Breeding resistant cultivars to reduce mycotoxin risks in oats

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A collaborative research effort

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The Research Council of Norway

Choice of resistant cultivars

Norwegian University of Life Sciences
FHB in Norway (1)

- Severe FHB epidemics in Norway since 2000
- Increased *F. graminearum* prevalence; major DON producer in Norway
- Reduced germination in certified seeds 2006-
- Cultivars *Gere, Nes and Lena* (Graminor) and *Bessin* (Norsaat) withdrawn from the market
FHB in Norway (2)

• Oat cannot be used as feed for monogastric animals in “FHB-years”

• For food 10% of the crop, but almost 1/3 imported every year due to FHB

• Due to FHB oat acreage should be reduced from 30 to 20%, but a key rotation crop in a specialized cereal production

• 22% in 2015, but no FHB!
Two types of trials

• Breeding trials:
  – Designed by Graminor primarily to identify new varieties that are resistant to FHB and DON

• Genetic trials
  – To study the resistance genetics of FHB, germination and DON in oats
Reliable field testing

• Spawn inoculation
• Mist irrigation in the evenings
• Field data (Flowering time, height, disease...)
• Parameters: FHB, Mycotoxin (DON), germination, seed infection
Parameter 1. FHB: Unreliable, confused with maturation - abandoned (Left cv. ‘Hurdal’, right ‘Z615’).
Parameter 2. Degree of fungal infection
left ’Belinda’, right ’615-4’: Less infected
Poor correlation with DON- abandoned
Parameter 3. Germination percentage used in breeding - but a composite problem:

- DON kills the seed at early infections
- Does not kill the healthy seed, but inhibits growth:

- Seedling blight: Reversible by dehulling and fungicides
Infection kills the seed.
Effects on the ultrastructure
Surface infections: seedling blight
Parameter 4. Mycotoxin content: used in breeding

- DON measured by GC-MS: direct and efficient
- Used since 2008
- NECESSARY to have strong infection levels
- DON-levels preferably between 3-30 ppm, but 1-10 may be significant
- Need several environments (two locations since 2011)
- Need repeated checks from the best to the worst
- Progress is possible: Recent varieties have 40% less DON
Varietal differences in DON 2007-2015
LSD=2.0 ppm

Oats DON mean (2008-2015)
LSD 5% = 2.0 (8 years vs 8 years)
LSD 5% = 3.0 (2 years vs 8 years)
Varietal differences in germination 2007-2015 (LSD = 4.6%)
Relationship between DON and germination:

$R^2 = 0.45$, but outliers exist

![Germination vs DON 2007-2015](image)
Part II. NIR calibration of DON and germination: useful in breeding?
NIR calibration and prediction also possible for germination

Correlation between Predicted and Actual = 0.85 (P = 0.000).
2015: 
Actual DON values and germination: -0.67 (P=0.000)

Predicted DON values and germination was -0.43 (P=0.000).
Limitation: to include enough environments in the calibration

Year and location effects dominate spectral signals
Part III. Genetic studies of resistance to DON and FHB

• QTL mapping populations
  – Hurdal x Z595-7 (184 lines) and Hurdal x Z615-4 (91 lines)
  – 3 years field testing
  – QTL mapping

• CORE spring oats from North America and Europe
  – 2 years field testing, N=432
  – GWAS

• Anther extrusion in relation to infection

He, et al., 2013
Bjørnstad et al, submitted
Hurdal x Z595-7: a strong QTL reduces DON by up to 25%

- **Qdon.umb-17A/7C**
  - Detected in all experimental years
  - independent of phenology
  - Verified in HZ615 population

- Other QTL were found on 5C, 9D, 13A, and 14D
  - <10% of DON variation
  - Not in all years
  - QTL on 9D and 13A co-localized with QTLs for agronomic traits

He, et al. , 2013
The Spring CORE panel

• 418 spring oats tested and analyzed for DON in 2011 and 2012
• SNPs using the 6k chip and GBS, 2974 polymorphic markers
• Analyzed for structure and trait relationships
1. Possible to score FHB if the variation is wide (bad) enough
2. Relationship between DON and FHB: Large variation - Midwest oats susceptible

Bjørnstad et al, submitted
Does less FHB and DON mean late and tall?

<table>
<thead>
<tr>
<th></th>
<th>DTF</th>
<th>DTM</th>
<th>PH</th>
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</thead>
<tbody>
<tr>
<td>FHB_M</td>
<td>-0.81***</td>
<td>-0.62***</td>
<td>-0.52***</td>
</tr>
<tr>
<td>DON_M</td>
<td>-0.28***</td>
<td>-0.14*</td>
<td>-0.24***</td>
</tr>
</tbody>
</table>

Klos et al 2016: Many QTLs for DTF coincide with FHB and DON

Bjørnstad et al, submitted
But low DON need not mean “late and tall”

• Mrg11/1C for FHB
  – *The previously identified Qdon-umb-17A/7C*

• The CORE correlations reflect the very susceptible, tall and daylength sensitive Midwest lines

• They do not represent the “truth” about genetics of DON resistance in oats

• Progress in Norwegian oats despite limited variation in earliness and height!

• Resistant cv. Leggett is early and not so tall
Anther extrusion (AE): a way to avoid FHB in oats, like in wheat and barley?
Variation of AE exists, but high AE rare in modern varieties

cv. Typhon: Closed  cv. Norum: Wide open
Transgressive segregation for AE and as heritable as days to flowering

Stormogul x Fia

Stormogul x Svea

<table>
<thead>
<tr>
<th></th>
<th>Wald</th>
<th>n.d.f</th>
<th>F</th>
<th>d.d.f</th>
<th>P</th>
<th>SE</th>
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<tbody>
<tr>
<td>Genotype (g)</td>
<td>3179.84</td>
<td>152</td>
<td>20.92</td>
<td>18</td>
<td>&lt;0.001</td>
<td>0.63</td>
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<tr>
<td>Year (y)</td>
<td>0.24</td>
<td>1</td>
<td>0.20</td>
<td>18</td>
<td>0.658</td>
<td>-</td>
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<tr>
<td>g×y</td>
<td>719.59</td>
<td>147</td>
<td>4.90</td>
<td>18</td>
<td>&lt;0.001</td>
<td>0.89</td>
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</table>
Sources of error: drought, and anthers remaining inside

Anthers may remain inside, even at high AE!

Beware of drought when scoring AE

AE and remaining anthers 2012

<table>
<thead>
<tr>
<th>AE</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td>% 1-3 anthers</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>% 0 anthers</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>

Avg. remaining anthers per floret

- Typhon 13
- Typhon 14
- GN9004 14
- Norum 13
- Norum 14

- Drought
- Control
Does AE protect against Fusarium? (1) Yes. In the field with spawn inoculation
The higher the AE, the less FHB and DON

<table>
<thead>
<tr>
<th>FXS</th>
<th>DTF</th>
<th>Height</th>
<th>FHB</th>
<th>AE*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHB</td>
<td>-0.54 (P=0.00)</td>
<td>-0.48 (P=0.00)</td>
<td></td>
<td></td>
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<tr>
<td>AE</td>
<td>0.33 (P=0.05)</td>
<td>0.53 (P=0.00)</td>
<td>-0.62 (P=0.00)</td>
<td></td>
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<tr>
<td>DON</td>
<td>-0.36 (P=0.02)</td>
<td>-0.34 (P=0.03)</td>
<td>0.64 (P=0.00)</td>
<td>-0.36 (P=0.03)</td>
</tr>
</tbody>
</table>

*16 RILs from each cross spawn inoculated in field 2015.
Anther extrusion data was scored in previous greenhouse experiment. ns-non significant.
Does AE protect against Fusarium? (2)

No. The higher the AE, the more FHB and DON in the greenhouse: spray inoculated
Conclusions

- **Significant breeding progress** possible in oats if testing is accurate and adequate.

- **NIR** may be possible to supplement or replace expensive measurements.

- **Anther extrusion** may have a role in infection, but confused with open flowering.

- **Anther retention** more accurate, but laborious to score.

- **Wide open flowers very susceptible** to spray.
Thank you