PATHOGEN BIOLOGY AND GENETICS

PRESENCE OF *FUSARIUM GRAMINEARUM* IN AIR ASSOCIATED WITH SORGHUM FIELDS

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ABSTRACT

Sorghum can be included in crop rotations with wheat. However, there are no known reports on the effects of sorghum grown in rotation with wheat on the epidemiology of head scab caused by *Fusarium graminearum*. Conidia in air samples within two sorghum fields were collected by passive spore trapping for two years at four plant stages (vegetative, anthesis, grain development or maturity) during the sorghum growing season. Spores were collected and germinated on a general fungal medium (potato dextrose agar; PDA) and a medium semi-selective for *Fusarium* spp. (pentachloronitrobenzene-containing agar; PCNB). Colonies cm^{-2} hr^{-1} on PCNB ranged from 31.0 to 85.7 percent of colonies cm^{-2} hr^{-1} on PDA, depending on environment and growth stage. A subsample of *Fusarium* isolates from PCNB traps were identified molecularly by comparing sequences from a portion of the translation elongation factor (*TEF*) 1α gene with those in the FUSARIUM-ID database (http://isolate.fusariumdb.org/index.php). Surprisingly, 26.8% were *F. graminearum*, the most numerous *Fusarium* species or genotype. Phylogenetic analyses of these isolates, as well as *F. graminearum* from sorghum leaf tissue and grain, using *TEF*, the rRNA internal transcribed spacer region and a portion of the histone-3 gene (*H3*), revealed that these isolates were highly similar to one another and to previously characterized *F. graminearum* isolates. Further research to determine whether isolates associated with sorghum production produce tricothecenes or zearalenone and are pathogenic to wheat, will need to be conducted to determine whether *F. graminearum* associated with sorghum production can affect head scab levels in wheat.

RESISTANCE MECHANISMS AND MANAGEMENT OF *GIBBERELLA ZEAE* TO BENZIMIDAZOLE FUNGICIDE CARBENDAZIM AND A NOVEL FUNGICIDE PHENAMACRIL (JS399-19)

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ABSTRACT

In China, *Gibberella zeae* is the dominant pathogen causing wheat head blight. Carbendazim (MBC) has been widely used to control this disease since the 1970s. However, the resistance to MBC is currently serious in *G. zeae*. Though the MBC-resistance was controlled by one major gene and is involved in mitotic division, no mutation in the target β-tubulin was found, which was different from other filamentous fungi. To identify the MBC-resistance mechanism of *G. zeae*, other tubulin genes were analyzed. Alterations at amino-acid codon 17 or 167 or 198 or 200 in β2-tubulin were found to correspond to the different phenotypes of MBC-sensitivities. Deletion/complementation of the β2-tubulin gene as well as mononucleotide displacement and affinity of MBC binding tubulins validated the point mutations conferring resistance of *G. zeae* to MBC. It is interested to find that MBC-resistance mutation leads to increased expression of deoxynivalenol (DON) biosynthesis genes. Compared to the wild-type MBC-sensitive strain, the resistant strain produced twice as much DON in infected grains. Novel chemical, phenamacril (development code no. JS399-19) 2-cyano-3-amino-3-phenylancryic acetate is recommended as a *Fusarium* specific fungicide to control MBC-resistance Fusarium head blight.

Phenamacril is a novel cyanoacrylate fungicide discovered and patented by the Jiangsu Branch of the National Pesticide Research & Development South Center of China. The fungicide exhibited specific activity against fungal plant pathogens of the genus *Fusarium* with which it strongly interferes with mycelial growth and it has an excellent control effect on Fusarium head blight. We have monitored phenamacril-resistance population in the field for 3 years and do not find any resistant mutants. In the lab, phenamacril-resistant mutants were obtained. Through the genome sequence of the sensitive strain and resistant mutants, and homologous double exchange between the gene locus of the sensitive strain and the resistant mutant, we found that the point mutations in the gene myosin-5 (at codon 216, 217, 418, 420, or 786) confer resistance to phenamacril.

FUSARIUM GRAMINEARUM INTERACTION WITH THE EPIDERMIS OF THE BARLEY PALEA

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ABSTRACT

The first step in disease initiation is the penetration by the pathogen of the host surface. Preventing entry of host targets by *Fusarium graminearum* is an intriguing goal that requires better understanding of the mechanisms of *F. graminearum* ingress of host tissues. We are investigating the role of the various epidermal cell types in the interaction between host and pathogen. The epidermis of the barley palea features several cell types including multiple phytolith (trichomes and cells of similar origin) morphotypes and stomata. We have used a histological approach to demonstrate that the fungus preferentially interacts with particular cell types on the palea of excised barley florets. In particular, one phytolith morphotype common on two-row barley appears to be a common point of contact between the fungus and the host. Elucidation of the initial targets of infection will aid future strategies for disease control.

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CO-PRODUCTION OF 3ADON AND 15ADON BY CULTURES OF *FUSARIUM GRAMINEARUM* 15ADON STRAINS, BUT NOT 3ADON STRAINS, IS DUE TO DIFFERENCES IN ACETYLTRANSFERASE ACTIVITY AND SUBSTRATE SPECIFICITY

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ABSTRACT

Fusarium graminearum strains can be assigned to chemotypes, e.g. 3ADON or 15ADON, on the basis of PCR analysis using polymorphisms in the trichothecene biosynthetic genes *TRI3* and *TRI12*. Trichothecene production in liquid culture is consistent with the chemotype predicted with PCR analyses, i.e., acetylated DON (3ADON or 15ADON) is produced but no DON is detected. In contrast, grain infected with 3ADON or 15ADON strains are predominantly contaminated with DON with small amounts of 3ADON or 15ADON, or sometimes both, detected. A mixture of acetylated deoxynivalenols is also sometimes found in rice cultures. Although *F. graminearum* 70E1, a 3ADON strain, produced DON and 3ADON, *F. graminearum* GZ3639, a 15ADON strain, produced a mixture of DON, 15ADON, 3ADON and 3,15-diADON. We have previously shown that differences in the *TRI8* esterase gene determine the 3ADON or 15ADON chemotype. Disruption of Tri8 in either 3ADON or 15ADON strains results in the accumulation of 3,15-diADON, the common precursor of 3ADON and 15ADON. Yeast expressing *TRI8* from a 3ADON strain removed the C-15 acetyl group from 3,15-diADON while yeast expressing *TRI8* from a 15ADON strain removed the C-3 acetyl group from 3,15-diADON. In order to determine if differences in trichothecene acetyltransferase genes might contribute to the production of both acetylated forms in the cultures of one chemotype, *TRI3* and *TRI101* from 3ADON and 15ADON chemotypes were expressed in yeast. In trichothecene biosynthesis, Tri101 acetylates at C-3, converting isotrichodermol into isotrichodermin, and Tri3 acetylates at C-15, converting 15-decalonectrin into calonectrin. Feeding experiments with yeast expressing Tri101 from a 3ADON or a 15ADON strain indicated that Tri101 can convert DON into 3ADON. Feeding experiments with yeast expressing Tri3 indicated that Tri3 from 3ADON or 15ADON strains were functional, i.e. able to convert 15-decalonectrin into calonectrin. Tri3 from a 15ADON strain was also able to convert DON into 15ADON but Tri3 from a 3ADON strain did not convert DON into 15ADON. These differences in Tri3 activity and substrate specificity can account for both 3ADON and 15ADON being produced in a 15ADON strain but not a 3ADON strain.

THE NIVALENOL-PRODUCING *FUSARIUM GRAMINEARUM* GENOTYPE IN SCABBY NORTH CAROLINA WHEAT SPIKES

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ABSTRACT

Fusarium head blight (or scab), which in the U.S. is caused primarily by *F. graminearum*, leads to drastic decreases in yield and test weight of small grains. In addition, *Fusarium* mycotoxins in grain heads can render the crop unsuitable for human or animal consumption. In livestock, scabby grain can lead to feed refusal and/or poor weight gain. Although this fungus produces various mycotoxins, the most important ones in small grains are deoxynivalenol (DON) and nivalenol (NIV). Both can cause severe toxicoses in humans and livestock; compared to DON, NIV has greater mammalian toxicity. While DON is the dominant wheat scab toxin in most of the U.S., a high proportion of *Fusarium* isolates from southern Louisiana wheat had been found by other researchers to be NIV producers. Although a 2006 wheat survey detected about 10% NIV producers in each of two NC counties, the distribution of NIV strains across the state was unknown. DON contamination is often measured in North Carolina grain crops, but NIV is not.

In this study, we sampled commercial wheat heads symptomatic for scab from 60 fields in 24 NC counties in the 2013-14 growing season. From each infected head, a single Fusarium strain was isolated and, using polymerase chain reaction (PCR), categorized as a 3-ADON, 15-ADON, or NIV genotype. Partial results showed that, of the 776 isolates that amplified successfully, 96% were 15-ADON. NIV types were 3% of the total sample, and up to 8% of strains from a single field; they did not seem to be concentrated in any county or region of the state. As in the 2006 survey, the 3-ADON genotype was found in several counties at a very low frequency (<1% of the total sample), raising the question of whether it will increase in frequency in North Carolina as it has in the northern U.S. and Canada, or alternatively if selective forces are keeping it rare. In practical terms, by assessing the distribution of NIV-producing *Fusarium* strains in North Carolina wheat fields, we will determine whether and where NIV may warrant monitoring in severe scab years.

GENOTYPING BY SEQUENCING FOR FOOTPRINTS OF SELECTION IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Previous population genetic studies of *Fusarium graminearum* have found evidence for frequent outcrossing as well as genetic clustering into populations associated with genotypes at the trichothecene gene cluster. Notably, several studies have reported the increase in frequency of isolates with the 3ADON chemotype from Fusarium head blight (FHB) infected wheat, though the specific cause of this shift is still uncertain. We argue that new population genomic studies will provide an important complement to experimental studies that investigate functional differences between populations. The sequencing of the *F. graminearum* genome has revealed how the genome is functionally organized and which regions are most dense with polymorphisms. However, there is an urgent need to use sequence-based markers on a genome-wide scale to describe patterns of variation along chromosomes and among different geographical regions. This information can lead to the identification of the genetic basis of functional differences between populations that can affect pathogen management and strategies for developing host plant resistance.

Here, we provide preliminary results from our FY14 USWBSI project that uses genotyping by sequencing (GBS) markers for the population genomic analysis of isolates from multiple regions in the Americas. Though our sample is still expanding, we have revisited population structure with nearly 300 isolates and asked how it relates to isolate collection location and trichothecene genotype. Our GBS markers let us investigate how patterns of genetic differentiation between populations vary across the genome, identifying candidates for local adaptation. To determine whether genome-wide association studies are feasible, we have characterized the decay of linkage disequilibrium with distance along chromosomes and how this varies by genome location. Finally, we are scanning the genome to determine whether footprints of selection support the hypothesis that natural selection acting directly on genetic changes at the FHB-related trichothecene loci has caused the 3ADON population shift. By investigating the cause of population shifts and their relationship to mycotoxin chemotypes, we may identify novel genes critical for fungal fitness against which we can develop strategies for toxin reduction and FHB control.

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SPREAD AND COLONIZATION OF *FUSARIUM GRAMINEARUM* DURING INFECTION IN A RESISTANT WHEAT CULTIVAR CARRYING *FHB1* RESISTANCE

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ABSTRACT

The spread and colonization of *F. gaminearum* in rachis of a resistant wheat cultivar carrying *FHB1* resistance was examined by fluorescence microscopy using a fungal strain constitutively expressing GFP. To preserve GFP fluorescence, a simple cryohistological process was employed. At 6 dpi in the upper portion of the rachis segment, immediately adjacent to the inoculation point, fungal hyphae were visible predominantly in the parenchyma and, to a lesser extent, in the vascular bundles. In the parenchyma, hyphae were observed inside the cells and in the apoplast. Fungal hyphae that appear in apoplast tend to surround cells that appeared to be healthy, with intact chloroplasts. In parenchymatous cells that had intracellular hyphae, chloroplasts were damaged. Histochemical investigation identified massive depositions of condensed catechol-type tannins, visible starting at 3 dpi, and the presence of pectin associated with the fungal hyphae in the upper portion of the rachis segment. Accumulation of phenolic compounds and pectin were detected within and along the vascular bundles, within the parenchyma cells, and in the apoplast. Our findings suggest that phenolic compounds represent a part of the response of the plant to fungal infection. Phenolic compounds and pectin may restrict fungal spread in the apoplast thus playing a role in controlling the spread of the infection. Investigation of the genes involved in this plant resistance response is in progress.

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MODIFICATION OF THE MYCOTOXIN DEOXYNIVALENOL WITH ENZYMES AND MICROORGANISMS

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ABSTRACT

New strategies are needed to mitigate deoxynivalenol (DON) contamination in wheat and barley. This research is aimed at discovering novel enzymes and microorganisms to degrade DON to improve food safety and decrease economic loss producers face. We have first developed a tool to screen for potential candidate DON enzymes by engineering several yeast strains to be sensitive to DON. One of the yeast strains is sensitive to DON at 100 ppm, but not sensitive to de-epoxy DON (the detoxified product) at the same concentration. Second, enzyme candidates to transform DON were selected using the BRENDA database, an enzyme repository, based on their functionality; promising epoxide hydrolases and cycloisomerases have been identified. Third, we bioprospected for DON detoxifying microorganisms from the environment and cultured the samples in the presence of 100 ppm DON. Three mixed cultures and one pure culture consistently detoxify DON in laboratory experiments; the organisms responsible for DON detoxification are in the process of being characterized. Organisms and genes that demonstrate DON detoxification will be tested in contaminated wheat and barley samples in future studies. This research will offer new strategies for detoxifying DON in wheat and barley.

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