

# CDFA PD/GWSS Research Program

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## Summary of PD/GWSS Research Scientific Review:

*Where are we with research efforts?  
How do we accelerate progress?*



November 2007

# Introduction

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- Seven year collaboration
  - Industry, Scientific Community and Government Agencies
- Expectation
  - Funding and scientific efforts will provide tools and technologies against Pierce's disease
- Fairly unique relationship
  - Significant long-term funding contributions
  - Intensive & focused research efforts against a specific need

# Assessing Research Progress

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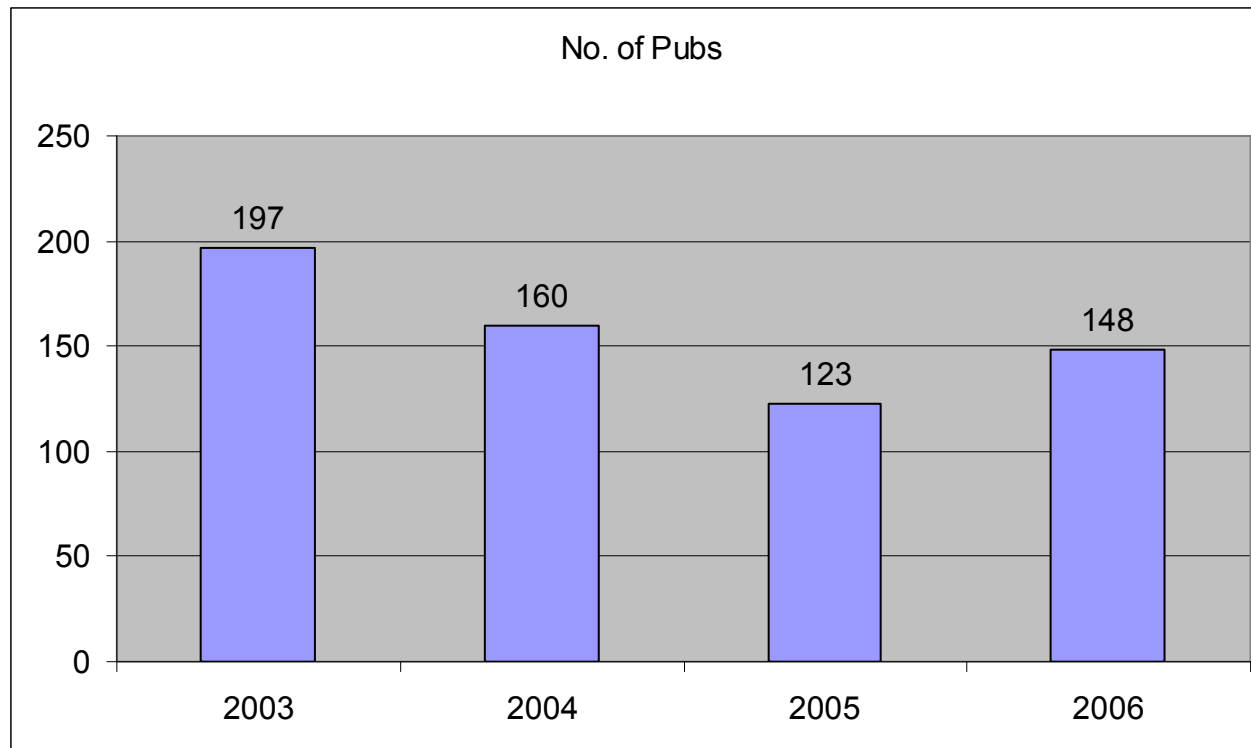


- Early scientific efforts (up to 2004) reviewed by National Academy of Sciences (NAS)
- NAS report published in 2004
  - Provided historical background on the disease and its insect vectors
  - Assessed the research completed at the time of the review
  - Set forth a broad list of PD research priorities to be targeted for subsequent research and funding activities

# Research Activities Continue



*Considerable research effort has occurred toward finding solutions to PD since the 2004 NAS report—  
Where are we now?*



# Research Progress Update

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- In 2006, the CDFA PD/GWSS Board requested a thorough independent scientific review, assessment and interpretation of all recent PD/GWSS research efforts and potential outcomes
- Scientific efforts reviewed within the context of:
  - The remaining three-year funding time limit (grape levy)
  - Recommendations put forward by the 2004 NAS report

# Research Scientific Advisory Panel (RSAP)

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- RSAP appointed by the Secretary of California Department of Food and Agriculture
  - 6 independent scientists
  - Relevant expertise and experience
- The research review and assessment was completed in May 2007 and the report published in August of 2007
  - [http://www.cdfa.ca.gov/pdcp/Documents/RSAP\\_Final\\_Report\\_08302007.pdf](http://www.cdfa.ca.gov/pdcp/Documents/RSAP_Final_Report_08302007.pdf)

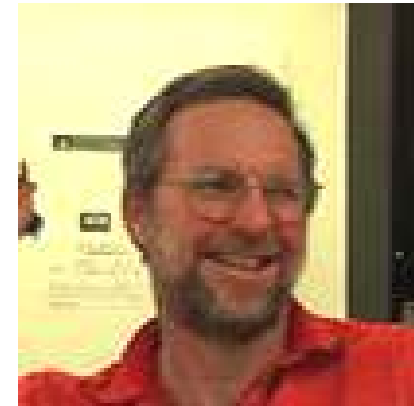
# RSAP Members



**Adam Bogdanove**



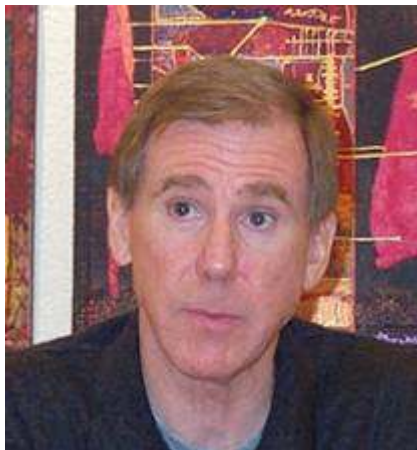
**Mike Davis**



**Greg Loeb**



**Roger Innes**



**Dan Sumner**



**George Sundin**

# Goals of the RSAP

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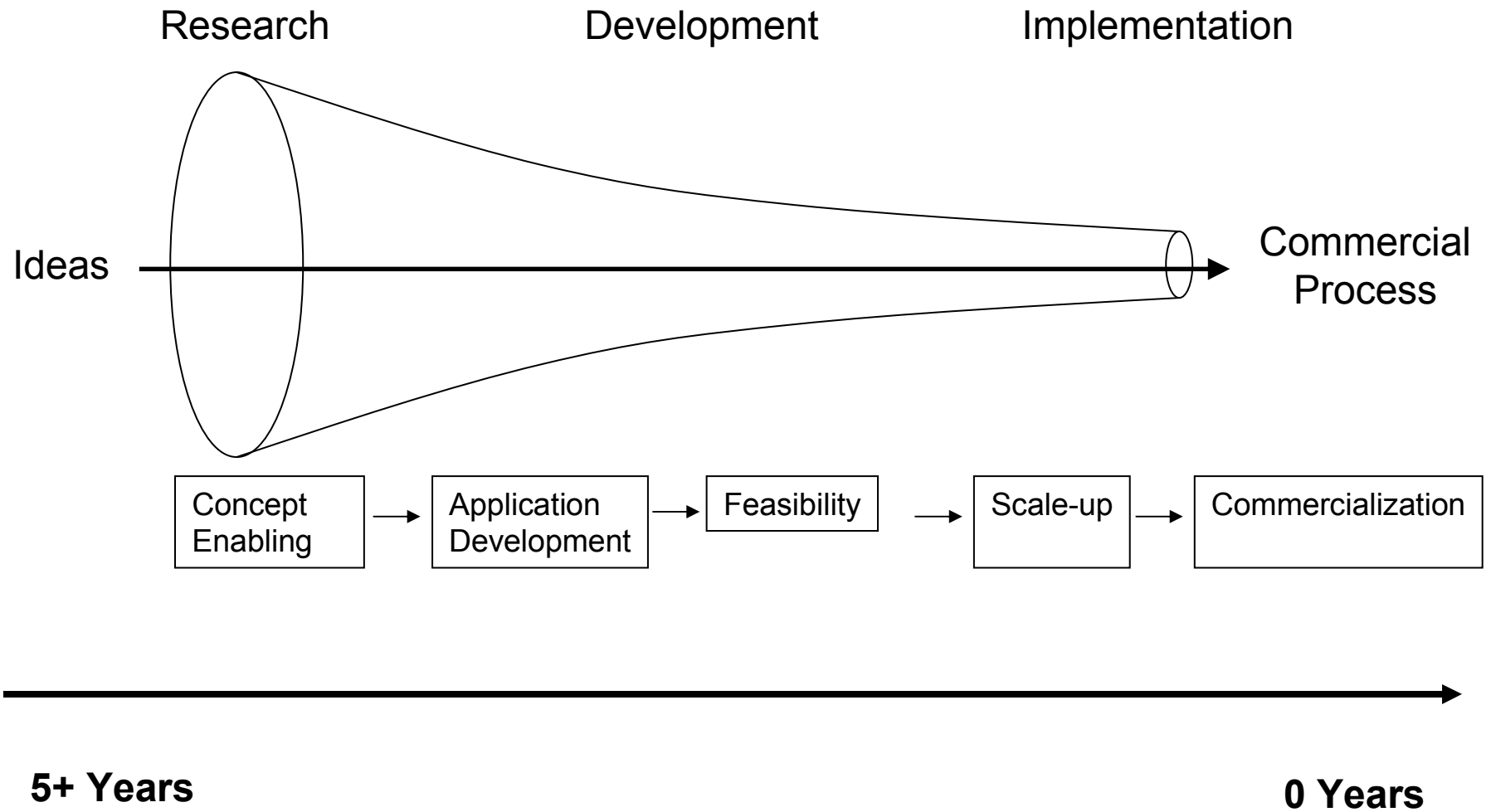
Evaluate progress made by PD/GWSS research program made 2003-present:

- Summarize areas of significant progress
- Identify research tracks that hold the most promise
- Examine potential barriers and determine gaps
- Make recommendations for next steps

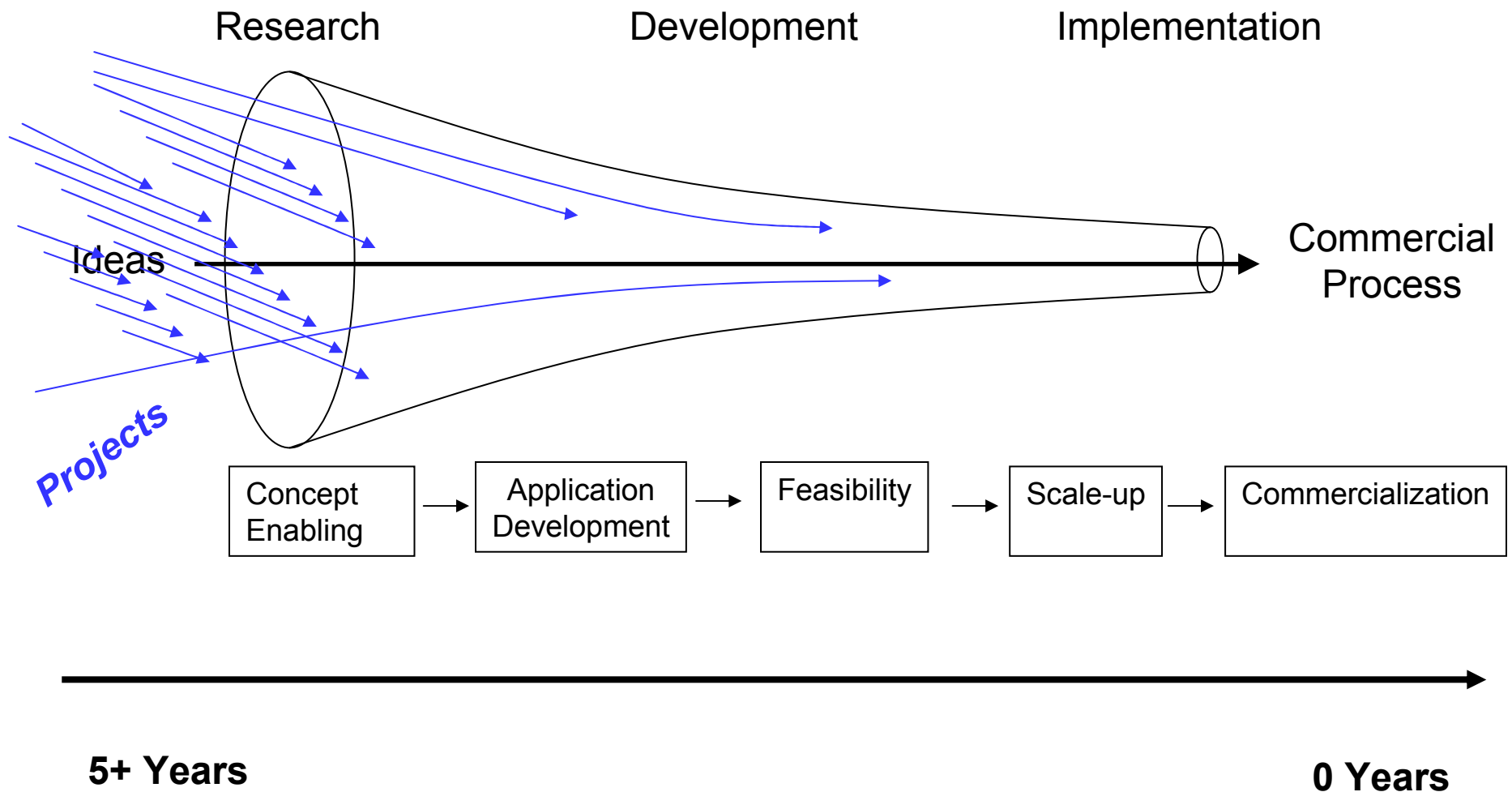


# Panel Research Review—Results

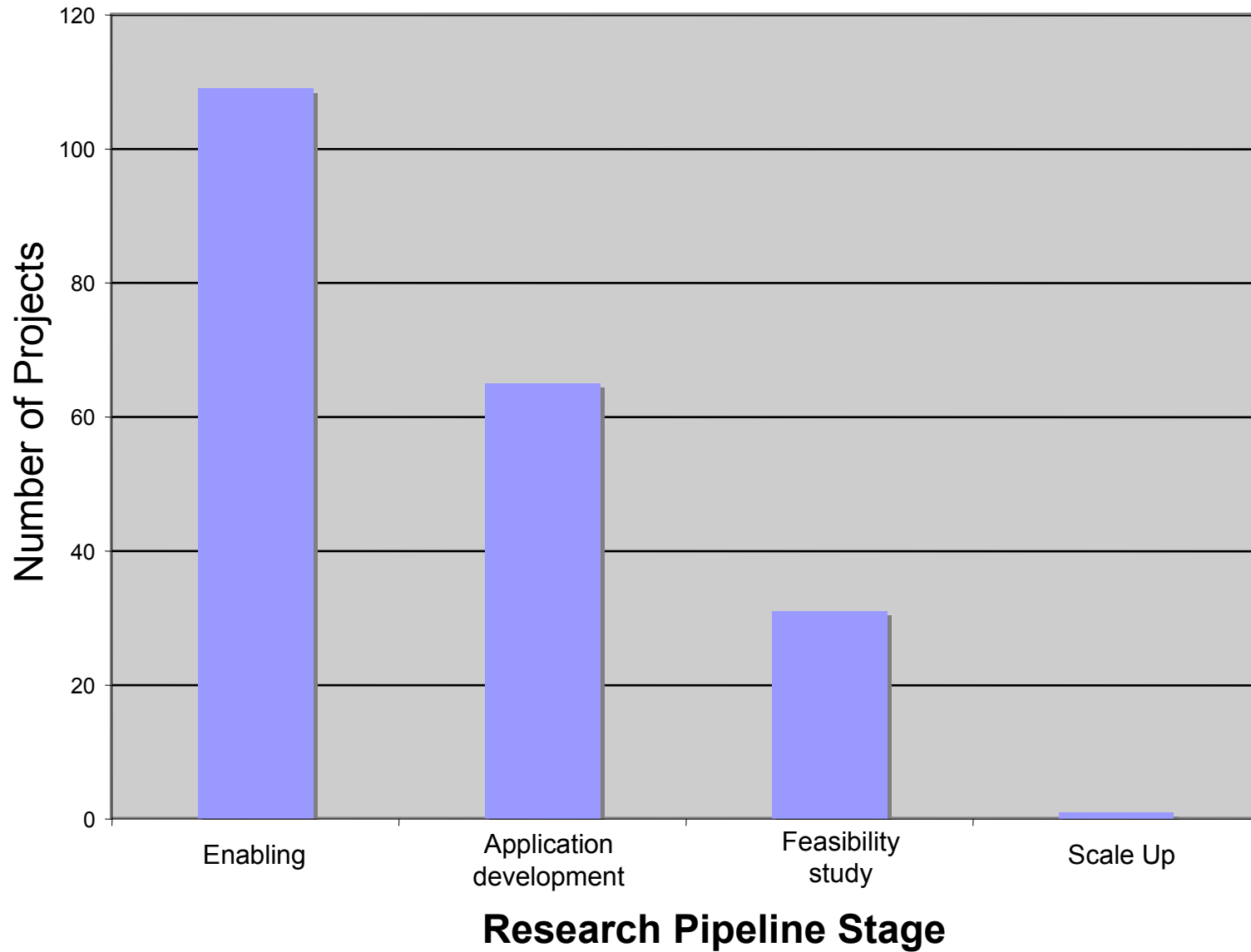
# Research Delivery Pipeline



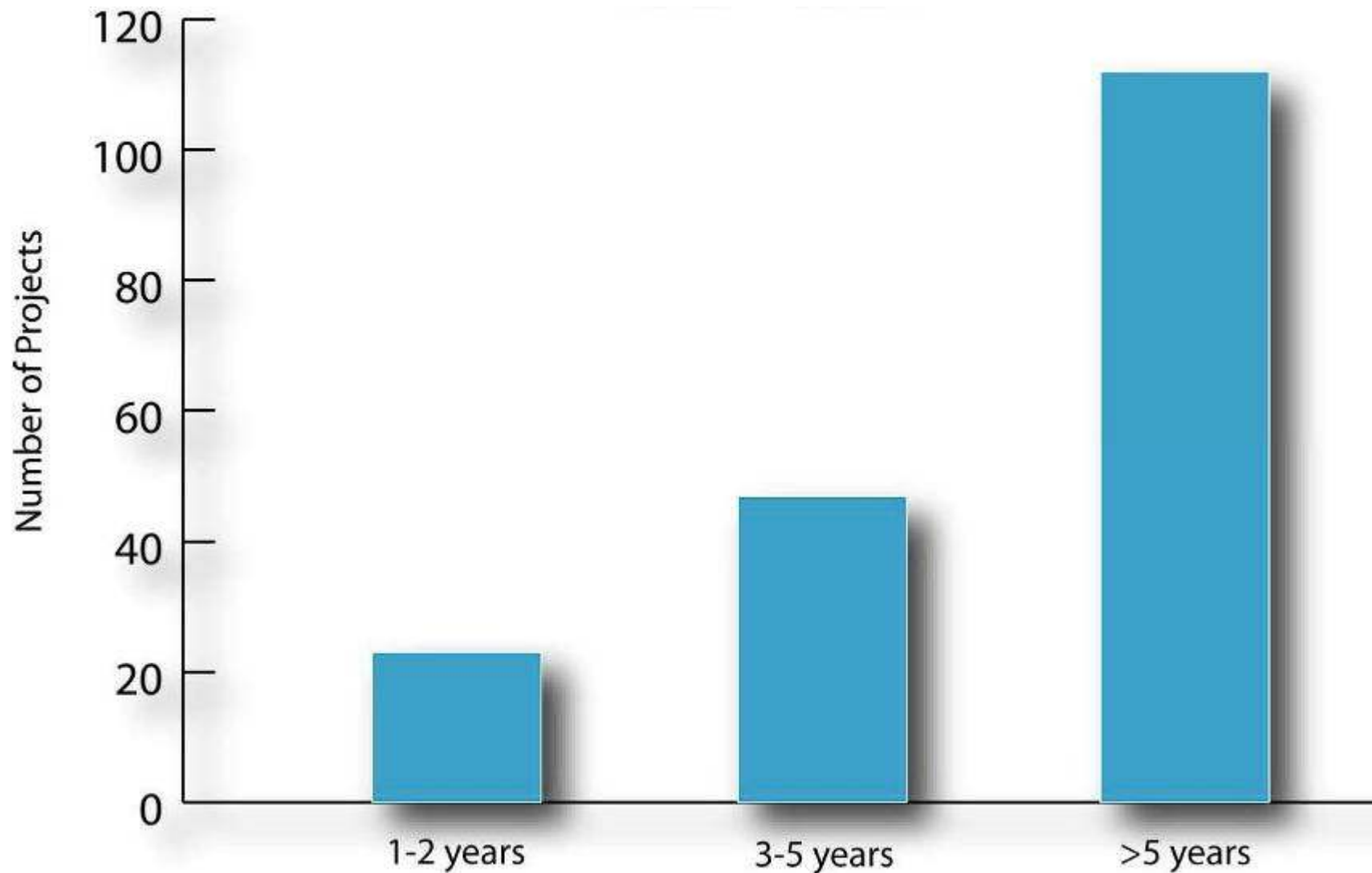
# Where is the PD/GWSS Program within this Process?



# Research Pipeline



# Time to application



# Areas of Significant Progress

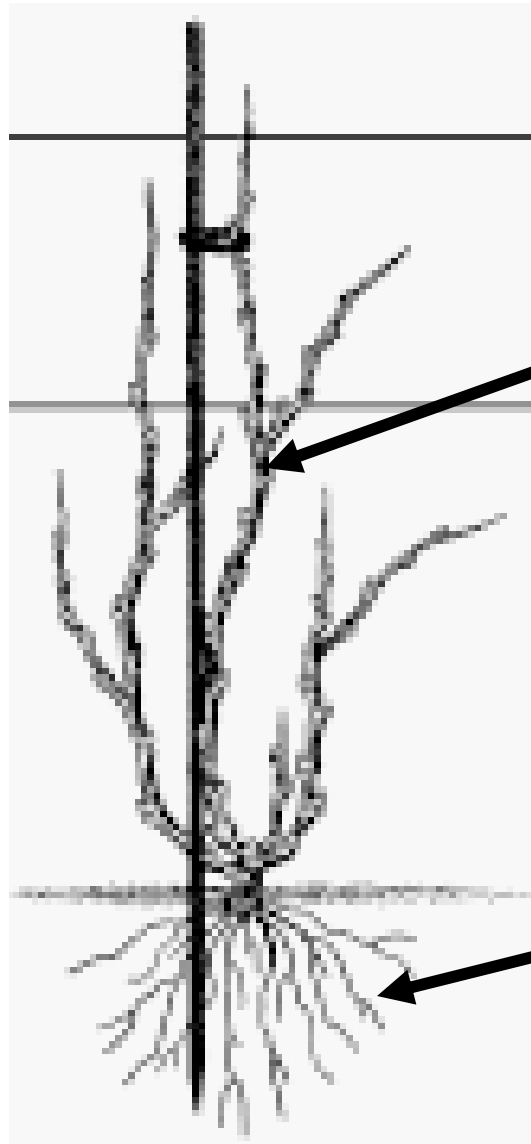


- Potential for using modified root stocks in combination with traditional scion varieties
- Identification of PD bacterial genes that control disease infection, spread, and severity in grape
- Identification of PD host-specificity determinants
- Insecticidal-based control of GWSS (area-wide control programs)
- Understanding the habits and movements of GWSS
- Managing GWSS with insect parasitoids
- Grape breeding for resistance/mapping PD resistance genes in grapes

# Proof-of Concept Experiment



**Commercial  
Variety**



**Gene product  
translocated to  
scion**



**Gene added  
to rootstock**

**Genetically  
Modified  
Rootstock**

# Advantages

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- Develop a rootstock that confers PD resistance to commercial scion varieties
- Avoid time constraints of long-term breeding programs
- Alternative to using transgenic scion varieties
  - Avoid regulatory hurdles
  - Consumer acceptance issues
  - Maintain varietal integrity

# Areas of Significant Progress



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# Significant Progress—Disease: Cause & Effect

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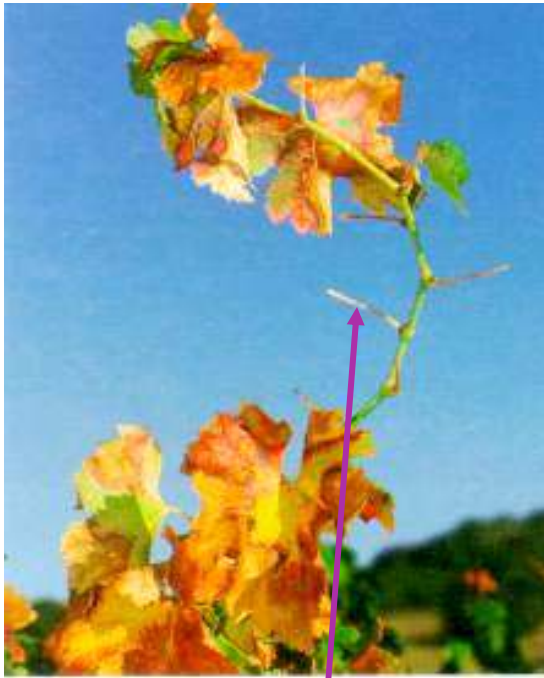
- Significantly better understanding of how PD infects and causes disease
- Id'ed and characterized various PD-produced factors that mediate disease process
- Understanding the fundamental mechanism provides opportunities a variety of systems/strategies to disrupt the disease cycle



# PD Basics

***Some background slides***

**Pierce's Disease (PD)** is caused by the bacterium *Xylella fastidiosa* (Xf).



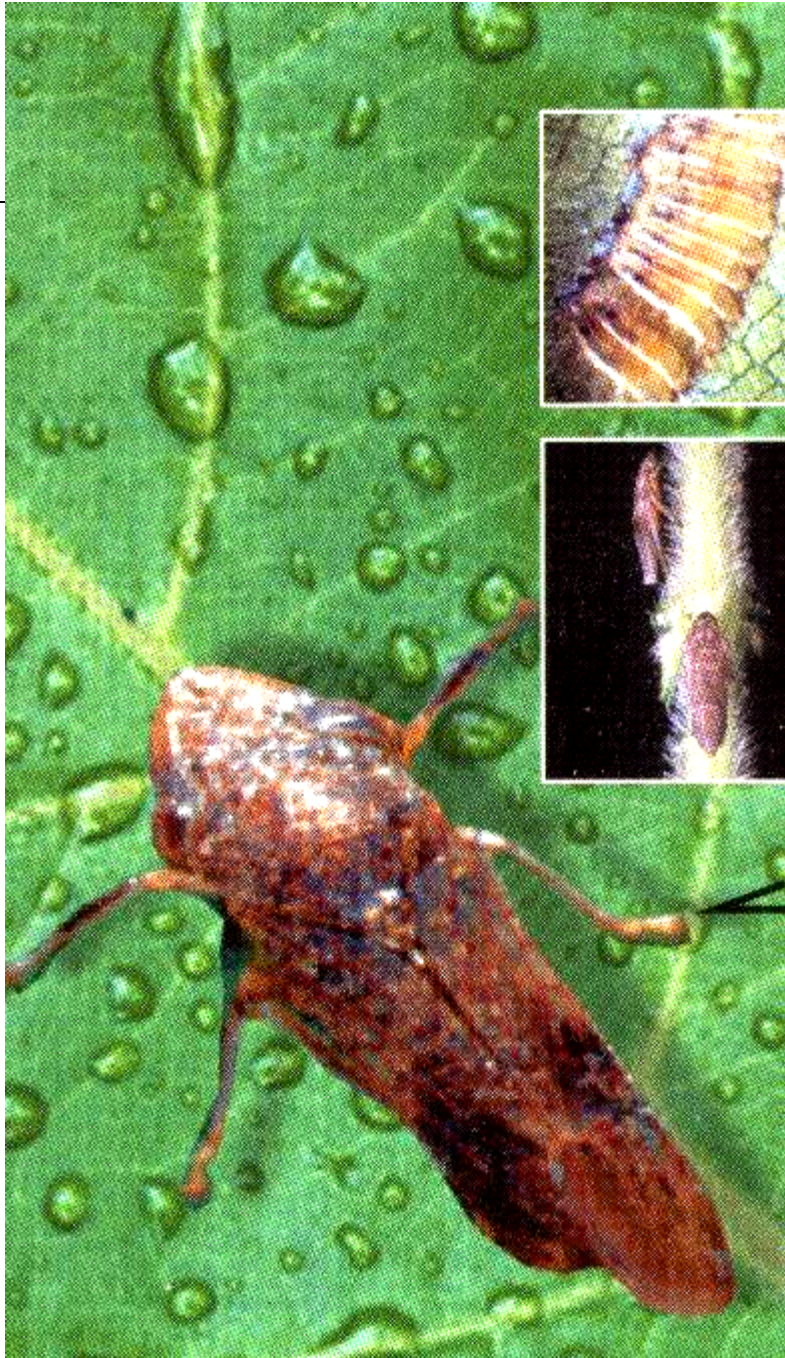
Petiole “matchsticks”



Berries become raisins



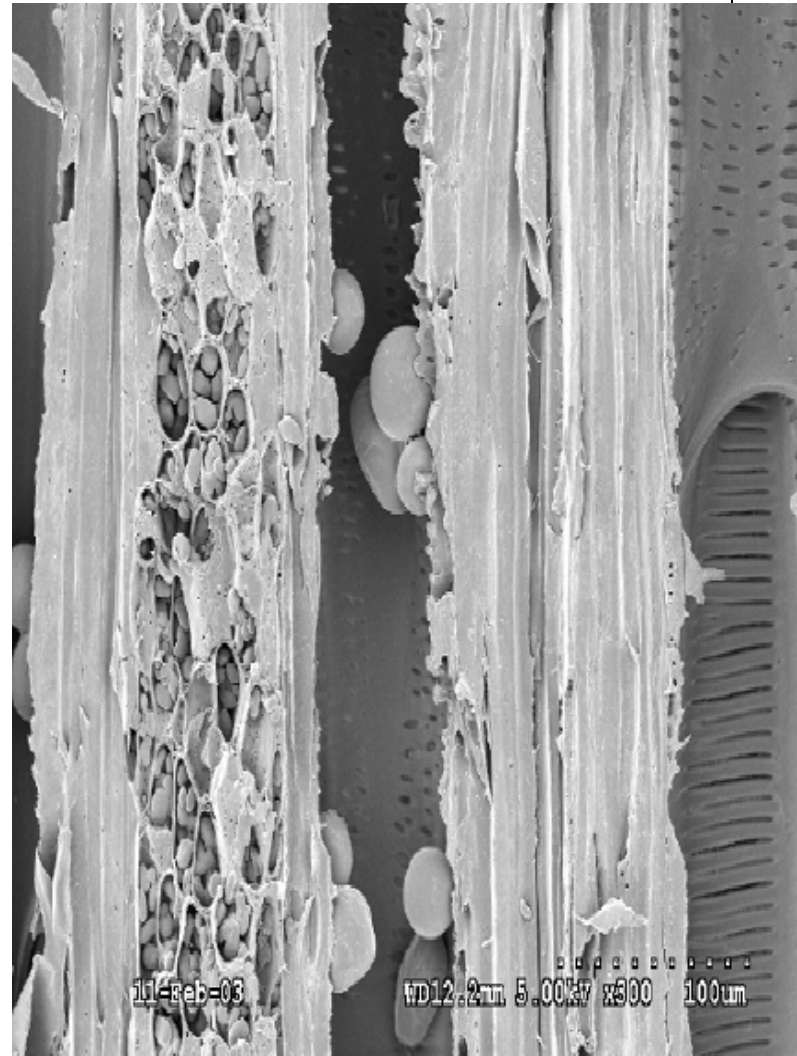
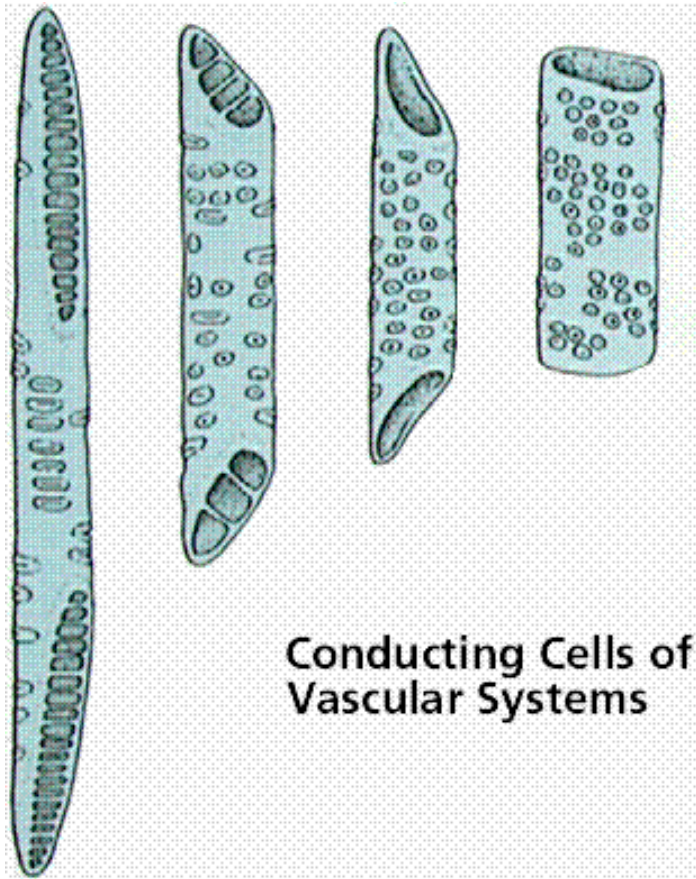
Leaves are scorched



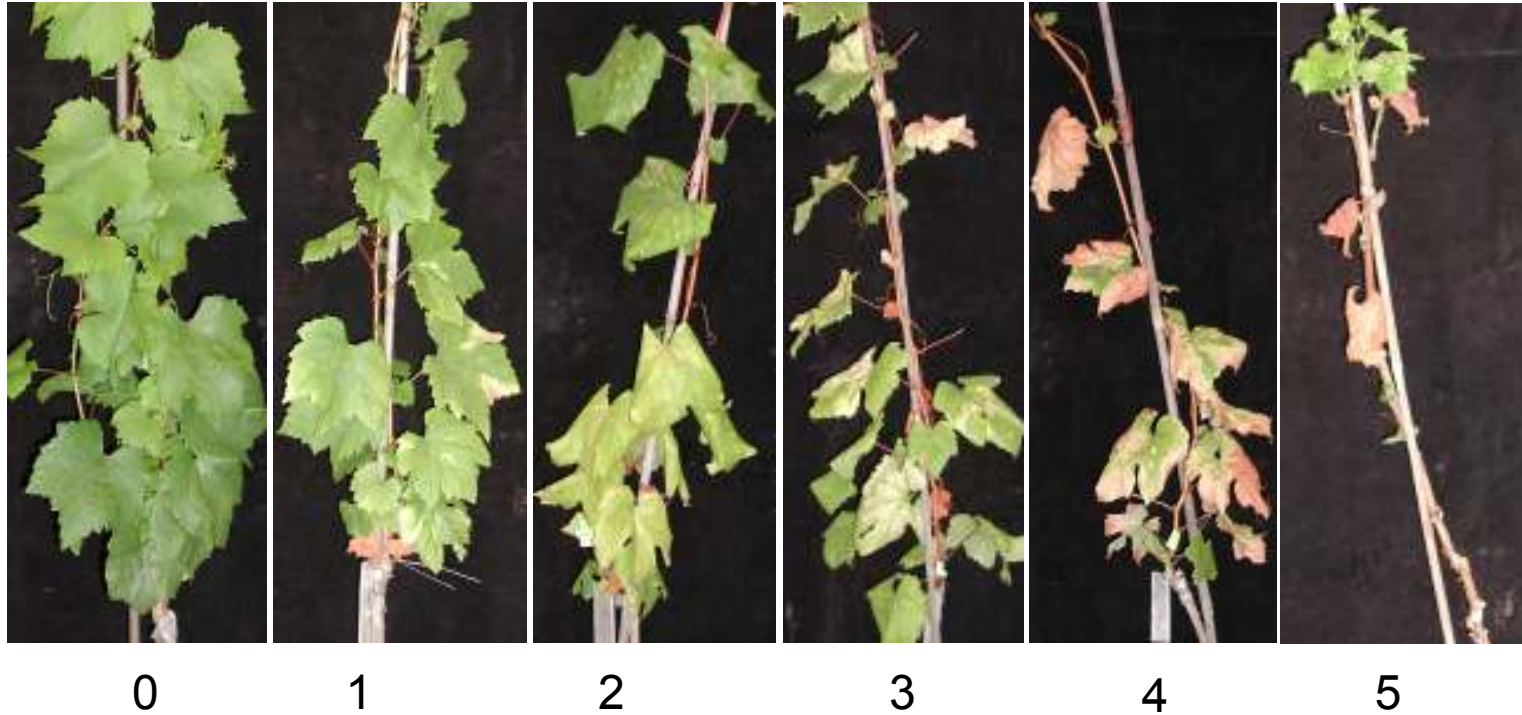
Vines are inoculated by the xylem-feeding vector, *Homalodisca coagulata* (GWSS, the glassy-winged sharpshooter)

The bacterium is introduced to the vine's xylem and spreads through the plant in the using the conductive tissues

# Xylella spreads through Plant by Conductive tissue: Xylem Transport



# Disease Rating Scale for Severity of PD Infection



# Examples of Recent Progress

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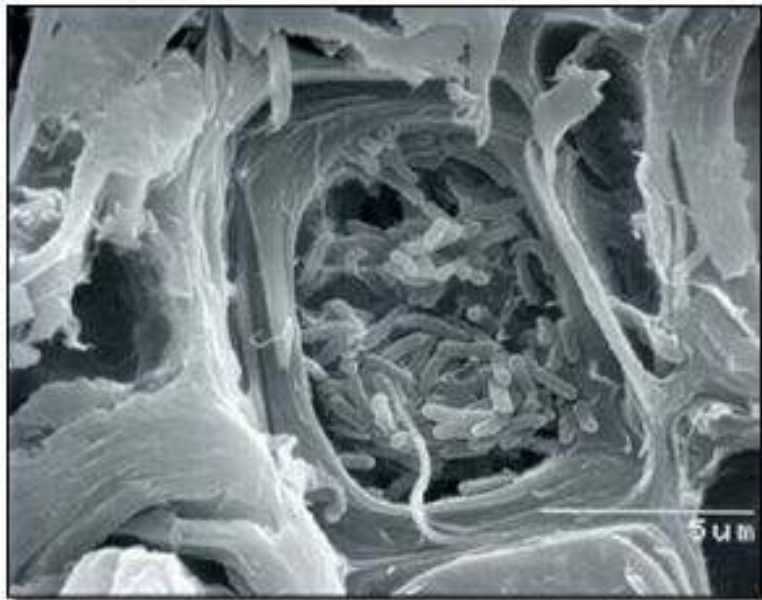


*PD bacterial genes that control disease infection, spread, and severity in grape*

# Example 1: Diffusible Signal Factor (Dsf)



...Selected technology "*Management of Pierce's Disease of Grape by Interfering with Cell-Cell Communication of Xf*" lead by Steven Lindow (UC Berkeley)



- PD virulence is partially attributed to biofilm production
- PD produces a diffusible-signal-factor (DSF) that regulates biofilm production.

**Delicate balance of DSF Concentration:**

**↑[DSF] = ↓[PD] & ↓Transmission**  
**↓[DSF] = ↓Transmission**



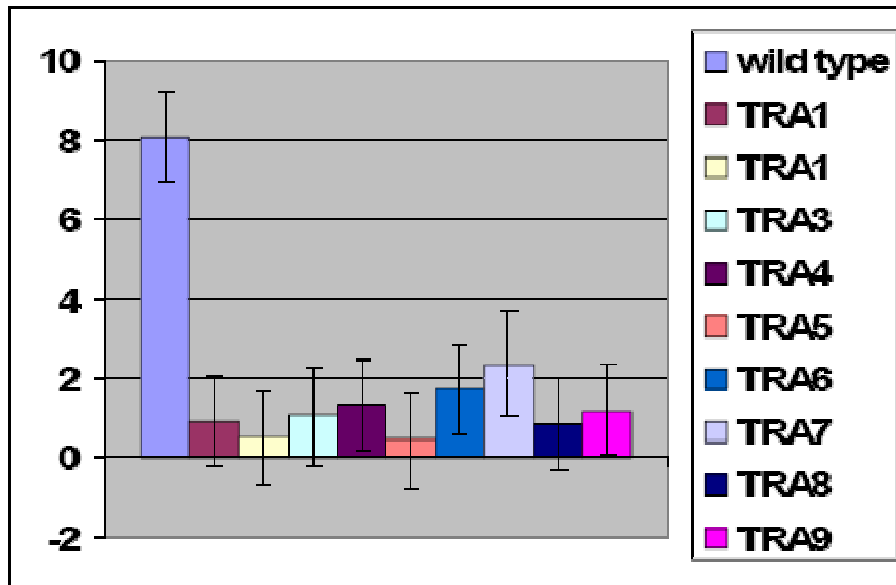
- Up or down regulation of DSF results in decrease of Transmission/PD

# Multiple Application Strategies



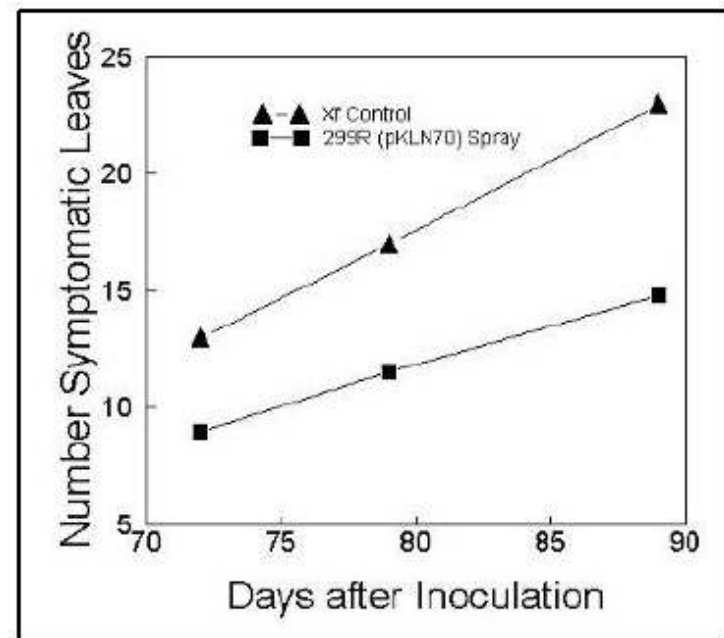
## I. DSF gene put into Grape Rootstocks

GE grape plant that over-expresses DSF exhibit a reduction in disease transmission and onset of symptoms



## II. Topical Application of GE bacteria expressing DSF

Spray grape plants with GM endophytes designed to over-express or degrade DSF (tested- *E. coli*, *E. herbicola*)

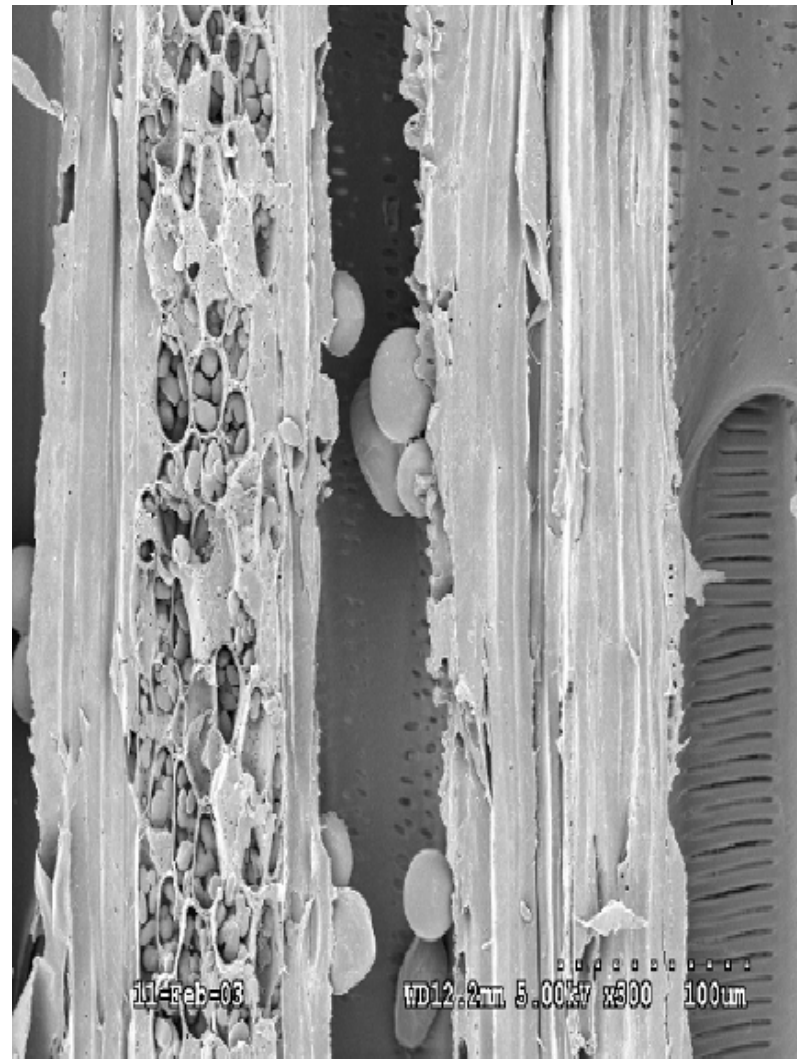
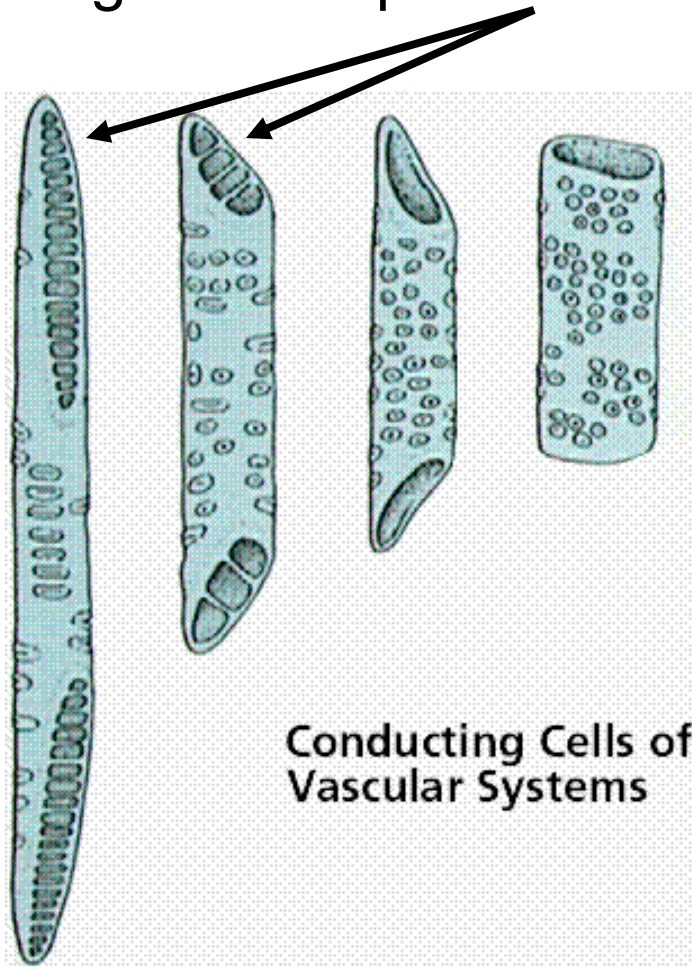


## III. Aerosol Topical Application of Purified DSF

# Example 2: Polygalacturonase (PG)



PG Enzyme helps PD spread throughout the plant



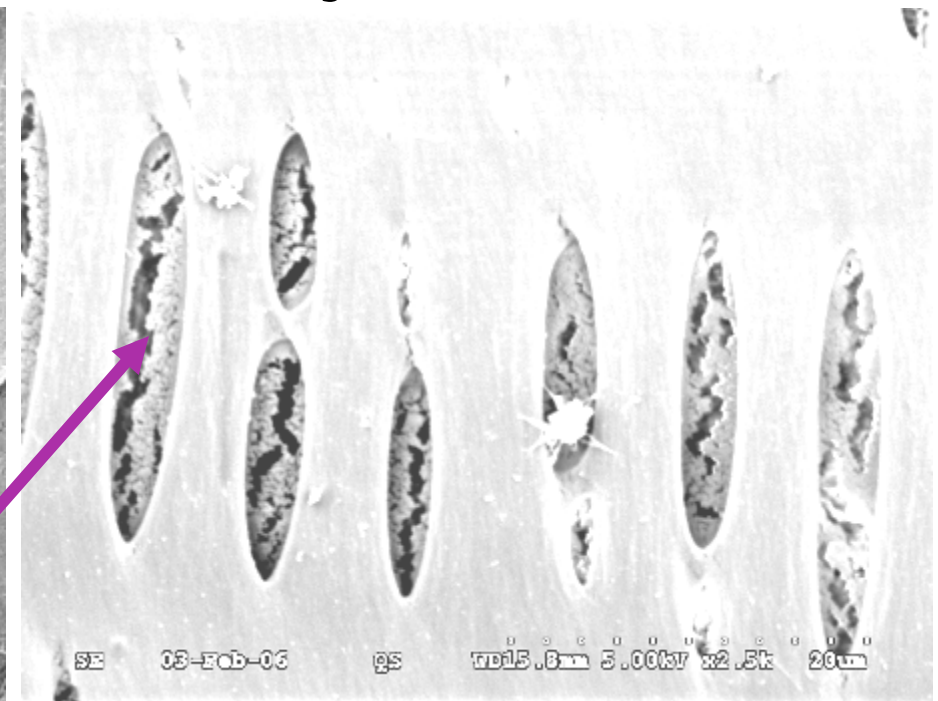
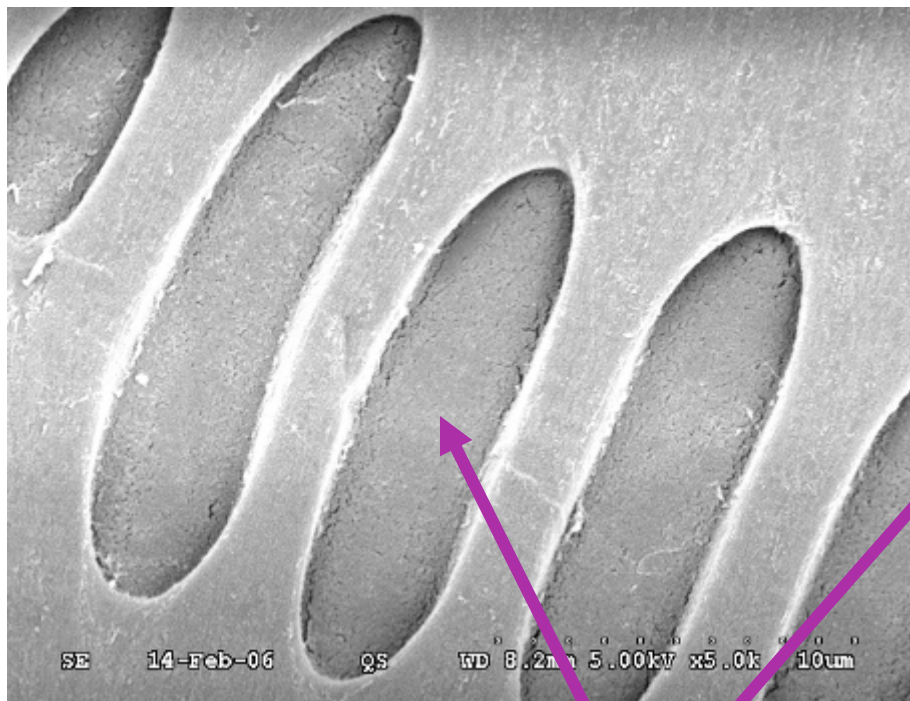
# PG Enzyme Breaks Down Membranes



*PD* produces an enzyme that allows it to breakdown membranes between xylem vessels and spread throughout the plant

***Control***

***Enzyme treated***



**Xylem Vessels (face-on view)**

# “Knocking out” PG Gene Effects Disease Severity



Wild Type *PD*



*PG* (-) mutant *PD*



H<sub>2</sub>O control

13 weeks post-inoculation

# 18 Weeks After Infection



Wild Type *PD*

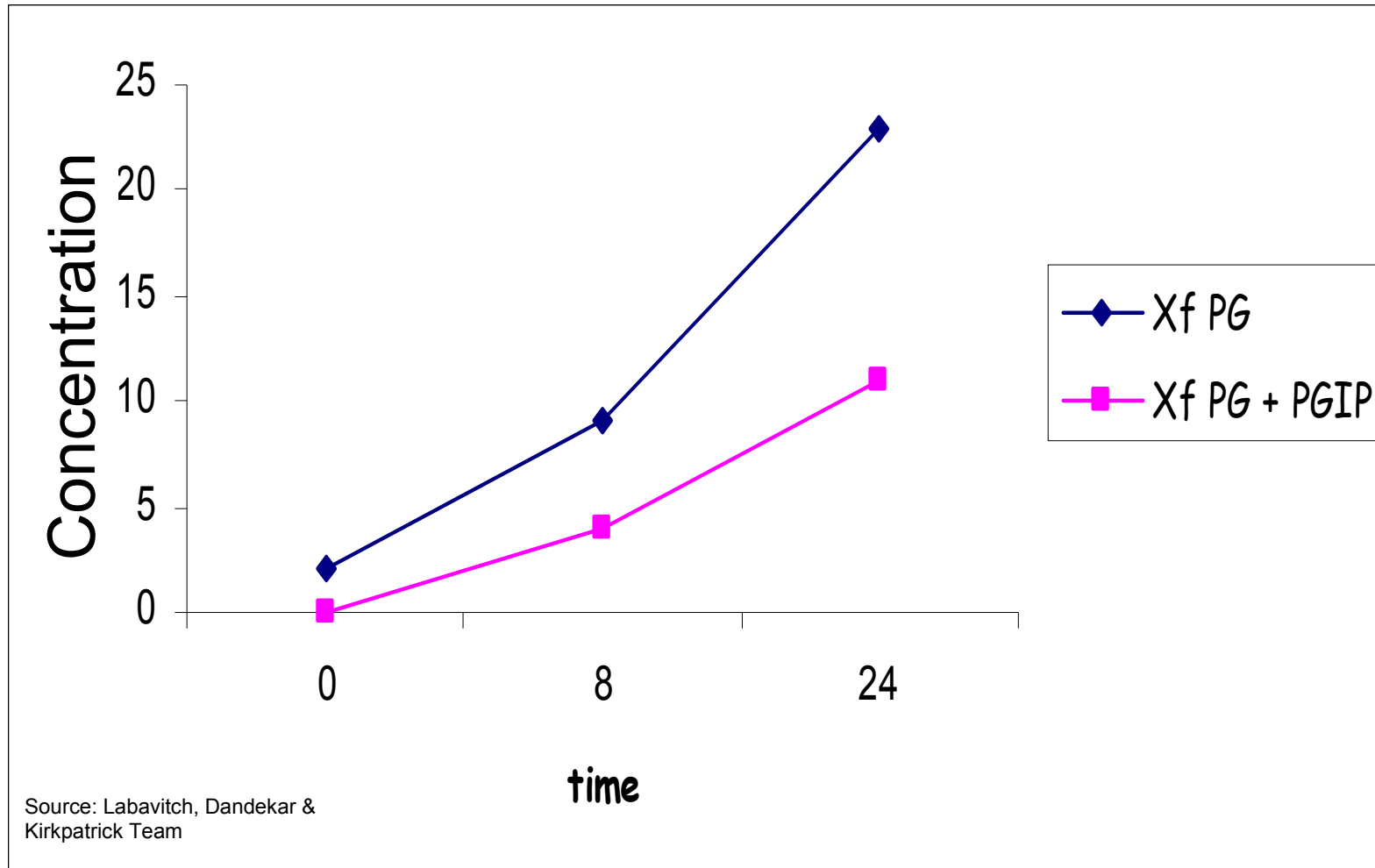


PG (-) *PD*



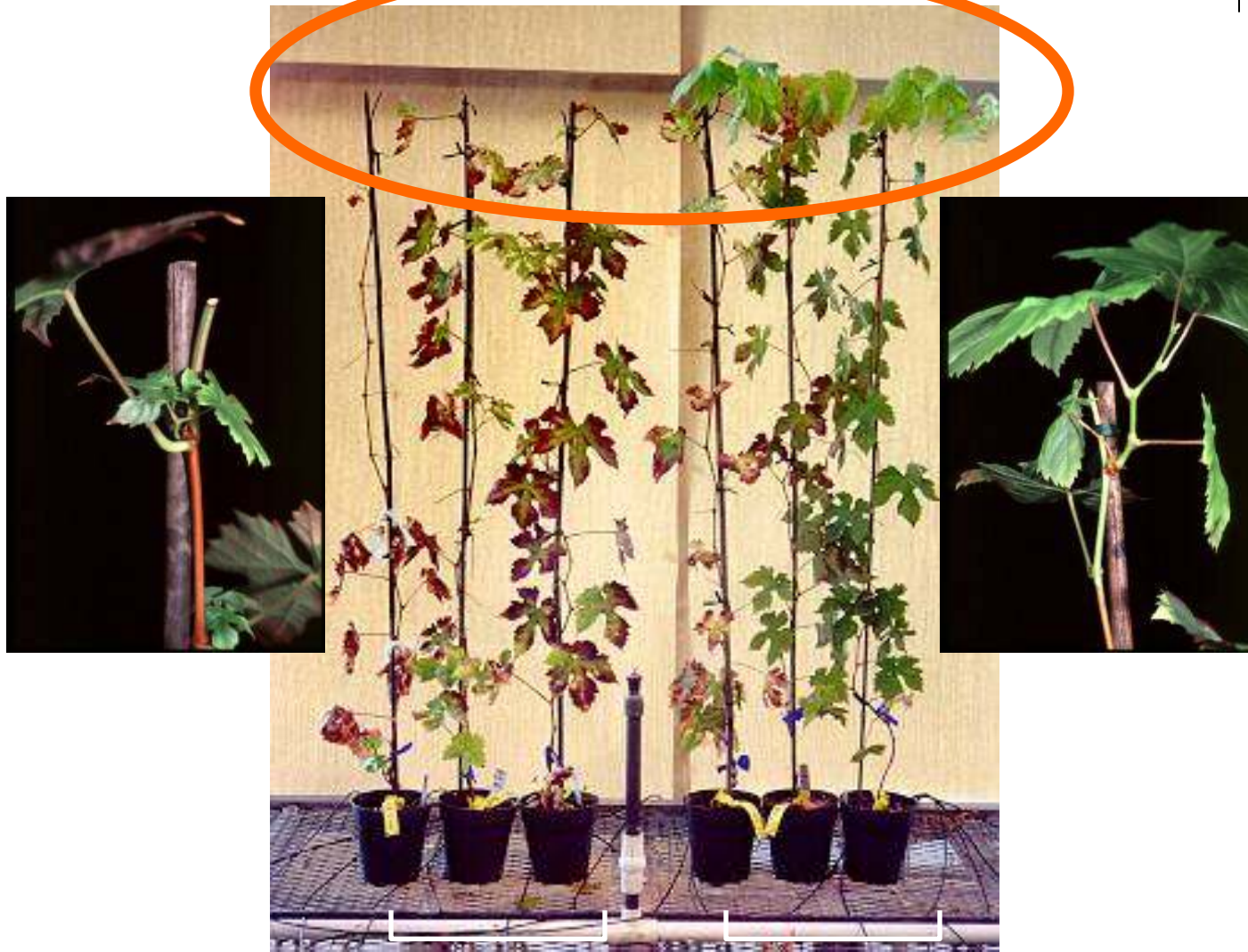
H<sub>2</sub>O control

# PD Control Strategy: PGIP



A Polygalacturonase Inhibiting Protein (*PGIP*)  
was found in Pear

# PGIP Gene Inserted in Grape Shows Reduced PD Symptoms



Source: Labavitch, Dandekar & Kirkpatrick Team

**Controls**

**Grape + PGIP**

Slide 32

# Areas of Significant Progress



- Potential for using transgenic root stocks in combination with non-transgenic scions
- Identification of PD bacterial genes that control disease infection, spread, and severity in grape
- Identification of PD host-specificity determinants
- Insecticidal-based control of GWSS (area-wide control programs)
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# PD Host-Specificity

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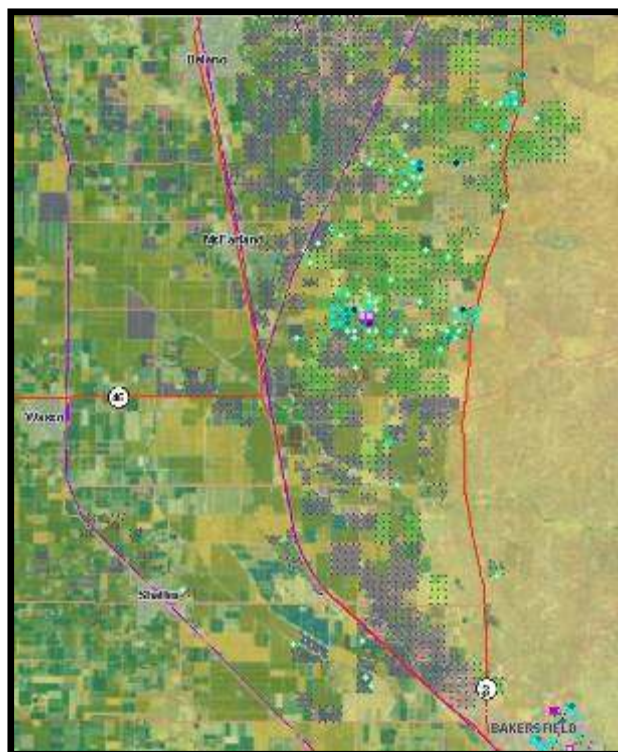
- Entire genetic code of several different PD strains is now available
- Info has lead to development of strain-specific diagnostic tools
- Can now pin-point sources of outbreaks
- Aid in eradication efforts

# Areas of Significant Progress



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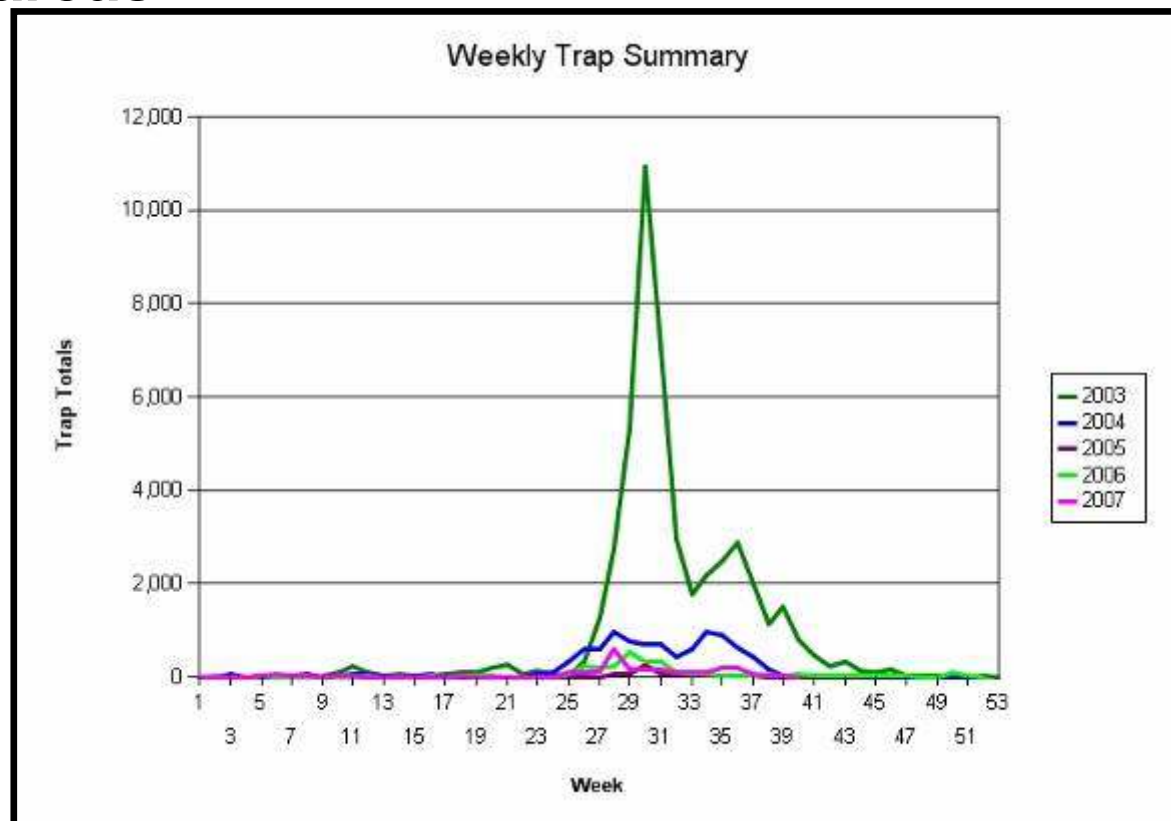
# GWSS Spread Currently Contained



- USDA-APHIS & CDFA PD Control Program
- Comprehensive program for monitoring GWSS populations in key areas

- Partnership with grape and citrus growers
- Strategic insecticide applications

Source: USDA APHIS

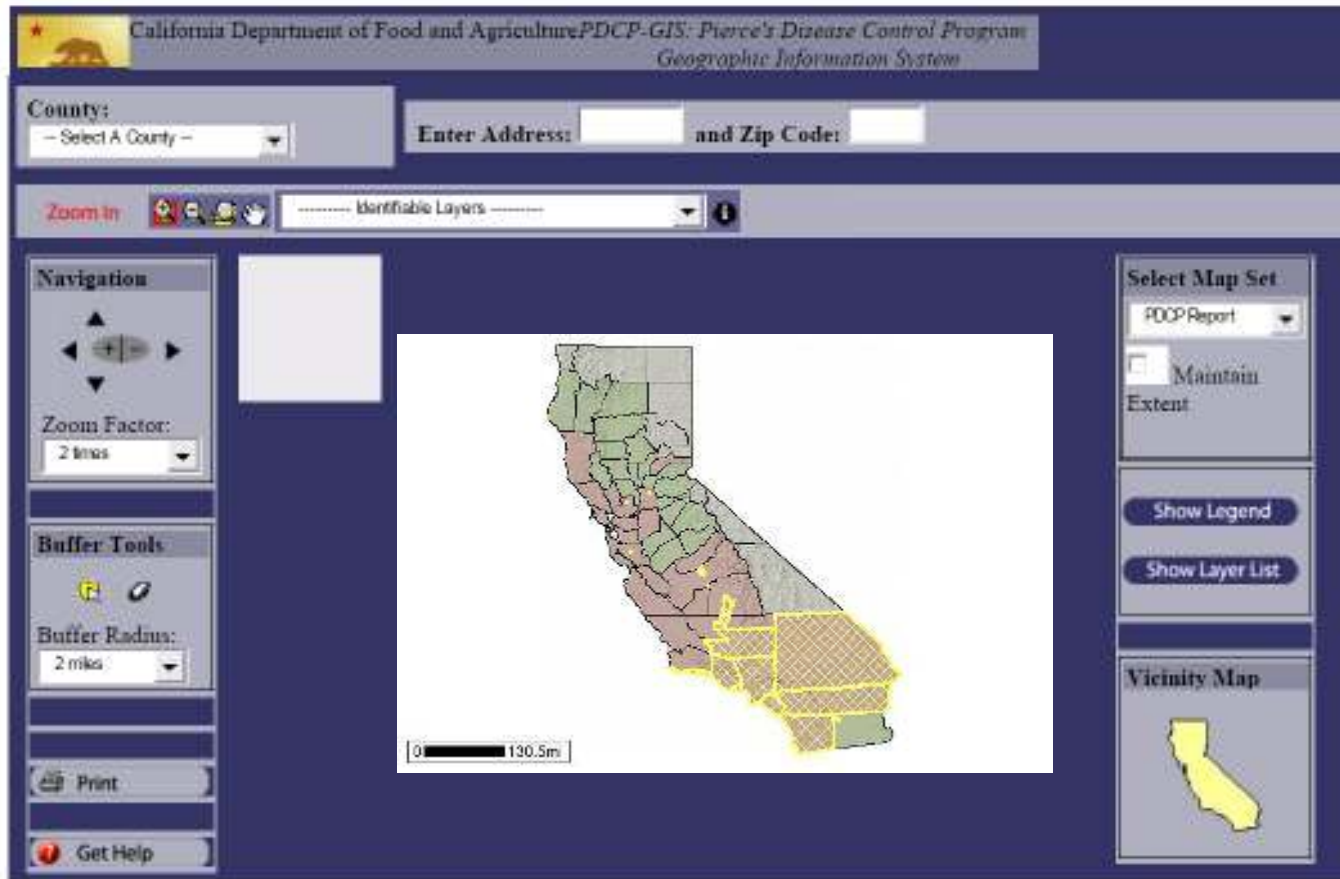


# Areas of Significant Progress



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# Geographic Information System (GIS)



Data on  
GWSS  
behaviors  
and  
movements  
loaded into  
GIS database

**Impact of environmental and biological factors  
on GWSS movement, feeding and reproduction**

# Areas of Significant Progress

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# GWSS Egg Parasitoids Hold Promise: *Recent Advances*



- **New molecular diagnostic tools**
- **Better identification keys for adult insects**

*Improved speed and accuracy in identifying species*

## **Current Applications—**

- Reduce contamination in rearing facilities
- Evaluate the success of field releases

# Areas of Significant Progress



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# PD Resistance Gene (*PDR1*) Found in Wild Grape



- Found by the Walker Lab
- Isolated from *Vitis arizonica*



# Research Gaps Identified by the RSAP

Areas of work that should be  
addressed to accelerate research  
progress

# Lack of Economic Research

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Need an assessment of

- Commercial impact of PD/GWSS
- Current control measures
- Commercial viability of proposed control strategies

*Information needed to validate impact, investment and potential return*

# Information Gaps in Current GIS System

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- Need to integrate additional data sets into GIS to improve utility of the system
  - GWSS population density info, Xylella genotype/strain distribution, PD distribution, vine replacement due to PD, crop information, temperature and geographical info
- Overlapping these additional details will allow researchers to more directly address the source of Xf strains causing PD outbreaks, and determine how it is being vectored

# Additional Gaps

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- Need to Enhance Data Access and Sharing
  - Quicker access to new data and developments
  - Increase sharing of gene discovery data
- More efficient parasitoid rearing methods
  - Time- and labor-intensive
  - Better means to mass produce GWSS eggs or suitable alternative
- Extension/Education Outreach
  - Update current management recommendations
  - Enhance current extension tools
- Need more effort in resistance breeding
  - Possibly multiple sources of PD resistance genes in wild species
  - Currently one gene isolated from one wild species by one laboratory



# RSAP Recommended Research Targets Going Forward

Target Research Areas to Accelerate  
Progress:

*Short-term (impact within 2 years)*

*Long-term (impact in 5 to 10 years)*

# Short-term Targets

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## Promising Tracks

- Refinement of Area Wide GWSS control program
- Id & track PD strains on alternative hosts in relation to strains causing adjacent PD outbreaks

## *Areas to Target (impact in 2 to 5 years)*

- Expansion and enhancement of the GIS database
- Address gaps in economic research and enhancement of extension

# Long-Term Targets

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- Disrupt Pierce's disease by disrupting virulence mechanism
  - DFS, PGIP, and other PD vir genes
  - Explore different delivery systems: modified vines, rootstocks, bacteria, external spray applications, natural and synthetic interfering molecules, etc
- Parasitoids of GWSS—development of better rearing methods
- Marker-assisted selection-based grape breeding for resistance



# Current Activities

Work underway to address RSAP  
Recommendations

# New Research Priorities of Next Research Grant Cycle

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The RSAP recommendations for biological research priorities are being incorporated into the next UC/CDFFA research grant funding cycle

- RSAP research priorities and recommendations integrated into the RFP language
- RSAP research funding priority list included in UC/CDFFA joint research funding program

# Addressing Identified Gaps

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- Economic Research
  - CDFA PD/GWSS Research Program will be accepting economic research proposals this year
  - RFP includes a separate section for economic research priorities and ad-hoc proposal review process



# Pierce's Disease Research & Impact Database

- About this Database
- Documents
- Advisory Panel
- Contact Us
- Login
- Logout

Database Sponsored by



## CDFA Demo Report Submission Form

**Project Leader Name \***

Title First Last Suffix

**Collaborators Names**

**Email \***

**Name of Institution and Address \***

Street Address

Address Line 2

City

State / Province / Region

Postal / Zip Code

Country

**Funding Source \***

CDFA PD/GWSS Board

**Topic Area \***

Vector Biology and Ecology

Reporting Period

The results reported here are from work conducted between the following dates:

**Reporting period start date \***

/  /    
MM DD YYYY

**Reporting period end date \***

/  /    
MM DD YYYY

# Addressing Gaps (cont.)

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## Enhancement of Geographic Information System

- CDFA a potential lead in partnership with research community
- Preliminary discussion underway to overlay additional datasets

# To Summarize

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- Significant research progress in last 4 years
- The majority of the work underway will take time to complete
- Key research areas have been targeted for focused efforts going forward
- Key gaps in economic data and extension components should be addressed
- RSAP recommendations will play a key role in setting research and funding priorities in the coming year



[http://www.cdfa.ca.gov/pdcp/Documents/RSAP\\_Final\\_Report\\_08302007.pdf](http://www.cdfa.ca.gov/pdcp/Documents/RSAP_Final_Report_08302007.pdf)