Development of a DNA Macroarray for Detection of Young Vine Decline Pathogens

Dan O’Gorman: Pacific Agriculture Research Centre, Summerland, BC
Development of a DNA Macroarray

Outline

• Snapshot of BC Vineyards and the Okanagan Valley
• Grapevine Trunk Diseases (GTD) & Young Vine Decline
• DNA Macroarray System
  a) Macroarray development
  b) Macroarray diagnostic results
  c) Macroarray testing
• Macroarray potential applications and future work
Pacific Agriculture Research Centre - Summerland BC
Okanagan Valley

Desirable conditions:
- Ideal growing degree days
- Abundant sunshine
- Low rainfall
- Low humidity

Extremes:
- Short growing season (compact)
- Low and variable winter temperatures (arctic outflow events)
Okanagan Valley

Geography

- narrow glaciated valley
- Coastal mountain rain shadow

Climatic influences

- continental summer heat
- temperature moderated by lakes
- warm Pacific weather systems
- cold arctic outflow events
### Red Wine Grape Varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>Acreage</th>
<th>% of Whites</th>
<th>% of Total Grapes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merlot</td>
<td>1,600.90</td>
<td>31.15%</td>
<td>16.25%</td>
</tr>
<tr>
<td>Pinot Noir</td>
<td>948.71</td>
<td>18.46%</td>
<td>9.62%</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>755.17</td>
<td>14.69%</td>
<td>7.65%</td>
</tr>
<tr>
<td>Syrah/Shiraz</td>
<td>546.50</td>
<td>10.63%</td>
<td>5.54%</td>
</tr>
<tr>
<td>Cabernet Franc</td>
<td>517.45</td>
<td>10.07%</td>
<td>5.24%</td>
</tr>
<tr>
<td>Gamay Noir</td>
<td>153.73</td>
<td>2.99%</td>
<td>1.56%</td>
</tr>
<tr>
<td>Malbec</td>
<td>137.30</td>
<td>2.67%</td>
<td>1.59%</td>
</tr>
<tr>
<td>Blattner Riesling</td>
<td>97.29</td>
<td>1.89%</td>
<td>0.99%</td>
</tr>
<tr>
<td>Melon</td>
<td>95.96</td>
<td>1.87%</td>
<td>0.97%</td>
</tr>
<tr>
<td>Petit Verdot</td>
<td>65.58</td>
<td>1.28%</td>
<td>0.67%</td>
</tr>
<tr>
<td>Zierfandler</td>
<td>41.72</td>
<td>0.81%</td>
<td>0.43%</td>
</tr>
<tr>
<td>Zinfandel</td>
<td>18.84</td>
<td>0.37%</td>
<td>0.19%</td>
</tr>
<tr>
<td>Pinotage</td>
<td>14.90</td>
<td>0.29%</td>
<td>0.15%</td>
</tr>
<tr>
<td>Tempranillo</td>
<td>13.91</td>
<td>0.27%</td>
<td>0.14%</td>
</tr>
<tr>
<td>Baco Noir</td>
<td>12.45</td>
<td>0.24%</td>
<td>0.13%</td>
</tr>
<tr>
<td>Pinot Meunier</td>
<td>11.33</td>
<td>0.22%</td>
<td>0.12%</td>
</tr>
<tr>
<td>Lemberger</td>
<td>10.10</td>
<td>0.20%</td>
<td>0.10%</td>
</tr>
<tr>
<td>Sangiovese</td>
<td>8.17</td>
<td>0.16%</td>
<td>0.09%</td>
</tr>
<tr>
<td>Leon Millot</td>
<td>7.20</td>
<td>0.14%</td>
<td>0.07%</td>
</tr>
<tr>
<td>Chancellor</td>
<td>7.10</td>
<td>0.14%</td>
<td>0.07%</td>
</tr>
<tr>
<td>Dunkelfelder</td>
<td>6.16</td>
<td>0.12%</td>
<td>0.06%</td>
</tr>
<tr>
<td>Agria</td>
<td>5.71</td>
<td>0.11%</td>
<td>0.06%</td>
</tr>
<tr>
<td>Castal</td>
<td>5.52</td>
<td>0.10%</td>
<td>0.05%</td>
</tr>
<tr>
<td>Carmenere</td>
<td>4.43</td>
<td>0.09%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Grenache</td>
<td>4.18</td>
<td>0.08%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Mourvedre</td>
<td>3.82</td>
<td>0.07%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Dornfelder</td>
<td>3.17</td>
<td>0.06%</td>
<td>0.03%</td>
</tr>
<tr>
<td>Barbera</td>
<td>3.13</td>
<td>0.06%</td>
<td>0.03%</td>
</tr>
<tr>
<td>Rotberger</td>
<td>2.95</td>
<td>0.06%</td>
<td>0.03%</td>
</tr>
<tr>
<td>Michurineitz</td>
<td>1.00</td>
<td>0.02%</td>
<td>0.01%</td>
</tr>
<tr>
<td>St. Laurent</td>
<td>0.90</td>
<td>0.02%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Nebbiolo</td>
<td>0.50</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Miscellaneous Red</td>
<td>33.70</td>
<td>0.66%</td>
<td>0.34%</td>
</tr>
</tbody>
</table>

**Total Red Hybrids (Acres):** 280.46  5.5%
**Total Red Vinifera (Acres):** 4,858.82  94.5%
**Total Red Grapes (Acres):** 5,139.28  100.00%  52.09%

### Reported Tonnage by Production Area

- Fraser Valley: 0.51%
- Penticton: 9.58%
- Okanagan: 5.84%
- Kelowna: 0.64%
- seismic: 0.09%
- Salmon Arm: 0.06%
- Oliver: 45.59%
- Similkameen: 3.95%
- Westbank: 4.39%
- Okanagan: 4.45%
- Kootenays: 0.12%
- Vancouver Island: 0.49%
- Salmon Arm: 0.06%
- Gulf Islands: 0.05%
Grapevine Trunk Diseases (GTD)

- Caused by fungi (Ascomycetes and Basideomycetes)
- Infect grapevines through wounds (pruning wounds)
- Endophytes or facultative parasites
- Colonize the vascular tissue (spurs, cordon, trunk, roots)

- Cause either a rapid or slow decline and eventual death
- Significant economic impact on the grapevine industry
GTD in young vines (< 5-6 years)

Young Vine Decline Complex

- Petri Disease
- Black Foot Disease
GTD in mature vines (> 6 years)

- Esca
- Eutypa dieback
- Botryosphaeria dieback
- Phomopsis dieback
Current status and significance of GTD in B.C.

Disease survey: 173 Vineyards surveyed and 393 Samples collected

Okanagan Valley
Total Vineyards Surveyed = 147
Total Samples Collected = 338

Lower Mainland
Total Vineyards Surveyed = 13
Total Samples Collected = 26

Vancouver and Gulf Islands
Total Vineyards Surveyed = 13
Total Samples Collected = 29
Disease survey
Overall GTD vascular symptoms observed in B.C.

All 173 vineyards surveyed showed GTD infected vines

<table>
<thead>
<tr>
<th>Type of Infection</th>
<th>Number of Samples</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Samples</td>
<td>393</td>
<td></td>
</tr>
<tr>
<td>Dark Streaking Wood</td>
<td>125</td>
<td>31.8%</td>
</tr>
<tr>
<td>Wedge-Shape canker</td>
<td>113</td>
<td>28.8%</td>
</tr>
<tr>
<td>Central Circular Canker</td>
<td>50</td>
<td>12.7%</td>
</tr>
<tr>
<td>Mix Infections</td>
<td>105</td>
<td>26.7%</td>
</tr>
</tbody>
</table>
Disease survey
GTD vascular symptoms based on vineyard age

65 young vineyards surveyed (< 6 years)

- 87.3% Roots and rootstock
- 12.7% Scion

Total Samples Collected: 393
- Samples From Young Vineyards: 128 (32.6%)
- Dark Streaking Wood: 86 (67.2%)
- Wedge-Shape canker: 25 (19.5%)
- Central Circular Canker: 17 (13%)

Total number of samples distribution:
- 72% Scion
- 28% Roots and rootstock
Disease survey
GTD vascular symptoms based on vineyard age

108 mature vineyards surveyed (> 6-years)

These symptoms were more prevalent above the graft-union (trunk, cordons and spur positions)
Disease survey: GTD field symptoms monitoring

114 Vineyards monitored during the 2011 growing season
Disease survey: GTD field symptoms monitoring

114 Vineyards monitored during the 2011 growing season

Over 56,000 vines monitored
Disease survey: GTD field symptoms monitoring

- 44 young vineyards monitored (< 6-years)
- Only 4 out of 44 did not show GTD Symptomatic Vines

Total young vines monitored: 20,695

- YVD = 437 vines (2.1%)
- Esca = 281 vines (1.4%)
- Cankers = 72 vines (0.3%)
- 83 (18.9%) Collapsed vines

Healthy Looking: 20,231 vines (98.2%)

3.8% of total young vines monitored showed symptoms.
Disease survey: GTD field symptoms monitoring

44 young vineyards monitored (< 6-years old)

Disease severity varied significantly among vineyards monitored

% of YVD symptomatic vines per vineyard

- Total Vineyards Monitored: 44
- No Symptomatic Vines: 7
- 0-5% Symptomatic Vines: 34
- 5-10% Symptomatic Vines: 2
- >10% Symptomatic Vines: 1 (19.7% of vines showing YVD symptoms)
Disease survey: GTD field symptoms monitoring

44 young vineyards monitored (< 6-years old)

Disease severity varied significantly among vineyards monitored

% of Esca symptomatic vines per vineyard

- Total Vineyards Monitored: 44
- No Symptomatic Vines: 36
- 0-5% Symptomatic Vines: 2
- 5-10% Symptomatic Vines: 1
- >10% Symptomatic Vines: 5 (13.3 to 18.8% of vines showing Esca symptoms)
Young Vine Decline

1) Traditional plating and morphological identification

2) Molecular identification (DNA - PCR - Sequencing)
- Phylogenetic studies

**ITS1-5.8S-ITS2**

Length = 1432  
CI = 0.596  
RI = 0.888  
HI = 0.404

**Botryosphaeriaceae**  
"Botryosphaeria dieback"

**Mitosporic Ascomycota**  
"Esca" & "Petri Disease"

**Mitosporic Heliotiales**  
"Petri Disease"

**Diatrypaceae**  
"Eutypa dieback"

**Mitosporic Amphisphaeriaceae**  
Grapevine cankers

**Valsaceae**

*Phomopsis viticola*  
"Phomopsis cane and leaf spot" & "Phomopsis dieback"

*Cytospora* spp.  
Cankers on fruit trees

**Calosphaeriaceae & Mitosporic Calosphaeriaceae**  
"Esca" & "Petri Disease"

**Nectriaceae & Mitosporic Neonectria**  
"Black-foot disease"
Disease survey: pathogens identification

2) Molecular identification (ITS, BTUB, EF, ACTIN)

1. Cadophora luteo-olivacea
2. Phaeomoniella chlamydospora
3. Togninia minima
4. Togninia fraxinopennsylvanica
5. Togninia viticola
6. Phaeoacremonium angustius
7. Phaeoacremonium iranianum
8. Phaeoacremonium sp. nov.
9. Cylindrocarpon pauciseptatum
10. Ilyonectria liriodendri
11. Ilyonectria macrodydima
12. Ilyonectria radicicola
13. Botryosphaeria dothidea
14. Diplodia mutila
15. Diplodia seriata
16. Dothiorella sp. nov.
17. Neofusicoccum parvum
18. Neofusicoccum ribis
19. Eutypa lata
20. Eutypa flavovirens
21. Eutypa laevata
22. Cryptosphaeria pullmanensis
23. Cryptovalsa ampelina
24. Diatrype stigma
25. Diatrype whitemanensis
26. Diatrypella sp.
27. Diatrypella sp. nov.
28. Phomopsis sp.

Young Vine Decline (Petri disease / Esca)

Young Vine Decline (Black-foot disease)

Botryosphaeria dieback
Eutypa dieback
Phomopsis dieback
Disease survey: Pathogens Identification

2) Molecular identification (ITS, BTUB, EF, ACTIN)

1. Cytospora cincta
2. Cytospora chrysosperma
3. Cytospora sp. nov.
4. Valsa sp. nov.
5. Cadophora melinii
6. Cadophora malorum
7. Phialophora dancoi
8. Neofabraea alba
9. Neofabraea malicorticis
10. Phomopsis amygdali
11. Phomopsis quercina
12. Phomopsis vacinii
13. Phomopsis sp. nov.
14. Diaporthe eres
15. Diaporthe viticola
16. Truncatella angustata
17. Fusarium spp.

Some of these fungal species are well-known pathogens causing dieback in perennial hosts, including fruit trees (apple, cherry, pear, peach, apricot, and plum) and cane berries (blueberries, blackberries, and raspberries)
**Disease survey: pathogen identification and incidence**

### Dark streaking of the wood

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaeomoniella chlamydospora</td>
<td>32.3%</td>
</tr>
<tr>
<td>Cylindrocarpon spp.</td>
<td>21.7%</td>
</tr>
<tr>
<td>Phaeoacremonium spp.</td>
<td>10.4%</td>
</tr>
<tr>
<td>Botryosphaeriaceae spp.</td>
<td>9.8%</td>
</tr>
<tr>
<td>Cytospora spp.</td>
<td>2.8%</td>
</tr>
<tr>
<td>Phomopsis sp.</td>
<td>1.8%</td>
</tr>
<tr>
<td>Cadophora spp. / Phialophora spp.</td>
<td>0.8%</td>
</tr>
<tr>
<td>Truncatella angustata</td>
<td>0.8%</td>
</tr>
<tr>
<td>Verticillum sp.</td>
<td>0.8%</td>
</tr>
<tr>
<td>Other fungi</td>
<td>18.8%</td>
</tr>
</tbody>
</table>

### Wedge-shape cankers

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytospora spp.</td>
<td>27.3%</td>
</tr>
<tr>
<td>Diatrypaceae spp.</td>
<td>23.3%</td>
</tr>
<tr>
<td>Botryosphaeriaceae spp.</td>
<td>23.1%</td>
</tr>
<tr>
<td>Phaeomoniella chlamydospora</td>
<td>7.4%</td>
</tr>
<tr>
<td>Phaeoacremonium spp.</td>
<td>6.1%</td>
</tr>
<tr>
<td>Phomopsis sp.</td>
<td>4.3%</td>
</tr>
<tr>
<td>Truncatella angustata</td>
<td>3.4%</td>
</tr>
<tr>
<td>Neofabraea spp.</td>
<td>2.1%</td>
</tr>
<tr>
<td>Cadophora spp. / Phialophora spp.</td>
<td>1.7%</td>
</tr>
<tr>
<td>Cylindrocarpon spp.</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
Disease survey: pathogen identification and incidence

Central necrosis

1. Diatrypaceae spp.  (22.7%)
2. Cytospora spp.  (22.1%)
3. Phaeomoniella chlamydospora  (12%)
4. Botryosphaeriaceae spp.  (11.3%)
5. Phaeoacremonium spp.  (10.7%)
6. Truncatella angustata  (8.4%)
7. Neofabraea spp.  (4.8%)
8. Cadophora spp. / Phialophora spp.  (3%)
9. Phomopsis sp.  (1.8%)
10. Cylindrocarpon spp.  (1.2%)
DNA Macroarray Development

• **A DNA array** (a.k.a. microarray, macroarray, gene chip, or DNA chip,) is a collection of microscopic DNA spots attached to a solid surface.

• Each DNA spot contains a specific DNA sequence, known as probes
  - hundreds to thousands spots/probes can be placed in known locations on a single DNA array
  - sample DNA binds specifically to individual probes and is detected by a chemiluminescent signal

• DNA arrays are of great use for:
  - simultaneous pathogen detection (disease complex or multiple infections)
  - detection of many different targets (large number of different pathogen species)
**DNA multiscan**

The DNA multiscan: A multiplex method, that means that in one analysis different pathogens can be tested. At this moment Relab den Haan offers only fungal DNA multiscan. This will change soon!! At this moment we are working hard on the development of a membrane with more fungi, bacteria and nematodes.

**Fungi detected with the DNA multiscan**

- Oomyceten
- Athelia (Sclerotium) rolfsii
- Botrytis cinerea
- Colletotrichum spp.
- Colletotrichum coccodes
- Colletotrichum gleosporioides
- Colletotrichum acutatum
- Didymella spp.
- Fusarium spp.
- Fusarium solani
- Fusarium oxysporum
- Penicillium spp.
- Phytophthora capsici
- Phytophthora cinnamomi
- Phytophthora drechsleri
- Phytophthora cryptogea
- Phytophthora infestans
- Phytophthora nicotianae
- Phytophthora ramorum
- Phytophthora fragariae
- Phytophthora cactorum
- Plectosphaerella cucumerina
- Pyrenochaeta lycopersici
- Pythium spp.
- Pythium aphanidermatum
- Pythium dissotocum
- Pythium polystomum
- Pythium sylvaticum
- Pythium ultimum
- Pythium irregulare
- Rhizoctonia solani
- Sclerotinia spp.
- Sclerotinia minor
- Sclerotinia sclerotiorum
- Sclerotinia trifoliorum
- Trichoderma spp.
- Trichoderma asperellum
- Trichoderma harzianum
- Trichoderma hamatum
- Verticillium spp.
- Verticillium albo-atrum
- Verticillium dahliae

![Image of DNA multiscan](http://www.denhaan.nl/ukdna.html)
DNA Macroarray Development

Petri Disease / Esca
1. Phaeomoniella chlamydospora
2. Phaeoacremonium aleophilum
3. Phaeoacremonium amstelodamense
4. Phaeoacremonium angustius
5. Phaeoacremonium argentiniense
6. Phaeoacremonium armeniacum
7. Phaeoacremonium austroafricanum
8. Phaeoacremonium cinereum
9. Phaeoacremonium croatiense
10. Phaeoacremonium dauci
11. Phaeoacremonium ducedens
12. Phaeoacremonium flaccidum
13. Phaeoacremonium griseolivacea
14. Phaeoacremonium hispanicum
15. Phaeoacremonium hungaricum
16. Phaeoacremonium inflatipes
17. Phaeoacremonium iranianum
18. Phaeoacremonium krajdenii
19. Phaeoacremonium mortoniae
20. Phaeoacremonium nova-zelandiae
21. Phaeoacremonium occidentale
22. Phaeoacremonium pallidum
23. Phaeoacremonium prunicola
24. Phaeoacremonium scolyti
25. Phaeoacremonium sicilianum
26. Phaeoacremonium tardicrescens
27. Phaeoacremonium theobromatis
28. Phaeoacremonium tuscanum
29. Phaeoacremonium venezuelense
30. Phaeoacremonium viticola
31. Phaeoacremonium vivatilis
32. Phaeoacremonium vivratilis

Black-foot
1. Ilyonectria liriodendri
2. Cylindrocarpon obtusisporni
3. Cylindrocarpon lucidum
4. Cylindrocarpon olidum
5. Ilyonectria faginata
6. Cylindrocarpon theobromica
7. Ilyonectria radiciola
8. Cylindrocarpon ianthothele
9. Cylindrocarpon cylindroides
10. Cylindrocarpon gamsii
11. Cylindrocarpon europaea
12. Campylocarpon fasciculare
13. Campylocarpon pseudofasciculare
14. Ilyonectria macrodydima
15. Cylindrocarpon pauciseptatum
16. Cylindrocarpon sp.

32 species of Phaeomoniella and Phaeoacremonium spp.
16 species of Cylindrocarpon spp.
DNA Macroarray Development

- Species-specific oligonucleotide probes design
  
  115 probes (pathogens and related species)

  **86 YVD pathogen-specific probes**
  
  12 - *Phaeomoniella*
  46 - *Phaeoacremonium*
  28 - *Cylindrocarpon, Campylocarpon, Ilyonectria*
  6 - Control probes (Bt2a & Bt2b)

- *Fusarium, Botrytis, and Alternaria* species were included
DNA Macroarray Development

- YVD fungal pathogens targeted (No. isolates used - Origin)

1. Phaeomoniella
   - Pa. chlamydospora (9-Canada, 3-Spain, 3-USA)
   - Pa. capensis (1-South Africa)
   - Pa. dura (1-South Africa)
   - Pa. effusa (1-South Africa)
   - Pa. pinifoliorum (1-South Korea)
   - Pa. prunicola (1-South Africa)
   - Pa. tardicola (1-South Africa)
   - Pa. zymoides (1-South Korea)

2. Phaeoacremonium
   - Pm. aleophilum (10-Canada, 2-USA, 1-Italy)
   - Pm. angustius (2-USA)
   - Pm. argentiniense (1-Argentina)
   - Pm. armeniacum (1-New Zealand)
   - Pm. australiense (1-Australia)
   - Pm. austroafricanum (1-South Africa)
   - Pm. alvesii (2-USA)
   - Pm. cinereum (1-Iran)
   - Pm. croatiense (1-Croatia)
   - Pm. globosum (1-New Zealand)
   - Pm. hispanicum (1-Spain)
   - Pm. hungaricum (1-Hungary)
   - Pm. iranianum (4-Canada, 1-Italy)
   - Pm. krajdenii (1-Canada)
   - Pm. mortoniae (4-Canada, 1-USA)
   - Pm. occidentale (1-New Zealand)
   - Pm. scolyti (1-South Africa)
   - Pm. sicilianum (1-Italy)
   - Pm. subulatum (1-South Africa)
   - Pm. tuscanum (1-Italy)
   - Pm. venezuelense (1-Venezuela)
   - Pm. viticola (1-France)

3. Campylocarpon, Cylindrocarpon, Ilyonectria
   - Campylocarpon fasciculare (1-South Africa)
   - Cylindrocarpon pauciseptatum (1-Canada, 1-Slovenia)
   - Ilyonectria europaea (1-Germany)
   - Ilyonectria liriodendri (5-Canada, 3-USA, 2-South Africa)
   - Ilyonectria macrodydima (5-Canada, 2-USA, 1-South Africa)
   - Ilyonectria robusta (4-Canada, 1-Netherlands)
   - Cylindrocarpon iantothele (2-Germany)
   - Cylindrocarpon olidum (1-Germany)
   - Cylindrocarpon theobromicola (1-Papua New Guinea)
   - Ilyonectria faginata (1-Canada)
   - Ilyonectria gamsii (1-Netherlands)
   - Ilyonectria radicicola (1-Sweden)
   - Cylindrocarpon cylindroides (1-Norway)
   - Cylindrocarpon obtusisporium (1-Germany)

13 - 22 species (represent all species known in the genus)
22 - 58 isolates

00 - includes the ex-type specimen
DNA Macroarray Development

- Pathogen specific oligonucleotide probes design
- DNA amplification and sequencing using primers Bt2a & Bt2b

**Beta-tubulin nuclear gene**

forward and reverse sequences
DNA Sequence Alignment for Probe Selection and Design

Probes are selected from unique regions of DNA.

Probes are designed to match and bind the DNA of a single species.
DNA Sequence Alignment for Probe Selection and Design

DNA sequence alignment

Probe selection

Probe for species A

Probe for species B

species A(1) CGCTGTC TTCCGT--CTATTTTCATAGTCTGCCC
species A(2) CGCTGTC TTCCGT--CTATTTTCATAGTCTGCCC
species B(1) CGCGG TCGTCCG------TTTC--TTCGTCC
species B(2) CGCTG TCGTCCG------TTTC--TTCGTCC
DNA Macroarray: Attaching probes to a nylon membrane

2) Membrane printing

384 well plate and transfer pins

Transferring probe solution from the 384 well plate to the membrane

Printed membrane

Schematic of probe attached to membrane

O

NH

GCATACGTGGATCGAATGGCT

linker arm

nylon membrane

DNA probe
DNA Macroarray: new membrane

Example of freshly printed membrane

<table>
<thead>
<tr>
<th>species 1</th>
<th>species 2</th>
<th>species 3</th>
<th>species 4</th>
<th>species 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
</tr>
<tr>
<td>species 6</td>
<td>species 7</td>
<td>species 8</td>
<td>species 9</td>
<td>species 10</td>
</tr>
<tr>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
</tr>
<tr>
<td>species 11</td>
<td>species 12</td>
<td>species 13</td>
<td>species 14</td>
<td>species 15</td>
</tr>
<tr>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
</tr>
<tr>
<td>species 16</td>
<td>species 17</td>
<td>species 18</td>
<td>species 19</td>
<td>species 20</td>
</tr>
<tr>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
<td>○ ○ ○</td>
</tr>
</tbody>
</table>

○ = O

DNA probe: GCATACTGGATCGAATGGCT

linker arm

nylon membrane
DNA Macroarray: example of a positive reaction

Positive reaction
Sample DNA binds to specific probe(s)

Negative reaction
Sample DNA does not bind to the rest of the probes

amplified sample DNA

TU*TACGCGCTAU*TAATGCG
CGTATGACCTAGCTTACCGA
TU*TACGCGCTAU*TAATGCG

nylon membrane

DNA probe

linker arm

GCATACGGAGATCGAATGGCT

linker arm

nylon membrane
DNA Macroarray: positive reaction

DNA probe

linker arm

nylon membrane

chemiluminescence

alkaline-labile ester bound

dUTP

Spacer

DIG = Digoxigenin

CSPD / GDP-Star

X-Ray film or Imaging System

TU*TACGCCGCTAU*TAATGCG
CGTATGACCTAGCTTACCGA
TU*TACGCCGCTAU*TAATGCG

GCATACTGGATCGAATGGCT
DNA Macroarray: positive test results

Petri Disease / Esca pathogens

Positive reaction with sample PARC70
\textit{Phaeoacremonium iranianum}

Positive reaction with sample ICMP 17037
\textit{Phaeoacremonium occidentale}

[Diagram showing DNA Macroarray with positive test results for various species]
DNA Macroarray Results

- Pathogen detection

*Phaeoacremonium aleophilum*
CBS 631.94

*C. C. C*

*Pm. griseorubrum*
CBS 111657

*Pm. africanum*
CBS 120863

*Phaeoacremonium canadensis sp. nov.*
PARC392
DNA Macroarray Results

- Pathogen detection

*Ilyonectria liriodendri*
CBS 110.81

*Cylinodidyma pauciseptatum*
CBS 120675
DNA Macroarray Results

- Pathogen detection

**Phaeomoniella chlamydospora**
- CBS 117179
- PARC50
- PARC100

**Pa. tardicola**
- CBS 121757

**Pa. zymoides**
- CBS 114904
DNA Macroarray Detection Limit

- DNA dilutions

<table>
<thead>
<tr>
<th>DNA Stock Solution</th>
<th>1 ng/ul</th>
<th>10^-1 ng/ul</th>
<th>10^-2 ng/ul</th>
<th>10^-3 ng/ul</th>
<th>10^-4 ng/ul</th>
<th>10^-5 ng/ul</th>
<th>10^-6 ng/ul</th>
<th>10^-7 ng/ul</th>
<th>10^-8 ng/ul</th>
</tr>
</thead>
</table>

Phaeomoniella chlamydospora (CBS 117179)

DNA ladder

10^-8 ng/ul

Phaeomoniella chlamydospora (CBS 117179)

ndecl | deci | 10^-1 | 0.1 |
centi | centi | 10^-2 | 0.01 |
milli | milli | 10^-3 | 0.001 |
micro | micro | 10^-6 | 0.000001 |
nano | nano | 10^-9 | 0.000000001 |
pico | pico | 10^-12 | 0.000000000001 |
femto | femto | 10^-15 | 0.00000000000001 | *
DNA Macroarray Proof of Concept

- Wood tissue inoculation by using mycelium plugs
  
  *Phaeomoniella chlamydospora* (CBS 117179)

- Soil inoculation by using spore suspension
  
  *Ilyonectria liriodendri* (CBS 110.81)
  *Ilyonectria macrodidyma* (CBS 112615)

- Total DNA isolation and Dig-11-dUTP PCR amplification (31 days after inoculation)

- Total DNA isolation and Dig-11-dUTP PCR amplification (10 days after inoculation)
DNA Macroarray Summary

• Identification of 59 different species
  - *Phaeomoniella*
  - *Phaeoacremonium*
  - *Campylocarpon*
  - *Cylindrocarpon*
  - *Ilyonectria*

• No cross-reaction seen among the 86 YVD probes designed

• Lower detection limit between $10^{-5}$ and $10^{-6}$ ng of DNA

• Positive detection from **direct processing** of infected wood and soil samples
Potential Applications

DNA Macroarray: scientific - diagnostic - management tool

Multiple pathogen/disease detection from a single sample with a single test

Diagnostic sample

Hybridize membrane

- taxonomy
- epidemiology
- diagnostics
- plant certification
DNA Macroarray Potential Applications

measurement of disease thresholds

A) **macro array** - screening for multiple pathogens

B) **QPCR** - quantification (disease/infection threshold)
**DNA Macroarray Ongoing Work**

**Development of a DNA Macroarray for Canker Pathogens**

**Next:** develop species specific probes for the canker pathogens

<table>
<thead>
<tr>
<th>Botryosphaeria dieback</th>
<th>Eutypa dieback</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Botryosphaeria dothidea</em></td>
<td>1. <em>Eutypa lata</em></td>
</tr>
<tr>
<td>2. <em>Diplodia corticola</em></td>
<td>2. <em>Eutypa leptoplaca</em></td>
</tr>
<tr>
<td>3. <em>Diplodia mutila</em></td>
<td>3. <em>Eutypa laevata</em></td>
</tr>
<tr>
<td>5. <em>Dothiorella sp.</em></td>
<td>5. <em>Eutypella microtheca</em></td>
</tr>
<tr>
<td>7. <em>Lasiodiplodia crassispora</em></td>
<td>7. <em>Eutypella sp. 1</em></td>
</tr>
<tr>
<td>8. <em>Lasiodiplodia missouriana</em></td>
<td>8. <em>Eutypella sp. 2</em></td>
</tr>
<tr>
<td>10. <em>Lasiodiplodia viticola</em></td>
<td>10. <em>Eutypella sp. 4</em></td>
</tr>
<tr>
<td>11. <em>Neofusicoccum australe</em></td>
<td>11. <em>Cryptosphaeria lygniota</em></td>
</tr>
<tr>
<td>12. <em>Neofusicoccum luteum</em></td>
<td>12. <em>Cryptosphaeria pullmanensis</em></td>
</tr>
<tr>
<td>15. <em>Neofusicoccum parvum</em></td>
<td>15. <em>Diatrype brunneospora</em></td>
</tr>
<tr>
<td>17. <em>Neofusicoccum viticlavatum</em></td>
<td>17. <em>Diatrype stigma</em></td>
</tr>
<tr>
<td>18. <em>Neofusicoccum vitifusiforme</em></td>
<td>18. <em>Diatrype whitmanensis</em></td>
</tr>
<tr>
<td>20. <em>Spencermartinsia viticola</em></td>
<td>20. <em>Diatrypella verrucaformis</em></td>
</tr>
<tr>
<td>21. <em>Diatrypella vulgaris</em></td>
<td></td>
</tr>
</tbody>
</table>
DNA Macroarray Ongoing Work

- **Macroarray for Canker pathogens**
  
  *Botryosphaeriaceae* - 20 spp.
  
  *Diatrypaceae* - 21 spp.
  
  *Phomopsis-Diaporthe*
  
  *Cadophora - Phialophora*
  
  (70 different species)

- **Semi-quantification of the signal obtained** *(ImageQuant TL)*

**DNA Macroarray Potential Applications**

- **Scientific tool** - taxonomy, epidemiology, disease thresholds, etc.

- **Diagnostic tool** - transfer to public/private diagnostic laboratories

- **Management tool** - nursery propagation, new plantings (soil analysis) and mature vineyards (disease monitoring)
Acknowledgements

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Paula Haag - Research Technician
Maeghan Sheepwash - Summer Student

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