six key mycotoxins – aflatoxins, fumonisins, deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin, and ochratoxin A (OTA) by using UPLC-MS/MS in 2021 and VICAM fluorometry in 2022. Fungal contamination was high, especially in the Lower Shire, where 83% of cobs had at least some visible fungal contamination and 20% were visibly completely covered by fungal growth. Fumonisins were the most prevalent toxin, detected in over 95% of samples. DON and ZEA also were frequently detected, with the highest levels occurring in a highland district, where DON reached up to 15,600 µg/kg and ZEA up to 2,000 µg/kg. Aflatoxins were found in 16% of the samples at levels up to 787.4 µg/kg, while OTA and T-2 toxin appeared infrequently (> 3% of samples) and at low levels. Co-contamination was frequent, with fumonisins co-occurring with deoxynivalenol (22%), DON and zearalenone (17%), aflatoxins (4%), and all three toxins combined (4%). In a Principal Component Analysis, downward-facing cobs with tight husks were consistently associated with lower fumonisin levels and fungal contamination, whereas upward-facing or angled cobs with loose husks were associated with higher levels of mycotoxin contamination. These traits varied by location and season and were correlated with localized mycotoxin risk. Our findings underscore the role of maize ear morphology in preharvest mycotoxin accumulation and suggest that integrating trait-based screening with visual grading of fungal contamination could enhance breeding, surveillance, and food safety strategies.

Incidence of Fumonisin B1 in small grains in Serbia

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Mycotoxins monitoring programs for small grains need to include fumonisins.

Abstract

A wide variety of commodities in the world have been analysed for fumonisins contamination. However, they have mostly been reported in maize and maize-based foods and feeds. There is a lack of data on the natural occurrence of fumonisins in small grains, which is not in accordance with the widespread occurrence of their producers and favourable conditions for their biosynthesis in some years in Serbia. Since small grains are important source of energy in human nutrition and in nutrition of monogastric animals, its quality is crucial.

Survey was carried out to determine Fumonisin B1 (FB1) contamination in 46 samples of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) samples collected during three harvest seasons (2020, 2021 and 2022) from 12 different locations in Serbia. The primary samples were homogenised and quartered to obtain a 1 kg sample for laboratory analyses. Concentration of FB1 were analysed with the Enzyme-Linked Immunosorbent Assay (ELISA). Positive results were found in 50.7%, 31.6% and 10.1% samples in 2020, 2021 and 2022, respectively. FB1 concentration varied from 750 to 1300 µg kg⁻¹, and the mean levels recorded were: 1005.1 µg kg⁻¹ in (wheat grain) and 753.7 µg kg⁻¹ (barley grain). In 2021, the highest concentrations of fumonisins were recorded in wheat and barley, due to favorable environmental conditions during field growth.

Surveillance of emerging fusariotoxins in the food chain in France: presentation of FUSÉ, a working group of the SCA Platform



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The Food Chain Surveillance Platform (SCA) has launched in 2023 the FUSÉ Working Group to address concerns about emerging mycotoxins (beauvericin, enniatins, moniliformin) in the food chain. Despite the lack of toxicological values, the EFSA has raised health concerns related to their presence in cereal products. The group gathers sixteen experts from both public and private sectors, aiming to improve surveillance practices and develop recommendations for enhanced food and feed monitoring of these emerging mycotoxins.

Abstract

The French Food Chain Surveillance Platform (SCA) is a multidisciplinary and multi-stakeholder consultation space that offers methodological and operational support to managers of surveillance systems, aiding in the design, implementation, management, enhancement, and evaluation of French surveillance systems. Coordinated by the General Directorate for Food (DGAL), the French Agency for Food, Environmental and Occupational Health & Safety (Anses) and the French National Research Institute for Agriculture, Food and Environment (INRAE), it brings together thirteen public and private partners¹ involved in food chain surveillance in France in a shared public-private governance model. Following its 2022 focus on cadmium surveillance, SCA Platform launched the FUSÉ Working Group in December 2023 to specifically address enniatins, beauvericin, and moniliformin. The EFSA has indeed expressed concerns about the presence of these mycotoxins in cereal products, although no reference toxicological values have been established to date [1, 2]. Recent toxicological studies have prompted the EFSA to review its previous opinions, with a reassessment of the genotoxicity of beauvericin published in October 2024 [3] and a comprehensive reassessment of enniatins expected for 2026. The Working Group, composed of sixteen experts from public and private organisations and co-led by INRAE and Intercéréales, aims to assess current surveillance practices and develop recommendations for improvement. This work includes reviewing existing surveillance plans and compiling knowledge on these mycotoxins, focusing on toxicity, producing fungal species and food chain contamination. The group also plans to document contamination levels observed in food and

feed, by analyzing data from literature as well as data submitted to EFSA by Member states. Finally, the group will formulate recommendations spanning all stages of surveillance, from refining sampling strategies to enhancing analytical tools and fostering essential collaborations.

ACTA, ACTIA, Adilva, ANIA, Anses, CGAD, DGAI, DGS, FCD, INRAE, La Coopération Agricole, Oqualim, Santé publique France.

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Diversity of *Fusarium* species and their mycotoxins in major cereals



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Different *Fusarium* species infect different cereals, leading to varied mycotoxin contamination due to weather conditions and cereal nutrient differences.

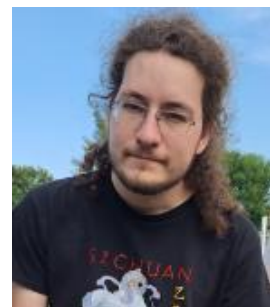
Abstract

Fusarium species are among the most common grain pathogens, known for producing a variety of mycotoxins harmful to both humans and animals. Given the central role grains play in human and animal nutrition, monitoring and controlling mycotoxin contamination and toxigenic *Fusarium* overgrowth is of critical importance. During the 2024 harvest season, 140 grain samples—including maize, barley, wheat, oat, and triticale—from three Croatian regions (eastern, northern and central) were collected immediately after harvest and analyzed for the presence of *Fusarium* species and 15 associated mycotoxins and their metabolites. *Fusarium* identification was performed using PCR with primers targeting the *TEF1α* and *RPB2* genes. Mycotoxins concentrations were determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS). *Fusarium verticillioides* was the most frequently isolated species in maize, while *F. sporotrichioides* dominated in other cereals, particularly wheat. In total, nine *Fusarium* species were isolated from maize, followed by eight from barley and seven from wheat. Oat and triticale were contaminated with four and three *Fusarium* species, respectively. The occurrence of mycotoxins varied depending on the type of cereal. Interestingly, zearalenone (ZEN) was not detected in any of the samples; however, its metabolite, zearalenone-14-sulfate, was present in all grain types, as well as deoxynivalenol (DON). Fumonisin B1 appeared in 85% of maize samples and 8.3% of oat samples, while fumonisin B2 was detected only in maize (26%). Diacetoxyscirpenol (DAS) was found exclusively in 25% of oat samples. This study has shown that different species of *Fusarium* colonize different types of cereals, and that different cereals are contaminated with different mycotoxins. This can be explained by varying weather conditions during growth and harvest, as well as by differences in the nutritional composition of the cereals, as pointed out by recent studies. Further comprehensive research is needed to confirm the possible relationship.

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Non-host colonization by *Fusarium oxysporum*: common weeds as potential disease reservoirs



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Common weeds act as reservoirs for *Fusarium oxysporum* in crop rotations.

Abstract

Fusarium oxysporum is a pathogen on many different plant hosts and causes mainly wilting and rotting diseases. More than 200 *formae speciales* of *F. oxysporum* have been described, in which each *forma specialis* causes disease on one or a limited few species. However, generally just Koch's postulates are tested for pathogenicity or pathogenicity is tested on a limited number of (related) species.

Fusarium oxysporum f.sp. *cepae* causes dieback of onion (*Allium cepae*) in the field and fusarium basal rot in stored onion bulbs. *F. oxysporum* f.sp. *cepae* seems to have a worldwide spread and is the major cause of Fusarium basal rot in the Netherlands. With increasing disease problems and a limited numbers of control options as many registered fungicides are becoming candidates for substitution, a system approach for the control of *Fusarium* in onion is needed to mitigate disease problems. The aim of this project was to get better insight in the epidemiology of *F. oxysporum* f.sp. *cepae* during crop rotations including onions.

Isolates of *Fusarium oxysporum* f.sp. *cepae* have been sequenced and compared to over 600 other *Fusarium oxysporum* f.spp. and non-pathogenic isolates. This resulted in the identification of 7 genes/alleles that seem specific for the onion pathogen. Based on two of these genes, a TaqMan detection method was developed for recognition and quantification of *F. oxysporum* f.sp. *cepae* in plants and other substrates. This detection method has been used to test different common weeds collected in onion fields with a history or clear symptoms of Fusarium wilt in onion and on collected weeds grown in pots with clayey field soil inoculated with *F. oxysporum* f.sp. *cepae*. Several of the common weed species proved to be reservoir plants for the pathogens. Pathogens reisolated from weeds and tested in a whole onion disease assay proved to retain their pathogenicity.

Ambrosia (2024-2027): Bridging Knowledge, Communication, and Action for Food Safety in a Changing Climate



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The project focuses on understanding and mitigating the risks posed by *Fusarium* mycotoxins, a significant hazard in grains like wheat, oats, barley, and corn, and their derived products. Using predictive modelling, AMBROSIA will analyse spatio-temporal climate projections to anticipate how changing climate variables—such as heatwaves, extreme rainfall, or prolonged droughts—might influence the prevalence and spread of mycotoxins in different biogeographical regions across Europe.

Abstract

The Ambrosia project aim to transform the European approach to food safety by addressing the increasing challenges posed by climate change. The project adopts a holistic, systemic methodology to assess and mitigate food safety risks across the entire supply chain, from primary production to consumption. By leveraging advanced digital technologies, such as artificial intelligence (AI) and predictive analytics, AMBROSIA integrates climate change projections with food safety hazard models to address emerging and cumulative risks.

A core focus of the project lies in understanding and mitigating the risks posed by *Fusarium* mycotoxins, a significant hazard in grains like wheat, oats, barley, and corn, and their derived products. Using predictive modelling, AMBROSIA will analyse spatio-temporal climate projections to anticipate how changing climate variables—such as heatwaves, extreme rainfall, or prolonged droughts—might influence the prevalence and spread of mycotoxins in different biogeographical regions across Europe (Atlantic, Boreal, Continental, and Mediterranean).

Furthermore, AMBROSIA extends this predictive framework to fresh produce, examining risks associated with enteric pathogens like *Salmonella* and *Escherichia coli*, which are also expected to evolve due to climatic shifts. By integrating data from diverse sources, including real-time climate models, microbiological data, and agrifood supply chain analytics, the project will develop robust predictive tools to forecast food safety risks at both regional and EU-wide scales.

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Non-destructive detection of deoxynivalenol and zearalenone in individual oat grains using spectroscopy



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Mycotoxin contamination in oats is driven by a very small fraction of highly contaminated grains, while most remain safe. By applying non-destructive spectroscopy, these high-risk grains can be rapidly identified and selectively removed. This approach significantly reduces toxin levels in bulk batches, improves food safety, and prevents unnecessary waste of otherwise healthy product.

Abstract

Mycotoxins, such as deoxynivalenol (DON) and its modified forms, as well as zearalenone (ZEN), produced by *Fusarium* spp., pose a persistent threat to food safety. Oats are particularly affected, yet contamination is highly heterogeneous: while most grains fall below regulatory thresholds, a small fraction can contain extreme toxin levels, leading to the rejection of entire batches and considerable food waste.

To address this challenge, 200 individual oat grains from two industry-rejected samples with high DON levels were analysed. Liquid chromatography-tandem mass spectrometry revealed that only 8–12% of grains exceeded European Union (EU) limits for DON (1,750 µg/kg) and/or ZEN (100 µg/kg). This highlights the potential of targeted removal to recover a safe product.

Therefore, non-destructive alternatives as visible-near-infrared spectroscopy (Vis-NIR, 350–2500 nm) and near-infrared hyperspectral imaging (NIR-HSI, 900–1700 nm) were explored. Classification models built on spectral data correctly identified grains above EU legal thresholds with accuracies up to 99.8% for DON, 100% for ZEN, and 99.2% when detecting grains exceeding either limit. The most informative wavelengths were between 1000 and 1250 nm, associated with protein, fibre, and hull compositional changes linked to *Fusarium* infection. For industrial feasibility, model complexity was reduced by selecting 20 key wavelengths, maintaining accuracies above 95%. Simulation of grain removal based on predictions showed substantial reductions in toxins: DON by 87% and ZEN by nearly 60%.

This study demonstrates, for the first time, real-time, non-destructive classification of naturally DON and ZEN-contaminated oat grains. Integrating spectroscopy into grain processing could enable the selective elimination of high-risk grains, enhance consumer protection, regulatory compliance, and sustainability by reducing unnecessary food waste.

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