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Grape DIAP-Activity 3

Diagnostic Technologies and Management Strategies for Trunk Diseases of Grapevines in British Columbia

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Canada

Grape DIAP-Activity 3

Diagnostic Technologies and Management Strategies for Trunk Diseases of Grapevines in British Columbia

Research Team

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OUTLINE

- Introduction and significance of grapevine trunk diseases
- *Sub Activity 3.1.* Development of molecular diagnostic tools
  - DNA macro-array for GTD pathogens
- *Sub Activity 3.2.* Status and Significance of GTD in B.C.
  - Disease survey
  - GTD pathogens identification
- *Sub Activity 3.3.* Evaluation of available and new disease management strategies

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What are grapevine trunk diseases (GTD)?

- Caused by fungi (primarily ascomycetes)
- Infect grapevines through wounds and openings
  - Primarily through pruning wounds
  - Wounds caused by mechanical damage, hail, freezing,…
  - Fungi may be present in endophytic or latent phase
- Symptoms include
  - Slow or rapid decline
  - Grapevine dieback
  - Eventual death of the plant

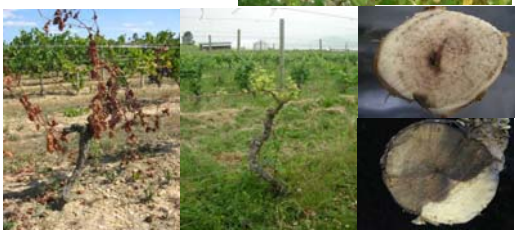
  -Interruption of xylem conductivity
  -Toxin production

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



Introduction and significance of GTD

- GTD are found in all the grape-growing regions of the world
- GTD presents significant disease problems worldwide, limiting both vineyard longevity and productivity

- Overall loss to the wine industry in California is estimated in $260 million/year (*Only considering Eutypa and Botryosphaeria dieback*)
- In the Okanagan we only started seeing this problem in 2007
- Major challenge resides in the lack of effective control strategies

SUB ACTIVITY 3.2 – STATUS OF GTD IN B.C.

- Current status and significance of GTD in B.C.

Disease survey

- GTD pathogen identification

  Morphological studies

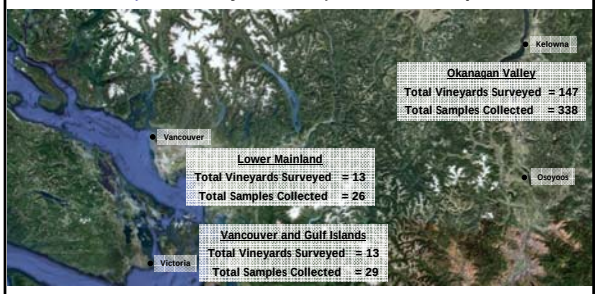
  Molecular and phylogenetic studies (DNA-based ID)

- incidence of GTD and potential impact to BC vineyards

  GTD field symptoms monitoring

Current status and significance of GTD in B.C.

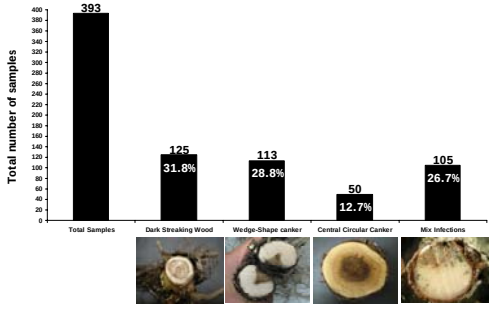
Disease survey: **173 Vineyards** surveyed and **393 Samples** collected



Disease survey

Overall GTD vascular symptoms observed in B.C.

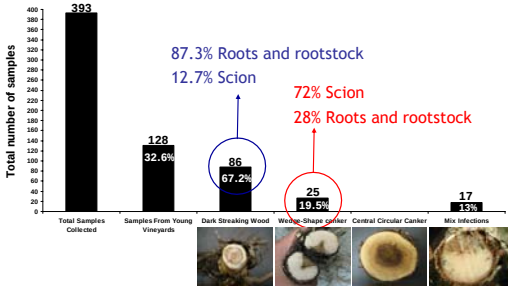
All 173 vineyards surveyed showed GTD infected vines



Disease survey

GTD vascular symptoms based on vineyard age

65 young vineyards surveyed (**< 6 years**)

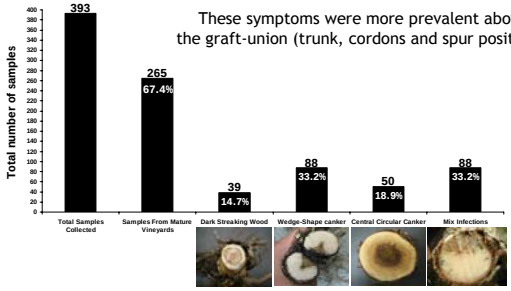


Disease survey

GTD vascular symptoms based on vineyard age

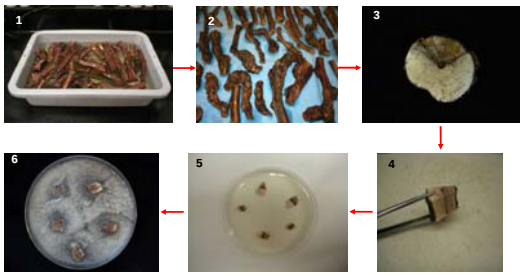
108 mature vineyards surveyed (**> 6-years**)

These symptoms were more prevalent above the graft-union (trunk, cordons and spur positions)



Disease survey: GTD pathogens identification

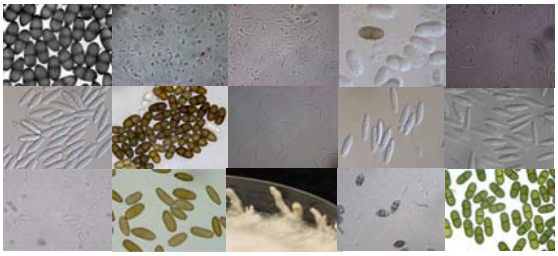
1) Fungal isolation and morphological identification



Long process (some fungi need up to 5 weeks to grow on culture media)

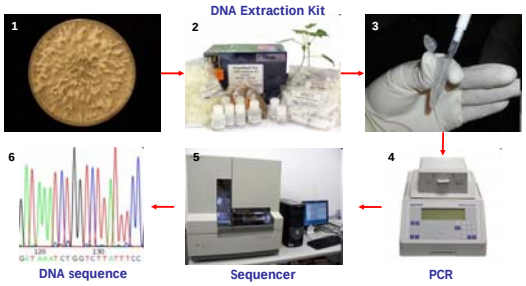
Disease survey: GTD pathogens identification

1) Fungal isolation and morphological identification



Disease survey: GTD pathogens identification

2) Molecular identification (DNA-based)



Disease survey: GTD 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. *Ilyionectria 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

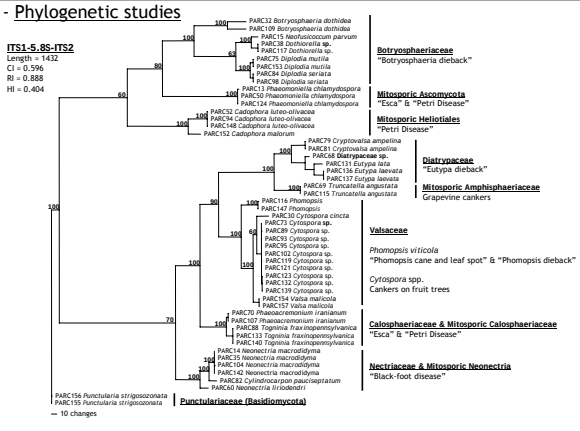
Disease survey: GTD Pathogens Identification

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.*
13. *Diaporthe eres*
14. *Diaporthe viticola*
15. *Truncatella angustata*
16. *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)

- Phylogenetic studies



Disease survey: GTD pathogen identification and incidence

Dark streaking of the wood

1. *Phaeomoniella chlamydospora* **(32.3%)**
2. *Cylindrocarpon* **spp. (21.7%)**
3. *Phaeoacremonium* **spp. (10.4%)**
4. **Botryosphaeriaceae spp. (9.8%)**
5. *Cytospora* spp. (2.8%)
6. *Phomopsis sp.* (1.8%)
7. *Cadophora* spp. / *Phialophora* spp. (0.8%)
8. *Truncatella angustata* (0.8%)
9. *Verticillum* sp. (0.8%)
10. Other fungi (18.8%)

Wedge-shape cankers

1. *Cytospora* **spp. (27.3%)**
2. *Diatrypaceae* **spp. (23.3%)**
3. *Botryosphaeriaceae* **spp. (23.1%)**
4. *Phaeomoniella chlamydospora* (7.4%)
5. *Phaeoacremonium* spp. (6.1%)
6. *Phomopsis sp.* (4.3%)
7. *Truncatella angustata* (3.4%)
8. *Neofabraea spp.* (2.1%)
9. *Cadophora* spp. / *Phialophora* spp. (1.7%)
10. *Cylindrocarpon* spp. (1.3%)

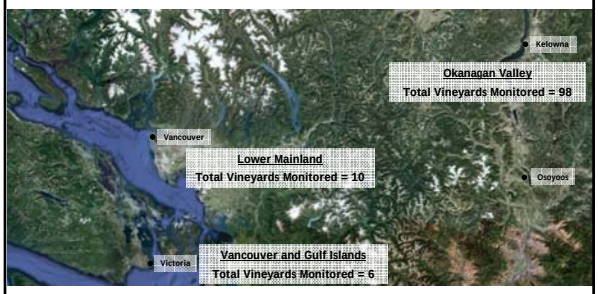
Disease survey: GTD pathogen identification and incidence

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%)

Disease survey: GTD field symptoms monitoring

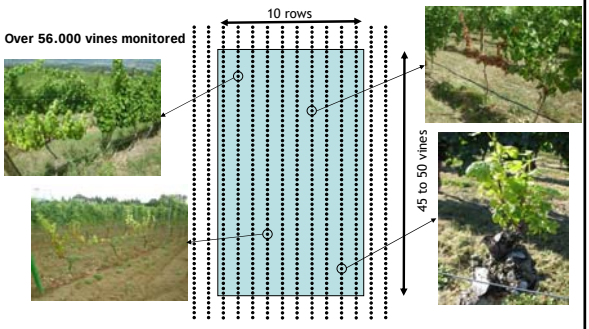
114 Vineyards monitored during the 2011 growing season



Disease survey: GTD field symptoms monitoring

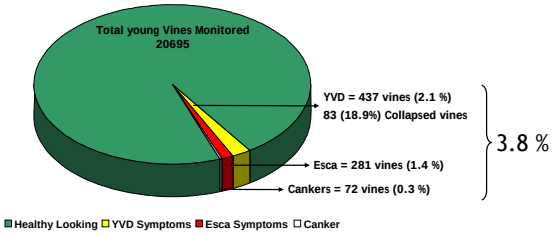
114 Vineyards monitored during the 2011 growing season

Over 56.000 vines monitored



Disease survey: GTD field symptoms monitoring

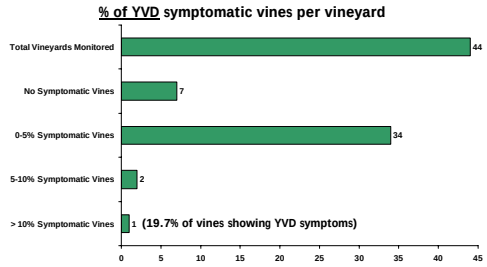
- 44 young vineyards monitored (< 6-years)
- Only 4 out of 44 did not show GTD Symptomatic Vines



Disease survey: GTD field symptoms monitoring

44 young vineyards monitored (< 6-years old)

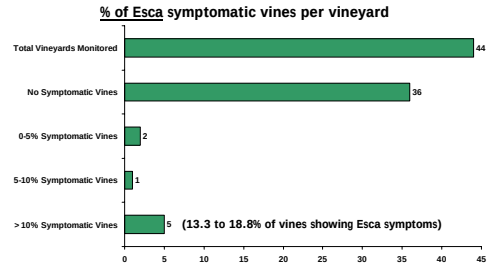
Disease severity varied significantly among vineyards monitored



Disease survey: GTD field symptoms monitoring

44 young vineyards monitored (< 6-years old)

Disease severity varied significantly among vineyards monitored



Disease survey: Additional analysis (ongoing)

- GTD status on Mature Vineyards in B.C.

  70 mature vineyards monitored (> 6-year-old)

- The effects of:

  Geographical location
  Rootstock
  Cultivar
  Age
  } Severity of GTD in B.C.

SUB ACTIVITY 3.1 – MOLECULAR DIAGNOSTIC TOOLS

Development of a DNA macro-array for GTD pathogens

- A **DNA array** (a.k.a. micro-array, macro-array, 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)

Development of a DNA macro-array for GTD pathogens

- DNA sequencing of *rDNA*, *ß-tubulin* and EF genes are targeted for design of pathogen specific probes

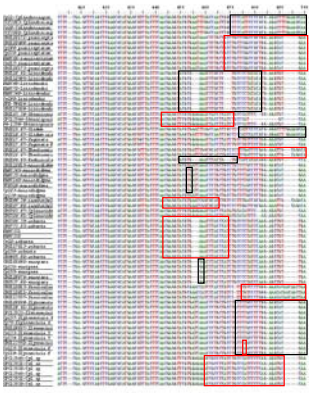
- Fungal isolates collected through disease survey were sequenced for ID and aligned with reference sequences (from PARC culture collection and Genbank database)

- PARC fungal culture collection:
  - GTD disease pathogens (BC isolates)
  - live ex-type cultures (GTD pathogens and related species)
  - DNA samples from California and Australia

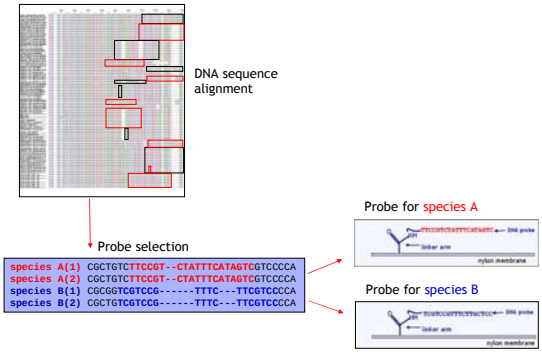
DNA sequence alignment for probe selection and design

Probes are selected from unique regions of DNA

Probes are designed to match and bind the DNA of a single species

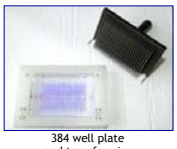


DNA sequence alignment for probe selection and design



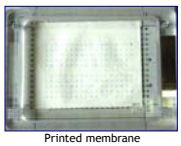
DNA macro-array: Attaching probes to a nylon membrane

2) Membrane printing



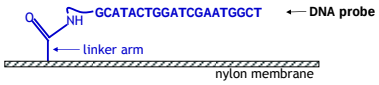
384 well plate
and transfer pins

Transferring probe solution
from the 384 well plate to
the membrane



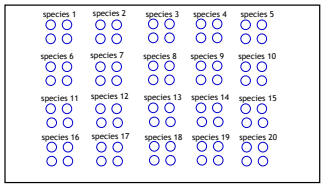
Printed membrane

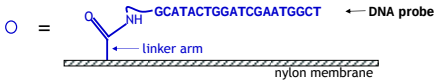
Schematic of probe attached to membrane



DNA macro-array: new membrane

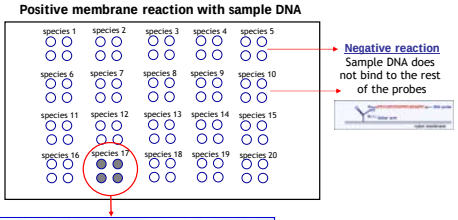
Example of freshly printed membrane





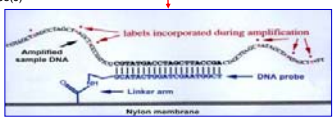
DNA macro-array: example of a positive test result

Positive membrane reaction with sample DNA



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

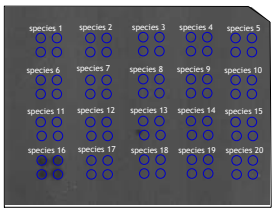
Positive reaction
Sample DNA binds to specific probe(s)



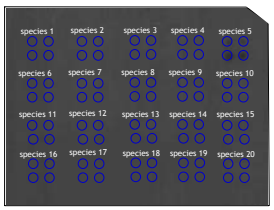
DNA macro-array: positive test results

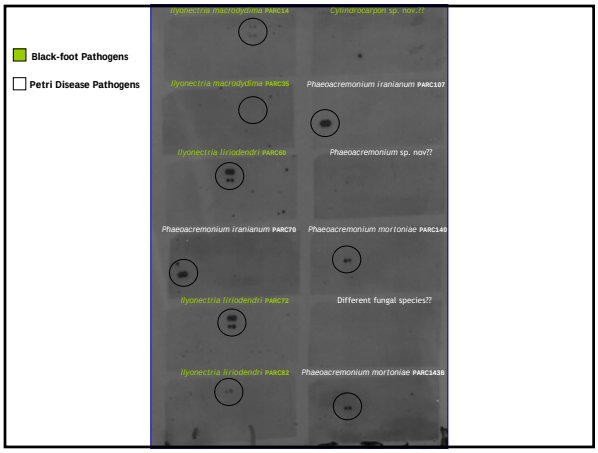
Petri Disease / Esca pathogens

Positive reaction with sample PARC70
Phaeoacremonium iranianum



Positive reaction with sample ICMP 17037
Phaeoacremonium occidentale





Development of a DNA macro-array for YVD pathogens

Petri Disease / Esca

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

● Species present in B.C.

Specific probes for the detection of **28** out of 32 *Phaeomoniella* and *Phaeoacremonium* spp.

Black-foot

1. *Ilyonectria liriodendri*●
2. *Cylidrocarpon obtusisporium*
3. *Cylindrocarpon lucidum*
4. *Cylindrocarpon olidum*
5. *Ilyonectria faginata*
6. *Cylindrocarpon theobromicola*
7. *Ilyonectria radicicola*●
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.

Specific probes for the detection of **9** out of 16 *Cylindrocarpon* spp.

Development of a DNA macro-array for canker pathogens

Next: develop species specific probes for the canker pathogens

Botryosphaeria dieback

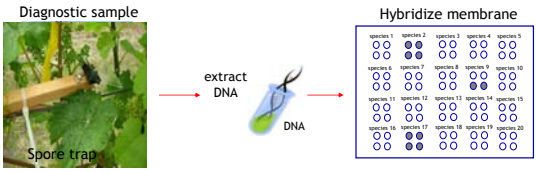
1. *Botryosphaeria dothidea*
2. *Diplodia corticola*
3. *Diplodia mutila*
4. *Diplodia seriata*
5. *Dothiorella* sp.
6. *Dothiorella americana*
7. *Lasiodiplodia crassispora*
8. *Lasiodiplodia missouriana*
9. *Lasiodiplodia theobromae*
10. *Lasiodiplodia viticola*
11. *Neofusicoccum australe*
12. *Neofusicoccum luteum*
13. *Neofusicoccum macroclavatum*
14. *Neofusicoccum mediterraneum*
15. *Neofusicoccum parvum*
16. *Neofusicoccum ribis*
17. *Neofusicoccum viticlavatum*
18. *Neofusicoccum vitifusiforme*
19. *Phaeobotryosphaeria porosa*
20. *Spencermartinsia viticola*

Eutypa dieback

1. *Eutypa lata*
2. *Eutypa leptoplaca*
3. *Eutypa laevata*
4. *Eutypella cryptovalsoidea*
5. *Eutypella microtheca*
6. *Eutypella vitis*
7. *Eutypella* sp. 1
8. *Eutypella* sp. 2
9. *Eutypella* sp. 3
10. *Eutypella* sp. 4
11. *Cryptosphaeria lygniota*
12. *Cryptosphaeria pullmanensis*
13. *Cryptovalsa ampelina*
14. *Cryptovalsa rabenhorstii*
15. *Diatrype brunneospora*
16. *Diatrype oregonensis*
17. *Diatrype stigma*
18. *Diatrype whitmanensis*
19. Diatrypaceae sp.
20. *Diatrypella verrucaeformis*
21. *Diatrypella vulgaris*

DNA macro-array

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



SUB ACTIVITY 3.3- EVALUATION OF MANAGEMENT STRATEGIES
(Black-foot and Bot-canker)

1) Assessment of rootstock and biologicals to manage black-foot disease

- rootstock trial with Chardonnay vines:
  rootstocks: 3309, 101-14, S04, and self-rooted
  biological treatments: mycorrhizae x mycorrhizae/*Trichoderma* and control

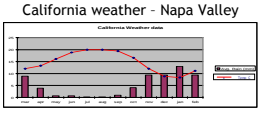
  (treatment variables = rootstock x mycorrhizae x mycorrhizae/Trichoderma)

2) Biology of *Botryosphaeria* species causing bot-canker

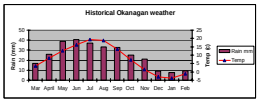
- design of Q-PCR test for the detection of *Botryosphaeria spores*
- monitoring of *Botryosphaeria* spore release using spore traps
- combining spore trap results with weather data to identify potential infection periods

Culture control recommendations for bot-canker and the weather

California weather - Napa Valley



Okanagan Valley weather

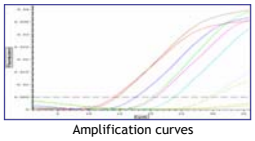


Biology of *Botryosphaeria* spp. causing bot-canker

Spore trap samples collected weekly to determine:

- date of spore release in Okanagan vineyards
- weather conditions required for spore release and potential infection period

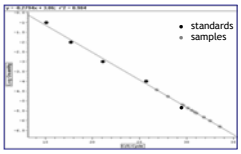
Q-PCR: Detection and quantification of *Botryosphaeria* from spore traps



Amplification curves

Detection

DNA amplification curves provide detection of *Botryosphaeria* spores from vineyard spore trap samples.



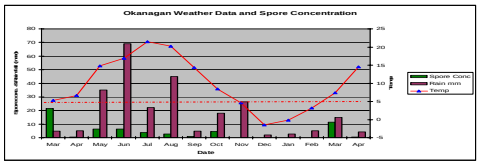
Standard curve

Quantification

standard curve calculates the number of spores from individual spore trap samples.

Spore trap data

Detection and quantification of *Botryosphaeria* spores



Goal:

- Identify potential recommendations for local conditions
  (e.g. pruning recommendation; and dormant spray timing (lime sulphur)

Summary

Disease survey

- nearing completion with some follow-up in 2012 to look at potential disease spread and sample collection for DNA array testing

DNA macro-array for GTD disease pathogens

- validation of the esca/YVD membrane and development of the bot-canker/eutypa membrane is continuing

Evaluation of management strategies

- spore trapping will continue, to identify spore release and infection periods (bot-canker)
- Root zone sampling will continue to identify any potential colonization and protection of root stocks by biologicals from pathogens (black-foot)

Agriculture and Agri-Food Canada / Agriculture et Agroalimentaire Canada

Questions

BC Wine Grape Council

Agriculture and Agri-Food Canada
Developing Innovative Agri-Products Initiative (DIAP)

NSERC CRSNG **Visitor Fellowship Program**

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