The diversity and population structure of *Fusarium verticillioides* in South African maize (MTM11_15)

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INTRODUCTION

- *Fusarium verticillioides* is widespread throughout maize producing areas in the world
- This fungus is only pathogenic on maize
- *Fusarium verticillioides* produces the mycotoxin fumonisin
- The fungus can grow as endophytes in the stem, roots and kernels and can become pathogenic
- Reproduces sexually and asexually
- Sexual reproduction – recombination occurs and the genetic material of the fungus is highly diverse
- Asexual reproduction – clonal and genetic material is less diverse
INTRODUCTION

• In order to control *F. verticillioides*, knowledge of
  – the fungus,
  – its genetics,
  – reproduction,
  – pathogenicity and toxicity under different environmental conditions is required
  ✓ Fusarium ear rot symptoms ≠ fumonisins produced, *vice versa*
AIM

1) Understand the genetic and genotypic diversity of *Fusarium verticillioides* in South Africa
2) Understanding the relationship between pathogenicity and toxin production

- Help to test control methods against diverse range of fungi
- Help predict durability of control methods
- Sexual – diverse – overcome resistance (fungicide resistance)
MATERIALS AND METHODS

Collection of *F. verticillioides* isolates from stem, root and kernels
MATERIALS AND METHODS

Identification of *F. verticillioides* from maize plants

- **Morphological characterisation**
  - Microconidia is carried in long chains, presence of monophialides, no chlamydospores are produced

- **Molecular identification**
  - Species specific primers (Mulé *et al.*, 2004)

- **Phylogenetic identification**
  - Translocation elongation 1-α (*tef1*) (O’Donnell *et al.*, 1998)
  - Mitochondrial small subunit (*mtssu*) (White *et al.*, 1990)
  - Beta-tubulin genes (O’Donnell *et al.*, 2000)
  - Bayesian Interference and Maximum likelihood analysis
RESULTS

Morphological identification
RESULTS

Molecular identification

- *Fusarium verticillioides* specific primers
- *Fusarium proliferatum* specific primers
- *Fusarium subglutinans* specific primers

[Image of gel electrophoresis showing bands for different Fusarium species]
Phylogenetic tree

Fusarium verticillioides

Gibberella fujikuroi spp.
MATERIALS AND METHODS

Production of fumonisins

- The presence or absence of the *FUM1* and *FUM19* genes were determined.
- HPLC analysis of fumonisins of different isolates were determined (López-Errasquín et al. 2007)
RESULTS

Presence and absence of *FUM* genes

![Image of gel electrophoresis with markers and bands labeled as FUM1 and FUM19]
The percentage of *F. verticillioides* isolates in the South African population that produced different levels of fumonisin *in vitro*.
MATERIALS AND METHODS
Virulence of *F. verticillioides* isolates

- Three maize cultivars
- Five *F. verticillioides* isolates

**Growth-chamber experiment:**
- Seed inoculation treatment
- Soil inoculation treatment

**Field trial:**
- Silk channel inoculation
- Toothpick inoculation
MATERIALS AND METHODS
Virulence of *F. verticillioides* isolates

- **Harvesting**
  - Percentage ear infection was visually quantified.

- **Toxin analysis**
  - 50g maize powder
  - HPLC analysis using the Fumonitest™ HPLC method on the reverse-phase HPLC/fluorescence detector system.

Disease severity rating scales and percentage of visibly infected kernels for Gibberella ear rot after silk channel (left) and kernel (right) inoculations with *Fusarium graminearum*.
## RESULTS

### Growth cabinet trial

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Root length m.s.</th>
<th>P-value</th>
<th>m.s.</th>
<th>P-value</th>
<th>Shoot length m.s.</th>
<th>P-value</th>
<th>Root Wet weight m.s.</th>
<th>P-value</th>
<th>Shoot wet weight m.s.</th>
<th>P-value</th>
<th>Root Dry weight m.s.</th>
<th>P-value</th>
<th>Shoot Dry weight m.s.</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>ISOLATE</td>
<td>5</td>
<td>756.31</td>
<td>0.009</td>
<td>560.6</td>
<td>0.126</td>
<td>0.0432</td>
<td>0.048</td>
<td>0.048</td>
<td>0.156</td>
<td>0.0001</td>
<td>0.859</td>
<td>0.0002</td>
<td>0.178</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CULT</td>
<td>2</td>
<td>4624.93</td>
<td>&lt;.001</td>
<td>4847.5</td>
<td>&lt;.001</td>
<td>0.3859</td>
<td>0.034</td>
<td>0.313</td>
<td>0.0014</td>
<td>0.0014</td>
<td>0.14</td>
<td>0.0014</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMT</td>
<td>1</td>
<td>3559.90</td>
<td>&lt;.001</td>
<td>6073.6</td>
<td>&lt;.001</td>
<td>0.2632</td>
<td>0.179</td>
<td>0.015</td>
<td>0.0020</td>
<td>0.0013</td>
<td>0.13</td>
<td>0.0062</td>
<td>&lt;.001</td>
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<tr>
<td>ISOLATE.CULT</td>
<td>10</td>
<td>238.45</td>
<td>0.402</td>
<td>246.3</td>
<td>0.641</td>
<td>0.0264</td>
<td>0.925</td>
<td>0.012</td>
<td>0.936</td>
<td>9.02E-05</td>
<td>0.982</td>
<td>5.03E-05</td>
<td>0.957</td>
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<td></td>
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<tr>
<td>ISOLATE.TMT</td>
<td>5</td>
<td>388.75</td>
<td>0.138</td>
<td>67.3</td>
<td>0.955</td>
<td>0.0984</td>
<td>0.168</td>
<td>0.010</td>
<td>0.876</td>
<td>0.0001</td>
<td>0.865</td>
<td>0.0001</td>
<td>0.273</td>
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<tr>
<td>CULT.TMT</td>
<td>2</td>
<td>614.36</td>
<td>0.072</td>
<td>75.6</td>
<td>0.786</td>
<td>0.0091</td>
<td>0.861</td>
<td>0.036</td>
<td>0.291</td>
<td>0.0001</td>
<td>0.594</td>
<td>0.0001</td>
<td>0.393</td>
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<tr>
<td>ISOLATE.CULT.TMT</td>
<td>10</td>
<td>178.63</td>
<td>0.635</td>
<td>239.9</td>
<td>0.66</td>
<td>0.0335</td>
<td>0.849</td>
<td>0.016</td>
<td>0.839</td>
<td>0.0001</td>
<td>0.834</td>
<td>6.94E-05</td>
<td>0.881</td>
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</table>

The growth cabinet trials that were performed using three cultivars, five *Fusarium verticillioides* isolates and two inoculation techniques. The root length, shoot length, wet and dry weight of the root and shoots were measured. Statistical analysis was performed and a P-value of < 0.05 is significant.
The effect of *Fusarium verticillioides* isolates on root length pooled for all cultivars in a greenhouse experiment. Data was analysed using multifactor ANOVA and means were separated using Fisher’s LSD. Values with different alphabetical letters indicates significance at P < 0.05.
Maize field trials were performed using three cultivars, five isolates and three inoculation techniques. Fusarium ear rot symptoms were measured by percentage disease severity and the resultant fumonisin levels were measured with HPLC (ppm). Statistical analysis was performed and a P-value of 0.05 is significant.
## RESULTS

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Silk channel inoculations</th>
<th>Toothpick inoculations</th>
<th>Silk channel inoculations</th>
<th>Toothpick inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC826</td>
<td>6.925abc</td>
<td>5.574bcdef</td>
<td>7.222ab</td>
<td>4.921def</td>
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<tr>
<td>GCI77</td>
<td>6.778bc</td>
<td>6.543bcd</td>
<td>6.617bcd</td>
<td>5.389cdef</td>
</tr>
<tr>
<td>GCI212</td>
<td>4.054fg</td>
<td>5.331cdef</td>
<td>8.615a</td>
<td>5.301cdef</td>
</tr>
<tr>
<td>GCI445</td>
<td>2.471gh</td>
<td>4.637ef</td>
<td>6.625bcd</td>
<td>6.997abc</td>
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<tr>
<td>GCI2006</td>
<td>6.369bcde</td>
<td>4.722ef</td>
<td>5.796bcdef</td>
<td>5.851bcde</td>
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<tr>
<td>Control</td>
<td>2.325gh</td>
<td>2.325gh</td>
<td>2.005h</td>
<td>2.005h</td>
</tr>
</tbody>
</table>

The average percentage Fusarium ear rot development Significant results were obtained for season X isolate X inoculation method interaction with a P value of <0.001.
## RESULTS

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Season 1 (2009/10)</th>
<th>Season 2 (2010/11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC826</td>
<td>6.049bcd</td>
<td>4.969cde</td>
</tr>
<tr>
<td>GCI77</td>
<td>7.114abc</td>
<td>4.469cde</td>
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<tr>
<td>GCI212</td>
<td>3.701de</td>
<td>3.642de</td>
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<td>GCI445</td>
<td>3.582def</td>
<td>6.717abc</td>
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<tr>
<td>GCI2006</td>
<td>7.754ab</td>
<td>8.773a</td>
</tr>
<tr>
<td>Control</td>
<td>2.475ef</td>
<td>0.960f</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Silk channel inoculation</th>
<th>Toothpick inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC826</td>
<td>8.155ab</td>
<td>2.863e</td>
</tr>
<tr>
<td>GCI77</td>
<td>8.713ab</td>
<td>2.869de</td>
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<td>GCI212</td>
<td>5.158cd</td>
<td>2.185e</td>
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<tr>
<td>GCI445</td>
<td>7.994ab</td>
<td>2.305e</td>
</tr>
<tr>
<td>GCI2006</td>
<td>10.321a</td>
<td>6.206bc</td>
</tr>
<tr>
<td>Control</td>
<td>1.718e</td>
<td>1.718e</td>
</tr>
</tbody>
</table>

The average fumonisin production (ppm). Significant results were obtained for the isolate X season interaction with a P value of 0.045.

The average fumonisin production (ppm). Significant results were obtained for the isolate X inoculation method interaction with a P value of 0.02.
MATERIALS AND METHODS
Determine pathogenicity and resultant fumonisin levels of maize isolates

- Field trial planted at Potchefstroom in a randomised block design
- Only silk inoculations
- Percentage ear infection was quantified and HPLC analysis performed
- Nine *F. verticillioides* isolates were used:
  - GCI 282 (0ppm)
  - GCI 2004 (5.655ppm)
  - GCI 1608 (5.281ppm)
  - GCI 309 (7.699ppm)
  - MRC 826 (7.869ppm)
  - GCI 438 (11.165ppm)
  - GCI 51 (11.606ppm)
  - GCI 434 (21.324ppm)
  - GCI 340 (28.146ppm)
RESULTS

Pathogenicity of *F. verticillioides* isolates
RESULTS

Disease severity and fumonisin levels of *F. verticillioides* isolates
MATERIALS AND METHODS

Determine mating type distribution

- The presence of mating type genes
  - $MAT1$ gene
  - $MAT2$ gene
  - de Oliveira Rocha et al. (2011) and Steenkamp et al. (2000)
RESULTS

Mating type distribution

MAT1 gene

MAT2 gene
RESULTS

Mating type distribution

The percentage *F. verticillioides* isolates in the South African population that contains either the *MAT1* or *MAT2* genes
DISCUSSION

• The *F. verticillioides* isolates also differ in virulence and toxin production

• The *F. verticillioides* isolates collected is able to reproduce sexually
  • MAT1:MAT2 = 1:1
  • The population genetic material is diverse

• Thus the *F. verticillioides* pathogen can easily adapt
• Newly developed control methods might be easily overcome
FUTURE RESEARCH

• Simple sequence repeats (SSR’s) – Population structure
  ✓ Reproduction system (genotypic diversity) – sexual leads to new combinations of alleles and to different genotypes
  ✓ Genetic drift – random process and leads to unpredictable changes in pathogen populations in a short period of time – leads to fixation of alleles and genotypes in population
  ✓ Gene flow/migration – introduces new genes/genotypes into fields distant from the site of original mutation (how much genetic info has been exchanged)

• Field trials were planted in Natal, Mpumalanga and the North West province for the 2011/12 season
  ✓ determine if high fumonisin producing fungi remain high and vice versa
  ✓ Determine if fumonisin production is a stable function of the fungus or if it is dependant on localities
ACKNOWLEDGEMENTS

• Maize Trust for funding

• Belinda Janse van Rensburg, Monica Kwele and Edson Ncube (ARC-GCI) for technical assistance

• Gerda Fourie (University of Pretoria) for technical assistance with molecular work

• Liesl Morey (ARC-Biometry Unit, Pretoria) for assistance with statistical analysis of data