Crown Gall on Grapevines: Management strategies based on current understanding of pathogen biology

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Specificity of \textit{Agrobacterium vitis} on grape
Systemic colonization in grapevines
Infections are initiated at wound sites; freeze injuries, disbudding points, graft unions, etc.

Crown Gall Disease on Grape

Initiated at grafts in nursery and vineyard
At base and disbudded sites of rootstocks
Crown gall following top grafting

Cross Section of Crown Gall

Agrobacterium
**A. vitis Causes Necrosis on Grape**

Grape necrosis
- Affects root development
- Facilitates survival of *A. vitis* in soil
- Inhibits graft take

**Grape Crown Gall Disease Cycle**

In spring, bacteria can be detected in the apex of budding roots. Bacteria are disseminated in apparently healthy cuttings. Freeze injuries are common sites for infection. Bacteria survive systemically; they infect xylem vessels throughout the plant. Path of disease occurs, wounded shoot dies. Bacteria can cause lesions on roots and pedicel in wire baskets in the soil when the vine is removed. Galls develop at wounds of graft unions.
Improved Detection of *A. vitis*

- Magnetic Capture Hybridization (MCH) allows precise detection of specific gall-forming types of *A. vitis*.
- A capture probe was designed to selectively trap the target DNA sequence (*virD2 gene*) that is required for *A. vitis* to cause crown gall. Final identification by Real-time PCR.

Sample Preparation for Detection of *A. vitis* in Grapevines

- Cut grapevine nodes (4-6) in 4 slices. May sample any tissues or environmental samples.
- Vacuum extract bacteria to help remove bacteria from xylem of woody tissues.
Sample preparation for *A. vitis* detection using MCH

1. Incubate in Nutrient Broth with cycloheximide for 2-3 days, collect bacteria by centrifugation
2. DNA extracted from total bacteria by mechanical lysis
3. Incubate total DNA extract with bead/capture probe complex
4. Real-time PCR

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Magnetic Capture Hybridization

**Steps in MCH**

1. Binding of capture probe to streptavidin coated beads

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Streptavidin coated magnetic beads + A. vitis (virD2) capture probe -> 1h @ 37°C -> Bead/capture probe complex
Magnetic Capture Hybridization

1. Magnetic capture probe complex
2. Hybridize DNA to capture probe @ 37°C for 2h
3. Rinse beads to eliminate PCR inhibitors and non-target DNA
4. Use magnetic beads with captured DNA in real-time PCR

Summary of MCH assay

- MCH is more sensitive than previous methods for A. *vitis*. (at least 1000 times)
  - can detect about 10 cells of pathogen per sample
- Is being used in the NCPN program to index accessions from various sources
- To verify effectiveness of shoot tip and meristem culture for elimination of pathogen
- To study A. *vitis* in environment
Summary of MCH assay

- The real-time PCR primers are specific for wide range of *A. vitis* strains (*virD2*).
- 3-4 days to complete assay compared to weeks for previous methods
- Any questionable samples are further tested by traditional PCR. If still questionable counted as negative.

Considerations When Indexing Grapevines for *A. vitis*

- Proper vine sampling procedure
  - Relative distribution of *A. vitis* in vines
- Genetic diversity of *A. vitis* strains
- Specificity and sensitivity of assay
  - Cost and time required for assay
Distribution of *A. vitis* in Canes

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* Collected from vines with crown gall

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Can We Produce and Maintain *A. vitis* –free Grapevines for Commercial Production?

- Shoot tip and meristem culture as means to eliminate pathogens from plant material.
- How effective?
- Environmental sources of pathogen that may contaminate the clean plants?
Can Shoot Tips and Meristems Carry *A. vitis*?

- 2013 – Two replications of experiment to determine if *A. vitis* could be detected from shoot tips from cuttings taken in infected vineyard. Other crown gall work was being done in the greenhouse.
  - Replication one, 18 of 29 positive
  - Replication two, same vines that were cut back and shoots regrown, 4 of 29 positive

Can Shoot Tips and Meristems Carry *A. vitis*?

- 2014 – Again, shoots from cuttings taken from infected vineyard.
  - 49 samples of meristems and shoot tips, all tested negative for *A. vitis*.

Currently in research funded by NCPN, vines being propagated from tips and meristems cuttings taken from infected vineyard. Will be assayed to confirm that clean vines can be generated.

Can they be kept clean?
“New” Sources of *A. vitis* in Environment

- **Shoot tips in vineyards**
  - 2013, 11 of 30 tips from vineyard with crown gall were positive
  - 2014, 16 of 240 tips from two vineyards with crown gall were positive

- **Leaves in vineyard with crown gall**
  - Two leaves from 10 vines

*Suggestive that *A. vitis* survives on surfaces of grape shoots and leaves*

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“New” Sources of *A. vitis* in Environment

- **Wild grapes, NY – V. riparia**
  - 2013 – 18 of 54 positive for *A. vitis*
  - 2014 – 27 of 100 positive for *A. vitis*

- **Wild grapes, CA – species?**
  - 2014 – 7 of 34 positive

- **Others to investigate**
  - Dormant buds
  - Water
Where Does *A. vitis* Live in the Environment?

- **In Grapevines**
  - Cultivated and wild grapevines
  - Trunks, canes, roots
  - In dormant buds
- **On Grapevines**
  - On surfaces of shoots and leaves
- **Others to investigate**
  - Water, soil, other plants

Current Management Practices

- **Cultivar choice.** Plant varieties and rootstocks that are tolerant of the disease.
- **Site Selection.** Plant vineyards on sites that have good air drainage and well drained soils to minimize freeze injury.
- **Hilling up.** Mounding soil over the graft union in the fall protects it from extreme cold events, and ensures survival of scion buds for trunk renewal.
Current Management Practices

- **Multiple trunks.** Establishing multiple trunks allows growers to remove and replace galled trunks while maintaining production.

- **Regular monitoring and replacement or renewal.** Evaluate trunk and vine health on a regular basis, mark and replace trunks and vines promptly.

- **Cropping levels and fertility.** Manage cropping levels, irrigation and nutrition such that active vegetative growth ceases or slows by veraison.

Crown Gall Management

- **Hot water treatments**
  - 50 to 53 C for 30 min
    - Reduces >90% of pathogen in cuttings

- **Treating galls with antibacterial compounds**
  - *A. vitis* persists internally in vines
Relative Susceptibility of Grape Rootstocks to Crown Gall

**Highly resistant:** Paulsen 775, R. gloire

**Resistant:** 3309 C, 101-14 Mgt, Freedom, Harmony, Kober 5BB

**Moderately susceptible:** Teleki 5C, SO4,

**Susceptible:** Paulsen 1103

**Highly susceptible:** 110R, Ramsey, K5140

*Even highly resistant rootstocks may carry A. vitis internally*

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Biological Control of Crown Gall

*A. vitis strain F2/5 is non-tumorigenic and inhibits gall formation on grapes when inoculated on wounds at same time or prior to pathogen*
F2/5 Inhibits Infection But Not Pathogen Growth at Grape Wound Sites

Populations of CG49 and F2/5 on grape wound sites

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*F2/5 gall inhibition is specific to grape

A. vitis (Including F2/5) Causes Necrosis on Grape

Grape necrosis
- Affects root development
- Facilitates survival of A. vitis in soil
- Inhibits graft take
PPTase (F-avi5813) Required for Necrosis But Not for Gall Inhibition

Effect of F2/5 and ΔF-avi5813 on Root Development and Grafts
ΔF-avi5813 is Necrosis-Negative and Retains Biological Control

CG49 inoculated

F2/5 treated (necrosis)

ΔF-avi5813 treated cutting

CG49 + ΔF-avi5813

Effect of A. vitis on Graft Take and Shoot Growth

Tumorigenic strain CG49

CG 49 plus ΔF-avi5813
Future Directions

- Verify that *A. vitis* free vines can be produced via tissue culture.
- Further identify environmental sources of *A. vitis*.
  - Epiphytic nature in vineyards
  - Survival and spread in wild grapes, soil, water
- Further clarify disease triggers
- Develop commercially viable biological control for grape crown gall.

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