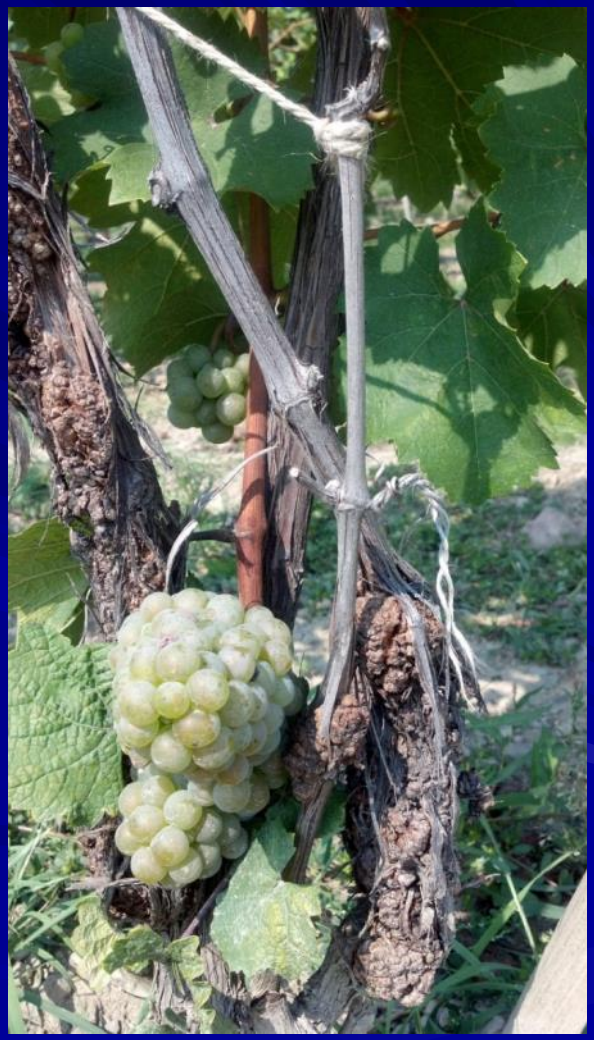
Crown Gall on Grapevines: Management strategies based on current understanding of pathogen biology

Tom Burr
Cornell University, NYSAES
Geneva, NY



Specificity of *Agrobacterium vitis* on grape

Systemic colonization in grapevines

Infections are initiated at wound sites; freeze injuries, disbudding points, graft unions, etc.

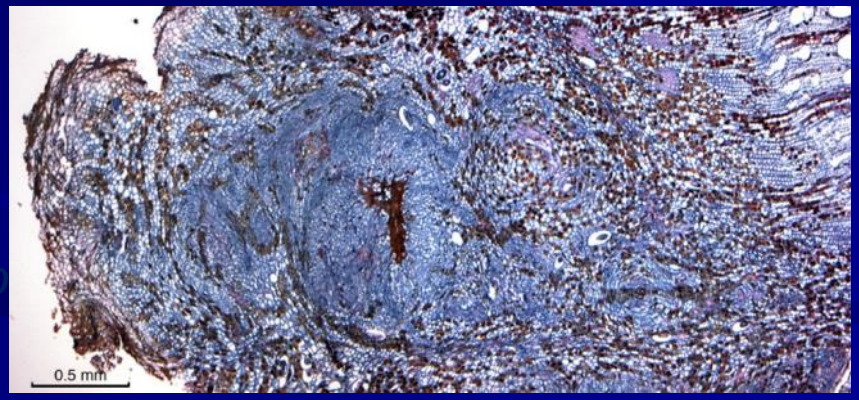
Crown Gall Disease on Grape

Initiated at grafts in nursery and vineyard

At base and disbudded sites of rootstocks

Crown gall following top grafting

Initiation of Crown Gall at Wounds



No *Agrobacterium*

Vine vigor and yield are significantly reduced when 50% or greater of trunk circumference is covered with gall.

Schroth et al. 1988. Plant Disease

A. vitis Causes Necrosis on Grape

Grape necrosis

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

Effects of *A. vitis* on Graft Unions of Grapevines

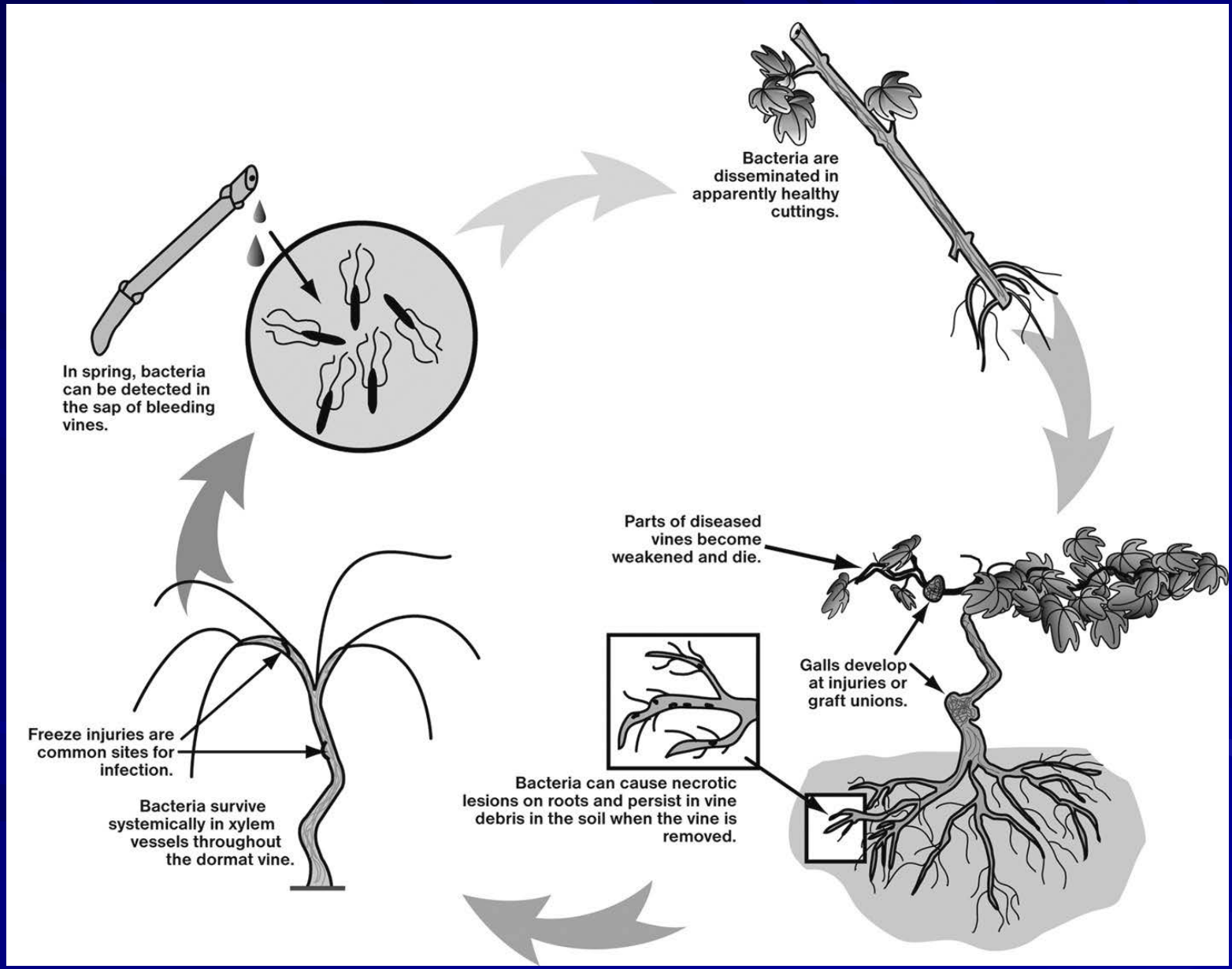
Disease in grape nursery

water

tumorigenic strain

Non-tumorigenic strain

Grape Crown Gall Disease Cycle



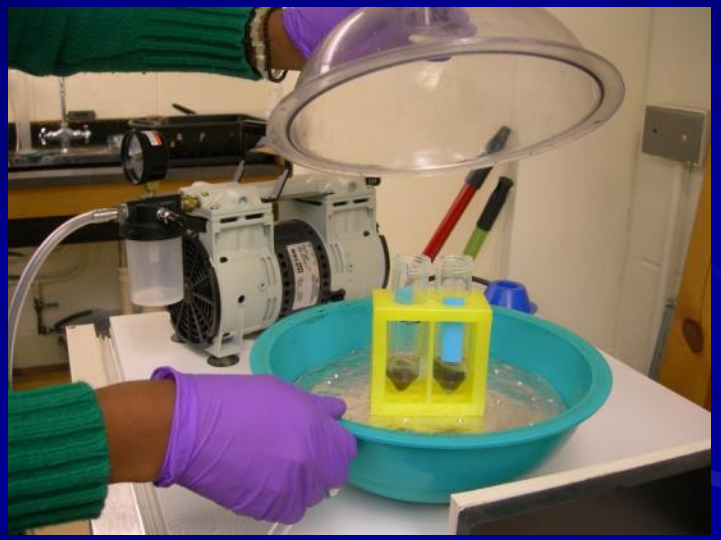
Improved Detection of *A. vitis*

- 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

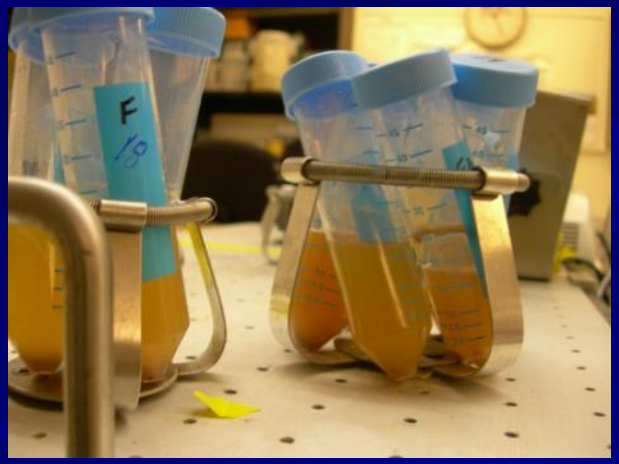
Portion of dormant cutting cut into small pieces. May sample any tissues or environmental samples.

Vacuum extract bacteria to help remove bacteria from xylem of woody tissues.



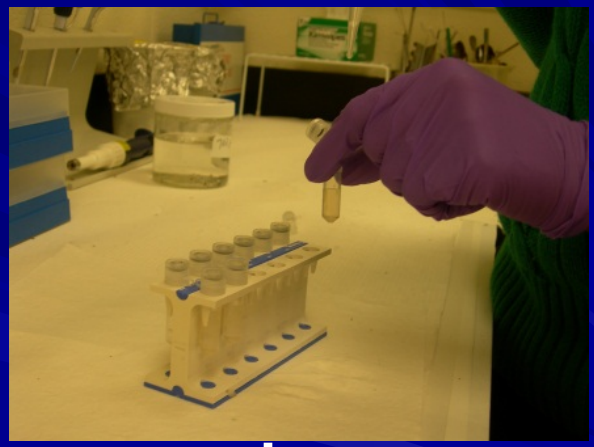
Sample preparation for *A. vitis* detection using MCH

Incubate in Nutrient Broth with cycloheximide for 2-3 days, collect bacteria by centrifugation



DNA extracted from total bacteria by mechanical lysis

Incubate total DNA extract with bead/capture probe complex

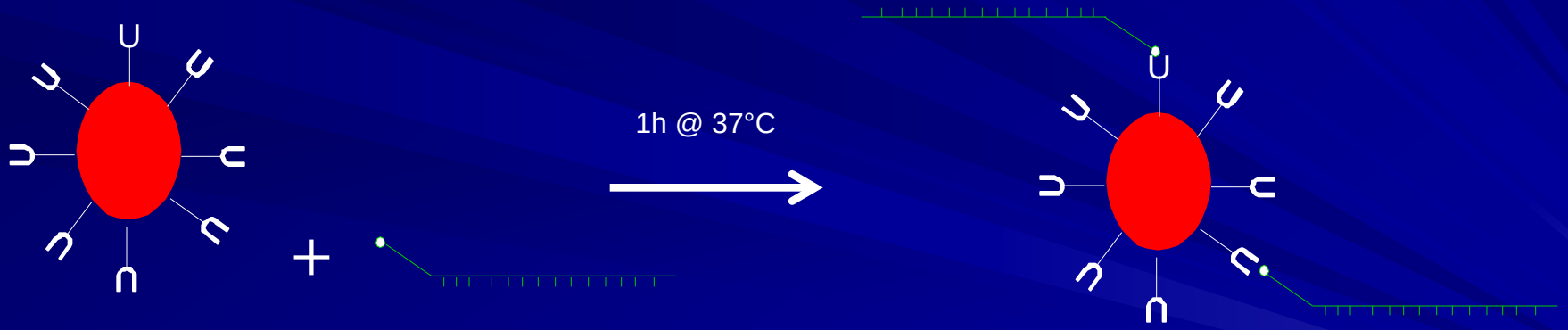


Real-time PCR

Magnetic Capture Hybridization

- Steps in MCH

1. Binding of capture probe to streptavidin coated beads

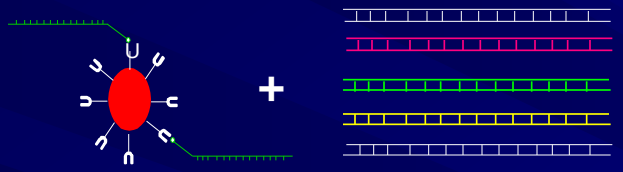


Streptavidin coated magnetic beads

A. *vitis* (*virD2*) capture probe

Bead/ capture probe complex

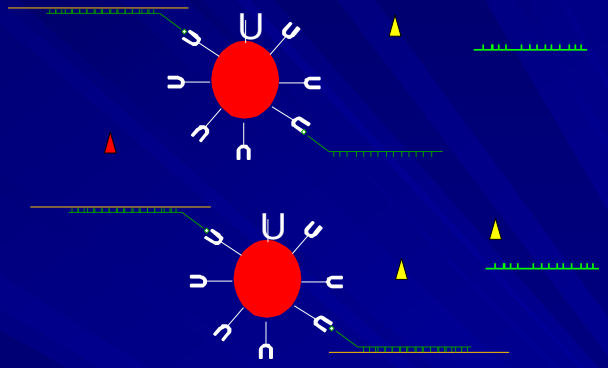
Magnetic Capture Hybridization



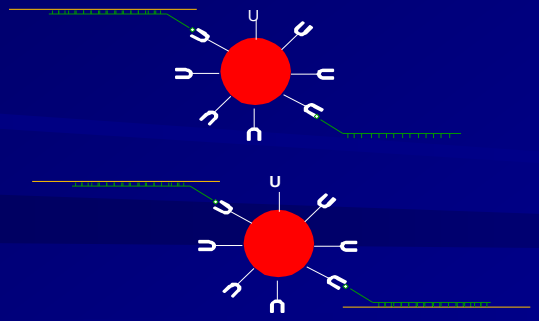
Bead/ capture probe complex

Crude DNA prep.

2. Hybridize DNA to capture probe @ 37°C for 2h

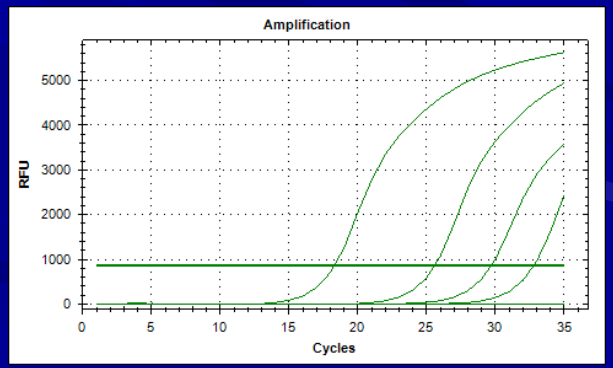


3. Rinse beads to eliminate PCR Inhibitors and non-target DNA



Beads with target DNA attached

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 procedures for clean plant production
- 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.
- Non-tumorigenic *A. vitis* are not detected.

Considerations When Indexing Grapevines for *A. vitis*

- Genetic diversity of *A. vitis* strains
- Specificity and sensitivity of assay
  - Cost and time required for assay
- Proper vine sampling procedure
  - Relative distribution of *A. vitis* in vines (canes)
  - Hypothesis: *pathogen at highest level near cane base and at nodes*

Distribution of *A. vitis* in Canes

Vine number	Grapevine cane segment																	
	1N	1I	2N	2I	3N	3I	4N	4I	5N	5I	6N	6I	7N	7I	8N	8I	9N	9I
1A	-	-	-	-	-	-	-	-	-	-	-	+	+	-				
1B	-	-	-	-	+	+	-	-	-	-	+	-						
2A	-	-	+	-	+	+	-	-	-	+	-	-						
2B	-	-	-	-	-	+	-	-	-	-	-	-						
3A	+	+	+	+	-	+	-	-	-	-	-	-						
3B	-	-	-	-	-	+	-	-	+	-	-	-						
4A	-	-	+	-	-	-	-	+	-	-	-	-						
4B	+	+	+	-	+	+	-	+	-	+	+	+	+	+				
5A	+	+	-	-	+	-	-	-	+	-	-	+	+	-	+	-		
5B	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
6A	-	+	+	+	-	-	-	-	-	+	-	+						
6B	-	+	-	+	+	-	-	+	-	-								
7A	-	+	+	+	-	-	-	+	+	-	+	-	+	-				
7B	-	-	-	-	+	-	-	-	-	+								
8A	-	+	+	-	-	-												
8B	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
9A	+	-	-	-	-	-	-	-	+	-								
9B	+	-	+	+	-	+	+	+	+	-								
10A	-	+	-	-	-	+	+	+										
10B	+	-	-	-	-	+	-	-										

* Collected from vines with crown gall

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*.
- 2014-15
  - Vines were propagated from tips and meristems.
  - Thus far all vines in tissue culture have been free of pathogen.
- 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
  - Preliminary results show presence of pathogen on grape leaves

**Does A. vitis survive on surfaces of grape shoots and leaves?*

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

- Wild grapes, NY – *V. riparia*
  - 2013 – 18 of 54 positive for *A. vitis*
  - 2014 – 12 of 41 positive for *A. vitis*
- Wild grapes, CA
  - 2014-15 – 25 of 87 positive for *A. vitis*

Where Does *A. vitis* Live in the Environment?

- In Grapevines
  - Cultivated and wild grapevines
  - Trunks, canes, roots
- On Grapevines
  - On surfaces of shoot tips and leaves
- Others to investigate
  - Water, soil, other plants

Current Management Practices

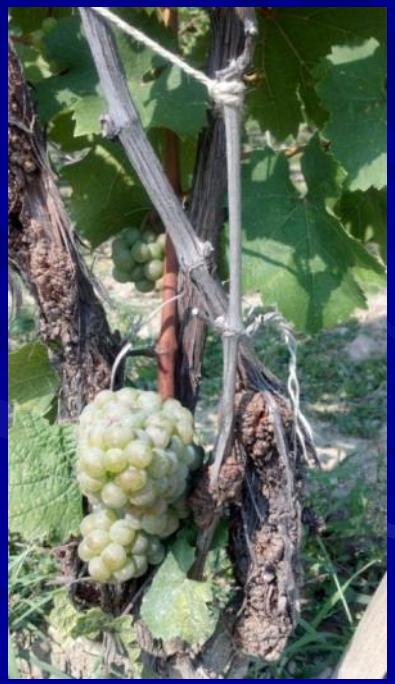
- **Cultivar choice.** Plant varieties and rootstocks that are tolerant of the disease.
- **Site Selection.** Plant vineyards on sites that have good air drainage and well drained soils to minimize freeze injury.
- **Hilling up.** Mounding soil over the graft union in the fall protects it from extreme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

Current Management Practices

- **Multiple trunks.** Establishing multiple trunks allows for removal and replacement of 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.
- **Cropping levels and fertility.** Manage cropping levels, irrigation and nutrition such that active vegetative growth slows by veraison.
- Water management. Prevention of vigorous growth late in season

Crown Gall Management

- Hot water treatments
  - 50 to 53 C for 30 min
    - Reduces >90% of pathogen in cuttings
- Treating galls with antibacterial compounds (Gallex, Cu, etc.)
  - *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 (V. riparia – wild grapes)*

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**

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

Grape necrosis

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

A Mutant of F2/5 Was Made, Δ*avi5813*, that is Necrosis-Minus and Biological Control-Positive

CG49 inoculated

F2/5 treated (necrosis)

Δ*F-avi5813* treated cutting

CG49 + Δ*F-avi5813*

Effect of F2/5 and Δ*F-avi5813* Mutant on Grape Roots

Δ*F-avi5813*

F2/5

Effect of *A. vitis* on Graft Take and Shoot Growth

Tumorigenic strain CG49

CG 49 plus ***ΔF-avi5813***

Conclusions

- *A. vitis* can be randomly distributed in dormant grape canes.
- Vines free of the pathogen can be developed through tissue culture*.
  - Preventing crown gall in the early years of a vineyard will reduce economic impact
- Wild grapevines are a significant source of the pathogen in nature.

Conclusions

- Site selection and vine growth management are key considerations in disease management.
- Environmental sources of *A. vitis* will contribute to infection of vineyards.
- Biological control will be effective for reducing infections particularly at grafts and base of rooted cuttings.

Acknowledgements

- USDA-APHIS NCPN
- USDA Federal Capacity Funds
- SCBG – USDA – New York State
- New York Wine and Grape Foundation
- Collaborators
  - Desen Zheng
  - Kameka Johnson
  - Cherie Reid
  - Tim Martinson
  - Marc Fuchs