Genetic mapping of resistance to Aphanomyces root rot in alfalfa

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Aphanomyces root rot

Seedling “corpses”

Acute phase

Adult plants lack lateral and fibrous roots, no nodules. Foliage stunted, yellow. Chronic phase.
Aphanomyces root rot
Research questions

- Survey of MN and NY soils for APH and PRR
  - What races are found in alfalfa fields?
- Two races identified by host genetics
  - Can markers be identified linked to resistance for marker assisted breeding?
- Possible failure of race 1+2 resistant cultivars
  - Are there additional races?
Bioassay for APH

Plant checks in field soil sample. After plants germinate, flood soil for 4 days.

Score symptoms at 21 d after planting.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>PRR</th>
<th>APH Race 1</th>
<th>APH Race 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saranac</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Agate</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>WAPH-1</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>WAPH-5</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>
Race 2 is most common

Minnesota
N=44

Wisconsin
N=227

New York
N=40

Gibbs, 2009
## Results from atypical soils

<table>
<thead>
<tr>
<th>State</th>
<th>Soil</th>
<th>WAPH-1 resistant plants (%)</th>
<th>WAPH-5 resistant plants (%)</th>
<th>Predominant race</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN</td>
<td>GRE</td>
<td>6</td>
<td>15</td>
<td>Undetermined</td>
</tr>
<tr>
<td>MN</td>
<td>JIM</td>
<td>0</td>
<td>5</td>
<td>Undetermined</td>
</tr>
<tr>
<td>MN</td>
<td>WAS</td>
<td>14</td>
<td>28</td>
<td>Undetermined</td>
</tr>
<tr>
<td>NY</td>
<td>CAR</td>
<td>13</td>
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<td>Undetermined</td>
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<tr>
<td>NY</td>
<td>ROY</td>
<td>0</td>
<td>21</td>
<td>Undetermined</td>
</tr>
</tbody>
</table>
60 pure Aphanomyces strains tested

- 4 check cultivars, 4-6 reps, 30 seeds/rep
- All strains read as either race 1 or race 2
- Resistance in WAPH-5 was not overcome
- What is the nature of resistance?
APH Susceptible Reaction

[Image showing plants labeled Agate and WAPH-1, along with tissue samples labeled Agate 7 dai and Agate 14 dai.]
APH Resistant Reaction

Race-specific resistance

Hypersensitive response indicates defense is triggered by NBS-LRR type resistance gene.
Pre-infection resistance?
Developing MAS for APH

- Selected resistant and susceptible seedlings
  - WAPH-1 (race 1), WAPH-5 (race 1+2), 53V54 (race 1+2)
  - Retested with vegetative cuttings

- Symptoms correlated with oospores counts and Aphanomyces DNA content
Identify DNA markers associated with race-specific resistance to APH

1. Identify resistant and susceptible plants
2. Confirm phenotypes: strong R or S
3. Make R x S biparental crosses
4. Phenotype F1 populations for disease reaction
5. Isolate DNA, process, submit for genotyping by sequencing (GBS)
6. Marker/trait analysis and identify potential SNPs associated with trait
7. Confirm marker association with parents and progeny
Genotyping by sequencing to map R genes

MF1 (race 1)

MER4 (race 2)
### Reaction of strains on F1 population (L85)

<table>
<thead>
<tr>
<th>MER4</th>
<th>GRE5</th>
<th>ROY8</th>
<th>STE2</th>
<th>JIM1</th>
<th>Number of plants (of 269)</th>
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<tbody>
<tr>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>S</td>
<td>S</td>
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<tr>
<td>S</td>
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<tr>
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<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>3</td>
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</table>

- Suggests that there are multiple R genes in WAPH-5.
- Intercross plants with resistance to a single strain to develop new checks.
Genes near MF-1 (race 1) marker
Pennsylvania field soil
Flooded for 4 days (6-9 days after planting)
Photo at 12 days after planting

Commercial race 1 resistant variety
WAPH-5 race 1+2 resistant check
WAPH-1 race 1 resistant check
Susceptible checks
Commercial race 1+2 resistant variety
Conclusions

- Resistance appears to be conditioned by dominant R-genes with additional pre- and post-infection defense mechanisms
- R-genes may be clustered on chromosome 1 and 2
- WAPH-5 may have multiple R-genes for APH
- Strains of A. euteiches with diverse virulences are found in field soil
- Developers of resistant varieties should use diverse isolates to have sufficient field resistance to Aphanomyces root rot
Thank you!