Fighting necrotrophs
Practical Application of Necrotrophic Effectors to Assist Breeders Develop Resistant Cultivars

ICRPMC Denmark 2015

Centre for Crop and Disease Management

ACNFP

Curtin University

Grains Research & Development Corporation
Wheat necrotrophic diseases
Necrotrophic effectors
Breeding for tan spot resistance – simple in Australia
Breeding for nodorum resistance
  Effector multiplicity
  Effector-effector interactions – epistasis
Pf2 – a regulator of multiple effectors

Fighting necrotrophs in the ROW.
### Pleosporales Diseases of Wheat

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan spot</td>
<td><em>Pyrenophora tritici-repentis</em></td>
</tr>
<tr>
<td>Yellow spot</td>
<td><em>Helminthosporium tritici-repentis</em></td>
</tr>
<tr>
<td>Yellow leaf spot</td>
<td><em>Dreschlera tritici-repentis</em></td>
</tr>
<tr>
<td>Septoria nodorum blotch</td>
<td><em>Parastagonospora nodorum</em></td>
</tr>
<tr>
<td>Glume blotch</td>
<td><em>Phaeosphaeria nodorum</em></td>
</tr>
<tr>
<td></td>
<td><em>Leptosphaeria nodorum</em></td>
</tr>
<tr>
<td></td>
<td><em>Stagonospora nodorum</em></td>
</tr>
<tr>
<td></td>
<td><em>Septoria nodorum</em></td>
</tr>
<tr>
<td>Septoria tritici blotch</td>
<td><em>Zymoseptoria tritici</em></td>
</tr>
<tr>
<td>Septoria leaf blotch</td>
<td><em>Mycosphaerella graminicola</em></td>
</tr>
<tr>
<td></td>
<td><em>Septoria tritici</em></td>
</tr>
</tbody>
</table>

**Promise and reality of effector-assisted breeding**
Dominant diseases in Australia
Spreading Worldwide

Murray GM and Brennan JP (2009); Oliver

- **Tan Spot**
  - *Pyrenophora tritici-repentis*
  - $212m
  - LGT around 1941

- **Septoria nodorum Blotch**
  - *Parastagonospora nodorum*
  - $108m

- **Yellow rust**
  - $127m

- **Rhizoctonia**
  - $59m

- **Fusarium**
  - $79m

- **All other fungi Inc stem and leaf rust**
  - $102m

Zymoseptoria tritici
STB - <$1m
Wheat leaf blotch disease status in rest of world

<table>
<thead>
<tr>
<th>Disease</th>
<th>Status and Requirements</th>
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</thead>
<tbody>
<tr>
<td>SNB</td>
<td>Used to be major disease in NW Europe – declined since 1970s. Rarely seen in UK (Fera), Rothamsted Broadbalk. Common in France, Norway, SE USA. Needs heavy sporadic rainfall, stubble retention.</td>
</tr>
<tr>
<td>Tan spot</td>
<td>3rd commonest disease (after STB and PM) in UK. Major in Denmark, Germany. Needs warmer climate, stubble retention, baseline resistant to fungicides.</td>
</tr>
<tr>
<td>STB</td>
<td>Re-emergent in SE Australia. Dominant in EU. Needs regular light rainfall.</td>
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</table>
Effectors; Molecules produced by a pathogen that induce a response in a potential host plant.
Necrotrophic Effectors – the Naïve Model

- Interaction between a necrotrophic effector and the product of a host dominant sensitivity gene leads to disease
- Multiple effectors and recognisers in each disease
- The more matching effectors and recognisers, the more virulence
### Known SNB and Tan Spot Effectors

<table>
<thead>
<tr>
<th>Effector</th>
<th>Species</th>
<th>Recogniser</th>
<th>Function</th>
<th>Effector versions</th>
<th>Notes</th>
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<tr>
<td>ToxA</td>
<td>Tan spot</td>
<td>Tsn1</td>
<td>5BL</td>
<td>NBS-LRR-PK</td>
<td>1 Ubiquitous in Australia</td>
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<tr>
<td>ToxB</td>
<td>Tan spot</td>
<td>Tsc2</td>
<td>2BS</td>
<td>?</td>
<td>Gene copy number varies; absent in Australia</td>
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<tr>
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<td>Tsc1</td>
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<td>?</td>
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<td>Snn1</td>
<td>1BS</td>
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<td>SNB</td>
<td>Snn3</td>
<td>5BS</td>
<td>?</td>
<td>6 Ubiquitous in Australia</td>
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</table>

**Evidence for 15+ effector/recognisers pairs in both pathosystems**
Naïve Model for Necrotrophic Effector-Assisted Breeding

- Screen primary germplasm for sensitivity to all effectors
- Avoid use of sensitive germplasm
  - Use sensitive germplasm but select for insensitive progeny
- Finished cultivar should be at least as resistant as most resistant current cultivar (but with more freedom to incorporate other traits)
The Role of ToxA in Tan Spot Disease
Field resistance strongly correlated with ToxA insensitivity

- More than 100,000 breeding lines tested with ToxA by Australian breeders since 2009
- UK, EU, ROW. ??
• Additional evidence for new effectors from ToxA knockout strain

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>toxa-1</th>
<th>toxa-2</th>
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<td>Sunguard</td>
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<td><img src="image2.png" alt="Image" /></td>
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<td>Estoc</td>
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<td><img src="image5.png" alt="Image" /></td>
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<tr>
<td>Scout</td>
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<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
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</table>
Is the Australian tan spot situation typical?

• One major effector – one recogniser
• All pathogen isolates carry the effector.

• Several effectors
  – Epistasis in effector sensitivities
  – Variation in effector activities
  – Pathogen isolates that don’t carry just the known effectors

• Effectors that are recognised by > 1 gene
  – Alleles that vary in activity
No cultivars that lack sensitivity to all three effectors
Sensitivity varies to 1 and 3
Sensitivity also to culture filtrate lacking A, 1 and 3
?? Sensitivity to any one effector is enough to give susceptibility
Effector sensitivity and disease loci distribution

1A
- Snn4

2A
- SN::tox13 culture filtrate (GladiusxDrysdale) (IGW2471xWyal)

3A
- Snn3A

4A
- Snn5A
- SN::toxa culture filtrate (GladiusxDrysdale)

5A
- Tox1 effector & SN::toxa culture filtrate/SN::tox13 culture filtrate/SN::toxa3 culture filtrate (2074x6HR)/SNB adult field (CalingirixWyal)

6A
- SN::tox13 WPS (CS) Toxa3CF (IGW2471xWyal)

7A
- Snn4b

1B
- Tox1 effector/SN::toxa3 culture filtrate/ SNB adult field (CalingirixWyal)

2B
- Snn2B

3B
- QSng.sfr-3BS from Shatalina et al 2013

4B
- Tox1 effector (CalingirixWyal)

5B
- Snn3B-1

6B
- Snn6B

7B
- Snn7B

1D
- Tox1 effector (GladiusxDrysdale)

2D
- Snn2

3D
- SN::toxa3 culture filtrate (GladiusxDrysdale)

4D
- SN::toxa3 culture filtrate (2074x6HR)

5D
- Snn5D
- Snn3-1

6D
- Snn6D

7D
- Snn7D
**P. nodorum** deleted in *ToxA, 1 and 3*

1. Genetic mapping of novel SNB QTLs
   - Optimise and validate SNB-friendly markers for use on effector phenotypes

2. Unmasking novel effector production
   - Identification and delivery of expressed effectors for wheat breeding

**P. nodorum toxa** (Friesen et al. 2006)

**P. nodorum toxa3**

**P. nodorum toxa13**

Collaborators: Assoc. Prof. Peter Solomon & Dr. Yit Heng Chooi
*P. nodorum* deleted in *SnToxA*, 1 and 3 can still infect

*Calingiri*  
*SN15*  
*toxa13*  
*Gelatin*  
*Emu Rock*  
*SN15*  
*toxa13*  
*Gelatin*  
*Halberd*  
*SN15*  
*toxa13*  
*Gelatin*

*P. nodorum toxa13* still produces necrosis-inducing factor(s)
Our protein chromatography workflow

Crude culture filtrate → Stepwise anion exchange → Assay for active fractions → Size exclusion chromatography

Reducing protein complexity

Candidate effector genes → Proteomics to identify proteins → Active fractions identified → Assay for active fractions

Activity identified → Correlation to SNB QTL? → Delivery to breeders (M1)

E. coli expression (M4)
QTL detected using culture filtrates from strain lacking A, 1 and 3

Crude culture filtrate QTLs

<table>
<thead>
<tr>
<th>Effector QTL</th>
<th>LOD</th>
<th>Phenotype explained (%)</th>
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<tbody>
<tr>
<td>2AS</td>
<td>3.7</td>
<td>7</td>
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<tr>
<td>2B</td>
<td>3.1</td>
<td>6</td>
</tr>
<tr>
<td>3BS</td>
<td>3.9</td>
<td>10</td>
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<tr>
<td>2DL</td>
<td>9</td>
<td>19</td>
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<tr>
<td>5DL</td>
<td>3.5</td>
<td>6</td>
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Ion exchange pooled fractions

<table>
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<th>Effector QTL</th>
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<th>Phenotype explained (%)</th>
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<td>2AS</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>3BS2</td>
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<tr>
<td>3BS1</td>
<td>3.3</td>
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<tr>
<td>Fraction 2</td>
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<tr>
<td>2B</td>
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<tr>
<td>Fraction 3</td>
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<td>2B</td>
<td>3.7</td>
<td>9</td>
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<tr>
<td>Fraction 4</td>
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<td></td>
</tr>
<tr>
<td>2B</td>
<td>3.6</td>
<td>9</td>
</tr>
</tbody>
</table>

New Quantitative Trait Loci in Wheat for Flag Leaf Resistance to Stagonospora nodorum Blotch

Flag leaf resistance QTL on ‘2AS’

Two glume resistance QTLs on ‘3BS’
Is tan spot typical?

• One major effector – one recogniser
• All pathogen isolates carry the effector.

• Several effectors
  – Australian isolates of SNB carry > 9 effectors
  – > 30 QTL detected in wheat
  – Pathogen isolates that don’t carry just the known effectors

• Effectors that are recognised by > 1 gene
  – Alleles that vary in activity
Is tan spot typical?

• One major effector – one recogniser
• All pathogen isolates carry the effector.

• Several effectors
  – Epistasis in effector sensitivities
  – Variation in effector activities
  – Pathogen isolates that don’t carry just the known effectors

• Effectors that are recognised by > 1 gene
  – Alleles that vary in activity
We infiltrated the population with expressed SnTox1 and SnTox3 proteins, scored the symptom and performed QTL analyses.

<table>
<thead>
<tr>
<th></th>
<th>SnToxA</th>
<th>SnTox1</th>
<th>SnTox3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calingiri</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>+</td>
<td>+++</td>
<td></td>
</tr>
</tbody>
</table>

0 = insensitive
1 = slight chlorosis
2 = chlorosis
3 = chlorosis with some necrosis
4 = necrosis

- Commercial WA wheat varieties.
- Double haploid population (~230 lines) from Intergrain.
- SSR, EST-SSR and DArT markers (AWBMMP).
We then examine the relationship between disease and effector sensitivity on a population subset.
### Population QTL analyses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chromosome Arm</th>
<th>QTL</th>
<th>Locus/QTL’s Flanking markers</th>
<th>LOD</th>
<th>$R^2$</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td><strong>SnTox1</strong></td>
<td>1BS</td>
<td>QSnb.fcu-1BS</td>
<td>$Snn1$</td>
<td>21</td>
<td>44</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>SnTox3</strong></td>
<td>5BS</td>
<td>QSnb.fcu-5BS</td>
<td>$Snn3$</td>
<td>80</td>
<td>89</td>
<td>1.79</td>
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<tr>
<td></td>
<td>4BL</td>
<td>QSnb.cur-4BL</td>
<td>Xcfa2194-Xbarc163</td>
<td>4</td>
<td>10</td>
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<tr>
<td><strong>Seedling SNB (SN15)</strong></td>
<td>1BS</td>
<td>QSnb.fcu-1BS</td>
<td>$Snn1$</td>
<td>7</td>
<td>17</td>
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<tr>
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<td>2AS</td>
<td>QSnb.cur-2AS</td>
<td>Xgpw5281 -</td>
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<td><strong>Adult SNB (SN15)</strong></td>
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<td>18</td>
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<td>6B</td>
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<tr>
<td><strong>Seedling SNB (SN15::tox1)</strong></td>
<td>2AS</td>
<td>QSnb.cur-2AS</td>
<td>Xgpw5281 –Xwmc522</td>
<td>6.8</td>
<td>16</td>
<td>0.59</td>
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<td>5BS</td>
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<td>$Snn3$</td>
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<td>Xwmc695 – wPT4859</td>
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<td>8</td>
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</table>

SnTox1-$Snn1$ interaction masks SnTox3-$Snn3$ interaction
1. Up-regulation of \( SnTox1 \) on \( Snn1 \) wheat? \( \times \)

2. Increased \( SnTox3 \) expression in \( P. nodorum tox1 \). \( ? \)

3. Inhibition of \( SnTox3 \) activity by \( SnTox1 \). \( \checkmark \)
Prelim data: co-infiltration of SnTox1 and SnTox3

### SnTox1 (dil)

<table>
<thead>
<tr>
<th>Lines</th>
<th>Genotype</th>
<th>0x</th>
<th>4x</th>
<th>10x</th>
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<tbody>
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<td>Calingiri</td>
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### SnTox3 (20x):SnTox1 (dil)

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</tbody>
</table>

**Key**
- Red: Necrosis
- Orange: Chlorosis with some necrosis
- Yellow: Chlorosis
- Blue: Mild chlorosis
- White: No symptom
Is tan spot typical?

- One major effector – one recogniser
- All pathogen isolates carry the effector.

- Several effectors
  - Epistasis in effector sensitivities
  - Variation in effector activities
  - Pathogen isolates that don’t carry just the known effectors

- Effectors that are recognised by > 1 gene
  - Alleles that vary in activity
### SNB Effectors; +/- alleles

<table>
<thead>
<tr>
<th></th>
<th>% Present (n = 1065)</th>
<th>Highest %</th>
<th>Europe % (n =206)</th>
<th>Lowest %</th>
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</thead>
<tbody>
<tr>
<td>ToxA</td>
<td>40</td>
<td>97 (Australia)</td>
<td>12</td>
<td>6 (China)</td>
</tr>
<tr>
<td>Tox1</td>
<td>84</td>
<td>96 (Australia)</td>
<td>89</td>
<td>70 (US)</td>
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<tr>
<td>Tox3</td>
<td>61</td>
<td>97 (Australia)</td>
<td>67</td>
<td>40 (China)</td>
</tr>
</tbody>
</table>

European isolates
1992 to 2005; some from triticale
England 9; Dk 54; Sweden 55; Switzerland 113
Tan spot??

Megan McDonald

New versions of Tox1 and Tox3 in France
Identification of an *Alternaria brassicola* putative transcription factor gene, *AbPf2*

**Fungal-specific transcription factor AbPf2 activates pathogenicity in *Alternaria brassicicola***

Yangrae Cho¹, Robin A. Ohm², Igor V. Grigoriev³ and Akhil Srivastava¹

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³US Department of Energy Joint Genome Institute, 2860 Mitchell Drive, Walnut Creek, CA 94596, USA

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Non-pathogenic on *A. thaliana* and cabbage

Down-regulation of genes that code for effector-like proteins
1. *AbPf2* ortholog present in *P. nodorum* (*SnPf2*)

2. Validated by RNAseq

3. Phylogenetic analysis of predicted protein alignment

*Ab* = *Alternaria brassicicola*, *Bc* = *Botrytis cinerea*, *Bgh* = *Blumeria graminis* f. sp. *hordei*, *Bgt* = *Blumeria graminis* f. sp. *tritici*, *Cc* = *Cochliobolus carbonum*, *Cgl* = *Colletotrichum gloeosporioides*, *Cgr* = *Colletotrichum graminicola*, *Ch* = *Cochliobolus heterostrophus*, *Cm* = *Cochliobolus miyabeanus*, *Cs* = *Cochliobolus sativus*, *Cv* = *Cochliobolus victoriae*, *Fg* = *Fusarium graminearum*, *Mo* = *Magnaporthe oryzae*, *Mf* = *Mycosphaerella fijiensis*, *Ptr* = *Pyrenophora tritici-repentis*, *Ptt* = *Pyrenophora teres* f. sp. *teres*, *Sa* = *Parastagonospora avenae*,

*Sb* = *Sclerotinia borealis*, *Sc* = *Saccharomyces cerevisiae*, *Sn* = *Parastagonospora nodorum*, *St* = *Setosphaeria turcica*, *Zt* = *Zymoseptoria tritici*
SnPf2 is required for virulence on Tsn1 and Snn3 wheat.
Next steps for TS and SNB in Australia

Discover further effectors
Deploy to breeders
Mechanism of epistasis
Next steps for TS and SNB in ROW

Presence of known effectors in local populations
Presence of known recognisers in local wheats
Eliminate relevant recognisers
Discover further relevant effectors and deploy

Interaction with STB and other breeding objectives
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Caroline Moffat

Postdocs

Pao Theen See

TBA

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Elyce Iagallo

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• Dr. Yit-Heng Chooi
• Shao-Yu Lin
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ETH
James Cockram
NIAB
Lise Jorgensen
Marc-Henri Lebrun
Morten Liliemo
The proportion of *Bgh* isolates with mutant T509 *CYP51* alleles collected in Australia over a five year period.